

# On the Average Coefficient of Dominance of Deleterious Spontaneous Mutations

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Manuscript received October 25, 1999

Accepted for publication April 27, 2000

## ABSTRACT

T. Mukai and co-workers in the late 1960s and O. Ohnishi in the 1970s carried out a series of experiments to obtain direct estimates of the average coefficient of dominance ( $\bar{h}$ ) of minor viability mutations in *Drosophila melanogaster*. The results of these experiments, although inconsistent, have been interpreted as indicating slight recessivity of deleterious mutations, with  $\bar{h} \approx 0.4$ . Mukai obtained conflicting results depending on the type of heterozygotes used, some estimates suggesting overdominance and others partial dominance. Ohnishi's estimates, based on the ratio of heterozygous to homozygous viability declines, were more consistent, pointing to the above value. However, we have reanalyzed Ohnishi's data, estimating  $\bar{h}$  by the regression method, and obtained a much smaller estimate of  $\sim 0.1$ . This significant difference can be due partly to the different weighting implicit in the estimates, but we suggest that this is not the only explanation. We propose as a plausible hypothesis that a putative nonmutational decline in viability occurring in the first half of Ohnishi's experiment (affecting both homozygotes and heterozygotes) has biased upward the estimates from the ratio, while it would not bias the regression estimates. This hypothesis also explains the very high  $\bar{h} \approx 0.7$  observed in Ohnishi's high-viability chromosomes. By constructing a model of spontaneous mutations using parameters in the literature, we investigate the above possibility. The results indicate that a model of few mutations with moderately large effects and  $\bar{h} \approx 0.2$  is able to explain the observed estimates and the distributions of homozygous and heterozygous viabilities. Accounting for an expression of mutations in genotypes with the balancer chromosome Cy does not alter the conclusions qualitatively.

**A**LTHOUGH a great deal of research effort is currently devoted to elucidating the rate and distribution of effects of deleterious mutations (see recent reviews by García-Dorado *et al.* 1999; Keightley and Eyre-Walker 1999; and Lynch *et al.* 1999), not so much attention has been given to their degree of dominance. Yet, the heterozygous effects of mutations are critical for many evolutionary issues, such as the genetic load concealed in heterozygous condition in natural populations or the maintenance of genetic variation through mutation-selection balance.

Different methods have been used for estimating the coefficient of dominance of mutations ( $h = 0, 0.5$ , and  $1$  for recessive, additive, and dominant gene action, and  $h < 1$  or  $h > 1$  for over- or underdominance). Some of these are based on the analysis of chromosomes extracted from natural populations [see García-Dorado *et al.* (1999), for a recent review of the most important methods], but they rely heavily on the assumption of mutation-selection balance, which does not necessarily hold. In principle, the most reliable estimates should be obtained from mutation-accumulation (MA) experiments. In these, spontaneous mutations are allowed to

accumulate under relaxed selection in chromosome lines derived from the same uniform genetic background, and the viability of heterozygotes is compared to that of the corresponding homozygotes. Analogous estimates from inbred MA lines are not available at present. The main challenge in MA experiments concerns the estimate of the properties of nonseverely deleterious mutations, so that only chromosomes with viability  $> \sim 2/3$  [called quasinormals (QN)] are usually considered. As far as we know, the only estimates from QN chromosomes derive from the two classical MA experiments from T. Mukai and co-workers (Mukai 1964; Mukai *et al.* 1965; Mukai and Yamazaki 1968), and Ohnishi (1974, 1977a,b,c). Houle *et al.* (1997) recently obtained direct estimates of the average degree of dominance ( $\bar{h}$ ) for spontaneous mutations on several fitness traits in *Drosophila melanogaster*, but the analysis included non-QN chromosomes (except those carrying lethals). Because the average coefficient of dominance of highly deleterious mutations tends to be small (see Caballero and Keightley 1994), an estimate including chromosomes other than QN is likely to be a downward estimate of the  $\bar{h}$  of mildly deleterious mutations.

In one type of experiment carried out by Mukai and Ohnishi, the MA chromosome was paired with a putative "original chromosome," supposedly carrying very few or no new mutations. Therefore, all mutations that arose

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during the experiment at the corresponding MA line were coupled in the same chromosome in these so-called "coupling heterozygotes." We note that in Mukai's experiments (Mukai and Yamazaki 1968) the original chromosome was a high-viability chromosome chosen after 32 MA generations, the assumption being that its high viability was due to the presence of few or no mutations. Mukai's coupling heterozygotes were also made by using nonisogenic chromosomes of high viability (Mukai *et al.* 1965). In the studies from Mukai and co-workers, the estimated  $\bar{h}$  for the coupling heterozygotes with an original chromosome was consistently negative (average  $-0.17$ , pooling estimates summarized by Simmons and Crow 1977), indicating overdominance, while the coupling results using nonisogenic chromosomes were positive but small (average  $0.11$ ; see Simmons and Crow 1977). In Ohnishi's experiments, the original chromosome, derived from a long-term inbred line, had been maintained by a few single-pair matings (O. Ohnishi, personal communication). Ohnishi's results were radically different, with a significantly larger estimated value of  $0.49$  (Ohnishi 1977c).

In other types of experiments, "repulsion heterozygotes" were made by pairing different MA chromosomes, the assumption being that mutations that arose during the experiment were distributed at different loci along both chromosomes. For repulsion heterozygotes, Mukai's and Ohnishi's results coincided, with an estimate of  $\sim 0.4$ . Given the discrepancy between Mukai's and Ohnishi's results for the coupling heterozygotes and other evidence (see Simmons and Crow 1977), the overdominance results were taken as spurious. Thus, the typical value cited for the dominance of minor viability mutations is  $\sim 0.4$ .

However, several arguments cast doubts on the validity of this widely accepted value. The discrepant results for coupling and repulsion heterozygotes obtained by Mukai are still an unresolved matter. Furthermore, the estimate of  $\sim 0.4$  from Mukai's repulsion heterozygotes was obtained after removing about one-fifth of the heterozygotes from the analysis on the basis that these showed overdominance (Mukai and Yamazaki 1968; Mukai 1969). The discarded heterozygotes corresponded to crosses in which at least one of the chromosomes had a high homozygous viability, the argument being that these heterozygotes were, in fact, coupling heterozygotes. It also must be noted that both Mukai's and Ohnishi's results were obtained by pooling results from different MA generations. Because mutations arising in the early generations are also present in the later ones, estimates obtained at different generations are not independent, and a pooled estimate gives excessive weight to mutations in early generations.

In addition to the above problems, we show here that the estimates from Ohnishi are not unquestionable. A reanalysis of his data shows estimates different from those obtained by him. We discuss the possibility that the estimates of Ohnishi are biased upward and suggest

that the average coefficient of dominance of deleterious mutations in QN lines may be substantially smaller than the generally accepted value.

