Dominance of Mutations Affecting Viability in *Drosophila melanogaster*

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ABSTRACT

There have been several attempts to estimate the average dominance (ratio of heterozygous to homozygous effects) of spontaneous deleterious mutations in *Drosophila melanogaster*, but these have given inconsistent results. We investigated whether transposable element (TE) insertions have higher average dominance for egg-to-adult viability than do point mutations, a possibility suggested by the types of fitness-depressing effects that TEs are believed to have. If so, then variation in dominance estimates among strains and crosses would be expected as a consequence of variation in TE activity. As a first test, we estimated the average dominance of all mutations and of *copia* insertions in a set of lines that had accumulated spontaneous mutations for 33 generations. A traditional regression method gave a dominance estimate for all mutations of 0.17, whereas average dominance of *copia* insertions was 0.51; the difference between these two estimates approached significance ($P = 0.08$). As a second test, we reanalyzed Ohnishi's (1974) data on dominance of spontaneous and EMS-induced mutations. Because a considerable fraction of spontaneous mutations are caused by TE insertions, whereas EMS induces mainly point mutations, we predicted that average dominance would decline with increasing EMS concentration. This pattern was observed, but again fell short of formal significance ($P = 0.07$). Taken together, however, the two results give modest support for the hypothesis that TE insertions have greater average dominance in their viability effects than do point mutations, possibly as a result of deleterious effects of expression of TE-encoded genes.

THE rates and effects of spontaneous deleterious mutation figure importantly in much evolutionary and ecological theory (reviewed in Lynch *et al.* 1999). One of the most important questions regarding deleterious mutations is whether they can account for the majority of genetic variation for fitness traits observed in natural populations. Under a simple model of mutation-selection balance (Charlesworth and Hughes 1999), the genetic variance for fitness maintained at equilibrium is given by the product of the diploid rate of deleterious mutation, U, and the average heterozygous effect of mutations on fitness. Most empirical studies, however, have focused on homozygous effects of mutations. These studies provide little information on how much genetic variance might be maintained by mutation-selection balance in outbreeding species.

Homozygous and heterozygous effects of mutations are related by the dominance coefficient, h. An h value of 0.5 implies additivity of mutations, while values of 0 and 1 imply complete recessivity and complete dominance, respectively. Most of the available information on dominance coefficients of deleterious mutations in higher eukaryotes comes from mutation-accumulation (MA) experiments in *Drosophila melanogaster* (reviewed in Simmons and Crow 1977; also see Houle *et al.* 1997; Chavarrías *et al.* 2001). In these experiments, spontaneous mutations were allowed to accumulate in sets of lines derived from a single isogenic progenitor by maintaining the lines at a very small population size, a situation that minimizes the effectiveness of selection. After multiple generations, the lines were assayed for one or more measures of fitness in both homozygous and heterozygous conditions. Although other components of fitness have been studied (*e.g.*, Houle *et al.* 1997), most of the studies have used egg-to-adult viability as the fitness measure.

If an appropriate control line is available—*i.e.*, one that preserves the characteristics of the progenitor stock before mutation-accumulation—then h can be estimated from the declines in mean viability of the MA lines in both heterozygous and homozygous conditions. The details depend on whether heterozygotes are generated by crossing to the control or other relatively mutant-free stock ("coupling" crosses), in which case the new mutations are on only one homolog, or by crossing different MA lines to each other ("repulsion" crosses), in which case mutations are on both homologs. The general formula is

$$h_{MD} = \frac{(M_{HET,C} - M_{HET,M})\,M_{HOM,C}}{z(M_{HOM,C} - M_{HOM,M})\,M_{HET,C}} \qquad (1)$$

(Simmons and Crow 1977), where M is mean viability, the subscripts C and M denote controls and MA lines,

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the subscripts HET and HOM denote heterozygous and homozygous viabilities, and $z = 1$ or 2 for coupling and repulsion crosses, respectively. An alternative method for estimating h is by regressing heterozygous viabilities of MA lines on their homozygous viabilities, correcting for sampling error of the homozygous line means (Mukai *et al.* 1972; Caballero *et al.* 1997; Chavarrías *et al.* 2001):

$$h_{\mathrm{REG}} = \frac{\sigma_{\bar{X},\bar{Y}}}{z\sigma^2_{\mathrm{G},X}}. \qquad (2)$$

Here, $\sigma_{\bar{X},\bar{Y}}$ is the covariance of coupling heterozygote viability with viability of the corresponding homozygote or the covariance of repulsion heterozygote viability with the sum of the viabilities of the corresponding homozygotes, and $\sigma^2_{\mathrm{G},X}$ is the variance component among lines for homozygous viability. The mean decline method gives an estimate of h weighted by s, the homozygous effect of mutations, while the regression method estimates h weighted by s^2. While the latter weighting is less desirable than the former, the regression method has the advantage of not requiring a control line.

