ABSTRACT

The effect of 250 generations of mutation accumulation (MA) on the second chromosome competitive viability of *Drosophila melanogaster* was analyzed both in homozygous and heterozygous conditions. We used full-sib MA lines, where selection hampers the accumulation of severely deleterious mutations but is ineffective against mildly deleterious ones. A large control population was simultaneously evaluated. Competitive viability scores, unaffected by the expression of mutations in heterozygosis, were obtained relative to a Cy/L2 genotype. The rate of decline in mean $\Delta M \approx 0.1\%$ was small. However, that of increase in variance $\Delta V \approx 0.08 \times 10^{-3}$ was similar to the values obtained in previous experiments when severely deleterious mutations were excluded. The corresponding estimates of the mutation rate $\lambda \approx 0.01$ and the average effect of mutations $E(s) \leq 0.08$ are in good agreement with Bateman-Mukai and minimum distance estimates for noncompetitive viability obtained from the same MA lines after 105 generations. Thus, competitive and noncompetitive viability show similar mutational properties. The regression estimate of the degree of dominance for mild-to-moderate deleterious mutations was $\sim \lambda$, suggesting that the pertinent value for new unselected mutations should be somewhat smaller.

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Genetics 158: 681–693 (June 2001)
jul [λ = 0.05, E(s) = 0.11]. However, the Fry et al. ΔM estimate was not much smaller than Mukai’s, although that of ΔV was larger. It should be noted that experiments with other species in which heterozygosis has not been forced during the MA process also showed a small (Schultz et al. 1999; Keightley and Bataillon 2000) or unappreciable (Staw et al. 2000) fitness decline.

A third design (“middle-class neighborhood”) was used by Shabalina et al. (1997). In this case, populations recently captured from the wild were maintained with single-pair random matings, each contributing a male and a female offspring to be parents in the next generation. Flies were kept under benign conditions but tested in competitive ones. In populations of size N = 200, after 30 generations of mutation accumulation, the average viability decline ΔM observed by Shabalina was 2% per generation, in agreement with Mukai’s data. Nevertheless, although middle-class neighborhood minimizes between-family selection, the effects of mutation accumulation, adaptation, and inbreeding on ΔM estimates cannot be disentangled in outbred populations (Keightley et al. 1998). Moreover, Gilligan et al. (1997) allowed mutations to accumulate in outbred populations of different sizes (N = 25, 50, 100, 250, and 500) and, after 40–50 generations, they did not detect a greater mutational load in the smaller populations, as would be expected if the reduction in fitness was due to deleterious mutations accumulating by drift.

Some aspects of the experimental results cited above raise a number of potential concerns. First, viability was measured in benign (uncrowded) conditions in the Fernández and López-Fanjul experiment, but in competitive conditions in the remaining ones. This may introduce a downward bias if mutations were quasi-neutral in good environments but deleterious in harsh ones (Kondrashov and Houle 1994). However, Fernández and López-Fanjul (1997) found that the mutational heritability due to nonsevere deleterious mutations did not increase with intensified environmental harshness; rather, a high degree of environmental specificity of mutations was detected.

Second, as noted above, a recurrent impediment in the analysis of MA data is the lack of a suitable control to allow unbiased estimation of ΔM. Thus, a fraction of the viability decline observed by Mukai and Ohnishi could be nonmutational, resulting in upwardly biased ΔM estimates (Keightley 1996; García-Dorado 1997). This problem has been obviated by Fry et al. (1999) by using a control where viability remained constant throughout the experiment. In parallel, Fernández and López-Fanjul kept a large control population, but it was only evaluated synchronously with a few MA lines.

Third, Bateman-Mukai bounds for λ and E(s) do not efficiently use the information available, and new statistical approaches have been proposed to compute unbiased estimates from the observed form of the distribution of line means [maximum likelihood (ML), Keightley 1996; minimum distance (MD), García-Dorado 1997]. Similar MD estimates of λ and E(s) (unconstrained by the observed ΔM) have been obtained from Mukai, Ohnishi, Fernández and López-Fanjul, and Fry data [λ = 0.015, E(s) = 0.17], in agreement with Bateman-Mukai bounds found for the two later data sets (García-Dorado et al. 1999).

The coefficient of dominance h of mildly deleterious mutations (i.e., the fraction of the effect s that is expressed in the heterozygote; h = 0, 0.5, and 1 for recessive, additive, and dominant gene action) is also an essential parameter for theoretical predictions in population and quantitative genetics, such as the amount of dominance variance for fitness or the inbreeding depression rate at mutation-selection balance. From Drosophila MA experiments (Mukai 1964; Ohnishi 1974), a widely accepted average E(h) = 0.4 was obtained. At mutation-selection balance, this value implies that a large deleterious mutation rate is required to account for the inbreeding depression of viability observed in natural populations. However, the validity of the previous estimate was recently questioned and a lower one [E(h) = 0.2] was proposed (García-Dorado and Caballero 2000). Obviously, there is substantial need for additional work on this issue.

In this study, the Fernández and López-Fanjul lines were reexamined after a further MA period of 150 generations (255 generations in total). To address the problems stated above, the experimental design was modified as follows. First, competitive viability scores were obtained relative to the Cy/L genotype (instead of the Cy/+ as in the Mukai-Ohnishi-Fry design). Thus, λ and E(s) estimates are independent of the average coefficient of dominance of the mutations involved. However, viability relative to the Cy/+ genotype was also analyzed for comparison. Second, contemporary viability evaluations of MA lines and control were made. Third, regression estimates of the average coefficient of dominance for nonsevere deleterious viability mutations were estimated.

MATERIALS AND METHODS

Base population and inbred lines: A D. melanogaster line isogenic for all chromosomes obtained by Caballero et al. (1991) was used as the base population. From this, 200 MA inbred lines were derived and subsequently maintained with high inbreeding (equivalent to full-sib mating; see Santiago et al. 1992 for further details). Thus, natural selection against mildly deleterious mutations will be ineffective.

The isogenic line carried the recessive eye-color marker sepia (se) in the third chromosome as an indicator of possible contamination from wild-type flies. It was also classified as Q (weak P) or M+ (pseudo-M) for the P-M system of hybrid dysgenesis.

Culture conditions: Flies were reared in the standard medium formula of this laboratory (Brewer’s yeast-agar-sucrose). All cultures were incubated at 25 ± 1°C, 45 ± 5% relative humidity, and maintained under continuous lighting. Flies were handled at room temperature under CO2 anesthesia.

Each inbred line was maintained by a single pair of parents per generation, kept in a glass vial (20 mm diameter, 100 mm...
height) with 10 ml medium added. Oviposition was allowed during 4 days, after which both parents were discarded. This implies that culture densities were low. At emergence, virgin male and female offspring were collected. All offspring of the same sex and line were maintained in the same vial until 4 days old, after which pair matings were individually made and kept in separate vials. One pair was used to perpetuate the line, but up to two spare matings were used when the first failed to reproduce, which can result in some natural selection. At specified generations (see below), lines were tested for viability. At those times, 92 lines survived.