#### ANALYSIS OF COEFFICIENTS OF DOMINANCE IN OHNISHI'S EXPERIMENT

In Ohnishi's experiment, mutations were allowed to accumulate for 40 generations in 136 copies of a single original chromosome II of *D. melanogaster*. The homozygous viability ( $v_{ii}$ ) of each copy  $i$  was assayed at different generations as the percentage of wild adults  $+_i/+_i$  in the offspring of a cross between five  $Cy/+_i$  females and five  $Cy/+_i$  males in a half-pint bottle. Viability of heterozygotes ( $v_{ij}$ ) for random pair chromosomes  $i, j$  (repulsion heterozygotes) was analogously assayed as the percentage of wild  $+_i/+_j$  adults in crosses between five  $Cy/+_i$  females and five  $Cy/+_j$  males. Furthermore, the viability of heterozygotes carrying a copy of the original chromosome II ( $+$ ), maintained as a control, was also assayed as the percentage of wild adults  $+_c/+_c$  from crosses between five  $Cy/+_i$  females and five  $Cy/+_c$  males. These are assumed to be coupling heterozygotes. However, the control  $+_c$  chromosome was maintained by a few single-pair matings between  $+_c/+_c$  individuals (O. Ohnishi, personal communication), and the extent to which it might have accumulated new mutations is unknown.

In all cases, the viability of each chromosome  $+_i$  was assayed by reference to that of the curly progeny. The interpretation of the results could be greatly complicated if deleterious mutational effects were expressed in the  $Cy/+_i$  heterozygotes (Cockerham and Mukai 1978). It was not possible to test for this possibility with Ohnishi's data. However, we have experimental results suggesting that mutations that accumulated in originally isogenic full-sib MA lines, showing deleterious effects in homozygous condition, do not show correlated effects on  $Cy/+_i$  heterozygotes (D. Chavarrías, A. García-Dorado and C. López-Fanjul, personal communication).

From now on we refer to all viability measurements relative to the original average  $\bar{v}_0$ , which had a value of  $31.71$  on Ohnishi's viability scale. Thus, denoting by  $+$  the original chromosome and by  $m$  a copy of it carrying a new mutation, relative viabilities for genotypes  $+/+$ ,  $+/m$ , and  $m/m$  are  $1$ ,  $1 - hs$ , and  $1 - s$ , where  $s$  and  $h$  are the mutation's homozygous deleterious effect and coefficient of dominance, respectively.

**Estimation of the average coefficient of dominance from Ohnishi's experiment:** Ohnishi (1977c) obtained estimates of the average coefficient of dominance of new mutations by comparing homozygous and heterozygous viability declines,

$$\bar{h}_{ms} = \frac{\bar{v}_0 - \bar{v}_{ij}}{(\bar{v}_0 - \bar{v}_{ii}) + (\bar{v}_0 - \bar{v}_{jj})} = \frac{\sum sh}{\sum s}, \quad (1)$$

where  $i$  and  $j$  indicate two given MA lines,  $j = c$  for

**TABLE 1**  
**Estimated average coefficient of dominance for mutants in QN chromosomes of Ohnishi's experiment**

Generation	$\bar{h}_{ws}$			$\bar{h}_{ws^2}$		
	Coupling	Repulsion	Average	Coupling	Repulsion	Average
10	0.443*	0.510*	0.476*	-0.018	0.169	0.075
	±0.033	±0.033	±0.023	±0.234	±0.236	±0.166
20	0.539*	0.457*	0.498*	0.231*	0.215*	0.223*
	±0.034	±0.034	±0.024	±0.127	±0.125	±0.089
30	0.538*	0.420*	0.479*	0.159*	0.205*	0.182*
	±0.034	±0.034	±0.024	±0.087	±0.110	±0.070
40	0.416*	0.280*	0.348*	0.100	0.029	0.065
	±0.035	±0.035	±0.025	±0.077	±0.082	±0.056
Overall mean <sup>a</sup>	0.484	0.417	0.450	0.118	0.154	0.136

\* Significantly >0 at a 5% level.

<sup>a</sup> Significance is not tested because of the dependence between estimates obtained at different generations.

coupling heterozygotes, and the summation is over all the accumulated mutations. As stated in the right-hand side of the expression, this is an estimate of the average  $h$  of mutations weighted by their homozygous effect,  $s$  (Mukai 1969). Estimates obtained by Ohnishi (1974) at different generations for the QN chromosomes (*i.e.*, those with a viability larger than  $20/31.71 = 0.63$ ) are given in Table 1. We have computed standard errors (SE) for  $\bar{h}_{ws}$  using the approximation for the standard error of the ratio of two variables and assuming that  $\bar{v}_0$  and  $\bar{v}_c$  are constant. Averaging over generations and schemes (coupling and repulsion) gives  $\bar{h}_{ws} = 0.45$ . The average estimate for non-QN chromosomes ( $v_{ij} < 0.63$  excluding those carrying lethals) is 0.085, and that for lethals is 0.046. It is also worthwhile to note that deleterious mutations in chromosomes with homozygous viability >0.95 (of which 29 lines remained by the end of the experiment) tended to appear as partially dominant, with  $\bar{h}_{ws} = 0.74$  and 0.67 for coupling and repulsion heterozygotes, respectively.

Additional information can be obtained from the regression of heterozygous on homozygous values (Mukai 1969). For repulsion heterozygotes, if  $x$  is the sum of the homozygous viabilities of two parental lines and  $y$  is the heterozygous viability, the covariance for a large set of  $n$  lines is

$$\sigma(x,y) \approx (\sum_k h_k s_k^2 + \sum_l h_l s_l^2) / n,$$

where summation is over all mutations accumulated,  $k$  and  $l$  refer to mutations at maternal and paternal chromosomes, respectively, and mutation is assumed to be nonrecurrent. For coupling heterozygotes, the maternal chromosome is the control one and does not contribute to the covariance. Thus,  $x$  is the homozygous viability of the MA paternal chromosome, and  $y$  is that of the heterozygote. Then, analogously to the repulsion case,  $\sigma(x,y) \approx \sum_k h_k s_k^2 / n$ , where  $k$  stands for the mutations accumulated in the paternal MA chromosome. The between-line component of variance for the homozygous

viability is  $\sigma_b^2 \approx \sum_k s_k^2 / n = \sum_j s_j^2 / n$ . Thus, an estimate of the average degree of dominance weighted by  $s^2$  can be obtained in any particular generation as

$$\bar{h}_{ws^2} = \frac{\sigma(x,y)}{z\sigma_b^2} = \frac{\sum s^2 h}{\sum s^2}, \tag{2}$$

where  $z$  is the number of homozygotic parental values used ( $z = 1$  for coupling heterozygotes and  $z = 2$  for repulsion heterozygotes). None of these estimates are given in Ohnishi's articles. However, we were able to obtain them from data provided in his Ph.D. thesis (Ohnishi 1974). The correlations  $r(x,y)$  between the heterozygous average viability ( $y$ ) and that ( $x$ ) of its homozygotic parent (coupling) or midparent (repulsion) are given for the group of QN homozygotic MA lines at generations 10, 20, 30, and 40 (Ohnishi 1974, Table 15).  $\sigma_x$  and  $\sigma_y$  can be estimated from the ANOVA given by Ohnishi (1974, Tables 2a and 12a) as  $\hat{\sigma}_x = \sqrt{\text{MSB}_{\text{hom}}/mz}$ ,  $\hat{\sigma}_y = \sqrt{\text{MSB}_{\text{het}}/m}$ , where  $\text{MSB}_{\text{hom}}$  and  $\text{MSB}_{\text{het}}$  are the between-line means of squares in the ANOVA for homozygotic and heterozygotic assays, respectively, and  $m$  is the number of observations per line (three for  $\hat{\sigma}_x$  and two for  $\hat{\sigma}_y$ ). Using these values, we can obtain  $\hat{\sigma}(x,y) = r(x,y)\hat{\sigma}_x\hat{\sigma}_y$  for QN chromosomes.