The above methods have been applied to data from three MA experiments by Mukai and co-workers (Mukai *et al.* 1964, 1965; Mukai and Yamazaki 1968), Ohnishi (1974, 1977b), and Chavarrías *et al.* (2001). Using both the mean decline and regression methods, Mukai and co-workers obtained a puzzling array of results (reviewed in Simmons and Crow 1977), including overdominance (negative h) when MA line chromosomes were made heterozygous against a chromosome from a high-viability MA line presumed to carry few or no new mutations (Mukai *et al.* 1964), near additivity when they were made heterozygous against more normal MA lines (Mukai and Yamazaki 1968), but near recessivity ($h \approx 0.1$) when they were made heterozygous against unrelated chromosomes (Mukai *et al.* 1965). Ohnishi (1974, 1977b) could not replicate the finding of overdominance. Applying the mean decline method to both coupling and repulsion crosses, he reported mutations to be close to additive. García-Dorado and Caballero (2000), however, pointed out that Ohnishi used viability at the beginning of the experiment in calculating control viability, and there is some evidence that the subsequent viability decline in the MA lines was partly nonmutational in origin (*e.g.*, due to a change in the environment). A nonmutational decline, by inflating both the numerator and the denominator of (1), would bias the estimate of h upward. New h estimates calculated by García-Dorado and Caballero (2000) from Ohnishi's (1974) data by the regression method were much lower, averaging 0.14. Finally, Chavarrías *et al.* (2001) obtained an estimate of $h \sim 0.3$ using the regression method.

One hypothesis for the variation in reported h estimates is that h varies among different types of mutations. Transposable element (TE) insertions account for roughly half of all spontaneous mutations in *D. melanogaster* (Finnegan 1992; *cf.* Yang *et al.* 2001), and activity of TEs can vary considerably among strains (Pasyukova *et al.* 1997; Nuzhdin 1999). If TE insertions have dominance properties different from those of other classes of mutations, especially base substitutions, then the average dominance of spontaneous mutations would be expected to differ among strains. Indeed, there is reason to suspect that TE insertions should have greater average dominance than do base substitutions. TE insertions, like base substitutions, can disrupt genes; if the affected gene is haplo-sufficient, such gene disruption effects will be largely recessive. TEs can potentially affect fitness in other ways, however. Expression of TE-encoded genes could directly reduce host fitness, either because the extra transcription and translation is energetically costly (Nuzhdin *et al.* 1996; Carr *et al.* 2002) or because of transpose-induced chromosome breaks or deletions (McCarron *et al.* 1994). In addition, TE-mediated ectopic exchange events (recombination between TEs of the same family present at different chromosomal locations) can lead to deleterious deficiencies or other rearrangements (Montgomery *et al.* 1991). In contrast to the effects of gene disruption, negative effects of TEs caused by expression of their genes should be approximately additive, while negative effects caused by ectopic exchange events, which occur mainly in individuals heterozygous for element positions (Montgomery *et al.* 1991), should be underdominant ($h > 1$).

In spite of the above considerations, there have been no tests of whether TE insertions show greater average dominance in their fitness effects than do other types of mutations. We report here two tests of this hypothesis for mutations affecting viability in *D. melanogaster*. For the first test, we used a set of MA lines from the experiment of Fry *et al.* (1999; also see Fry 2001; Fry and Heinsohn 2002). We have taken advantage of the natural activity of the retrotransposable element *copia* in these lines to estimate the effects of *copia* insertions on both heterozygous and homozygous viability and the average dominance of the insertions. We compare the average dominance of *copia* insertions to the average dominance of all spontaneous mutations in the lines, estimated by both of the above methods. Because the spontaneous mutations are likely to have included many point mutations, we predict that $h(\textit{copia}) > h$(all mutations).

Second, we have reexamined the data of Ohnishi (1974, 1977b) on dominance of spontaneous and ethylmethanesulfonate-induced mutations affecting viability. Because an appreciable fraction of the spontaneous mutations were likely to have been TE insertions, while EMS produces mainly base substitutions (Grigliatti 1998), we predict that h(spontaneous mutations) > h(EMS-induced mutations). Using the mean decline method, Ohnishi (1977b) reported that h was 0.4–0.5 for lines either not treated with EMS or treated with a

low concentration (0.1 mM), but was 0.1–0.3 for lines treated with a higher concentration (0.5 mM). All of these estimates, however, are likely to have been biased to an unknown extent by Ohnishi's choice of a control line (see above). For this reason, we have used García-Dorado and Caballero's (2000) method to produce new estimates of dominance for the two EMS treatments. We predicted that the average dominance of mutations should decline as the fraction of mutations caused by EMS increases.

