

SPONTANEOUS MUTATIONAL GENOTYPE-ENVIRONMENT INTERACTION FOR FITNESS-RELATED TRAITS IN *DROSOPHILA MELANOGASTER*

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Abstract.—Spontaneous mutations were allowed to accumulate for 104–161 generations in 113–176 inbred lines, independently maintained by a single brother-sister mating per generation, all of them derived from a completely homozygous population of *Drosophila melanogaster*. In each of two to three consecutive generations, all lines were scored for fecundity, egg-to-pupa and pupa-to-adult viabilities, both in the standard laboratory culture medium (ST) and in three harsh media differing from the former by a single factor: higher temperature (HT), higher NaCl concentration (HSC), or a much reduced concentration of nutrients (D). Relative to the standard medium, productivity (fecundity \times viability) decreased by 25% (HT), 66% (HSC), and 80% (D). In each medium, mutational variances of those traits and mutational covariances between all possible pairs were calculated from the between-line divergence (codivergence). Mutational correlations between character states in different media were also obtained. Because we used inbred lines, those estimates were mainly due to the accumulation of mildly detrimental mutations, deleterious mutations of large effect being underrepresented. For all traits, mutational heritabilities ranged from 1.41×10^{-4} to 11.24×10^{-4} , and did not increase with intensified environmental harshness. Mutational correlations between character states in different media were usually not large (average absolute value 0.31), reflecting a high degree of environmental specificity of the mutations involved. In our results, mutations quasi-neutral in ST conditions and mildly detrimental in more stressful media were not, as a class, important. Mutational correlations between fecundity and egg-to-pupa viability were small and positive in all media. Those involving pupa-to-adult viability were positive in HT, nonsignificant in HSC, and negative in ST and D, showing how the genetic covariance structure of quantitative traits in populations may change in variable environments.

Key words.—*Drosophila melanogaster*, fecundity, mutational covariance, mutational genotype-environment interaction, mutational variance, viability.

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Theoretical models considering the roles of mutation and natural selection in the maintenance of polygenic variation in populations have been frequently analyzed (see reviews by Bulmer 1989 and Barton 1990). In parallel, experiments have been carried out to provide estimates of parameters needed for prediction, particularly the input of variation and covariation due to spontaneous mutation per generation, quantified by the mutational variances and covariances.

Most information refers to *Drosophila melanogaster*, and both morphological (Santiago et al. 1992; review by Caballero and Keightley 1994) and life-history traits (Houle et al. 1992, 1994; Fernández and López-Fanjul 1996) have been studied. Mutational heritabilities (the mutational variance scaled by the environmental variance) of all traits cluster around a value of 10^{-3} . Mutational correlations between life history-traits were found to be either strongly positive (Yoshimaru and Mukai 1985; Houle et al. 1994) or small and of varying sign (Fernández and López-Fanjul 1996). In principle, this discrepancy can be attributed to the different experimental procedures used, deleterious mutations of large pleiotropic effects having a much larger influence in the first study.

However, all these estimates have been obtained under benign conditions and it is of interest to inquire to what extent they can be extrapolated in the case of stressful environments. Genotype-environment interactions may increase the genetic variance beyond that maintained by mutation-selection balance (Via and Lande 1987) and may also provide an alternative mechanism for the maintenance of genetic variation (Gillespie and Turelli 1989).

The modal pattern of reaction norms of mutations will

affect the magnitude of both mutational variances and covariances across environments. If reaction norms of fitness and fitness-related traits decrease monotonically with enhanced environmental harshness, correspondingly increasing mutational variances and covariances will result. On the other hand, if the genotypic ordering alters with environment, in such a way that the slopes of reaction norms are of different sign, changes of mutational variances and covariances across environments will be unpredictable both in sign and magnitude. Therefore, the phenomenon can only be approached empirically. Strong genotype-environment interactions for viability have been detected in natural populations of *D. melanogaster* (Tachida and Mukai 1985; Takano et al. 1987) and *D. pseudoobscura* (Gupta and Lewontin 1982).