A minor problem is that  $\text{MSB}_{\text{het}}$  should be the between-line mean of squares for QN heterozygotes. However, the only ANOVA given by Ohnishi for the heterozygous viability refers to nonlethal chromosomes (instead of QN chromosomes). Using this ANOVA, we obtain an overestimate of  $\hat{\sigma}_y$  and, therefore, of  $\hat{\sigma}(x,y)$ . However, the  $\text{MSB}_{\text{het}}$  for nonlethal chromosomes increased only slightly during the MA process, and the corresponding between-line component of variance was always very small and nonsignificant. Therefore, the bias should be very small.

Using  $\hat{\sigma}(x,y)$  and Equation 2, we have computed the estimates for  $\bar{h}_{ws^2}$  given in Table 1. Standard errors for the regression of heterozygous on homozygous values were computed using standard equations from linear

regression theory. Estimates of  $\bar{h}_{ws^2}$  are considerably and significantly smaller than the  $\bar{h}_{ws}$  estimates obtained by Ohnishi using Equation 1. As stated above, averaging estimates from different generations gives greater weight to early mutations. Therefore, here we refer to the estimates at generation 40, which are the most informative because more mutations will have accumulated and because they have the smallest standard errors. These are  $\bar{h}_{ws} = 0.348$  and  $\bar{h}_{ws^2} = 0.065$ , which are significantly different ( $P < 0.001$ ).

All estimates for  $\bar{h}_{ws^2}$  in Table 1 were also directly calculated as regression coefficients from the grouped data in Tables 13a and 14a of Ohnishi (1974). The calculations were corrected for the use of phenotypic, instead of genotypic, variances in the regression estimates (Caballero *et al.* 1997). The results were very similar to those presented in Table 1 from the correlations and are not given.

At first, it is tempting to explain the substantial difference between both types of estimates in Table 1 by invoking that there is a negative correlation between the homozygous effects and the degree of dominance of new mutations, in which case we should expect  $\bar{h}_{ws} > \bar{h}_{ws^2}$ . A negative correlation between  $s$  and  $h$  is a very likely possibility when severely deleterious mutations are involved (see review by Caballero and Keightley 1994) and, in fact, a sharp decrease in the average  $h$  is observed when estimates include non-QN chromosomes (see above). However, the bias of an estimate from Equation 2 is not necessarily large in the analysis of Ohnishi's lines because most severe mutations were excluded from the analysis. After 40 generations, 78 out of the 80 QN chromosomes assayed in heterozygous condition had homozygous viability  $> 0.85$ .

We suggest that an additional explanation could be, at least in part, responsible for the difference: If some of the change in homozygous and heterozygous average viabilities were due to nonmutational causes, as has been recently suggested (Keightley 1996; García-Dorado 1997; García-Dorado *et al.* 1999), the estimate from Equation 1 would be biased toward 0.5. Imagine a hypothetical extreme situation in which all the observed decline is due to unknown nonmutational causes affecting both homozygotes and heterozygotes. Then, no between-line variance would be detected (due to the isogenic origin of the lines), and the decline would be ascribed to a large number of mutations with very small deleterious effects (see the Bateman-Mukai method in Mukai *et al.* 1972). Using Equation 1, additive gene action ( $\bar{h}_{ws} = 0.5$ ) would be inferred for this spurious class of mutations. Since the viability decline is not used in Equation 2, such a bias would not occur with the estimates obtained by the regression method.

An indication that a nonmutational decline in viability might have occurred in Ohnishi's lines comes from the observed pattern of decline in viability. Figure 1 gives the average viability against generation number of

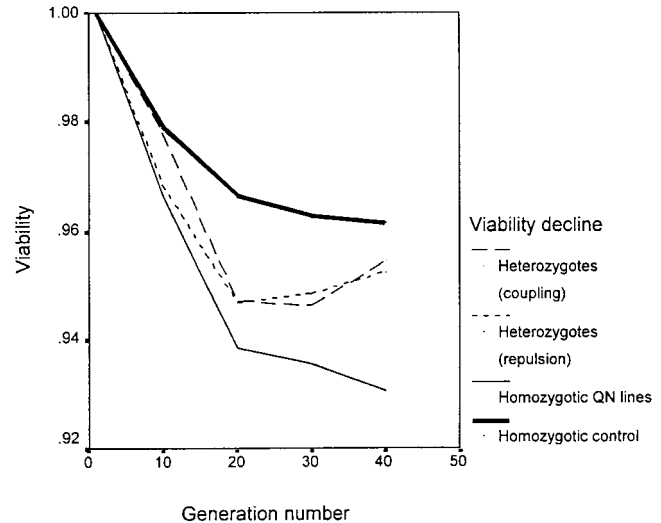


Figure 1.—Viability decline observed in Ohnishi's (1974) experiment.

the homozygotes for the control and QN lines, as well as for coupling and repulsion heterozygotes. In all cases the viability decline was much larger in the first half of the experiment than in the second half. In contrast, the proportion of lethals steadily increased during the experiment (Figure 2), being consistent with a steady mutation rate. Also, the between-line variance for QN homozygotes increased at a roughly constant rate (Figure 2), which suggests that the slower final decline in mean viability is not due to diminishing epistasis, to a reduction in the mutation rate, or to a relaxation of the environmental conditions.

Thus, an important fraction of the early viability decline could have been nonmutational. The cause of this hypothetical nonmutational decline is unknown, but it might be due to a simultaneous viability increase in the Cy chromosome by reference to which viability was assayed (Keightley 1996; García-Dorado 1997), to an increase in the ability of the experimenter to detect the Cy phenotype (Fry *et al.* 1999), or to an environmental effect.

Alternatively, the nature of deleterious mutations could vary between the first and second half of the experiment, with a large initial rate of mutations with very small deleterious effects causing a larger viability decline without appreciable additional increase in variance. It has been suggested (Keightley and Eyre-Walker 1999) that transposition could provide a mechanism for this behavior (see discussion).