MATERIALS AND METHODS

Rearing conditions and stocks: Flies were reared under continuous light in 2.5-cm-diameter shell vials containing 9 ml of cornmeal-molasses dead yeast-agar medium (Fry *et al.* 1996). Unless otherwise indicated, the temperature was 25° ± 1°.

The MA lines and control populations were made using the balancer stock *Cy/Pm*; *ve*, as described in Fry *et al.* (1999). Each MA line was propagated by crossing two (one in the first and final generations) *Cy/+* males to 5 *Cy/Pm* females each generation. Under this crossing scheme, selection against deleterious mutations on the + second chromosome should be largely ineffective. The work reported here makes use of lines in which mutations were accumulated for 33 generations. At the end of this period, two homozygous sublines were made from each MA line by independently crossing two sets of 10 *Cy/+* or *Pm/+* males from the MA line to 10 *Cy/Pm* females at a density of five pairs per vial for two consecutive generations. From the progeny of the second cross, *Cy/+* female and male progeny were mated to each other; their +/+ progeny were collected to establish the subline (August 1997). The two sublines from the same MA line (hereafter, A and B) have coancestries of 0 for the *X* chromosome and ~0.01 for the third and fourth chromosomes. The sublines were subsequently maintained by mass transfer in two vials per subline on 4-week generations at 18°. Some of the 34 original MA lines were infertile or difficult to maintain as homozygotes; as a result, only 25 lines were available by the time the work reported below was conducted.

While mutations were being accumulated in the MA lines, each of the three control populations was maintained at a population size of ~300 +/+ flies each, where + denotes the progenitor chromosome for the MA experiment. To further slow accumulation of mutations, the control populations were maintained on a longer generation interval than the MA lines (Fry *et al.* 1999). At about the same time that the MA sublines were made, four sublines were made from each control population using a modification of the above procedure. In the first cross to establish each subline, 35 +/+ males from the control population were mated to 35 *Cy/Pm* females in seven vials. Thirty-five *Cy/+* males were backcrossed to 35 *Cy/Pm* females for two more generations, and then homozygous sublines were established and maintained as above.

All MA and control lines were marked with the third chromosome marker *veinlet* (*ve*); no *ve*+ flies were observed in any of the lines.

Estimation of heterozygous viabilities: This experiment was performed in early 1999. Males from each subline of each MA and control line were first crossed to *Cy/Pm* females; the *Pm/+* male progeny were then used for the crosses to measure viability. In each cross, eight *Cy/Pm* females were mated to eight *Pm/+* males. Crosses were conducted in 12 blocks, each set up on a different day using a different randomized order of crosses, with one cross per subline per block. After 5 days, all flies were transferred to a second vial; after 5 more days, the flies were discarded. Progeny were counted on days 12, 14, and 19 after the crosses were set up.

The above crosses produce *Cy/Pm*, *Cy/+*, and *Pm/+* flies in equal expected numbers (the *Cy/Cy* combination is lethal). The *Cy/Pm* genotype is the same regardless of the MA or control line used and therefore can be used as a reference genotype for estimating the relative viability of the other two genotypes, which are heterozygous for MA or control line chromosomes. For each cross, counts from the two vials were pooled; the relative viability of *Cy* or *Pm* heterozygotes was then estimated as (no. of *Cy/+* or *Pm/+* flies)/(no. of *Cy/Pm* flies + 1). The 1 in the denominator is a slight bias correction (Haldane 1956). In addition, the mean of the two viability measures was used as a measure of average viability of the two heterozygous genotypes.

Data were analyzed using the MIXED procedure in SAS (Littell *et al.* 1996). For each group of lines (MA or control), effects were lines, sublines within lines, blocks, and the block × line interaction, all considered random. Means of each group and their standard errors were estimated by requesting the solution for the model intercept. Variance components were tested for significance using likelihood-ratio tests (Fry and Heinsohn 2002). A MIXED program was also used to estimate the genetic correlation between *Cy/+* and *Pm/+* viability and to test whether the among-line variances of the two measures differed significantly (for description of similar programs see Messina and Fry 2003).