Here we present estimates of mutational variances of three fitness-related traits, and of mutational covariances between all pairs of these traits, in four different culture media, as well as mutational correlations between character states expressed in different media. Those pertaining to standard laboratory conditions have been reported already by Fernández and López-Fanjul (1996). Data has been obtained from inbred lines started from the same homozygous population of *D. melanogaster*, in which mutations have been allowed to accumulate for over 100 generations.

Our approach provides information that differs from that obtained in experiments involving the accumulation of mutations in chromosomes maintained against a balancer (review by Mukai 1985). In this case, mutations are virtually sheltered from natural selection and observations are usually restricted to one chromosome. In our case, however, between-line differences were mostly due to the accumulation of mildly detrimental mutations occurring over the whole genome.

MATERIALS AND METHODS

Base Population and Inbred Lines

A *D. melanogaster* line isogenic for all chromosomes obtained by Caballero et al. (1991) was used as the base population. From this, 200 inbred lines were started. Half of them (B lines) were always maintained by a single brother-sister mating per line and generation. The other half (C lines) 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

Flies were reared in four different environmental conditions defined by the composition of the medium (ingredients added per liter of water by weight) and the culture temperature, as follows: (1) standard (ST): 100 g brewer's yeast, 100 g sucrose, 12 g agar, 2.5 g NaCl, 5 mL buffered propionic acid, 25°C ± 1; (2) high temperature (HT): ST medium, 28°C ± 1; (3) high saline concentration (HSC): ST medium except for higher NaCl content (20 g), 25°C ± 1; and (4) diluted (D): 12 g brewer's yeast, 40 g sucrose, 8 g agar, 2.5 g NaCl, 5 mL buffered propionic acid, 25°C ± 1.

In all cases, cultures were maintained under continuous lighting. Flies were handled at room temperature under CO₂ anaesthesia.

To determine limiting conditions in the different media, some pilot testing was carried out. Thus, it was found that matings were sterile or contributed less than 4–5 male or female progeny: (1) in more than 50% of cases if temperature rose to 29°C; (2) in more than 80% of cases with 25 g of NaCl added; (3) in nearly 100% of cases in an ultraimpoverished medium containing 8 g brewer's yeast, 20 g sucrose, and 8 g agar. In view of these results, HT, HSC and D conditions, as described above, were chosen as harsh environments.

Traits Scored

Each inbred line was maintained by a single pair of parents per generation, kept in a glass vial (20-mm diameter, 100-mm height) with 10 mL of ST medium added. Oviposition was allowed during four 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 four-days old, after which time pair matings were individually made and kept in separate vials. One pair was used to perpetuate the line (spare matings were used when the first failed to reproduce resulting in some natural selection). At specified generations (see below), four other were tested for fecundity, egg-to-pupa viability, 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 hours later (the 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. Underestimation of pupa-to-adult viability was negligible, since pilot data (not shown) indicated that the percentage of flies emerging after 12 days was lower than 1%. Viabilities were expressed as percentages.

Within media, all traits were simultaneously evaluated in all lines, individually scoring 4–5 females per line in each of two to three consecutive generations. All individuals scored were raised in the same medium from the egg stage. Between media, measurements were not done simultaneously, but at generations 104–106 (ST), 145–146 (HT), 153–155 (HSC), and 160–161 (D).

As stated above, when a pair of parents failed to reproduce, sibs were used to propagate the line. Notwithstanding, some lines were lost at different moments. From the original two hundred, the number of surviving lines was 181 (generation 106), 164 (generation 146), 160 (generation 155), and 157 (generation 161). The rate of line loss per generation was 0.18 (generations 0–106), 0.42 (generations 107–146), 0.44 (generations 147–155), and 0.50 (generations 156–161). This upward trend strongly suggests increasing accumulation of deleterious mutations. For specific treatments, some females failed to lay during the 24-hour testing period. Lines were included in the analysis when a minimum of three females per generation laid eggs. The number of lines included were 176 (ST), 154 (HT), 130 (HSC), and 113 (D). While 97% of lines could be evaluated in ST conditions, this percentage was reduced to 94% (HT), 81% (HSC), and 72% (D) due to the increasingly unfavorable quality of these media. In addition, all lines could not be tested in all environments. Therefore, calculations were either performed on the whole set of data or on the subset restricted to the 95 lines that could be evaluated for all treatments. Given that similar results were obtained in both cases, only the latter are presented.