Finally, another estimate of the average coefficient of dominance from Ohnishi's experiment, which we denote  $\bar{h}_{sd}$ , can be obtained using the ratio of heterozygotic to homozygotic rates of increase of the genetic standard deviations (both given by Ohnishi 1974, Table 12a). This ratio estimates the square root of the average of  $h^2$  weighted by  $s^2$  and, therefore, it is difficult to

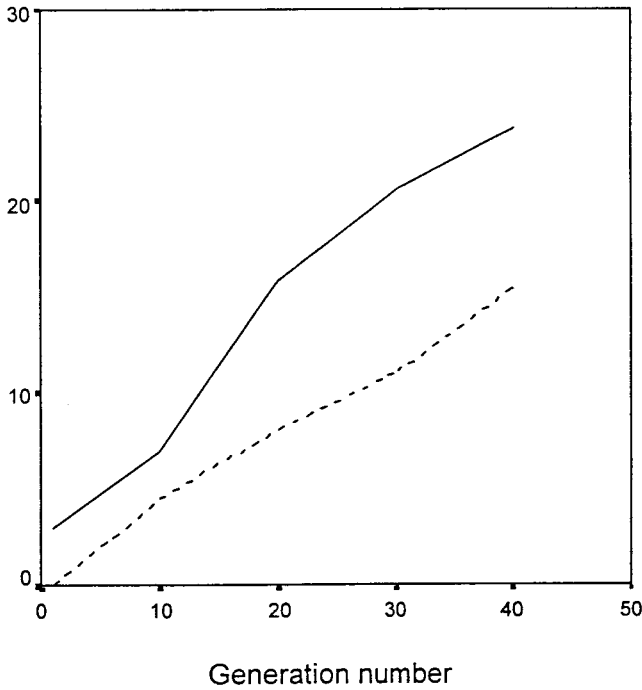


Figure 2.—Percentage of lethal mutations and variance between QN lines in Ohnishi's (1974) experiment. (---) Percentage of lethals, (—)  $Vg \times 10,000$ .

interpret. If  $s$  were constant but  $h$  were variable,  $\bar{h}_{sd}$  would be an upwardly biased estimate of the true  $\bar{h}$ . In addition to this, if  $s$  were variable, a downward bias is expected when there is a negative correlation between  $h$  and  $s$ . No estimates of this type could be obtained for QN chromosomes. For nonlethal chromosomes we obtained  $\bar{h}_{sd} = 0.058$  (variance for coupling and half-variance for repulsion pooled), which suggests small  $h$  values. Furthermore, it should be noted that both the rate of increase in variance ( $1.09 \times 10^{-6} \pm 6.26 \times 10^{-6}$ ) and the genetic variance at generation 40 ( $1.19 \times 10^{-4}$ ) for heterozygotes of nonlethal chromosomes were non-significant, pointing also to reduced values of  $h$ .

We carried out analytical work and simulations to investigate the possibility that the larger estimates of dominance obtained from the ratio method (Equation 1) were biased due to the hypothesis of a nonmutational decline in viability. These analyses and simulations are explained in what follows.

**Parameter estimations according to the two hypotheses:** Two different parameter estimations can be made. Bateman-Mukai (BM) estimates (Bateman 1959; Mukai *et al.* 1972) can be obtained by considering the observed drop in viability and, therefore, assuming that this drop is not affected by nonmutational factors. Minimum distance (MD) estimates (García-Dorado 1997) can also be obtained that are unconstrained by the observed change in mean and, therefore, would be appropriate whether or not nonmutational factors were involved in the observed decline.

TABLE 2

Mutational parameters for chromosome II nonlethal deleterious mutations under different models

Model	$\lambda$	$E(s)$	$E(s^2)$	$\alpha$	$\beta$
BM	0.05	0.0416	0.0046	0.612	14.7
BM <sup>s</sup>	0.12	0.0173	0.0019	0.187	10.8
MD	0.004	0.164	0.057	0.900	5.5

$\lambda$ , haploid mutation rate for chromosome II;  $E(s)$ , mean mutational effect;  $E(s^2)$ , mean squared mutational effect;  $\alpha$  ( $\beta$ ), parameter of shape (scale) for the gamma distribution; BM, Bateman-Mukai model; BM<sup>s</sup>, sharpened Bateman-Mukai model; MD, minimum distance model (see text for explanations).

*Model based on Bateman-Mukai estimates:* For QN chromosomes, relative viability declined at a rate  $\Delta M = 0.00173$ , and the corresponding rate for the increase of between-line variance was  $\Delta V = 0.594 \times 10^{-4}$ . From these, a BM lower bound for the rate of mutation per chromosome II and generation is  $\lambda$  (QN)  $\geq 0.05$ , and an upper bound for the average homozygous deleterious effect is  $E(s)$  (QN)  $\leq 0.034$  (Ohnishi 1974). Considering that chromosome II accounts for  $\sim 40\%$  of the *Drosophila* genome, these results suggest an important rate of mildly deleterious mutation, although smaller than those obtained in previous MA experiments by Mukai (1964) and Mukai *et al.* (1972), which gave  $\lambda$  (QN)  $\geq 0.14$  and  $0.17$ , respectively. The above estimates imply that, by generation 40, QN MA chromosomes II would carry on the average  $>2$  deleterious mutations and that the proportion of deleterious free QN lines is expected to be  $<13\%$ , assuming a Poisson distribution of mutations.

The BM estimates of  $\lambda$  and  $E(s)$  are only for QN chromosomes. However, to obtain quantitative predictions from these results (or to simulate the mutational process, see below) we need a more explicit model, specifying the distribution of effects for all deleterious mutations (QN and non-QN, lethals excluded), as well as a model of dominance. Homozygous deleterious effects are assumed to be gamma distributed (shape parameter  $\alpha$ , scale parameter  $\beta$ , expected value  $\alpha/\beta$ ), which allows us to handle different kurtosis. Noting that only 3 out of the 106 nonlethal chromosomes present at generation 40 were non-QN (which indicates a non-QN mutation rate of  $\sim 7 \times 10^{-4}$ ) we assume  $\lambda = 0.05$  for all deleterious mutations (QN and non-QN, lethals excluded). This value of  $\lambda$ , together with the  $\Delta M$  and  $\Delta V$  observed for nonlethal chromosomes ( $0.00208$  and  $2.28 \times 10^{-4}$ , respectively), leads to the values of  $E(s)$  and  $E(s^2)$  presented in Table 2. In turn, these values determine  $\alpha$  and  $\beta$  in the gamma distribution given in the same table [ $E(s) = \alpha/\beta$  and  $E(s^2) = \alpha(\alpha + 1)/\beta^2$ ]. Since this set of parameters is based on a BM estimate

of  $\lambda$ , we refer to the model they describe as the BM model.

*Model based on sharpened Bateman-Mukai estimates:* Because the above BM bounds approach unbiased point estimates as the variance of  $s$  decreases, we also consider the bounds obtained for the class of QN chromosomes when a few outliers are excluded. The QN set contains 100 lines showing a compact histogram and 3 lines whose mean viability is clearly below this group ( $\bar{v} < 0.8$ , *i.e.*,  $>3 \sigma_{\bar{v}}$  below the general average  $\bar{v}$ , where  $\sigma_{\bar{v}}$  is the standard deviation for mean viability in this compact group). Thus, BM bounds can be sharpened further by estimating the mutational rate from the change in mean and variance in the 100 lines belonging to the compact histogram (that we call QN<sup>s</sup> lines). These give  $\lambda$  (QN<sup>s</sup>)  $\geq 0.12$  and  $E(s)$  (QN<sup>s</sup>)  $\leq 0.013$ . This implies that, by generation 40, QN<sup>s</sup> chromosomes carry on the average at least 4.8 deleterious mutations, and only 0.8% of the QN<sup>s</sup> lines are expected to be deleterious-free. Following the same procedure as before, and using  $\lambda = 0.12$ , we analogously obtain the set of parameters presented in Table 2, which describe the BM<sup>s</sup> model.