Dominance of mutations was estimated by both the mean decline and regression methods. Data on homozygous viability came from the generation 33 data set of Fry *et al.* (1999); data from both the "ethanol" and "standard" treatments were used, because there was no evidence of genotype-environment interaction across these treatments (Fry *et al.* 1999; Fry and Heinsohn 2002). Mean viability and the variance component among lines were recalculated using only the 25 MA lines for which heterozygous viability was measured. For estimating the variance component, treatment was considered a fixed effect, and a random block effect and block × treatment interaction were included. Because of the small number of control lines, it was not appropriate to use resampling methods (*e.g.*, the bootstrap) to calculate a confidence interval for h_{MD}. Instead, a rough confidence interval was calculated by using the upper and lower bounds of a 95% confidence interval for $M_{HET,C} - M_{HET,M}$ (obtained by using the ESTIMATE statement in MIXED), regarding the other quantities in Equation 1 as estimated without error. While the confidence limit produced by this procedure is undoubtedly too narrow, $M_{HET,C} - M_{HET,M}$ is probably the greatest source of error in the estimation procedure.

For the regression method (Equation 2), $\sigma_{\bar{X},\bar{Y}}$ was calculated using mean viabilities that had been standardized by dividing by the corresponding control mean. Bootstrap 95% confidence intervals for h_{REG} were calculated by resampling lines with replacement 10,000 times, repeating the calculations (including the calculation of $\sigma^2_{G,X}$) each time. The 2.5th and 97.5th percentiles of the resulting distributions served as the upper and lower bounds of the confidence limit. $\sigma^2_{G,X}$ was negative in only a trivial fraction (0.11%) of the bootstrap replicates; in these cases it was set to 10^{-6}.

Determination of *copia* copy number and effects on viability: Insertion sites of *copia* were determined in both sublines of 24 of the 25 MA lines in late 1999. *copia* was scored by *in situ* hybridization of the plasmid *cDM5002* containing a full-length *copia* transposable element (Finnegan *et al.* 1978) to polytene salivary gland chromosomes of third instar larvae (Shrimpton *et al.* 1986). Probes were labeled with biotinylated dATP (bio-7-dATP; Bethesda Research Laboratories, Gaithersburg, MD) by nick translation. Hybridization was detected using the Vec-

tastain ABC kit (Vector Laboratories, Burlingame, CA) and visualized with diaminobenzidine. The element locations were determined at the level of cytological bands on the standard Bridge's map. Two larvae per subline were scored.

To estimate *copia* effects on heterozygous and homozygous viability, least-squares line means were calculated on log-transformed data and regressed on the number of *copia* sites shared by both sublines of a line. The resulting slopes can be interpreted as the proportional decline in viability due to the average *copia* insertion. *copia* sites not present in both sublines were ignored, because these most likely arose from insertions that occurred after the sublines were created. This would have been after the homozygous viability data were collected, and possibly after the heterozygous data were collected as well. The proportion of genetic variance explained by the regressions was calculated as the bias-adjusted R^2 of the model times the ratio $\sigma^2_{\bar{X}}/\sigma^2_{G,X}$ for log-transformed heterozygous or homozygous viability as appropriate, where $\sigma^2_{\bar{X}}$ is the variance among line means.

The average dominance of *copia* insertions, h_{COPIA}, was estimated as the ratio of heterozygous to homozygous effects. A 95% confidence limit for h_{COPIA} was calculated by taking 10,000 bootstrap samples, repeating both regressions for each sample. Replicates in which the regression of homozygous viability on *copia* number was positive (4.1% of the total) were excluded. For each bootstrap sample, the difference $h_{COPIA} - h_{REG}$ was calculated; the fraction of replicates in which this difference is negative was used as the empirical P value for testing the one-sided hypothesis $h_{COPIA} > h_{REG}$ against the null hypothesis $h_{COPIA} = h_{REG}$.

Reanalysis of data of Ohnishi (1974): Although Ohnishi (1974, 1977b) used only the mean decline method to estimate dominance, García-Dorado and Caballero (2000) pointed out that it is possible to calculate estimates by the regression method from information in Ohnishi's (1974) dissertation. They applied their method only to the non-EMS-treated lines, however. Here, we extend García-Dorado and Caballero's (2000) results by applying the method to the two EMS treatments. The relevant data come from Tables 2, 12, and 15 in Ohnishi (1974). These data are for "quasinormal" lines only, *i.e.*, those with at least half the viability of the controls. Lines treated with 0.5 mM EMS were assayed in both coupling and repulsion at generations 7 and 13 and in repulsion only at generation 3. Lines treated with 0.1 mM EMS were assayed in both coupling and repulsion at generations 7, 15, and 25, and in repulsion only at generation 3. Lines not treated with EMS were assayed in both coupling and repulsion at generations 10, 20, 30, and 40. The total numbers of dominance estimates are therefore 5, 7, and 8 for 0.5 mM EMS, 0.1 mM EMS, and spontaneous mutations, respectively. The number of lines on which the estimates are based ranges from 35 to 66 for the EMS treatments and from 58 to 90 for the spontaneous treatment.