The isogenic line was maintained as a control in bottles (250 ml with 50 ml medium added). The number of bottles was 8 (generations 0 to 200) or 25 (generation 201 onward). A circular mating scheme was used to ensure a large population size (~800–2500 potential parents per generation), which was considered sufficient to minimize the per generation rate of genetic change due to mutation. To make comparisons between lines and controls valid, control flies reared in vials under the same conditions as the inbred lines were used for evaluation.

**Viability assays:** A balanced stock \([\text{In}(2 \text{ L} 2R) \text{ O}, \text{ Cy} \text{ dp}^{b}_{1} \text{ pr} \text{ c}^{n}/\text{L}^{2}]\) marked by the Cy and \(L^{2}\) (Lobe) genes was used, abbreviated as \(\text{Cy} /L^{2}\). For a wild second chromosome, the competitive viability \((V)\) of homozygous \((+/+\)) or heterozygous \((+/+)\) genotypes was measured respectively as the ratio of wild type \((+/+)\) to \(\text{Cy} /L^{2}\) in the progeny of an intercross between five \(\text{Cy} /+\), females and five \(L^{2} /+\), males or five \(\text{Cy} /+\), females and five \(L^{2} /+\), males. The five pairs of parents were placed in a vial (with 10 ml medium added) and the females were allowed to lay during 10 days. Thus, viability assays were carried on in highly competitive conditions, comparable to those obtained by Mukai and colleagues. Our viability estimates are relative to that of the \(\text{Cy} /L^{2}\) genotype (instead of \(\text{Cy} /+\), as in Mukai’s experiments) and, therefore, they are not dependent on the expression of mutations in the \(\text{Cy} /+\) heterozygote. However, a Mukai-like viability measure \((V^{*})\), defined as the ratio of \(+/+\) to \(\text{Cy} /+\) progeny numbers, was also obtained from the data and the corresponding results were analyzed for comparison. All calculations were based on log-transformed data to achieve the normality of the residual errors required by ANOVA. Furthermore, if fitness is multiplicative between loci, log-transformed data are more suitable to compute Bateman-Mukai estimates, which are based on an additive model (see below). Notwithstanding, analyses of the Mukai-like viability measure were also performed on the untransformed data (relative to the corresponding panmictic control average).

As viability determinations are extremely demanding, the experiment was split in two parts as follows (Figure 1):

- **Experiment 1 (viability determination of control chromosomes, \(c^{n}\), in homozygosis and panmixia, simultaneous to that of MA line chromosomes, \(l^{2}\), in homozygosis):** At generation 250, 3 \(\text{Cy} /L^{2}\) males and 3 virgin control females were placed together in each of 100 vials. A single male offspring \(\text{Cy} /c^{n}\) \((i = 1, \ldots, 100)\) was chosen from each vial and was crossed to 4 \(\text{Cy} /L^{2}\) virgin females in a new vial (generation 251). From each of these vials, 20 \(L^{2} /c^{n}\) male and 20 \(\text{Cy} /c^{n}\) virgin female offspring were obtained and placed together in a bottle (generation 252). From the emerging offspring in each bottle (generation 253), 30 \(\text{Cy} /c^{n}\) virgin females and 30 \(L^{2} /c^{n}\) males were chosen and the following intercrosses were made (each replicated three times): (1) 5 \(\text{Cy} /c^{n}\) females by 5 \(L^{2} /c^{n}\) males and (2) 5 \(\text{Cy} /c^{n}\) females by 5 \(L^{2} /c^{n}\) males, to evaluate the viability of control chromosomes in homozygosis (1) and panmixia (2). In parallel, for each line \((j = 1, \ldots, 92)\), 20 virgin females and 20 \(\text{Cy} /L^{2}\) males were placed together in the same bottle (generation 252). From each bottle, 15 \(\text{Cy} /c^{n}\) virgin female and 15 \(L^{2} /c^{n}\) male offspring were obtained (generation 253) and three new vials were established, each with 5 males and 5 females, to evaluate the viability in homozygosis of the chromosome sampled from each line.

- **Experiment 2 (viability determination of control chromosomes in panmixia, simultaneously to that of MA line chromosomes in homozygosis and heterozygosis):** At generation 255, 20 \(\text{Cy} /L^{2}\) males and 20 virgin control females were placed together in each of 100 bottles. From the total offspring obtained (generation 256), 75 intercrosses were made, between each 5 \(\text{Cy} /c^{n}\) virgin females and 5 \(L^{2} /c^{n}\) males, to measure the viability of control chromosomes in panmixia. In parallel, for each line \((j = 1, \ldots, 92)\), 20 virgin females and 20 \(\text{Cy} /L^{2}\) males were placed together in the same bottle (generation 253). From each bottle, 25 \(\text{Cy} /c^{n}\) virgin female and 50 \(L^{2} /c^{n}\) male offspring were obtained (generation 256), and the following intercrosses were made (each replicated five times): (1) 5 \(\text{Cy} /c^{n}\) virgin females by 5 \(L^{2} /c^{n}\) males and (2) 5 \(\text{Cy} /c^{n}\) virgin females (see above) by 5 \(L^{2} /L^{2}\) males, to evaluate the viability of the chromosome extracted from each line both in homozygosis (1) and heterozygosis (2).

Viability \((V)\) data for specific chromosomes were excluded from the analysis if: (1) the total number of \(\text{Cy} /L^{2}\) and wild-type progeny from the three replicates of a given intercross at any experiment was less than 25 (experiment 1, line 21 and 4 \(c^{n}\) chromosomes; experiment 2, line 198); (2) the viability score departed from the mean by \(>3\) standard deviations (experiment 1, line 75; experiment 2, line 35); and (3) the assayed chromosome carried a lethal (experiment 1, 4 \(c^{n}\) chromosomes). Analogous criteria were applied to the analysis of \(V^{*}\) data, leading to the exclusion of lines 35 and 198.

In both experiments (1 and 2), a randomly chosen vial was assigned to each intercross, and the position of the vials in the stock room was randomized. A blind procedure was used for viability determinations.

**Bateman-Mukai estimates of the mutational rate and average effect of mutations:** Assume that: (1) the number of mutations per chromosome and generation is Poisson distributed with parameter \(\lambda\); (2) mutations act additively between loci, with the difference in relative viability between the mutant and the wild-type homozygotes being a random variable \(s\ (0 < s < 1)\), distributed with mean \(E(s)\) and variance \(\sigma^{2};\) (3) mutations accumulate during \(t\) generations in lines derived from a completely homozygous population; (4) the effective size of the lines is small, such that both the within-line genetic variance and the per generation selection effect on gene frequency can be safely ignored; and (5) a genetically invariant control is evaluated in the same environmental conditions as the lines.