*Model based on minimum distance estimates:* An alternative set of parameters unconstrained by the observed decline in mean viability could be obtained from MD estimation to explain Ohnishi's MA results. MD estimates were obtained by García-Dorado (1997) for a viability index (twice the ratio of wild to Cy numbers), using Ohnishi's data of MA lines at generation 40. In this method, mutational effects on viability were assumed to be sampled from a gamma distribution reflected about zero, thus allowing the occurrence of mutations increasing viability. However, the estimate of the probability of these favorable mutations turned out to be zero.

Results for heterozygous effects, available from Ohnishi (1974), referred to the percentage of wild-type flies instead of to twice the ratio of wild to Cy numbers. Thus, MD estimates obtained by García-Dorado (1997) had to be rescaled for the rate of increase in variance of the percentage measurement given by Ohnishi. This was accomplished by using the appropriate scale parameter ( $\beta$ ) in the gamma distribution of  $s$  to account for the mutational variance in Ohnishi's scale. These parameters describe the MD model and are presented in Table 2. The MD estimates imply that, by generation 40, a QN chromosome would carry 0.16 deleterious mutations on average, and  $\sim 85\%$  of the nonlethal chromosomes would be deleterious-free.

The nonmutational viability decline at generation 40 ( $D$ ) was estimated to be  $D = 0.056$ , and this value is considered later as a constant for both homozygotes and heterozygotes. The estimate was obtained from a comparison between the observed drop in viability and the expected one, assuming the MD model. It is strongly supported by the observation that the rate of decline from generations 0–20 exceeded by an amount of 0.00274 per generation that from generations 20–40

TABLE 3

Observed and expected estimates of the average coefficient of dominance

Observed <sup>a</sup>	BM	BM <sup>s</sup>	MD
$\bar{h}$ (QN)	0.44	0.47	0.22
$\bar{h}$ (NL)	0.44	0.47	0.20
$\bar{h}_{ws}$ (QN) $0.348 \pm 0.025$	0.361	0.345	$0.381^b$ and $0.112^c$
$\bar{h}_{ws^2}$ (QN) $0.065 \pm 0.056$	0.302	0.265	0.068
$\bar{h}_{ws^2}$ (NL) $0.020 \pm 0.018$	0.290	0.240	0.026

<sup>a</sup> For quasinormal (QN) chromosomes we give the average of coupling and repulsion estimates from Ohnishi's spontaneous mutation lines at generation 40 (see Table 1), while for all nonlethal (NL) chromosomes the estimate was obtained from regression of heterozygous on homozygous viabilities from Tables 13a and 14a of Ohnishi (1974).

<sup>b</sup> Expected estimate using Equation 1 considering a nonmutational decline in viability of 0.056.

<sup>c</sup> Expectation without considering a nonmutational decline in viability.

(see Figure 1). Assuming this difference is due to nonmutational causes, we obtain an overall  $D = 0.00274 \times 20 = 0.0548$ , in close agreement with the MD estimate (0.056).

The final viability decay in the control line ( $D = 0.038$ ) suggests a somewhat smaller nonmutational viability decline but, assuming the standard errors for the control evaluations were similar to those for the lines, this estimated decay was not significantly different from the MD estimate.

*Predictions of the average coefficient of dominance for the three models:* Ohnishi (1974) estimated  $\bar{h}_{ws}$ (QN) = 0.348 at generation 40 using Equation 1 (Table 1). From this estimate we need to guess the kind of  $h$  values to be expected under each model (BM, BM<sup>s</sup>, or MD) for mutants with different deleterious effects. To produce an inverse relationship between  $s$  and  $h$ , the latter is assumed uniformly distributed between 0 and  $\exp(-\rho s)$  (Caballero and Keightley 1994), where  $\rho$  is a constant chosen to give the desired unweighted arithmetic mean dominance ( $\bar{h}$ ) for the whole range of  $s$  values. This exponential model for  $h$  is used to account for the low dominance usually observed for rare severely deleterious mutations. The values of  $\bar{h}$  predicting the empirical  $\bar{h}_{ws}$ (QN)  $\approx 0.348$  have been found numerically for each model and are given in Table 3, together with the corresponding numerical results for the weighted averages  $\bar{h}_{ws}$  and  $\bar{h}_{ws^2}$ , and with the empirical estimates.

Under the BM model, the unweighted overall average  $h$  for nonlethals should be  $\bar{h} = 0.44$  to explain the empirical  $\bar{h}_{ws}$  (QN) estimate. However, the expected  $\bar{h}_{ws^2}$  (QN) is 0.302, which is considerably larger than the corresponding observed estimate (0.065) obtained in generation 40 (Tables 1 and 3). Similarly, using the BM<sup>s</sup>-based model, we need  $\bar{h} = 0.47$  to obtain an expected  $\bar{h}_{ws}$  (QN) about the empirical value, which again gives too

TABLE 4

Values of  $\bar{h}_{ws}$  estimated from Equation 1 for lines with homozygous viability >0.95, together with the expected values under the nonmutational decline hypothesis

Generations	No. of lines	Observed <sup>a</sup> $\bar{h}_{ws} (v > 0.95)$	Expected <sup>b</sup> $\bar{h}_{ws} (v > 0.95)$	Expected <sup>c</sup> $\bar{h}_{ws} (v > 0.95)$
		Coupling		
10	47	0.586	0.765	0.568
20	44	0.816	0.952	0.568
30	42	0.846	0.956	0.635
40	29	0.712	0.829	0.569
		Repulsion		
10	47	0.629	0.632	0.470
20	38	0.738	0.887	0.530
30	32	0.880	1.061	0.705
40	26	0.437	0.709	0.487

<sup>a</sup> Observed values obtained from Tables 16a and 17a of Ohnishi (1974).

<sup>b</sup> Using the MD estimate of the nonmutational decay (see text).

<sup>c</sup> Using the control estimate of the nonmutational decay (see text).

large an expected  $\bar{h}_{ws^2}$  (QN) of 0.265. These calculations suggest that different weighting factors ( $s$  for  $\bar{h}_{ws}$  and  $s^2$  for  $\bar{h}_{ws^2}$ ) are not wholly responsible for the difference between estimates from Equations 1 and 2.

For the MD model, the degree of dominance necessary to explain the observed average  $\bar{h}_{ws^2}$  (QN) at generation 40 was found to be  $\bar{h} = 0.20$  (Table 3). In the absence of nonmutational viability decline, this gives  $\bar{h}_{ws}$  (QN) = 0.112, well below the value estimated from Equation 1. However, when the expected nonmutational decline in viability of 0.056 is considered, the estimate from Equation 1 becomes  $\bar{h}_{ws^2}$  (QN) = 0.381, in agreement with the observed estimate. Alternatively, using the decline observed in the control line (0.038) as an estimate of the nonmutational viability decline, Equation 1 gives an estimate  $\bar{h}_{ws}$  (QN) = 0.344, again in good agreement with the empirical value. Therefore, the MD model with a nonmutational drop is able to explain both  $\bar{h}_{ws}$  (QN) and  $\bar{h}_{ws^2}$  (QN).

Table 3 also shows the results for all nonlethal (NL) chromosomes. The unweighted average for  $h$  is roughly the same as for QN chromosomes. The observed values of  $\bar{h}_{ws^2}$  (NL) were obtained as regression coefficients from the grouped data in Tables 13a and 14a of Ohnishi (1974). Observed estimates were considerably lower than the corresponding estimates for QN chromosomes. MD estimates were in close agreement with such low values.