For each treatment, we calculated the mean dominance estimate, weighted by the reciprocal of the squared standard errors (García-Dorado and Caballero 2000; weighted and unweighted means differed only slightly). The means were then regressed on an estimate of the fraction of mutations in each treatment that were spontaneous in origin. To calculate the latter, we used the per-generation rates of lethal mutations observed at each concentration: These were 0.0041, 0.0118, and 0.0556 for no EMS, 0.1 mM EMS, and 0.5 mM EMS, respectively (Ohnishi 1977a, Table 3). The fractions of spontaneous mutations are therefore 1, 0.0041/0.0118, and 0.0041/0.0556. The Y-intercept of this regression estimates the dominance of EMS-induced mutations (h_{EMS}), and the slope estimates $h_{SPONT} - h_{EMS}$; the slope plus the intercept therefore estimates h_{SPONT}. The standard error of h_{SPONT} was calculated from the variances and covariance of the slope and intercept estimates. Note that we used means rather than the individual dominance estimates in the regression, because the latter are not necessarily independent. Because there are only three points, the regression has low power.

RESULTS

Heterozygous viability of MA lines: Viability of *Cy/+* and *Pm/+* genotypes bearing + chromosomes from the MA or control lines was measured relative to the *Cy/Pm* standard. Although we tested only 25 MA lines, the replication within lines (24 crosses) was high compared to previous studies. Mean viability of either genotype did not differ between the MA lines and controls (Table 1). Variation among MA lines was marginally significant for *Pm/+* viability ($P = 0.079$), but not for *Cy/+* or average heterozygous viability ($P > 0.15$). Variation among sublines within lines was nonsignificant for all three viability measures ($P > 0.17$).

Cy/+ and *Pm/+* viabilities were highly correlated (product moment correlation of MA line means = 0.80, $P < 0.0001$; point estimate of genetic correlation = 1.12). In addition, among-line variances of the two measures were not significantly different ($P > 0.2$). For these reasons, dominance estimates and estimates of *copia* effects are presented for average heterozygous viability only.

The estimate of dominance by the mean decline method is −0.10, with a broad confidence limit of −0.58 to +0.37. The means give little information regarding dominance. The regression of heterozygous on homozygous viability was positive and marginally significant ($P = 0.085$, one-tailed; Figure 1). The estimate of dominance by the regression method (h_{REG}) is 0.16, with a bootstrap 95% confidence limit of −0.05 to +0.47. Taken together, the dominance estimates indicate that additivity of mutations can be rejected. Although the regression results suggest that mutations had some heterozygous effects, complete recessivity cannot be formally rejected.

Effects of *copia* on heterozygous and homozygous viability: Nine *copia* sites were shared by all MA lines; these were apparently present in the progenitor of the MA lines. *copia* transposed actively in the lines; there were an average of 2.2 new insertions per line (range 0–6).

The regression of heterozygous viability on *copia* copy number was significantly negative (Figure 2; $P = 0.04$, one-tailed), while that of homozygous viability on copy number was marginally significant ($P = 0.08$). Slopes (SE) were −0.013 (0.007) and −0.025 (0.017), respectively; insertion number explained 24% of the genetic variance of heterozygous viability but only 4.7% of that of homozygous viability. The ratio of the two slopes gives a dominance estimate for *copia* insertions of 0.51, suggesting approximate additivity. The bootstrap con-

TABLE 1

Basic statistics for heterozygous viability in the control and MA lines

Viability type	Means (SE)		Variances (SE)			
	Control lines	MA lines	Among MA lines	Among sublines within MA lines	Residual, MA lines	Residual, control lines[a]
Cy/+	1.337 (0.029)	1.337 (0.027)	0.766 (1.98)	2.05 (2.59)	80.8 (5.01)	86.7 (10.9)
Pm/+	1.447 (0.030)	1.473 (0.027)	3.20 (2.22)	0	102.4 (6.21)	66.1 (8.33)
Average	1.392 (0.027)	1.405 (0.026)	1.93 (1.98)	1.32 (2.19)	73.0 (4.53)	60.2 (7.58)

Variances and their standard errors have been multiplied by 1000. Viabilities are based on a mean of 229 (range 42–415) flies per cross.

