Spontaneous Mutational Variances and Covariances for Fitness-Related Traits in *Drosophila melanogaster*

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ABSTRACT

Starting from a completely homozygous population of *Drosophila melanogaster*, 176 lines were derived and independently maintained by a single brother-sister mating per generation. Three fitness-related traits were considered (fecundity, egg-to-pupa and pupa-to-adult viabilities). Mutational heritabilities of these traits and genetic correlations between all possible pairs were calculated from the between line divergence (codivergence), after 104–106 generations of mutation accumulation. Mutational heritabilities ranged from 0.60×10^{-3} to 0.82×10^{-3} and correlations from -0.11 to 0.25. These values are likely to be underestimates due to selection against deleterious mutations. The distribution of the means of the lines was asymmetric, positive for fecundity and negative for both viability components. The coefficients of asymmetry are also likely to be biased, again due to selection. Extreme lines from the two tails of the distribution were examined in detail. Homozygous line effects were all negative for viability traits but predominantly positive for fecundity, indicating the fixation of mutations with positive effects on the latter. Corresponding heterozygous line effects showed a variable degree of dominance.

There is a considerable amount of theoretical work aimed at understanding the role of mutation in the maintenance of polygenic variation in natural populations under selection (see reviews by BULMER 1989; BARTON 1990; HILL 1990). In finite populations, the outcome critically depends on the shape of the bivariate distribution of mutant effects on the quantitative trait of interest and fitness (KEIGHTLEY and HILL 1990). That shape can be characterized by the correlation of absolute values of mutant effects on the trait and fitness and the variances of the univariate marginal distributions. These are linearly related to the corresponding mutational rate of input of genetic variation (mutational variance, σ_m^2).

Experimental information on this parameter is largely restricted to both a single organism (*Drosophila melanogaster*) and a particular set of traits (morphological). For spontaneous mutations, estimates of mutational heritabilities (the mutational variance scaled by the environmental variance σ^2) cluster around a value of 10^{-3} (MACKAY *et al.* 1992, 1994; SANTIAGO *et al.* 1992; LÓPEZ and LÓPEZ-FANJUL 1993). However, these estimates have been based on a neutral infinitesimal model and are likely to underestimate the true value (KEIGHTLEY *et al.* 1993).

Information on the mutational heritability of fitness-related traits is very scarce. For *D. melanogaster* egg-to-adult viability, estimates of the order of 10^{-5} have been obtained from reanalysis of experimental data from MUKAI and coworkers and OHNISHI (LYNCH 1988). Recently, HOULE *et al.* (1994) reported estimates of the mutational heritabilities of life history traits (fecundity, male mating ability, productivity and longevity) ranging from 0.8×10^{-3} to 3.7×10^{-3}. In addition, HOULE *et al.* (1992) obtained an estimate of the mutational variance for a measure of fitness one order of magnitude larger than those previously reported for viability.

The data also suggest that the distribution of mutant effects is asymmetric (of variable sign for morphological traits and negative for viability) and leptokurtic (both for morphological traits and viability), with an intermediate correlation between the absolute value of mutant effects on morphological traits and on fitness (SANTIAGO *et al.* 1992; CABALLERO and KEIGHTLEY 1994; KEIGHTLEY 1994).

Here we present estimates of mutational variances and covariances for three fitness-related traits in *D. melanogaster*. Data has been obtained from inbred lines started from the same homozygous base population, in which mutations have been allowed to accumulate for >100 generations. The properties of mutations causing most of the divergence between individual lines have also been investigated. Our approach provides information that differs from that obtained in experiments involving the accumulation of mutations in chromosomes maintained against a balancer (see MUKAI 1985 for a review). In this latter case, mutations are virtually sheltered from natural selection, observations are usually restricted to one chromosome and further characterization of isolated mutations has not generally been pursued.

MATERIALS AND METHODS

Base population and inbred lines: A *D. melanogaster* line isogenic for all chromosomes obtained by CABALLERO *et al.*

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(1991) was used as the base population. From this, 200 inbred lines were started. Half of them (lines B1–B100) were always maintained by a single brother-sister mating per line and generation. The other half (lines C1–C100) were initially maintained by two double-first-cousin matings per line and generation (generations 0–47) and by one single brother-sister mating thereafter (see SANTIAGO *et al.* 1992 for further details).

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

Culture conditions and traits scored: Flies were reared in the standard medium formula of this laboratory (Brewer's yeast-agar-sucrose). All cultures were incubated at 25 ± 1° and maintained under continuous lighting. Flies were handled at room temperature under CO_2 anesthesia.

Each inbred line was maintained by a single pair of parents per generation, kept in a glass vial (20 mm diam, 100 mm height) with 10 ml medium added. Oviposition was allowed during 4 days, after which both parents were discarded. This implied that culture densities were low. At emergence, virgin male and female offspring were collected. All offspring of the same sex and line were maintained in the same vial until 4 day old, after which pair matings were individually made and kept in separate vials. One pair was used to perpetuate the line (spare mating pairs were used when the first failed to reproduce). Another four pairs were tested for fecundity, egg-to-pupa and pupa-to-adult viability as follows. On the third day after mating, each pair was transferred to a new vial with fresh medium and discarded 24 hr later (eighth day after adult emergence). For each female, the number of eggs laid in this period and the number of pupa (adults) present after an eight-day (12-day) incubation period were recorded. As some eggs may fail to hatch because of low paternal fertility, egg-to-pupa viability may be underestimated in some lines. Pilot data (not shown) indicated that the percentage of flies emerging after 12 days was <1%. Viabilities were expressed as percentages. All traits were simultaneously scored in samples of four females per line in each of three consecutive generations (104–106). At that time, 176 lines survived.