The nonmutational viability decay would also explain the high estimates of  $\bar{h}_{ws}$  obtained by Ohnishi (1974) at different generations using the ratio method (Equation 1) for the set of lines with homozygous viability >0.95 (Table 4). Under the nonmutational decline hypothesis, these lines would be a nonrandom subsample of those carrying few or no deleterious mutations. Then, the observed viability decline on their heterozygotic crosses would equal the nonmutational decline ( $D$ ) and,

since  $v_{ij}$  and  $v_{ji}$  are chosen close to one, the estimates of  $\bar{h}_{ws}$  from Equation 1 would be upwardly biased. The expected value of this estimate was computed under the hypothesis of a nonmutational decline using the observed homozygous viabilities for MA chromosomes with viabilities >0.95 (Tables 13a and 14a in Ohnishi 1974) and substituting  $\bar{v}_0 - \bar{v}_{ij} = D$  in Equation 1. We used the MD estimate of  $D$  (assuming that all the decline occurred linearly from generations 0-20, *i.e.*,  $D = 0.028, 0.056, 0.056,$  and  $0.056$  at generations 10, 20, 30, and 40, respectively) and, alternatively, the  $D$  value estimated from the control (0.021, 0.033, 0.037, and 0.038, respectively). The predictions are shown in Table 4. In general, large values for  $\bar{h}_{ws}$  are predicted, in agreement with empirical estimates. The best fitting corresponds to the mean between both alternative approaches, suggesting that the true final nonmutational viability decay could be some value between 0.038 and 0.056.

**Simulation results:** To get the expected distribution for chromosome viabilities for homozygotes and heterozygotes and to check the above analytical results, we performed Monte Carlo simulations. We simulated a Poisson distribution of the number of mutations per chromosome, with average number equal to  $40\lambda$ . Mutations accumulated over an original chromosome of viability  $\bar{v}_0 = 1$ . Viability deleterious effects were gamma distributed and were multiplicative across loci. Chromosome viabilities were analyzed in the (0, 1) scale, but using a log scale made no appreciable difference (data not shown). The model assumed for the dominance of mutations was the same as that used previously in the analytical method. We used the same sampling error obtained by Ohnishi ( $\sigma_e^2 = 7 \times 10^{-4}$  and  $\sigma_e^2 = 9 \times 10^{-4}$  for homozygotes and heterozygotes, respectively). These were obtained from the environmental variances in Tables 2a and 12a of Ohnishi (1974), divided by the number of observations per line and by the square mean

TABLE 5  
 Mutational rates  $\lambda$  per chromosome II for  $s$  within different ranges,  
 together with the corresponding unweighted average  $\bar{h}$

	BM		BM <sup>s</sup>		MD	
	$\lambda$	$\bar{h}$	$\lambda$	$\bar{h}$	$\lambda$	$\bar{h}$
$s < 0.001$	0.0042	0.500	0.055	0.495	0.0000	0.418
$0.001 < s < 0.05$	0.0317	0.472	0.052	0.472	0.0011	0.408
$0.05 < s < 0.1$	0.0085	0.397	0.0074	0.375	0.0008	0.242
$0.1 < s < 0.2$	0.0044	0.313	0.0040	0.285	0.0008	0.125
$0.2 < s < 0.4$	0.0010	0.209	0.0011	0.159	0.0007	0.040
$0.4 < s < 1$	0.00003	0.107	0.0090	0.077	0.0004	0.004

Simulation results for the different models considered.

viability at generation 0, *i.e.*, 31.71. Since the heterozygous distribution given by Ohnishi included chromosomes that were lethal when homozygous, additional lethal mutations were simulated in the heterozygous case. These were assumed to occur with the rate  $\lambda_L = 0.00466$  estimated by Ohnishi (1974) and with degree of dominance  $h_L = 0.02$  (Simmons and Crow 1977). A total of 100 chromosomes were simulated and results were averaged over 100 replicates.

Table 5 gives the number of mutations per chromosome II and generation within different  $s$  intervals obtained from the simulated data, as well as the corresponding average coefficient of dominance. Both BM and BM<sup>s</sup> models predict more frequent deleterious mutations than the MD model, the difference being much more important for small deleterious effects. The degree of dominance of mildly deleterious mutations ( $s < 0.05$ ) is similar ( $\bar{h} \approx 0.45$ ) under the three models. However, for the MD model, it decreases quickly for increasing deleterious effects.

The observed and simulated distributions for homozygous and heterozygous chromosome viabilities under the different models are presented in Figures 3 and 4, respectively.

Both BM and BM<sup>s</sup> models failed to predict the shape of the average viability distribution of the homozygous and heterozygous chromosomes. Although sharpening BM estimates (*i.e.*, reducing the variance of  $s$  by removing extreme lines) produces larger estimates of the rate of occurrence of mutations with small effect, BM and BM<sup>s</sup> models gave very similar distributions for the average viability, both in the homozygous and in the heterozygous condition. This suggests that MA experiments often lack the power to estimate the mutation rate for very small deleterious effects ( $s < 0.001$ , see Table 5).

MD parameters (including the rate of nonmutational viability decline) were originally estimated from the observed homozygous average viability distribution. Thus, it is not surprising that the observed and simulated distributions for homozygous viability agreed with each other to some extent. However, the model also ade-

quately predicted (although with a slight shift to the left) the shape of the observed distribution of average viabilities in the heterozygous state, which was not used in MD estimation.

The simulations were also used to obtain estimates for the average coefficient of dominance analogous to the numerical values computed from analytical expressions and given in Table 3. Simulated and analytical estimates were in close agreement (data not shown).

We also computed  $\bar{h}_{sd}$  (the ratio of heterozygotic to homozygotic rates of increase in genetic standard deviations) from our simulated nonlethal chromosome lines. Using the BM or BM<sup>s</sup> models, we obtained too large  $\bar{h}_{sd}$

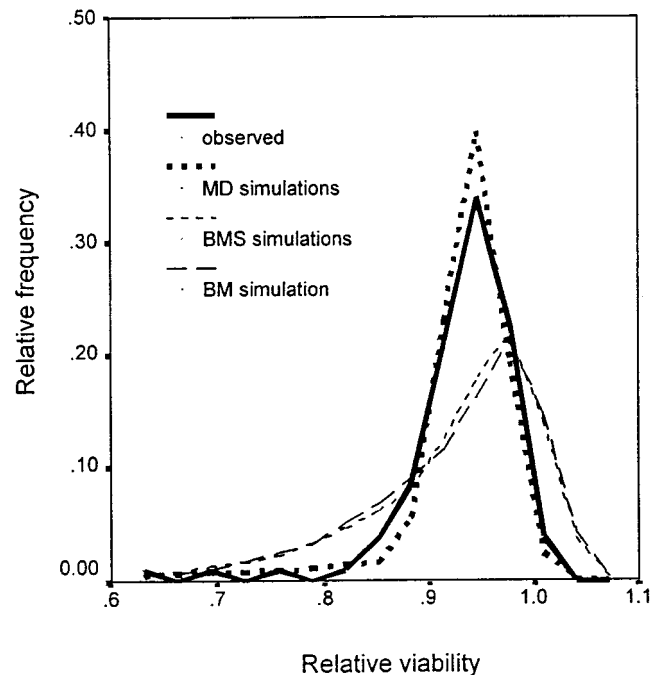


Figure 3.—Observed (Ohnishi 1974) and simulated distribution of chromosome homozygous viabilities using parameter estimates in Table 2 (see text for explanations). BM, Bateman-Mukai model; BM<sup>s</sup>, sharpened Bateman-Mukai model; MD, minimum distance model.