Estimation of Mutational Variances and Covariances

For each trait and environment, data were analysed following standard ANOVA techniques. The model adjusted was

$$y_{ijk} = l_i + g_j + (lg)_{ij} + e_{ijk}, \quad (1)$$

where l_i and g_j are, respectively, line and generation random effects, $(lg)_{ij}$ is the line-generation interaction effect, and e_{ijk} is the residual error corresponding to the ijk th individual.

Parallel analysis of the covariances between pairs of traits scored in the same conditions were also carried out. Thus, the variance (covariance) was partitioned into sources arising from variation (covariation) between lines (V_L, cov_L), between generations (V_G, cov_G), generation-line interaction (V_{LG}, cov_{LG}) and within lines (V_W, cov_W). Variance (covariance) components were estimated using the SAS statistical package, version 6.03 (SAS Institute 1988). All scored individuals from a line were raised in the same vial every generation, until separated for evaluation. The vial effects

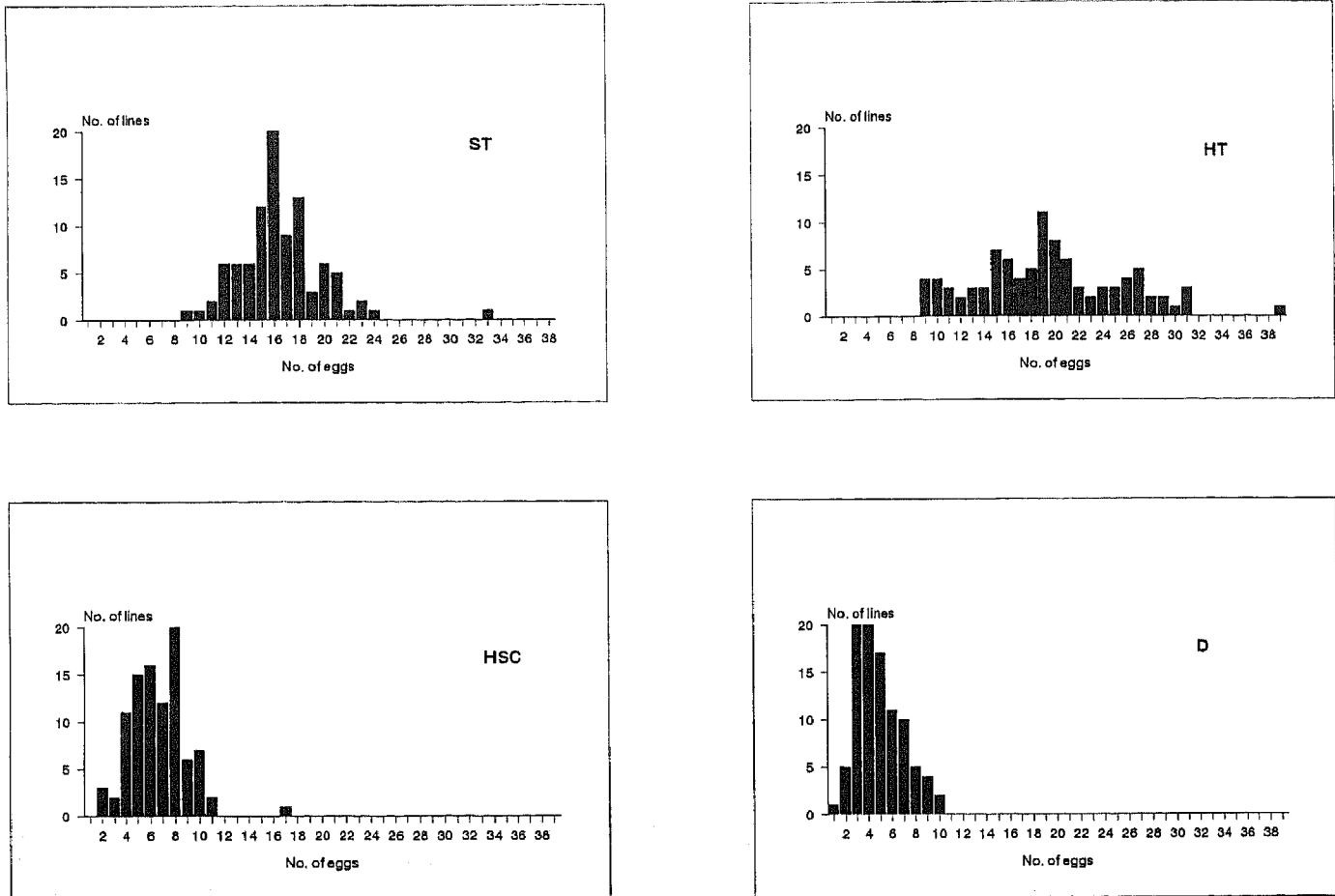


FIG. 1. Distribution of the mean fecundity of the mutation accumulation lines in different environments (ST, HT, HSC, and D). See text for environmental characteristics.

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 and additive, the mutational variance (V_m) can be obtained from

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

(Lynch and Hill 1986), where t is generation number and N is effective population size. A value $N = 2.5$ was used for both B and C lines, as the latter have been maintained by full-sib mating during 57–113 generations prior to the start of this experiment. The mutational heritability was calculated as V_m/V_w . Mutational covariances (cov_m) between