In this situation, the per generation rate of mutational decline in the mean \(\Delta M\) and that of increase in the between-line variance \(\Delta V\) of viability can be expressed as \(\Delta M = \lambda E(s)\) and \(\Delta V = \lambda E(s)^{2}\). Thus,

\[\Delta M^{2}/\Delta V = \lambda [1 - \sigma^{2}/E(s)^{2}],\]
\[\Delta V/\Delta M = E(s)[1 + \sigma^{2}/E(s)],\]

and an upper bound for \(\lambda\) and a lower bound for \(E(s)\), usually referred to as Bateman-Mukai estimates (MUKAI et al. 1972), are given by

\[\lambda \geq \Delta M/\Delta V,\]
\[E(s) \leq \Delta V/\Delta M.\]

These estimates will be unbiased only if all mutations affecting the trait have equal effects \((\sigma^{2} = 0)\).

After \(t\) generations of mutation accumulation, the rate of decline in mean can be estimated as \(\Delta M = (m_{a} - m_{c})/F_{c}^{t}\), where \(m_{a}\) and \(m_{c}\) are, respectively, the mean of the lines and the panmictic control for log-transformed viability data \((V)\) or \((V^{*})\), and \(F_{c}^{t}\) is the forward cumulated inbreeding coefficient (WRIGHT 1990: experiment 1, \(F_{c} = 0.245\); experiment 2, \(F_{c} = 0.245\).
Experiment 1. Viability determination of randomly sampled control chromosomes $c_1^+$ in homozygosis and panmixia ($i = 1, \ldots, 100$).

\[ \text{Gen} \]

\[ \begin{align*}
250 & \quad 3 \sigma_1^+ / c_1^+ \times 3 \sigma_1^+ Cy / L' \\
251 & \quad 1 \sigma_1^+ Cy / c_1^+ \times 4 \sigma_1^+ Cy / L' \\
252 & \quad 20 \sigma_1^+ Cy / c_1^+ \times 20 \sigma_1^+ L^2 / c_1^+ \\
253 & \quad 5 \sigma_1^+ Cy / c_1^+ \times 5 \sigma_1^+ L^2 / c_1^+
\end{align*} \]

Homozozogosity $^a$

\[ \begin{align*}
250 & \quad 3 \sigma_1^{i+1} / c_1^{i+1} \times 3 \sigma_1^{i+1} Cy / L' \\
251 & \quad 1 \sigma_1^{i+1} Cy / c_1^{i+1} \times 4 \sigma_1^{i+1} Cy / L' \\
252 & \quad 20 \sigma_1^{i+1} Cy / c_1^{i+1} \times 20 \sigma_1^{i+1} L^2 / c_1^{i+1} \\
253 & \quad 5 \sigma_1^{i+1} Cy / c_1^{i+1} \times 5 \sigma_1^{i+1} L^2 / c_1^{i+1}
\end{align*} \]

Panmixia $^a$

Experiment 1. Viability determination of MA line chromosomes $l_j^+$ in homozygosis ($j = 1, \ldots, 92$).

\[ \begin{align*}
252 & \quad 20 \sigma_1^+ l_j^+ / l_j^+ \times 20 \sigma_1^+ Cy / L' \\
253 & \quad 5 \sigma_1^+ Cy / l_j^+ \times 5 \sigma_1^+ L^2 / l_j^+
\end{align*} \]

Homozozogosity $^a$

Experiment 2. Viability determination of randomly sampled control chromosomes $c_1^+$ in homozygosis and MA line chromosomes $l_j^+$ in homozygosis and heterozygosis ($j = 1, \ldots, 92$).

\[ \begin{align*}
255 & \quad 20 \sigma_1^+ c_1^+ / c_1^+ \times 20 \sigma_1^+ Cy / L' \quad (100 \text{ bottles}) \\
256 & \quad 5 \sigma_1^+ Cy / c_1^+ \times 5 \sigma_1^+ L^2 / c_1^+ \\
257 & \quad 5 \sigma_1^+ Cy / c_1^+ \times 5 \sigma_1^+ L^2 / c_1^+
\end{align*} \]

Panmixia $^b$

\[ \begin{align*}
255 & \quad 20 \sigma_1^+ l_j^+ / l_j^+ \times 20 \sigma_1^+ Cy / L' \\
256 & \quad 5 \sigma_1^+ Cy / l_j^+ \times 5 \sigma_1^+ L^2 / l_j^+
\end{align*} \]

Heterozygosis $^c$

Homozozogosity $^c$

\[ ^a \text{3 replicates} \]
\[ ^b \text{75 replicates} \]
\[ ^c \text{5 replicates} \]

248). To study the expression of deleterious effects across generations, a joint analysis of both experiments (1 and 2) was performed using the average $\Delta M$.

In parallel, the rate of increase in variance can be estimated as $\Delta V = \sigma_i^2 / F_i$, where $\sigma_i^2$ is the between-line component of variance obtained from standard ANOVA techniques. The models adjusted were $y_{ik} = l_i + e_{ik}$ (in each experiment, 1 or 2) or $y_{ik} = l_i + g_j + (lg)_{ij} + e_{ik}$ (in the joint analysis of experiments 1 and 2), where $l_i$ and $g_j$ are, respectively, line ($i = 1, \ldots, 92$) and generation ($j = 1, 2$) random effects; $(lg)_{ij}$ is the line-generation interaction effect; and $e_{ik}$ and $e_{ik}$ are the residual errors corresponding to the $ik$th or the $ijk$th evaluation. Thus,

\[
\sigma_i^2 = (MSL - MSW) / K \quad \text{(one-way ANOVA)}
\]

\[
\sigma_i^2 = (MSL - MSLG) / JK \quad \text{(two-way ANOVA)}
\]

where $MSL$, $MSLG$, and $MSW$ are the between-line, interaction, and within-line mean squares, and $J$ and $K$ are the number of generations and observations per line and generation, respectively. Analogous ANOVAs were performed on the control viability estimates from experiment 1, where a randomly sampled control chromosome is statistically equivalent to a chromosome extracted from a MA line.

Standard errors for the components of variance were computed using standard ANOVA techniques. Those for $\Delta M$ and $\Delta V$ were derived from the variances of the corresponding means and variance components. Since $\lambda$ and $E(s)$ estimates are defined as ratios of variables, their approximate standard errors were obtained by the expansion method (Kendall et al. 1994).

**Average coefficient of dominance of mutations:** Denoting by $+$ the original second chromosome and by $m$ a copy of it
carrying a new mutation, relative viabilities for genotypes +/+,
+/m, and m/m are 1, 1 − h, s, and 1 − s, respectively, where s and h
are, respectively, the homozygous effect and the coefficient of
dominance of that mutation.