[a] Among the controls, all line and subline variances were zero.

fidence interval for h_{COPIA} is 0.09–5.0; recessivity of *copia* insertions can therefore be rejected. $h_{COPIA} > h_{REG}$ in all but 8.3% of the bootstrap samples. Thus, while we cannot formally reject the hypothesis $h_{COPIA} = h_{REG}$, the difference approaches significance.

Comparison of dominance of spontaneous and EMS-induced mutations in Ohnishi's (1974) experiment: Dominance estimates increased as the fraction of spontaneous mutations increased, as predicted (Figure 3). Unsurprisingly, given the small number of points, the regression is not quite significant ($P = 0.066$, one-tailed). The *Y*-intercept gives an estimate of the dominance of EMS mutations that is low and not significantly different from zero ($h_{EMS} = 0.021$; SE = 0.015; $P = 0.20$, one-tailed). In contrast, the estimated dominance of spontaneous mutations is significantly greater than zero ($h_{SPONT} = 0.141$, SE = 0.016, $P = 0.04$). There is no tendency for dominance estimates from coupling crosses to differ from those of repulsion crosses (Figure 3).

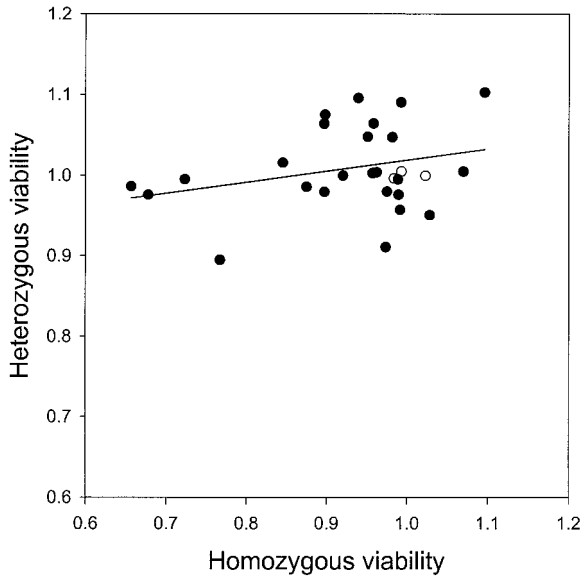

Figure 1.—Relationship between heterozygous and homozygous viabilities. Solid symbols, MA lines; open symbols, control lines; the regression line is for MA lines only. Viabilities have been standardized by the control means.

DISCUSSION

The hypothesis that transposable element insertions have effects on viability that are less recessive than those of base substitutions leads to the prediction that dominance coefficients should obey the inequalities h(TE insertions) $>$ h(all spontaneous mutations) $>$ h(EMS-induced mutations). In the experiment with the MA lines of Fry *et al.* (1999), estimates of dominance of spontaneous mutations based on the mean decline and regression methods were not significantly different from zero. In contrast, the estimate of the average dominance of spontaneous *copia* insertions was ~0.5, suggesting that *copia* had roughly additive effects on viability. A bootstrap comparison of the dominance of *copia* insertions with the regression method estimate of dominance of all mutations indicated that the difference approached significance ($P = 0.083$). In the reanalysis of Ohnishi's

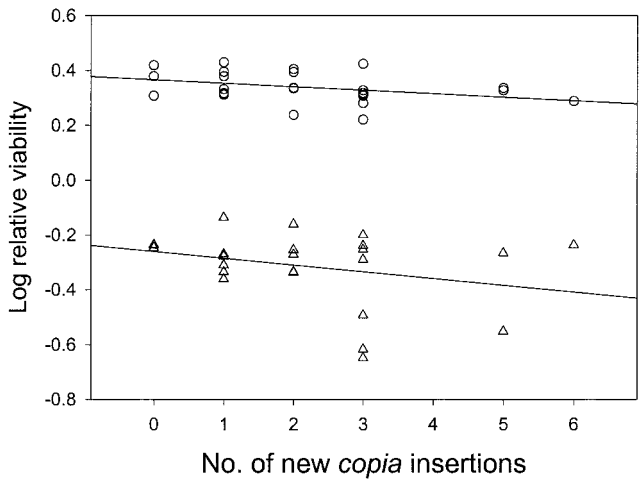

Figure 2.—Relationships between heterozygous (circles) and homozygous (triangles) viability and the number of *copia* insertions in the MA lines.