The isogenic line was maintained as a control in eight bottles (250 ml with 50 ml medium added). A circular mating scheme was used to ensure a large population size (~800 potential parents per generation), sufficient to allow the elimination of most mutations. To make comparisons between lines and control valid, control flies reared in vials under the same conditions as inbred flies were used for evaluation.

Estimation of mutational variances and covariances: For each trait, data were analyzed following standard ANOVA techniques. The model adjusted was

$$y_{ijk} = l_i + g_j + (lg)_{ij} + e_{ijk},$$

where l_i and g_j are, respectively, line ($i = 1, 176$) and generation ($j = 1, 3$) random effects, $(lg)_{ij}$ is the line × generation interaction effect and e_{ijk} the residual error corresponding to the ijkth individual ($k = 1,4$).

Parallel analyses of the covariances between pairs of traits were also carried out. Thus, the variance (covariance) was partitioned into sources arising from variation (covariation) between generations (V_G, cov_G), between lines (V_L, cov_L), generation × line interaction (V_{LG}, cov_{LG}) and within lines (V_W, cov_W). All scored individuals from a line were raised in the same vial every generation, until separated for evaluation. The vial effects are included in the corresponding V_{LG} (cov_{LG}) variance (covariance) component and, therefore, there is no confounding with line effects.

Estimates of mutational variances (covariances) were based on the V_L (cov_L) component, as follows. Starting from a completely homozygous population and assuming that all mutations are neutral, additive and of small effect, the mutational variance can be obtained from

$$V_L = 2\sigma_m^2\{t - 2N[1 - exp(-t/2N)]\}$$

(LYNCH and HILL 1986), where t is generation number and N is effective population size. A value of $N = 2.5$ was used for both B and C lines, as the latter had been maintained by full-sib mating during 57 generations before the start of the experiment. The mutational heritability was calculated as σ_m^2/V_W. Mutational covariances (cov_m) can also be obtained from the above formula, substituting V_L and σ_m^2 by cov_L and cov_m, respectively (HILL 1990). Mutational correlations (r_m) between two traits (1 and 2) were computed as $cov_m/\sigma_{m1}\sigma_{m2}$.

Approximate standard errors or confidence intervals were computed from standard multivariate analysis of variance techniques, as follows. From the analysis of variance

$$V_L = (MSL - MSLG)/JK$$

and

$$V_W = MSW,$$

where *MSL*, *MSLG* and *MSW* are between-line, interaction and within-line mean squares, and *I*, *J* and *K* are the number of lines, generations and individuals scored per line and generation, respectively. Taking variances,

$$V(V_L) = [V(MSL) + V(MSLG)]/(JK)^2$$

and

$$V(V_W) = V(MSW).$$

With balanced models, assuming normality,

$$V(MSL) = 2(MSL)^2/(I + 1),$$

$$V(MSLG) = 2(MSLG)^2/[(I - 1)(J - 1) + 2],$$

and

$$V(MSW) = 2(MSW)^2/[IJ(K - 1) + 2]$$

From the approximate formula for the variance of a ratio,

$$V(V_L/V_W) = [V_W^2V(V_L) + V_L^2V(V_W)]/V_W^4.$$

A parallel procedure was used to obtain the standard errors of the covariance components. All calculations were based on untransformed data.

Mutant effects and action: Lines showing an extreme mean for each trait were chosen for further analysis (generations 109–116). A total of 14 lines were considered (two to four lines from each tail of the three distributions). Reciprocal crosses were made between each line (P_1) and the control (P_0). In the next generation, the three traits were simultaneously scored in the reciprocal F_1s and the corresponding parental lines (100 individual observations per parental line or 50 for each reciprocal F_1 cross and trait). Fecundity was considered a property of the mother. However, viability can in principle be regarded as a property of both mother and offspring. Therefore, F_1 fecundity was measured by the number of eggs laid by F_1 females, but F_1 viabilities refer to the proportion of pupae (adults) developing from F_1 eggs (pupae).

Assuming that all mutations affecting a trait are fixed in the lines and absent in the control, estimates were obtained of homozygous $a = (\bar{P}_1 - \bar{P}_0)/2$ and heterozygous $d = \bar{F}_1 - (\bar{P}_1 - \bar{P}_0)/2$ effects summed over all mutations that occurred in each mutation accumulation line. Both a and d values were tested for significance ($P < 0.05$) separately for each trait

over lines, using the Bonferroni sequential comparison method (RICE 1989).

RESULTS

Distributions of the means of the lines: The distribution of the deviations of the means of the lines from the overall mean (averaged over generations 104–106) is shown in Figure 1 for each trait. The mean, variance and coefficient of asymmetry are given in Table 1. Unfortunately, no comparable control data was available, as this line was evaluated asynchronously (generations 109–116). Furthermore, generation effects have been shown to be as important as line effects (see Table 3 below).