The degree of dominance of mutations accumulated in the
dependence to mutation-selection balance and, therefore, that interpreta-
are not used. (56) is likely to be closer to mutation-selection-drift balance than in variance 
variance (Table 2) for MA chromosomes in homozygosis 
the sum of the genetic viability values for both homozygous In both experiments, the between-line component of 
the expression within brackets in the former equation; (2) 
weighted by b. Thus, 
where the summation is over all loci where mutations accumu-
Analogsously, the between-line variance of homozygous viability is 
that of the heterozygous on homozygous viability averages, and C is the ratio of the observed variance of homo-
yzygous viability to its genetic component (C = σ^2/σ^2). Approx-
imate standard errors (SE) of b_{j,00} were obtained as C × SE(b_{.0}).

The degree of dominance of viability mutations segregating in the control population was also investigated using data from generation 250 (experiment 1), where 100 second chromosomes sampled from the control were simultaneously assayed for viability, both in homozygosis and in panmictic pairs. The regression of the average viability of the panmictic pairs on the sum of the genetic viability values for both homozygous parental chromosomes is known to be

\[ b_{j,00} = \frac{\sum p_i q_i \hat{c}_i [h_i + q_i(1 - 2h_i)]}{\sum p_i \hat{c}_i} \]

(Mukai et al. 1972). If all deleterious mutations segregate at low frequency, this expression reduces to

\[ b_{j,00} = \frac{\sum q_i \hat{c}_i h_i}{\sum q_i \hat{c}_i} = E(h_{.0}). \]

Thus, b_{j,00} estimates the average degree of dominance weighted by \hat{c} for the set of copies of deleterious mutations segregating in the control population. This procedure will overestimate the E(h_{.0}) for new mutations as: (1) q could be large for mutations with small deleterious effects, inflating the expression within brackets in the former equation; (2) mutations with smaller deleterious effects, which would be overrepresented in the control population, could be expected to have larger \hat{h} values. For a population at mutation-selection balance, b_{j,00} estimates the harmonic mean of h for new mutations weighted by s (Mukai et al. 1972). However, our control population (with effective population size 100 < N_e < 1000) is likely to be closer to mutation-selection-drift balance than to mutation-selection balance and, therefore, that interpretation is not used.

Simulation procedure: To check whether our results are consistent with predictions from the available mutational models, we simulated the distribution of the average viability of a second chromosome in 10,000 MA lines after t generations of mutation accumulation, the number of deleterious mutations per line following a Poisson distribution of parameter \lambda. We used the procedure outlined by García-Dorado (1997) for gamma-distributed mutational effects. In all cases, the sampling error was made equal to that empirically estimated for viability effects averaged over generations 250 and 255 (\sqrt{MSLG}/\lambda = 0.157). More extensive simulations, studying the effects of natural selection and mutation accumulation both on the lines and the control, have been carried out by A. Caballero, E. Cusi, C. García and A. García-Dorado (unpublished data), and some of their conclusions are considered in the Discussion.

RESULTS

Experimental results: The distributions of second chromosome viabilities (V = ln(number of wild progeny/number of Gy/Lq progeny)) pooled over replicates at the specified generations are shown in Figures 2 (generation 250) and 3 (generation 255) for the MA lines (in homozygosis and heterozygosis, outliers not excluded) and the control (in homozygosis and panmixia), respectively. The corresponding means, variances, and coefficients of asymmetry and kurtosis are given in Table 1. All flies scored were sepia homozygotes, indicating that no contamination from wild-type flies occurred

In both experiments, the mean viability of chromosomes from MA lines (in homozygosis) was significantly lower than that of control chromosomes (in panmixia). In all instances, the coefficients of asymmetry and kurtosis did not significantly depart from the normal distribution values (g_3 = g_4 = 0). This is to be expected after a long period of mutation accumulation.

In both experiments, the between-line component of variance (Table 2) for MA chromosomes in homozygosis was significantly larger than zero, but it was nonsignificant in the remaining cases (control chromosomes in homozygosis and panmixia and MA chromosomes in heterozygosis). Within experiments, the mean viability of MA (or control) chromosomes in homozygosis was significantly lower than that in heterozygosis (or panmixia), and the corresponding between-line variance was larger (significant in the case of MA chromosomes). The control population showed a small but significant rate of inbreeding depression of 0.077%. Extrapolating to the whole genome, this is ~20% of the inbreeding depression rate observed in outbred populations (Mackay 1985; López-Fanjul and Villaverde 1989; García et al. 1994).

A summary of mutational parameter values is given in Table 3. On the whole, the rate of viability mutational decline for MA second chromosomes in homozygosis (ΔM) ranged from 0.04 to 0.15% and that of increase in variance (ΔV) was ~0.1 x 10^{-3}. Consequently, across-generation estimates of the mutational rate were low (~0.01). In parallel, the E(s) estimate for deleterious mutational effects averaged over generations was relatively small (~0.08). This result illustrates the absence
Figure 2.—Second chromosome mean viability ($V$) distributions at generation 250: (A) MA lines in homozygosis; (B) control in panmixia; (C) control in homozygosis.

Figure 3.—Second chromosome mean viability ($V$) distributions at generation 255: (A) MA lines in homozygosis; (B) control in panmixia; (C) MA lines in heterozygosis.
of mutations that drastically and consistently reduce viability in our MA lines.

Finally, estimates of the weighted average degree of dominance for the control (which overestimates that of new mutations) and the MA lines consistently suggested a value around 0.3, although only that for generation 255 reached statistical significance. This indicates partial recessivity of chromosomal effects, both in the MA lines and the control. It should be mentioned that estimates for generation 255 \( [E(h_{w,2}) = 0.328] \) and for generations 250–255 \( [E(h_{w,2}) = 0.323] \) are not independent, as they both rely on the same set of heterozygous evaluations. Moreover, for the MA lines, estimates of \( E(h_{w,2}) \) are associated with small \( E(s) \) values.

The moments of the empirical distribution of the average viability of the lines, measured as \( \bar{V}^* = \ln(\text{number of wild progeny/number of Cy/L}_2 \text{ progeny}) \), are given in Table 4. Means were always smaller than those for the viability estimate \( \bar{V} \), because the viability of \( \text{Cy/+} \) heterozygotes was larger than that of \( \text{Cy/L}_2 \) ones. However, for both viability measurements, comparison of means and variances between different groups gives a qualitatively similar picture, and the corresponding distributions did not depart significantly from normality in any case. Mutational parameters for \( \bar{V}^* \) are given in Table 5. Estimates of \( \Delta M \) were remarkably similar to those for \( \bar{V} \), but those for \( \Delta V \) were about one-half. This resulted in larger estimates of \( \lambda \) (about twice) and lower estimates of \( E(s) \) (about one-half) than those obtained for \( \bar{V} \) (Table 3). Mutational parameters estimated from untransformed data are also given in Table 5, and they did not substantially differ from those obtained for log-transformed data.