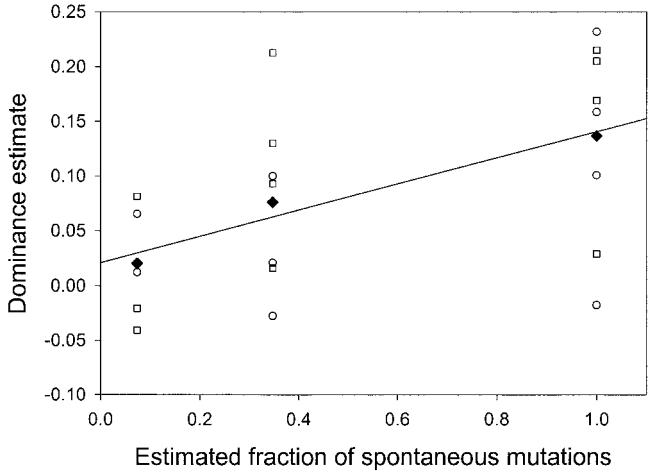

FIGURE 3.—Dominance estimates for quasinormal lines recalculated from data of OHNISHI (1974), using the method of GARCÍA-DORADO and CABALLERO (2000). The *x*-axis is the fraction of mutations estimated to be of spontaneous origin; from left to right, the three treatments are 0.5 mM EMS, 0.1 mM EMS, and no EMS. Open circles, coupling crosses; open squares, repulsion crosses; solid diamonds, means. The regression line is based on the means only. Estimates for the spontaneous mutation treatment come from GARCÍA-DORADO and CABALLERO (2000).

(1974) data, we found evidence for higher dominance of spontaneous mutations than of EMS-induced mutations (Figure 3), but this too fell short of formal significance ($P = 0.066$). Because both predicted inequalities derive from the same hypothesis, we can use Fisher's method of combining probabilities (SOKAL and ROHLF 1981) to combine the two tests. The result is significant ($\chi^2 = 10.41$, d.f. = 4, $P = 0.034$). Our results thus give modest support for the hypothesis that TE insertions have greater average dominance than do base substitutions.

The fitness effects of TEs are likely to be mediated through multiple pathways, rather than solely through harmful effects of their insertions on the expression of nearby genes. There are three potential mechanisms of selection against TEs (for review see NUZHDIN 1999). First, individual TE copies may be deleterious because they disrupt genes ("gene-disruption model"; FINNEGAN 1992). Second, transcription of TEs and translation of TE-encoded proteins may be costly, and these transcripts and proteins may generate deleterious effects by nicking chromosomes and disrupting cellular processes ("TE-product expression model"; NUZHDIN *et al.* 1996; CARR *et al.* 2002). Finally, a high copy number of TEs could be deleterious because ectopic recombination among dispersed and heterozygous TEs generates strongly deleterious chromosome rearrangements ("ectopic recombination model"; MONTGOMERY *et al.* 1987). While harmful effects of gene disruption by TEs should be largely recessive, those through TE expression should be additive, and those through ectopic recombination should be underdominant.

We believe our results can best be explained by the TE-product expression model, for the following reasons. Although ectopic exchange events among TEs present in multiple copies can result in chromosome rearrangements with negative fitness effects, such events are expected to occur primarily in female meiosis (MONTGOMERY *et al.* 1991). In our experiment to measure heterozygous viability, however, the MA line chromosome was contributed by the male parent. This was also true in Ohnishi's coupling crosses, which gave similar dominance estimates to his repulsion crosses (Figure 3; means in the spontaneous treatment were 0.134 and 0.140, respectively). Thus ectopic exchange events are not likely to have contributed to the dominance estimates in either experiment. (This does not mean that ectopic exchange events are not important as a force regulating TE copy number in natural populations.)

The view that TEs are closer to additive than to recessive in their fitness effects is indirectly supported by the abundance of TEs on *X* chromosomes *vs.* autosomes. If TEs were recessive, the *X* should contain ~13% of all inserts, rather than 20% when they are additive. While variable across TE families, actual distributions are closer to the latter number (reviewed by CHARLESWORTH *et al.* 1994; CARR *et al.* 2002).

We are unaware of other studies in which dominance coefficients of different types of mutations have been measured in the same genetic background. Two pairs of studies, however, have reported dominance estimates for EMS-induced mutations and *P*-element insertions, respectively. MUKAI (1970) and TEMIN (1978) measured heterozygous and homozygous viability of EMS-treated second chromosomes and found evidence for low dominance, consistent with our conclusions. MACKAY *et al.* (1992) and LYMAN *et al.* (1996) examined heterozygous and homozygous viability effects of new *P*-element insertions and reported somewhat contradictory results regarding dominance. On one hand, in MACKAY *et al.*'s (1992) experiment, heterozygous and homozygous viability of the lines with inserts (mean of 3.1/line) was lower than that of the control lines by 17 and 38%, respectively. This gives a dominance estimate by the mean decline method of 0.45. On the other hand, the regression of heterozygous viability on homozygous viability was close to zero (surprisingly, in view of the reported large viability reductions per insert under both heterozygous and homozygous conditions and the high variance in the number of inserts per line); this was also the case in LYMAN *et al.*'s (1996) study. Nonetheless, the *P* elements used by both studies are unlikely to be appropriate for testing the TE-product expression model. It is not known whether the defective Birmingham elements used by MACKAY *et al.* (1992) produce any transcript; if so, the product is not functional as either a transposase