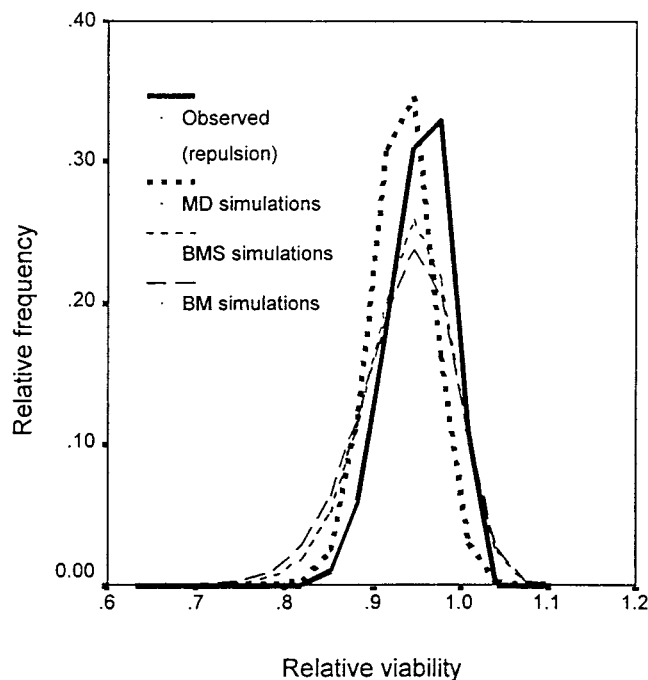


Figure 4.—Observed (Ohnishi 1974) and simulated distribution of chromosome heterozygous viabilities using parameter estimates in Table 2 (see text for explanations). See Figure 3 legend for definitions of abbreviations.

values (0.533 or 0.430, respectively), while using the MD model, we obtained a small  $\bar{h}_{sd} = 0.072$ , in agreement with the empirical value (0.058) estimated from Ohnishi's data.

A complication mentioned above and not considered in the previous analyses is the possible effect of mutations in  $Cy/+_i$  genotypes. The assumption so far has been that  $h = 0$  for all mutations in  $Cy/+_i$ . Although this is supported by new experimental evidence (D. Chavarrías, A. García-Dorado and C. López-Fanjul, personal communication), we evaluated this possible effect by simulation. We assumed that mutations expressed the same effect on  $Cy/+_i$  as on  $+_i/+_j$  genotypes. Thus, the viability of a homozygote ( $v_{ii}$ ) was scaled by the heterozygous effects of chromosome  $i$  ( $v_{ci}$ , where  $c$  is a chromosome with no mutations). The heterozygous viability ( $v_{ij}$ ) was scaled by the average heterozygous effects of chromosomes  $i$  and  $j$  ( $[v_{ci} + v_{cj}]/2$ ).

The simulation estimates corresponding to those of Table 3 for  $\bar{h}_{ws}$  (QN) and  $\bar{h}_{wsz}$  (QN) were 0.31 and 0.20 for BM, 0.31 and 0.18 for BM<sup>s</sup>, and 0.43 and 0.04 for MD, respectively. Therefore, the effect of an expression of mutations on  $Cy/+_i$  genotypes was a general reduction in the estimates of  $h$  (except when a nonmutational decline is accounted for; cf. Table 3), and the BM and BM<sup>s</sup> models were still unable to explain all the observations. Furthermore, an expression of mutations in the  $Cy$  genotype had a very small effect on the distributions of homozygous and heterozygous chromosome viabilities presented in Figures 3 and 4 (data not shown),

so the qualitative conclusions reached above are not altered. Finally, it is worth noting that an expression of mutations in the  $Cy$  genotype implies that Ohnishi's estimates of  $\bar{h}_{ws}$  from the ratio method (which are already high) are underestimations, so the true mean  $h$  would be exceedingly high.

## DISCUSSION

Estimates of the degree of dominance of new nonsevere deleterious mutations are only available from the classical MA experiments of Mukai and Ohnishi. Other estimates include chromosomes with highly deleterious mutations or are obtained as indirect inferences from natural populations, based on the assumption of mutation-selection balance. The direct estimates obtained from the classical experiments have traditionally rendered an average coefficient for new spontaneous mutations affecting viability in *Drosophila*  $\cong 0.4$ . However, this estimate is questionable.

Results obtained from Mukai's experiments are contradictory. A negative correlation was found between coupling heterozygous and homozygous viabilities ( $r = -0.25$  and  $r = -0.47$  at generations 32 and 60, respectively; Mukai and Yamazaki 1968). By generation 32 (60), the genetic variance for coupling heterozygotes was only 3% (1%) of that for homozygotes, suggesting a small degree of dominance. In contrast, for repulsion heterozygotes the regression estimate of  $h$  was  $\sim 0.4$ , the heterozygotic genetic variance being similar to that of homozygotes. Two additional regression estimates can be obtained for QN-coupling heterozygotes with nonisogenic chromosomes from Mukai *et al.* (1965). These can be computed from their homozygotic-heterozygotic genetic correlations (page 498, 0.5621 and 0.2698) multiplied by the appropriate genetic standard deviations from their Table 1 ( $\hat{\sigma}_{C^{het}}/\hat{\sigma}_{C^{hom}} = 0.126$  and 0.194, respectively). The estimates obtained for QN chromosomes are  $\bar{h}_{wsz} = 0.075$  and 0.053, respectively, close to those obtained from Ohnishi's data.

An even more striking feature observed in the results of Mukai and Yamazaki (1968) is that the average viability of coupling heterozygotes showed no reduction after 60 generations, while that of homozygotes and repulsion heterozygotes strongly declined. The original interpretation that the accumulated deleterious mutations behaved as overdominant in coupling and as partially recessive in repulsion is hard to understand. There is no simple way to reconcile these results, and explanations are doomed to be speculative. For example, we will never be able to rule out the possibility that the chromosome II (where mutations accumulated) had a tendency to produce recurrent recessive mutations. If this complication had occurred, repulsion heterozygotes could be homozygous for alleles with low viability shared by both parental lines, but this would not occur in coupling heterozygotes. This would explain why the average via-

bility of the repulsion heterozygotes was greatly reduced while that for coupling heterozygotes remained constant. Another explanation for this discrepancy is the following. Coupling heterozygotes were produced by crosses involving  $+_o/+_o$  females, while repulsion ones were produced by crosses involving  $Cy/+_i$  females (where  $+_i$  and  $+_o$  stand for the wild chromosome with and without accumulated mutations). It has been proposed that hybrid dysgenesis induced by crossing could produce segregation distortion in favor of  $Cy$ , biasing the viability estimates (Crow and Simmons 1983). This phenomenon is strain dependent and it is also known to be sex constrained. For example, the I-R system affects outcrossed females (Bregliano *et al.* 1995), although here the segregation distortion has not been investigated (J.-C. Bregliano, personal communication). Thus, hybrid dysgenesis could have caused the observed differences in average viability between coupling heterozygotes (produced by homozygotic females) and homozygotes or repulsion heterozygotes (produced by outcrossed females).