To investigate the expression of accumulated mutations on \( \text{Cy/+} \) genotypes, a two-way ANOVA was performed on the logarithm of the ratio of \( \text{Cy/+} \) to \( \text{Cy/L}_2 \) (generations 250–255), a nonsignificant between-line component of variance being obtained (\( \sigma^2 = 0.002 \), with \( P < 0.65 \)). Furthermore, a nonsignificant correlation (0.04) was calculated between \( \bar{V} \) (the logarithm of the ratio \( +/+ \) to \( \text{Cy/L}_2 \)) and the logarithm of the ratio \( \text{Cy/+} \) to \( \text{Cy/L}_2 \), measured in different generations. These results show that mutations affecting viability in the homozygous condition did not express a correlated effect in \( \text{Cy/+} \) heterozygotes.

For the degree of dominance in the control, estimates obtained for \( \bar{V}^* \) (untransformed and log-transformed data) behave much more erratically than those for \( \bar{V} \). However, the only ones significantly larger than zero were those for MA chromosomes at generation 255 (\( \bar{V} \) and \( \bar{V}^* \)), which were very similar to each other.

Simulation results: The three sets of mutational parameter values used to simulate the distribution of the average viability of second chromosomes at generation 250 are given in Table 6. The first set corresponds to minimum distance estimates obtained for noncompeti-

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### Table 1

<table>
<thead>
<tr>
<th>Generation</th>
<th>No. of lines</th>
<th>( \bar{X} )</th>
<th>( \sigma^2 \times 10^2 )</th>
<th>( \sigma )</th>
<th>( \sigma )</th>
</tr>
</thead>
<tbody>
<tr>
<td>MA lines (homozygosis)</td>
<td>250</td>
<td>86</td>
<td>0.341 ± 0.029</td>
<td>7.447</td>
<td>0.068 ± 0.260</td>
</tr>
<tr>
<td>Control (homozygosis)</td>
<td>250</td>
<td>83</td>
<td>0.370 ± 0.025</td>
<td>5.310</td>
<td>0.273 ± 0.266</td>
</tr>
<tr>
<td>Control (panmixia)</td>
<td>250</td>
<td>87</td>
<td>0.447 ± 0.027</td>
<td>5.814</td>
<td>0.468 ± 0.266</td>
</tr>
<tr>
<td>MA lines (homozygosis)</td>
<td>255</td>
<td>86</td>
<td>0.424 ± 0.026</td>
<td>6.037</td>
<td>−0.237 ± 0.260</td>
</tr>
<tr>
<td>MA lines (heterozygosis)</td>
<td>255</td>
<td>86</td>
<td>0.867 ± 0.025</td>
<td>5.203</td>
<td>0.014 ± 0.260</td>
</tr>
<tr>
<td>Control (panmixia)</td>
<td>255</td>
<td>25</td>
<td>0.799 ± 0.069</td>
<td>12.000</td>
<td>0.210 ± 0.464</td>
</tr>
</tbody>
</table>

See text for further explanation. \( \bar{V} = \ln(\text{number of wild progeny/number of Cy/L}_2 \text{ progeny}) \), replicates pooled.

### Table 2

Between-line (\( \sigma^2_1 \)) and residual (\( \sigma^2_2 \)) components of variance for second chromosome viability (\( \bar{V} \))

<table>
<thead>
<tr>
<th>Generation no.</th>
<th>MA lines (homozygosis)</th>
<th>Control (homozygosis)</th>
<th>Control (panmixia)</th>
<th>MA lines (heterozygosis)</th>
<th>MA lines (homozygosis)</th>
<th>MA lines (heterozygosis)</th>
<th>MA lines (panmixia)</th>
</tr>
</thead>
<tbody>
<tr>
<td>250</td>
<td>0.0272*</td>
<td>0.0166</td>
<td>0.0088</td>
<td>0.0321***</td>
<td>0.0064</td>
<td>0.0186***</td>
<td></td>
</tr>
<tr>
<td>255</td>
<td>0.142</td>
<td>0.133</td>
<td>0.146</td>
<td>0.195</td>
<td>0.275</td>
<td>0.177</td>
<td></td>
</tr>
</tbody>
</table>

\( \bar{V} = \ln(\text{number of wild progeny/number of Cy/L}_2 \text{ progeny}) \), *P < 0.05; **P < 0.005; ***P < 0.001.

*Line \times generation component of variance was not significant (\( \sigma^2_{1g} = 0.0039 \)).
tive relative viability measured at generations 104–106 in the same MA lines used in this experiment (GARCÍA-
DORADO et al. 1998; adjusted for the second chromo-
some by multiplying the gametic mutation rate by 0.4).
The remaining sets roughly assume the rate of muta-
tional decline and the increase in variance obtained by
MUKAI et al. (1972) for second chromosome competitive
relative viability ($\Delta M = 4 \times 10^{-3}$, $\Delta V = 8 \times 10^{-3}$ for
quasi-normal lines, i.e., those with relative viability >2/3).
These values imply Bateman-Mukai estimates $\lambda \geq 0.2$
and $E(s) \leq 0.02$. Two different shape parameters
for the gamma distribution of mutational effects were used
($\alpha = 1$, $\alpha = 0.1$), giving different $E(s')$ values for the
whole group of nonlethal mutations.

For each set of mutational parameter values, the distri-
bution of the average relative viability (deviated from the
original average) of 10,000 simulated MA lines is
given in Figure 4. The observed distribution of the mean
viability of the MA lines, averaged over generations 250
and 255 and deviated from the corresponding control
average, is also given in Figure 4. It should be stressed
that the mutational parameters used have been ob-
tained from different data sets, in which viability was
estimated either as the proportion of emerged adults
(MD estimates), or from the proportion of wild-type
genotypes (competitive estimates). In spite of this, it is
remarkable that the only simulation result reasonably
fitting the empirical data for a competitive viability mea-
sure (generations 250–255) was that obtained using the
mutational parameter values for noncompetitive viability
previously estimated in the same MA lines (MD esti-
mates from generations 104–105).


discussion

After 250 generations of mutation accumulation, the
long-term second chromosome competitive viability
changes observed in our inbred MA lines were charac-
terized by the corresponding mutational parameters.
These were remarkably similar to those observed for
noncompetitive viability in the same MA lines after 105
generations (FERNÁNDEZ and LÓPEZ-FANJUL 1996; GARCÍA-
DORADO et al. 1998). In both instances, the results indi-
cate that mild deleterious mutations occurred at a low
rate ($\lambda = 0.01$), with homozygous effects distributed
around a mean value $E(s) = 0.1$. These values are at odds
with classical Mukai-Ohnishi estimates, these implying
a 10-fold greater rate of mutations with a smaller average
effect and gene action close to additive. The validity of
our experimental approach is examined in the first part
of this section, the second and third parts concentrating
on the analysis of the results.

the experimental design: The number of mutations
accumulated in the lines will increase with the length of
the period considered and, therefore, the experimental
power to detect the rate of viability decline due to mild
or even tiny deleterious mutations will also increase with
time. On the other hand, with the accumulation of

<table>
<thead>
<tr>
<th>TABLE 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutational parameters for second chromosome viability ($\lambda$)</td>
</tr>
<tr>
<td>Generation</td>
</tr>
<tr>
<td>250</td>
</tr>
<tr>
<td>255</td>
</tr>
<tr>
<td>Joint analysis</td>
</tr>
</tbody>
</table>

See text for further explanation. $\lambda = \ln(\text{number of wild progeny/number of } C_y/L^2 \text{ progeny})$. *P < 0.05; ***P < 0.005; ***P < 0.001 (one-tailed tests).