pairs of traits scored in the same environment can also be obtained from the above formula, substituting V_L and V_m with cov_L and cov_m , respectively (Hill 1990). Mutational correlations (r_m) between traits 1 and 2 were computed as $cov_m/(V_{m1} V_{m2})^{1/2}$. Measurements of a trait in two different environments (1 and 2) were not done simultaneously, but after t_1 and t_2 generations of mutation accumulation. At equilibrium, the covariance between the means of the lines in these environments (averaged over generations) is

$cov(\bar{x}_1, \bar{x}_2) = 2t_1 cov_m$, and the between-line component of variance in each environment are $V_{L1} = 2t_1 V_{m1}$ and $V_{L2} = 2t_2 V_{m2}$. Therefore, the corresponding mutational correlation between the same trait scored in the two environments can be calculated as

$$(t_1 t_2)^{1/2} cov(\bar{x}_1, \bar{x}_2)/t_1 (V_{L1} V_{L2})^{1/2}. \quad (3)$$

The significance of variance (covariance) components was established by the appropriate F -tests. Significant between-line variances and covariances imply that the corresponding mutational variances, covariances, heritabilities and correlations, will also be significant. In specific cases, approximate standard errors were computed from standard multivariate analysis of variance techniques as outlined in Fernández and López-Fanjul (1996). All estimates are given in the original scale of measurement.

RESULTS

Distribution of the Means of the Lines

The distribution of the means of the lines (averaged over generations) is shown in Figures 1–3 for each trait and environment. The mean and the coefficients of variation and asymmetry of those distributions are given in Table 1. The distributions were always unimodal and departures from nor-