Results recently obtained by Houle *et al.* (1997) for different life-history traits suggest a pooled average coefficient of dominance of 0.12 with broad confidence limits (-0.17-0.41). This estimate includes all nonlethal chromosomes and, therefore, is expected to be lower than those for QN chromosomes, but the broad confidence intervals preclude any valid comparison. In any case, these are regression estimates, *i.e.*, weighted by  $s^2$ . For this reason, we believe that the average over traits should be made by weighting the  $\bar{h}_{ws^2}$  estimate for each fitness component by the corresponding  $\lambda E(s^2)$  estimate (the squared mutational coefficients of variation given by Houle *et al.*). This would provide an  $\bar{h}_{ws^2}$  estimate, where the appropriate contribution of the traits to the expected  $s^2$  for global fitness is considered. When this is done using data from Houle *et al.* (1997, Tables 4 and 5), the average coefficient of dominance over traits is 0.05. The estimate is consistent with the expected estimates for NL chromosomes with the MD model (see Table 3).

To the above uncertainties, we add in this article the possibility that Ohnishi's estimates for the degree of dominance are biased upward through the occurrence of nonmutational changes in viability in his MA lines. Ohnishi's published estimates were obtained from the same expression of the ratio of viability decline previously used by Mukai (1969; Equation 1). However, no estimates were made using the regression method, also used by Mukai and co-workers in previous articles. Here we found that the estimates from the two methods are radically different, the latter ones being considerably smaller than the former. Although some difference is expected because of the different weighting of the estimates, such a large difference is difficult to explain, as the data are obtained from chromosomes with a high viability. We suggest that a nonmutational decline in viability over the first half of Ohnishi's experiment

(see Keightley and Eyre-Walker 1999 and García-Dorado *et al.* 1999, for possible nonmutational causes of decline) may have caused overestimation of the average dominance through the method of the ratio. Such a nonmutational decline would not affect the regression estimates.

Alternatively, the larger initial viability decline in Ohnishi's lines could indicate mutational properties varying through the experiment: mutations with small deleterious effects being more common in the first half and accumulating also in the control. It has been proposed that the high rates of mild deleterious mutation found in Mukai's and Ohnishi's experiments could be due to an increased transposition rate induced by outcrossing (Keightley and Eyre-Walker 1999). However, in the case of Ohnishi's experiment, the large viability decline found during the early generations was also shown by the control chromosome, which was not outcrossed. Furthermore, it has been argued that, during mutation accumulation, transposition rates should increase (instead of decrease) with increasing number of inserts (Nuzhdin *et al.* 1996; Pasyukova *et al.* 1998). Regarding the control chromosome, it should be pointed out that it was maintained with a few single pairs (O. Ohnishi, personal communication), so nonsevere deleterious mutation could have accumulated in the control. Thus, under the BM or BM<sup>s</sup> models, the average viability of the control chromosome would be expected to decline as much as that of any other QN line. On the contrary, under the MD model, the control chromosome would likely be one of the 85% of nonlethal lines carrying no deleterious mutations by generation 40, its viability decline being nonmutational.

The simulations show that a model (MD) of low deleterious mutation rate, moderate kurtosis of the deleterious effects, and unweighted degree of dominance  $\sim 0.2$  fits the data better than a model (BM) derived from the large  $\lambda$  and  $h$  values previously estimated by Ohnishi. The MD model predicts that most detected deleterious mutations have  $s > 0.05$  and  $h < 0.25$ , mutations with  $s > 0.2$  having expected  $h < 0.04$  and occurring at an appreciable rate (0.0027 in the whole genome). Mild deleterious mutations would have  $\bar{h} \approx 0.4$ , a value slightly smaller than that from Ohnishi's estimates. Thus, although homozygous effects can be large, expected heterozygous effects ( $sh$ ) are  $< 0.02$  for any  $s$  value. This would produce long average persistence of mutations in natural populations ( $t > 80$ ), in rough agreement with other published estimates (Houle *et al.* 1996; Lynch *et al.* 1999). Using this relatively small  $\bar{h}$  value, mutation-selection balance could account for considerable inbreeding depression. However, since most deleterious mutations have important homozygotic effects in this model, the inbreeding load could be efficiently purged under slow inbreeding.

Of course, the above results are model dependent and other models can be constructed that fit the data adequately (Keightley 1996). For example, additional

mutations could have occurred from generations 0 to 20 with such a small effect that they do not appreciably affect the shape or the between-line variance of the distribution of line average viabilities. Thus, they would pass undetected in MD estimation (when this is unconstrained by  $\Delta M$ ), but they could still account for the excess in viability decline. Since estimates of  $\Delta V$  for QN are on the order of  $10^{-5}$  we can assume that an excess in  $\Delta V < 10^{-5}$ , parallel to the excess in  $\Delta M$ , could have passed undetected. Then, applying the Bateman-Mukai estimation method, the additional  $\Delta M$  observed during generations 0–20 (0.00274) would be ascribed to additional mutations occurring at a rate  $> 0.00274^2/10^{-5} = 0.75$  per chromosome II, with effects below 0.0036. This mutation rate seems to be exceedingly high, and the effects are smaller than the values usually considered for mild deleterious mutations (a few percent).

It is interesting to note that the MD model does not predict appreciable mutations with deleterious effects  $< 0.001$  (see Table 5). In fact, BM-based models are also quite insensitive to this class of mutations, for which the mutation rate is estimated as 0.0042 (BM model) and 0.055 (BM<sup>s</sup> model). However, in all three cases (MD, BM, and BM<sup>s</sup>), the scale parameter is small ( $\alpha < 1$ ), so that the mode of the distribution of effects is at  $s = 0$ . The class  $s < 0.001$  includes mutations whose frequency drifts as if they were neutral, as well as others whose effect is so large that natural selection makes their fixation extremely difficult (*i.e.*, constrained mutations, whose homozygous effects are larger than, say, 10-fold the inverse of the evolutionary population size). The frequency of this class is not expected to be  $> 0.02$  per chromosome II if just mutations producing amino acid changes are considered, but can be substantially increased due to selection at silent coding positions (biased codon usage) and to transposition (Keightley and Eyre-Walker 1999). However, due to limitation on the experimental power, standard mutation accumulation experiments are not expected to allow the study of this class ( $s < 0.001$ ) of mutations.

In this article, we have shown that current estimates of the degree of dominance are, at best, questionable. Given the importance of these estimates, it is clear that further experiments and reanalysis of previous ones are still necessary to get a better picture of quantitative genetic variation.

We are grateful to O. Ohnishi for allowing us to use his data and for kind support and to S. Otto, R. Shaw, and P. Keightley for helpful suggestions on the manuscript. This work was supported by grants PB95-0909-C02-01 (A.G.-D.) and PB96-0343 (A.C.) from the Ministerio de Educación y Cultura and by grant 64102C003 (A.C.) from Universidad de Vigo.

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