$^*$ Since the distributions of $\lambda$ and $E(s)$ estimates are unknown, significance tests were not performed.

$^*$ For the pool of mutant copies in the control population.

<table>
<thead>
<tr>
<th>TABLE 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (X), variance ($\sigma^2$), and coefficients of asymmetry ($g_1$) and kurtosis ($g_2$) of the distribution of Mukai-type second chromosome viability ($\lambda^*$) of MA lines and control</td>
</tr>
<tr>
<td>Generation</td>
</tr>
<tr>
<td>MA lines (homozygosis)</td>
</tr>
<tr>
<td>Control (homozygosis)</td>
</tr>
<tr>
<td>Control (panmixia)</td>
</tr>
<tr>
<td>MA lines (homozygosis)</td>
</tr>
<tr>
<td>MA lines (heterozygosis)</td>
</tr>
<tr>
<td>Control (panmixia)</td>
</tr>
</tbody>
</table>

See text for further explanation. $\bar{X} = \ln(\text{number of wild progeny/number of } C_y \text{ progeny})$, replicates pooled.
increasing numbers of deleterious mutations, the shape of the distribution of line means will approach that of a normal curve, thus hindering the study of the properties of individual mutations. For instance, after removing just a single outlier in generations 250 or 255, none of the distributions of the mean viability of MA lines departed significantly from normality. This precluded the estimation of mutational parameters by methods based on the information contained in the shape of those distributions (minimum distance or maximum likelihood). In these circumstances, we chose to exclude those outliers from the analysis, restricting our study to nonsevere deleterious mutations resulting in a normal distribution of the average viability of the MA lines.

After a long period of mutation accumulation, natural selection could become increasingly relevant. Simplifying the situation, we can consider selection acting at two levels: (1) reducing the fixation rate of mutations with increasingly deleterious effects; (2) bringing to extinction those lines with an excessive fixation load.

The first phenomenon will take place from the beginning of the experiment and, in principle, its intensity will be constant through time. However, as overall fitness deteriorates, the occurrence of new nonmild deleterious mutations will be more likely to force the use of the spares kept for the replacement of failed vials. This would increase the efficiency of natural selection against moderate or severe deleterious mutations occurring at later stages, hampering the accumulation of nonmild deleterious mutations in our inbred MA lines. Thus, we are not estimating the original rate and distribution of effects of all deleterious mutations, but those of mutations escaping natural selection.

The second one, line extinction, is expected to increase in importance with time. Once an important fraction of lines has been lost due to mutational load, the number of deleterious mutations in the surviving ones could no longer be considered Poisson distributed. This might render even the Bateman-Mukai method inappropriate. In particular, if the number of mutations accumulated per line is Poisson distributed, and lines are lost when that number exceeds a fixed value, the estimate of $\Delta V$ will be reduced by a larger factor than that of $\Delta M^2$. Therefore, Bateman-Mukai estimates of $\lambda$ [or $E(s)$] for the subset of nonsevere deleterious mutations accumulating in our lines will be biased upward (or downward).

On the other hand, our estimates of $\Delta M$ rely on the validity of the control population. This was maintained with large effective size (say $N_e > 100$ to be conservative), but some mild deleterious mutations could have accumulated. Furthermore, tiny mutations (say those with $s < 5 \times 10^{-4}$) will accumulate freely. There is no information on the behavior of the control population for the competitive viability measure obtained in this experiment. However, data are available for noncompetitive viability (measured as the proportion of adults emerged from the eggs laid). For this trait, there is no indication of a temporal decrease of the control average (0.66 in generations 104–106, Fernández and López-Fanjul 1996; and 0.62 in generation 210, A. Caballero, E. Cusi, C. García and A. García-Dorado, unpublished results), suggesting that mutations of mild or tiny deleterious effects did not cause a detectable viability decline after 210 generations.

### Table 5

<table>
<thead>
<tr>
<th>Generation</th>
<th>$\Delta M \times 10^6$</th>
<th>$\Delta V \times 10^6$</th>
<th>$\lambda^*$ (lower bound)</th>
<th>$E(s)^*$ (upper bound)</th>
<th>$h_{v,2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>250*</td>
<td>0.046 ± 0.013***</td>
<td>0.051 ± 0.038</td>
<td>0.0041 ± 0.0088</td>
<td>0.111 ± 0.089</td>
<td>0.117 ± 0.205*</td>
</tr>
<tr>
<td>255*</td>
<td>0.140 ± 0.023***</td>
<td>0.087 ± 0.029***</td>
<td>0.0226 ± 0.0106</td>
<td>0.062 ± 0.023</td>
<td>0.351 ± 0.165*</td>
</tr>
<tr>
<td>Joint analysis*</td>
<td>0.093 ± 0.014***</td>
<td>0.029 ± 0.020</td>
<td>0.0297 ± 0.0436</td>
<td>0.031 ± 0.022</td>
<td>0.037 ± 0.577</td>
</tr>
<tr>
<td>Joint analysis*</td>
<td>0.090 ± 0.016***</td>
<td>0.025 ± 0.013</td>
<td>0.0315 ± 0.0235</td>
<td>0.0285 ± 0.015</td>
<td>0.243 ± 0.548</td>
</tr>
</tbody>
</table>

See text for further explanation. *$P < 0.05$; ***$P < 0.001$ (one-tailed tests).

* $\ln($number of wild progeny/number of $Cy$ progeny).

* Number of wild progeny/number of $Cy$ progeny.

* Since the distributions of $\lambda$ and $E(s)$ estimates are unknown, significance tests were not performed.

* For mutations accumulated in the control.

### Table 6

<table>
<thead>
<tr>
<th>Source</th>
<th>$\Delta M^*$</th>
<th>$\lambda^*$</th>
<th>$\alpha$</th>
<th>$E(s)$</th>
<th>$E(s)^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>MD</td>
<td>0.0006664</td>
<td>0.0064</td>
<td>3.35</td>
<td>0.103</td>
<td>0.0137</td>
</tr>
<tr>
<td>Mukai</td>
<td>0.004</td>
<td>0.2</td>
<td>1.00</td>
<td>0.020</td>
<td>0.0008</td>
</tr>
<tr>
<td>Mukai</td>
<td>0.004</td>
<td>0.2</td>
<td>0.10</td>
<td>0.020</td>
<td>0.0044</td>
</tr>
</tbody>
</table>

See text for further explanation.

* MD estimates from generations 104–105 (García-Dorado et al. 1998).

* Bateman-Mukai estimates from Mukai et al. (1972) with two different $\alpha$’s assumed.