All distributions were asymmetric, positive for fecundity and negative for both viability traits. This is consistent with results from a further test (see below), where homozygous effects of extreme lines reestimated on a much larger data set were generally positive for fecundity but invariably negative for viability.

All flies scored were *sepia* homozygotes, indicating that no genetic contamination from external sources occurred in any of the mutation accumulation lines or the control line. Of course, that observation does not preclude between-line contamination. However, lines showing extreme means for morphological (SANTIAGO *et al.* 1992; LÓPEZ and LÓPEZ-FANJUL 1993) or fitness-related traits (this paper) had different homozygous and/or heterozygous effects on the traits considered. This observation suggests that between-line contamination has not occurred.

Mutational variances and covariances: The variance (covariance) components from the analyses of variance (covariance), which were used to estimate the mutational variances (covariances), are given in Table 2 for all traits (pairs of traits). Significant between-line and between-generation variance (covariance) components were found in all cases (excepting the between-generation variance component for egg-to-pupa viability). This implies that the corresponding mutational variances and covariances, as well as the mutational heritabilities and correlations were also significant. In some instances, a significant generation × line interaction variance component was also obtained, but it was always smaller than the former components.

Estimates of the mutational variances of the three traits, as well as those of the corresponding mutational covariances, are given in Table 3. Mutational heritabilities and their standard errors, as well as mutational correlations and their confidence interval, are also given. All mutational heritabilities were of comparable magnitude. Mutational correlations were small and of variable sign. Their values and significance were not substantially altered once data from extreme lines were excluded from the analysis (data not shown).

Individual lines: This analysis refers only to the two

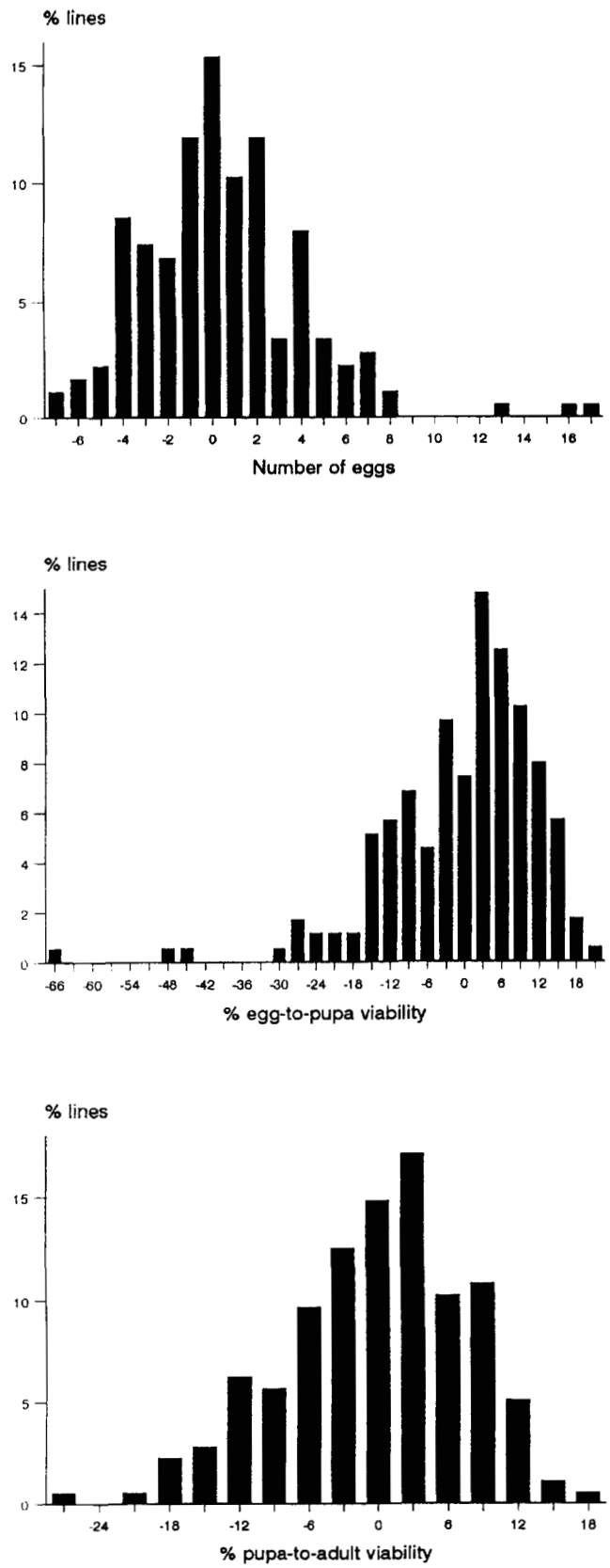

FIGURE 1.—Distributions of the deviations of the means of the mutation accumulation lines from the overall mean after 104–106 generations of inbreeding.