or a repressor (LeMaitre and Coen 1991). Similarly, the elements used by Lyman *et al.* (1996) were artificial constructs devoid of any *P*-element genes. We also note that the regression method for estimating dominance is based on the assumption of a Poisson distribution of mutations among lines. It is therefore not appropriate for the case where all lines have exactly one mutation, as in Lyman *et al.*'s (1996) study.

Our results add to the growing body of evidence that *copia* insertions in laboratory lines in which *copia* transposes at a high rate reduce fitness traits on the order of 1% per insert (D. Houle and S. V. Nuzhdin, unpublished results; E. Pasyukova, S. V. Nuzhdin, T. V. Morozova and T. F. C. Mackay, unpublished results). These negative effects may be related to the putative *copia* virus-like particles observed in cell nuclei of lines in which *copia* is active (Nuzhdin *et al.* 1996). Regardless of its origin, such an appreciable effect per insert at first glance seems incompatible with the much lower indirect estimates of selection coefficients against TE insertions from data on site occupancies in wild populations (Charlesworth and Langley 1989; Nuzhdin 1999). The TE-product expression model provides a possible resolution to this paradox. Under this model, the fitness effect of an insert should increase with increasing transcription of the element. Mutations in several genes have been shown to affect *copia* transcription (see references in Nuzhdin *et al.* 1998), and *copia* transcript abundance varies up to 10-fold among laboratory lines and natural isolates of *D. melanogaster*, even after correcting for *copia* copy number (Csink and McDonald 1990; Nuzhdin *et al.* 1998). Transcription is expected to be high in lines in which *copia* transposes at an unusually high rate (Nuzhdin *et al.* 1998); therefore these lines should also exhibit higher-than-normal fitness effects per insert. This prediction could be tested by measuring the fitness effects of chromosomes with a wide range of *copia* copy numbers in two genetic backgrounds, one restrictive and one permissive for *copia* transcription.

We speculate that genetic modification of TE transcription could account for some of the puzzling results regarding dominance of spontaneous mutations from Mukai's first mutation-accumulation experiment (Mukai *et al.* 1964, 1965; Mukai and Yamazaki 1968). The majority of MA lines (the "group 2 lines," in the terminology of Mukai and Yamazaki 1968) showed a rapid and apparently accelerating decline in homozygous viability, while eight (the "group 1 lines") showed relatively little change in viability (Mukai and Yamazaki 1968; see also García-Dorado and Caballero 2002). When group 2 lines were crossed to each other to generate repulsion heterozygotes, there were strong and highly significant correlations between heterozygous and homozygous viabilities, giving rise to dominance estimates of ~0.4 (Mukai and Yamazaki 1968). In contrast, when males from group 2 lines were crossed to females from a group 1 line or to an unrelated line from either the same or a different population, correlations between heterozygous and homozygous viability were weak (see Figure 2 in Mukai *et al.* 1965; the two right-most points, which include the group 1 lines, should be ignored). These results are consistent with the possibilities that (1) the rapid decline in viability of the group 2 lines was caused by unusually high activity of one or more families of TEs and (2) the other lines were restrictive for transcription of these TEs, with the restrictive phenotype being either partially dominant or maternally inherited. Under these assumptions, TEs would have been highly expressed in the repulsion crosses, giving rise to negative, nearly additive fitness effects, but much less expressed in the coupling crosses. Although we will never know the explanation with certainty, this hypothesis seems more plausible to us than Mukai and Yamazaki's (1968) hypothesis that the effects of nonallelic mildly deleterious mutations differ depending on whether they are located *in cis* or *in trans*. Our hypothesis does not account for the apparent overdominance of mutations in crosses between the group 1 and group 2 lines (Mukai and Yamazaki 1968; see especially their Figure 6); heterosis in these crosses would be expected, however, if the group 1 lines resulted from a contamination event early in the experiment, as suggested by García-Dorado and Caballero (2002).

Our results give evidence that the average dominance of mutations affecting viability varies among mutation categories, with transposable element insertions having greater dominance than base substitutions. This is consistent with the view that expression of TE-encoded genes negatively affects host fitness. Additional studies that compare dominance of different mutation categories, as well as direct tests of the TE-product expression model, are needed.

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