* Adjusted for the second chromosome when necessary.
Figure 4.—Second chromosome homozygous mean viability distributions. (A) Observed distribution for $\Psi$, averaged over generations 250–255 and deviated from the control mean. Relative viability distributions deviated from the original value, simulated using (B) MD estimates of mutational parameters, (C) Mukai’s mutational parameters with $\alpha = 1$, and (D) Mukai’s mutational parameters with $\alpha = 0.1$ (see text for explanation).

The effect of within-line selection and selective line extinction, as well as that of mutation accumulation in the control population, has been explored by A. Caballero, E. Cusi, C. García and A. García-Dorado (unpublished results) through extensive simulation. They used two models, both assuming gamma-distributed mutational effects, which were qualitatively similar to our Mukai and MD models (Table 6), respectively: (1) the “common mild deleterious mutations” model [$\lambda = 0.17$ for the second chromosome, $E(s) = 0.026$, $\alpha = 0.5$]; and (2) the few mutations model, with larger deleterious effects [$\lambda = 0.0044$ for the second chromosome, $E(s) = 0.191$, $\alpha = 3.12$]. Fitness was made multiplicative across loci and mutations accumulated on the whole Drosophila genome. Adjusting log-transformed results for the number of generations (250) of mutation accumulation on the second chromosome in our experiment, simulation showed that under the few mutations model, the final decline in the control population log-fitness was very small (0.02), but under the common mutation model it was more substantial (0.25). Even so, the rate of MA log-fitness decline [computed by reference to the control population, i.e., $\Delta M = (m_t - m_0)/F_t$] simulated under the common mutation model was 0.33% in the absence of selection and 0.29% when selection occurred both within and between lines, accounting for line extinction. These values were larger than our empirical estimate (0.09%), which was more consistent with results simulated using the few mutations model ($\Delta M = 0.02\%$ and $\Delta M = 0.08\%$ for the cases with and without selection, respectively).

At the beginning of the experiment (generations 0–20), line losses occurred at a rate $r = 0.0015$. Taking this value as a constant rate of accidental loss, we can compute the expected number of surviving lines at generation $t$, in the absence of purging selection, as $N_t = N_{t-1} \exp(-rt)$ ($N_{t-1}$ being the number of surviving lines in a previous generation where records are available, and $t$ the number of generations elapsed). From the 200 initial lines, the expected and observed numbers of lines
surviving at those generations where records were available are shown in Figure 5. Up to generation 161, line extinction can be wholly attributed to accidents, no purging of lines being detected. Part of the line losses between generations 161 and 208 are known to be due to a bacterial infection that occurred at about generation 200 and, therefore, they are not directly related to the fixation load (GARCÍA-DORADO et al. 2000). From generation 208 to 255, the number of lines declined from 111 to 93, instead of the expected 103. This suggests that 9% of the lines present at generation 208 could have been purged by generation 255. Alternatively, line extinction could be due partly to selection acting from the beginning of the experiment. Even in this case, the roughly constant rate of line extinction, illustrated in Figure 5, suggests that the effect of extinction on the estimates of mutational parameters does not necessarily increase at later stages. Thus, we have computed Bateman-Mukai estimates for $\lambda$ and $E(s)$, assuming that their respective upward or downward bias, attributable to purging selection, will not be large.

The effectiveness of selection against deleterious mutation can be calculated from classical diffusion theory (KIMURA 1962). Although derived for large populations and small deleterious effects, this theory gives good approximations even for full-sib lines and deleterious effects up to $s = 0.4$ (simulation results by CABALLERO et al. 1996). Following Kimura, selection will be ineffective against most mutations with $s < 1/4N_c$. In full-sib MA lines ($N_c = 2.5$, initial frequency 0.25), the fixation probability of mutations with $s = 0.1$ or $s = 0.05$ will be 0.82 and 0.91 times that of neutral mutations, respectively. This result is also supported by simulation results obtained by A. CABALLERO, E. CUSI, C. GARCÍA and A. GARCÍA-DORADO (unpublished results), even for the case in which selection accounts for all observed line extinction. Thus, we consider that mutations with a mildly detrimental effect on fitness accumulate in our lines roughly as if they were neutral, with a large fraction of mutations with fitness $s > 0.2$ being removed by selection. It should be noted that, although diffusion results apply to deleterious effects for overall fitness, in our MA lines we only measure viability. Therefore, mutations that are mild for viability but more severe for fitness could be eliminated by selection, thus escaping our analysis. However, this does not undermine the validity of our conclusions, either from the evolutionary or the conservationist viewpoint, where interest focuses on deleterious mutations with a mild effect on fitness.

Our estimate of viability $[\mathcal{V} = \ln(\text{number of wild progeny/number of } Cy/L^2 \text{ progeny})]$ is not affected by the expression of deleterious mutations in heterozygosis with the $Cy$ chromosome, but part of these effects could be masked when using the Mukai-like viability measurement $[\mathcal{V}^* = \ln(\text{number of wild progeny/number of } Cy \text{ progeny})]$. We found that the use of $\mathcal{V}^*$ (untransformed or log-transformed data) overestimates $\lambda$ and underestimates $E(s)$, as part of the increase in the between-line variance is concealed. However, no substantial differences were found between the estimates of mutational parameters for both viability measurements. Hereafter, we refer only to estimates obtained for the safer viability measurement ($\mathcal{V}$).

The rate and average effect of mutations: The rate of viability decline at generations 250–255 ($\Delta M = 0.094\%$) was very similar to that calculated at generations 104–106 ($\Delta M = 0.072\%$ from comparison with the control average, $\Delta M = 0.066\%$ from MD estimation, both adjusted for the second chromosome; GARCÍA-DORADO et al. 1998). These estimates were substantially lower than that obtained by Mukai et al. (1972; $\Delta M = 0.4\%$). The rate of mutational increase in variance at generations 250–255 ($\Delta V = 0.076 \times 10^{-3}$) was also very close to those computed at generations 104–106 ($\Delta V = 0.092 \times 10^{-3}$, adjusted for the second chromosome) or 208–209 (adjusted estimate $\Delta V = 0.104 \times 10^{-3}$) and it is in good agreement with that reported by Mukai et al. (1972) for quasi-normal chromosomes ($\Delta V = 0.094 \times 10^{-3}$). However, somewhat smaller estimates were obtained for the Mukai-like measure relative to $Cy/+\text{ (} \Delta V = 0.031 \times 10^{-3} \text{ and } \Delta V = 0.029 \times 10^{-3} \text{ for untransformed or log-transformed relative viability } \mathcal{V}^*, \text{ respectively).}$ Simulation results show that MD estimates of mutational parameters calculated at generations 104–106 for non-competitive relative viability accurately predict the empirical distribution obtained at generations 250–255 for competitive relative viability (Figure 4). This means that, in our lines, the mutational properties of competitive and noncompetitive viability are not qualitatively different. Furthermore, the genetic correlation between non-competitive (generations 104–106) and competitive (generations 250–255) viabilities was large and significant (0.77, with $P < 0.012$). Simulation results from

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**Figure 5.**—Observed (●) and expected (□) number of MA lines at different generations (see text for explanation).
models accounting for the mutational viability decline observed by Mukai do not fit our empirical distribution. Thus, the pictures emerging from Mukai’s experiment and ours are different, and this cannot be ascribed to the competitive levels involved in the viability assays of both experiments. The main discrepancy is the higher viability decline computed by Mukai, which was not accompanied by a larger $\Delta V$. This might be due to real differences between experiments but, in this case, the excess in viability decline obtained by Mukai should be ascribed to many deleterious mutations with very small effects. Otherwise, a larger mutational variance would have been observed. Mukai’s larger viability decline could also be attributed to some nonmutational source. A discussion of this possibility, embracing other mutation-accumulation experiments, can be found in García-Dorado et al. (1999).