TABLE 1

Mean, variance and coefficient of asymmetry of the distribution of the means of the mutation accumulation lines

Trait	$\bar{X}$	V	g_1
Fecundity	17.01 ± 0.22	13.70	1.09*
Egg-to-pupa viability (%)	73.83 ± 0.74	155.92	−1.58*
Pupa-to-adult viability (%)	76.27 ± 0.46	62.35	−0.39*

$\bar{X}$, mean; V, variance; g_1, asymmetry.
* $P < 0.05$.

to four lines in each tail of the distribution of line means for each trait. These were: B78, B86, C6 and C10 (positive fecundity); C59 and C70 (negative fecundity); C10, C50 and C52 (positive egg-to-pupa viability); B32, B33 and B52 (negative egg-to-pupa viability); B32 and C60 (positive pupa-to-adult viability); and B42, B78 and C47 (negative pupa-to-adult viability). Although the procedure chosen was based on a small number of observations per line, it clearly identified those lines with the largest effects on any trait (see below) with a single exception (line C70, which had a positive effect on fecundity when retested).

The performance of each line ($\bar{P}_1$), the control line ($\bar{P}_0$) and the corresponding reciprocal F_1s are shown in Tables 4–6 for each trait. From these values, estimates of homozygous (a) and heterozygous (d) line effects were obtained and are also presented in Tables 4–6 (in environmental standard deviation units). Significant a values were predominantly positive for fecundity, but invariably negative for both viability components. This is in agreement with the sign of the coefficient of asymmetry of the corresponding distributions of line means (Table 1).

In two generations, the control mean fecundity ($\bar{P}_0$) deviated strongly from the remaining estimates. This is an apparent anomaly because the between-generation variance of control means was larger than the between-generation component of variance of line means shown in Table 2. However, the corresponding homozygous line effects calculated from those extreme control values (lines B33, B52, C6 and C59) were very small and nonsignificant, excepting that for line C59. This line showed a negative deviation from the overall mean in each of three previous evaluations (generations 104–106) and was known to carry a deleterious mutation with a large effect on morphological traits (see below)

TABLE 2

Variance (covariance) components

Trait	V_L or cov_L	V_G or cov_G	V_{LG} or cov_{LG}	V_W or cov_W
F	7.86*	5.62*	5.43*	48.37
EP	88.63*	−1.05	15.22	746.55
PA	33.00*	27.21*	22.87*	226.62
F-EP	6.48*	0.69*	−4.91	47.73
F-PA	−1.77*	−11.08*	−0.82*	−10.56
EP-PA	1.67*	1.83*	5.66	−14.40

V_L (cov_L), V_G (cov_G), V_{LG} (cov_{LG}) and V_W (cov_W) are, respectively, variance (covariance) components among lines, between generations, line × generation interaction and within-line. Respective degrees of freedom are 175, 2, 350 and 1584 (1416 for pupa-to-adult viability). F, fecundity; EP, egg-to-pupa viability; PA, pupa-to-adult viability.
* $P < 0.05$.

TABLE 3

Estimates of mutational variances, covariances, heritabilities and correlations

Trait(s)	σ^2_m (× 10^{-3}) or cov_m (× 10^{-3})	h^2_m (× 10^{-3}) or r_m
F	39.68	0.82 (0.16)
EP	447.63	0.60 (0.12)
PA	166.66	0.74 (0.12)
F-EP	32.74	0.25 (0.11, 0.38)
F-PA	−8.93	−0.11 (−0.25, 0.04)
EP-PA	8.46	0.03 (−0.12, 0.18)

All estimates significant at the 5% level. Estimates of environmental variance (V_W): fecundity (48.37), egg-to-pupa viability (746.55), pupa-to-adult viability (226.62). σ^2_m, mutational variances; cov_m, covariances; h^2_m, heritabilities; r_m, correlations. Values ± SE or confidence interval.

In the calculation of d values, the means of the reciprocal F_1s were pooled when they did not differ significantly. This was the rule in lines having significant a values for fecundity or pupa-to-adult viability, as well as in those instances where a was not significant (all traits). In a number of cases, however, particularly for egg-to-pupa viability, the means of the reciprocal F_1s were clearly different, each of them approaching that of the corresponding male parent line. One possible explanation can be the occurrence of male-sterile mutations defective at some stage of spermatogenesis (CASTRILLÓN *et al.* 1993), resulting in some unfertilized eggs being counted. Recently, a paternal-effect gene has been described in *D. melanogaster* causing developmental arrest after sperm entry into the egg (YASUDA *et al.* 1995). Consequently, d values were not calculated in those instances, as very different results would have been obtained depending on the F_1 mean used. No obvious distorted sex ratio suggesting *Y*-linked mutations was noticed in those lines.

Heterozygous effects on both viability components were always nonsignificant. Nevertheless, those of lines with a significant homozygous effect on egg-to-pupa viability could not be calculated for the reasons stated above. On the other hand, heterozygous effects on fecundity were generally significant, irrespective of the corresponding a values being significant or not. In about half these cases, the mean of the heterozygote was outside of the range limited by the two parental values. No indication of directional dominance was present for any trait.

TABLE 4

Contemporary performance of mutation accumulation lines and control and their corresponding reciprocal F_1s (scale of measurement) and homozygous and heterozygous line effects on fecundity

Line	$\bar{P}_1$	$\bar{P}_0$	$\bar{F}_1$		a	d
			P_0 male parent	P_1 male parent		
B32	15.16 ± 0.92	16.19 ± 0.80	18.92 ± 1.16	18.38 ± 1.21	−0.07 ± 0.09	0.43 ± 0.15*
B33	8.77 ± 0.73	7.80 ± 0.77	10.30 ± 0.84	10.49 ± 1.08	0.07 ± 0.08	0.30 ± 0.12*
B42	18.36 ± 0.62	12.77 ± 0.76	18.70 ± 1.38	15.78 ± 0.76	0.40 ± 0.07*	0.24 ± 0.13
B52	7.38 ± 0.78	7.80 ± 0.77	3.34 ± 0.47	4.79 ± 0.85	−0.03 ± 0.08	−0.50 ± 0.11*
B78	26.00 ± 0.82	12.77 ± 0.76	14.70 ± 1.14	22.18 ± 1.36	0.95 ± 0.08*	
B86	30.99 ± 1.06	14.12 ± 0.73	12.16 ± 0.73	14.31 ± 0.95	1.21 ± 0.09*	−1.34 ± 0.13*
C6	24.46 ± 1.03	25.17 ± 0.82	21.77 ± 1.11	21.94 ± 1.34	−0.05 ± 0.09	−0.42 ± 0.16*
C10	23.82 ± 0.96	14.12 ± 0.73	9.39 ± 0.67	8.60 ± 0.73	0.70 ± 0.09*	−1.43 ± 0.11*
C47	17.83 ± 0.88	15.96 ± 0.83	18.69 ± 1.27	20.92 ± 1.05	0.13 ± 0.09	0.42 ± 0.15*
C50	9.40 ± 0.67	13.20 ± 0.82	13.60 ± 0.87	12.71 ± 0.92	−0.27 ± 0.08*	0.27 ± 0.12*
C52	21.76 ± 0.77	16.19 ± 0.80	16.45 ± 1.16	18.49 ± 1.23	0.40 ± 0.08*	−0.22 ± 0.15
C59	19.59 ± 0.96	25.17 ± 0.82	15.34 ± 0.94	18.02 ± 1.16	−0.40 ± 0.09*	−0.82 ± 0.14*
C60	15.61 ± 0.80	15.96 ± 0.83	16.45 ± 1.16	16.08 ± 1.00	−0.02 ± 0.08	0.07 ± 0.14
C70	25.33 ± 1.17	13.20 ± 0.82	26.57 ± 1.37	15.92 ± 1.57	0.87 ± 0.10*	

Values of a and d are in environmental standard deviation units; estimate of environmental standard deviation: 6.95. $\bar{P}_1$, mutation accumulation lines; $\bar{P}_0$, control; a, homozygous line effect; d, heterozygous line effect.

* $P < 0.05$, based on the sequential Bonferroni test.

Six lines showed a significant homozygous effect on a single trait and another six on two traits, commonly fecundity and pupa-to-adult viability. Lines B52, B78, C10 and C59 have been previously detected as carriers of mutations affecting morphological traits (SANTIAGO *et al.* 1992; LÓPEZ and LÓPEZ-FANJUL 1993). Thus, line C59 carried a deleterious mutation with a very large effect on abdominal (5σ) and sternopleural bristle number (1.4σ) and smaller effects on wing length and wing width (0.3σ). The remaining lines carried quasi-neutral mutations with small effects ($0.2–0.4\sigma$) on abdominal bristles (B52 and C10) or both on sternopleural bristles and wing length (B78).

DISCUSSION

We have studied the accumulation of spontaneous mutations affecting fecundity and viability in a set of inbred lines, all of them derived independently from the same homozygous base population. The analysis of the data revealed: similar mutational heritabilities for the three traits considered; small mutational correla-

TABLE 5

Contemporary performance of mutation accumulation lines and control and their corresponding reciprocal F_1s (scale of measurement) and homozygous and heterozygous line effects on egg-to-pupa viability

Line	$\bar{P}_1$	$\bar{P}_0$	$\bar{F}_1$		a	d
			P_0 male parent	P_1 male parent		
B32	23.55 ± 3.14	85.74 ± 2.72	76.92 ± 4.13	30.33 ± 4.99	−1.14 ± 0.08*	
B33	39.38 ± 3.83	69.55 ± 3.96	75.18 ± 4.97	29.91 ± 5.25	−0.55 ± 0.10*	
B42	84.32 ± 2.33	77.20 ± 3.07	78.84 ± 4.00	74.33 ± 4.30	0.13 ± 0.07	−0.15 ± 0.13
B52	44.82 ± 3.65	69.55 ± 3.96	73.85 ± 5.31	34.74 ± 4.78	−0.45 ± 0.10*	
B78	84.14 ± 1.78	77.20 ± 3.07	77.13 ± 3.62	80.48 ± 3.81	0.13 ± 0.06	−0.07 ± 0.12
B86	86.54 ± 2.19	86.97 ± 1.96	84.60 ± 2.81	83.64 ± 4.06	−0.01 ± 0.05	−0.09 ± 0.10
C6	78.65 ± 3.55	89.84 ± 1.88	88.41 ± 2.88	80.48 ± 4.24	−0.20 ± 0.07	0.24 ± 0.12
C10	83.16 ± 2.43	86.97 ± 1.96	90.94 ± 2.00	78.65 ± 4.79	−0.07 ± 0.06	−0.01 ± 0.20
C47	73.01 ± 2.41	84.28 ± 2.16	78.27 ± 3.94	74.21 ± 5.52	−0.21 ± 0.06*	−0.08 ± 0.14
C50	82.60 ± 2.96	80.54 ± 3.00	82.52 ± 3.50	81.03 ± 4.03	0.04 ± 0.08	0.07 ± 0.13
C52	87.91 ± 2.01	85.74 ± 2.72	88.83 ± 2.95	85.17 ± 3.66	0.04 ± 0.06	0.01 ± 0.11
C59	56.25 ± 3.78	89.84 ± 1.88	85.97 ± 2.83	60.71 ± 5.33	−0.61 ± 0.08*	
C60	79.53 ± 3.10	84.28 ± 2.16	82.68 ± 4.12	82.79 ± 3.75	−0.09 ± 0.07	0.03 ± 0.12
C70	83.53 ± 2.55	80.54 ± 3.00	84.68 ± 3.19	77.64 ± 4.27	0.05 ± 0.07	−0.04 ± 0.12