Our Bateman-Mukai estimate for the rate of deleterious mutation at generations 250–255 was low ($\lambda \geq 0.01$), although somewhat larger than that obtained at generations 104–106 (Bateman-Mukai estimate 0.0056, MD estimate 0.0064, both adjusted for the second chromosome). The estimate of $E(s)$ (0.08) was slightly smaller than that computed at generations 104–106 (Bateman-Mukai estimate 0.13, MD estimate 0.10). These differences could be due to sampling error, to bias induced by purging selection (see above), to a downward bias in the estimate of $\Delta M$ due to mutations accumulated in the control, or to a slightly larger proportion of mutations behaving as deleterious in more competitive conditions. In any case, the results indicate a small rate of mild deleterious mutation. Our estimates suggest that, by generation 250, the expected genomic number of deleterious mutations per line was 6.5 (2.5 at the second chromosome), with an average effect of $\sim 0.08$. As explained above, these estimates refer mainly to nonseverely deleterious mutations ($s < 0.2$).

The degree of dominance of mutations: Our regression estimates for the degree of dominance give the average value of $h$ weighted by $s^2$. These will underestimate $E(h)$ value if the degree of dominance is inversely related to the magnitude of the deleterious effect, although the bias will be small if severely deleterious mutations have been lost. However, the probability of fixation of mutations in our MA lines will increase with decreasing $s$ values and this may, therefore, result in overestimation of the average degree of dominance of new unselected mutations. Thus, the overall bias depends on the shape of the distribution of deleterious effects, the relationship between $k$ and $s$, and the strength of both within-line and purging selection. Assuming that the degree of dominance is uniformly distributed between 0 and $\exp(-ks)$ (Caballero and Keightley 1994) and using the MD distribution of $s$ values estimated from our lines at generations 104–106, we have numerically obtained that $k = 4$ gives $E(h_{\omega 5}) = 0.3$ for nonseverely deleterious mutations ($s < 0.2$). This $k = 4$ value gives $E(h_{\omega 5}) > 0.3$ in our data where, due to the longer MA period, mutations with larger effects (say $0.1 < s < 0.2$) will be underrepresented.

It should be noted that a given relationship between $h$ and $s$ will result in different $E(h)$ values for different distributions $f(s)$ of mutational effects and, therefore, the estimates of $E(h)$ from any experiment are expected to depend on the corresponding $f(s)$. Thus, MD estimates obtained from Mukai or Ohnishi experiments, where selection was virtually absent, gave a low rate of mild deleterious mutation but a relatively large $E(s) = 0.2$ (García-Dorado et al. 1998). Thus, using the corresponding estimate of $f(s)$ and $k = 4$ gives average degrees of dominance $E(h) = 0.25$ and $E(h) = 0.27$ for new unselected mutations accumulated in Mukai’s or Ohnishi’s experiments, respectively. However, when the large rates of viability decline found by Mukai or Ohnishi are considered, the distributions of mutational effects imply a high probability of mild deleterious mutations with $E(s) = 0.02$ (for which a larger $k$ is expected). Thus, for $k = 4$, the Mukai-like models in Table 6 give $E(h) = 0.46$, and a similar model based on Ohnishi’s data gives $E(h) = 0.43$. Details on this later model as well as on the argument below can be found in García-Dorado and Caballero (2000). Mukai’s original estimates of the degree of dominance have important inconsistencies, with opposing results from coupling and repulsion crosses. In Ohnishi’s data, the ratio of heterozygous to homozygous viability declines suggests $E(h) = 0.45$, but this estimate would be biased upward if part of the viability decline was nonmutational. On the contrary, the low regression coefficient of heterozygous on homozygous viability obtained from Ohnishi’s data suggests $E(h) = 0.20$. This is closer to $E(h) = 0.27$, obtained using the $k = 4$ value inferred from our data and the $f(s)$ estimated by MD from Ohnishi’s data.

In summary, the long-term study of our MA lines does not reveal an important decline for competitive viability due to mild deleterious mutation, the corresponding estimates of $\lambda$ and $E(s)$ being in good agreement with those obtained for noncompetitive viability from the same lines after 105 generations of mutation accumulation. In parallel, our results suggest that the average degree of dominance of mutations detected in MA experiments (i.e., tiny mutations excluded) could be lower than previously accepted.

We thank A. Caballero for helpful comments on the manuscript. This work was supported by grant PB98-0814-C03-01 from the Ministerio de Educación y Cultura.

LITERATURE CITED


mutational properties of viability


Communicating editor: R. G. Shaw

APPENDIX

Let $x$ be the viability of a MA chromosome in homozygosis and $y$ that of its heterozygous combination with a randomly sampled control chromosome. Consider a single accumulated deleterious mutation with effects $s$ in homozygosis and $sh$ in heterozygosis. Since mutation is nonrecurrent, this mutation is fixed in a single line, denoted by $l$, in the set of $n$ available lines. The estimated covariance between $x$ and $y$ can be expressed as

$$
\sigma(x, y) = \frac{1}{n-1} \left( (x_i - \overline{x})(y_i - \overline{y}) + \sum_{j=1}^{n} (x_j - \overline{x})(y_j - \overline{y}) \right),
$$

where the overbar denotes sampling average. Ignoring other sources of variability,

$$
\overline{x} = E(x) - s/n, \quad \overline{y} = E(y) - sh/n,
$$

$$
x_i = E(x) - s, \quad y_i = E(y) - sh,
$$

$$
x_{ij} = E(x), \quad y_{ij} = E(y).
$$

Therefore,

$$
\sigma(x, y) = \frac{1}{n-1} \left( \frac{s^2}{n} \right) \left( \frac{1}{n} \right) + \frac{s^2h}{n^2},
$$

which can be expressed as

$$
\sigma(x, y) = \frac{s^2h}{n} \left( \frac{1}{n-1} \right) + \frac{1}{n(n-1)},
$$

which approaches $s^2h/n$ for increasing $n$. Adding up the contributions of all mutations accumulated, and assuming that there is no environmental covariance, we obtain $\sigma(x, y) = \Sigma s^2h_i/n$. 

Mutational Properties of Viability 693