Estimate of environmental standard deviation: 27.32. See Table 4 for abbreviations.

* $P < 0.05$, based on the sequential Bonferroni test (arcsine transformed data).

TABLE 6

Contemporary performance of mutation accumulation lines and control and their corresponding reciprocal F_1s (scale of measurement) and homozygous and heterozygous line effects on pupa-to-adult viability

Line	$\bar{P}_1$	$\bar{P}_0$	$\bar{F}_1$		a	d
			P_0 male parent	P_1 male parent		
B32	79.39 ± 3.17	84.11 ± 1.47	80.06 ± 2.16	84.87 ± 3.17	−0.16 ± 0.12	0.02 ± 0.17
B33	87.92 ± 2.56	93.46 ± 1.70	92.67 ± 2.71	93.54 ± 3.21	−0.18 ± 0.10	0.15 ± 0.17
B42	76.70 ± 1.66	91.53 ± 1.17	81.14 ± 1.75	83.03 ± 2.02	−0.49 ± 0.07*	−0.14 ± 0.11
B52	89.51 ± 2.12	93.46 ± 1.70	86.52 ± 2.92	95.64 ± 2.01	−0.13 ± 0.09	
B78	74.04 ± 1.59	91.53 ± 1.17	88.04 ± 1.45	89.85 ± 1.34	−0.58 ± 0.07*	0.41 ± 0.09*
B86	79.06 ± 1.40	88.71 ± 1.19	82.43 ± 2.21	80.42 ± 1.48	−0.29 ± 0.06*	−0.19 ± 0.11
C6	77.34 ± 1.83	86.80 ± 1.17	83.79 ± 1.67	83.10 ± 1.42	−0.31 ± 0.07	0.92 ± 0.10*
C10	85.32 ± 1.18	88.71 ± 1.19	79.40 ± 1.97	84.47 ± 1.71	−0.11 ± 0.05	−0.35 ± 0.11*
C47	60.53 ± 2.02	82.49 ± 1.37	72.84 ± 2.96	75.74 ± 2.17	−0.73 ± 0.08*	0.17 ± 0.15
C50	86.63 ± 1.61	89.20 ± 1.26	88.79 ± 2.43	89.47 ± 1.39	−0.08 ± 0.07	0.08 ± 0.11
C52	83.28 ± 1.53	84.11 ± 1.47	83.43 ± 1.87	83.98 ± 1.83	−0.03 ± 0.07	0.00 ± 0.11
C59	86.43 ± 2.04	86.80 ± 1.17	83.22 ± 1.57	88.51 ± 2.75	−0.01 ± 0.08	−0.07 ± 0.13
C60	83.29 ± 1.86	82.49 ± 1.37	80.50 ± 2.23	76.95 ± 2.67	0.03 ± 0.08	−0.28 ± 0.14
C70	69.88 ± 1.43	89.20 ± 1.26	91.03 ± 1.26	85.97 ± 1.33	−0.64 ± 0.06*	

Estimate of environmental standard deviation: 15.05. See Table 4 for definitions.
* $P < 0.05$, based on the sequential Bonferroni test (arcsine transformed data).

tions of variable sign between any two traits; an asymmetric distribution of the means of the lines (positive for fecundity and negative for egg-to-pupa and pupa-to-adult viability); extreme homozygous line effects that were all negative for both viability components but predominantly positive for fecundity; and corresponding heterozygous line effects showing a variable degree of dominance.

Our experimental design introduces the potential for covariances between traits, due to uncontrolled larval density in generations previous to the assays, and this factor may contribute to the mutational correlation between fecundity and viability. In *D. melanogaster,* the effect of competition among immatures on their adult fecundity has been experimentally studied by PROUT and MCCHESNEY (1985). They concluded that a negative correlation between viability and fecundity could be expected, as less viable cultures tend to produce larger sized offspring that will consequently lay more eggs. Nevertheless, this effect was not found in low density cultures (<150 eggs per 15 ml medium, 0.85 egg-to-adult viability). Our lines are all within this class, even that with the highest lay (line B86: 124 eggs in 4 days per 15 ml medium; 0.68 egg-to-adult viability). In parallel, SANG (1949) reported that *D. melanogaster* adult weight decreases asymptotically with increasing larval density. However, densities ranging from 60 to 140 larvae/vial (which include all lines in this experiment) resulted in adults weights of great similarity, although lower than that recorded in the case of no competition (one larva/vial). Therefore, important effects of culture density on adult weight resulting in fecundity changes can in principle be disregarded in our case. This concords with the observation that none of the six lines with a positive significant effect on fecundity had a significant effect on egg-to-pupa viability. Four of these lines had a significant effect on pupa-to-adult viability. However, their effects on fecundity are unlikely to be due to competition as this was not manifested at the egg-to-pupa stage. Furthermore, the mutational correlation between fecundity and egg-to-pupa viability was positive.

Mutational heritabilities and correlations: In the same set of lines used in this experiment, the effect of mutation accumulation has also been studied for morphological traits (abdominal and sternopleural bristle number, wing length and wing width; SANTIAGO *et al.* 1992; LÓPEZ and LÓPEZ-FANJUL 1993). Estimates of mutational heritabilities and coefficients of variation of these traits together with those obtained in the present experiment are shown in Table 7. On the whole, mutational heritabilities of morphological traits were larger than those of life history traits and the corresponding mutational coefficients of variation smaller. This pattern can be attributed to the high degree of homozygosity of the lines, affecting both sets of traits differentially. Life-history traits are expected to show, more intensely than morphological traits, both a larger depression of the mean and a larger increase of the environmental variance, the latter due to reduced developmental homeostasis (FALCONER 1989). Thus, their mutational coefficients of variation will tend to be larger than those of morphological traits and their mutational heritabilities smaller.

The mutational variance is a function of the mutation rate, the number of loci involved, the variance of mutational effects on the trait and fitness and the degree of dominance of mutations. Our calculations have been

TABLE 7

Mutational heritabilities and coefficients of variation estimated from the between-line variance in the same set of mutation accumulation lines

Trait	h_m^2 ($\times 10^{-3}$)	CV_m (%)
Abdominal bristle number[a]	0.8–12.3	0.1–0.7
Sternopleural bristle number[b]	0.7–3.3	0.2–0.5
Wing length[b]	2.6–2.7	0.1
Wing width[b]	1.3–1.5	0.1
Fecundity[c]	0.8	1.2
Egg-to-pupa viability[c]	0.6	0.8
Pupa-to-adult viability[c]	0.7	0.5

All values significantly different from zero ($P < 0.05$). CV_m, coefficients of variation.

[a] Revised estimates from LÓPEZ and LÓPEZ-FANJUL (1993).

[b] SANTIAGO *et al.* (1992).

[c] Present work.

made by assuming an infinitesimal model of many neutral genes of additive effects. Strictly, none of these assumptions will hold in practice. With inbreeding, the fixation probability of neutral mutations is independent both of their effects and gene action. Nevertheless, that of deleterious mutations will be reduced and extreme ones (lethals) will be eliminated. However, the importance of selection relative to drift decreases with the magnitude of the effective size, and will be minimal in our case. Using information from *P*-element-induced mutations affecting bristle number in Drosophila, KEIGHTLEY *et al.* (1993) concluded that the infinitesimal model greatly underestimates the underlying mutational variance, deleterious mutations being the main factor. Consequently, the bias incurred in the estimates of mutational heritabilities of fitness components will be even larger. Thus, the differences between the estimates in Table 7 may partly be attributed to the experimental procedure used and the values of original mutational heritabilities of fitness related traits may well exceed those for morphological traits, these presumably subjected to weaker selection pressure.

Inbreeding will also result in biased estimates of mutational covariances, for the same reasons stated above. In addition, selection on unconditionally deleterious mutations will be stronger than on those showing antagonistic pleiotropy. Thus, underlying mutational covariances will be larger than their realized estimates, which will tend to become negative.

In *D. melanogaster,* the accumulation of second chromosome mutations affecting egg-to-adult viability maintained against a balancer chromosome has been studied by MUKAI and coworkers and OHNISHI (see LYNCH 1988 and HOULE *et al.* 1992 for references). Their estimates of the mutational variance extrapolated to the whole genome were in the range $(1.3–2.6) \times 10^{-4}$. Although the corresponding sampling errors cannot be calculated, they are larger than those obtained in the present experiment (egg-to-pupa viability: 0.45×10^{-4}, pupa-to-adult viability: 0.17×10^{-4}, expressing viabilities as proportions). This reduction is expected as mutations accumulate in the virtual absence of natural selection in the first case but not in the second. Furthermore, the number of loci at which mutations affecting egg-to adult viability can occur is presumably larger than that for any of its component traits. However, mutational heritabilities computed by LYNCH from MUKAI-OHNISHI's data were in the range $(1–6) \times 10^{-5}$, one to two orders of magnitude lower than our estimates for both viability components. Consequently, the environmental variance in MUKAI-OHNISHI's experiments should be correspondingly higher. The discrepancy can be attributed to a number of nonexclusive causes, among them differences in the environmental conditions (including the evaluation procedure) and in the background genotype appear likely.

Estimates of mutational variances and covariances of *D. melanogaster* life history traits (fecundity, male mating ability, productivity and longevity) have been recently reported by HOULE *et al.* (1994). Significant mutational heritabilities extrapolated to the whole genome [range $(0.8–3.7) \times 10^{-3}$] and correlations (range 0.6–0.9) were considerably larger than those obtained in our experiment [ranges $(0.6–0.8) \times 10^{-3}$ and (−0.11–0.25), respectively]. For early fecundity, the only trait coincident in both studies, the mutational variance (2.97) and heritability (1.8×10^{-3}) were also larger (0.4 and 0.8×10^{-3}, respectively, in our case) These differences imply that the environmental variance of the trait was also three times larger in HOULE *et al.* experiment.

Mutations accumulating against a balancer chromosome are effectively sheltered from natural selection and, therefore, they include: unconditionally deleterious mutations of large effect, mildly detrimental mutations, as well as those showing antagonistic pleiotropy. Thus, the first class of mutations will have a disproportionate influence on the magnitude of mutational variances and covariances (and on the sign of the later) of fitness-component traits. In inbred lines, however, the fixation probability of that class of mutations will be small and mutational variances and covariances will be mostly due, therefore, to the second and third kind of mutations, those having a major role on population survival (LANDE 1995). Consequently, mutational variances and covariances obtained from the first type of experiment are expected to be larger than those from the second. Summarizing, both methods result in partially overlapping descriptions: accumulation against a balancer chromosome gives a better representation of the original distribution of mutant effects, whereas the description obtained from inbreeding is more relevant to the genetic variation in natural populations after purging selection acts (LÓPEZ and LÓPEZ-FANJUL 1993).

Analysis of line effects: In *D. melanogaster,* the distribution of homozygous chromosomal effects on egg-to-

adult viability has been found to be negatively skewed, both for third chromosomes extracted from a natural population (MACKAY 1985) and for second chromosomes where mutations were allowed to accumulate (reviewed by MUKAI 1985). To the best of our knowledge, comparable fecundity data has not been previously reported. Nevertheless, our measurements had been taken in a low density condition and their relationship to those in a more competitive environment may not be linear.

In our experimental design, a significant departure of a line mean from the control mean must be attributed to previously fixed mutations affecting the trait considered. However, more than one mutation may be responsible as a large number of generations had elapsed by the time the lines were evaluated. Therefore, homozygous and heterozygous line effects cannot be unequivocally equated to additive and dominance effects of single mutations. Nevertheless, the data provides useful information on these, as follows.

First, significant positive and negative homozygous line effects on fecundity have been detected, implying the existence of mutations affecting the expression of the trait in both directions. For viability, all homozygous effects were negative and therefore, the existence of positive mutations cannot be ascertained from our data.

Second, significant dominant or recessive heterozygous line effects have been found, and they are evidence of at least one single mutation of that same action fixed in the line, although we cannot be certain of the number of mutations carried by any given line. This assertion relies on the effects of different mutations on the same trait being independent. Synergistic effects for viability have been described but were not large (MUKAI 1969; SIMMONS and CROW 1977). On the other hand, additive homozygous line effects are not necessarily due to additive mutations as fixation of recessive and dominant mutations in the same line could also produce the same global result. In natural populations of *D. melanogaster,* however, significant upward responses to artificial selection have been obtained both for early fecundity (ROSE 1984) and egg-to-pupa viability (GARCÍA *et al.* 1994), implying the segregation of alleles with additive effects on those traits in the corresponding populations. Moreover, the additive variance of viability maintained in natural populations has been shown to be much larger than the dominance variance (MUKAI 1985).

Third, significant heterozygous line effects of varying sign were found for fecundity. This implies that dominance effects of single mutations must also be of varying sign even though the degree of directional dominance cannot be established.

Fourth, d values significantly exceeding the range $a < d < -a$ have not been observed for viability but were relatively common for fecundity (seven out of twelve). Fixation of two (or more) mutations per line may result in apparent overdominance or underdominance, provided their effects are of opposite sign and their gene actions differ. Both conditions have only been observed for fecundity. This provides the simplest explanation for an odd phenomenon. In those lines showing nonsignificant homozygous effects, this interpretation also implies that the effects of the mutations fixed will cancel each other.

It is commonly accepted that most mutations affecting net fitness are deleterious. However, pleiotropic effects of those mutations on different fitness components do not need to be uniformly harmful. Strictly, pleiotropy cannot be studied with our data as several mutations, each of them affecting a different trait, may be fixed in the same line. Nevertheless, the results show patterns that are worth mentioning. Generally, when a line had significant homozygous effects on two traits, they were positive for fecundity and negative for pupa-to-adult viability. This is in agreement with the observed negative mutational covariance between these traits. In parallel, significant homozygous line effects both on fecundity and egg-to-pupa viability or on both viability components were only detected once and were invariably negative. This is also consistent with the corresponding mutational correlations being small and positive. In all instances, the inferences can be extended to those mutations not individually analyzed, as the estimates of mutational correlations were not appreciably altered after extreme lines were excluded from the calculations. Summarizing, there is a suggestion of mutational correlations being negative between fecundity and pupa-to-adult viability and positive in the other two cases considered. ROFF and MOUSSEAU (1987) examined the pattern of genetic correlations between life history traits in natural populations of *D. melanogaster.* Estimates were very variable in magnitude and sign, effectively covering the whole definition range. As far as we know, estimates of genetic correlations between the traits studied in our experiment have not yet been reported for natural populations. However, indirect evidence points to a small positive correlation between fecundity and egg-to-pupa viability (GARCÍA *et al.* 1994), in agreement with the mutational correlation obtained in this experiment.

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