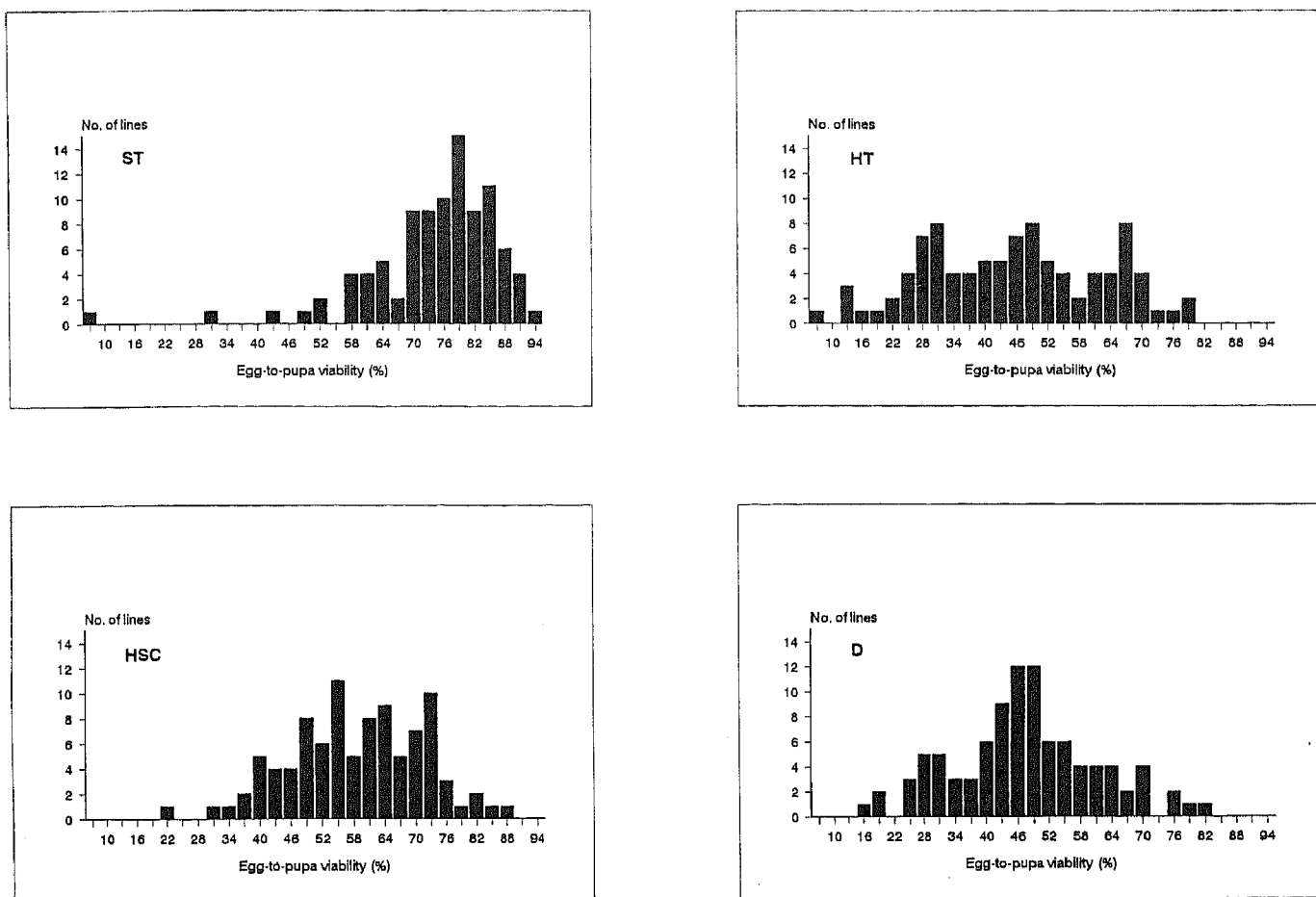


FIG. 2. Distribution of the mean egg-to-pupa viability of the mutation accumulation lines in different environments (ST, HT, HSC, and D). See text for environmental characteristics.

mality were not large, although they were significant in most cases.

Changing conditions affected traits differently. Compared to ST medium, productivity (fecundity \times viability) decreased, relatively in HT (by 25%), but drastically in HSC and D (by 66% and 80%, respectively).

Rising temperature from 25°C to 28°C increased mean fecundity, in agreement with previous observations (David and Clavel 1969). On the other hand, fecundity drastically decreased in HSC and D environments (to 42% and 32% of that observed in ST, respectively). These results suggest that females discriminate between egg laying sites (Del Solar and Palomino 1966), effectively detecting HSC and D as unsuitable environments for that purpose.

Egg-to-pupa viability was strongly reduced in HT, HSC, and D (to 60–80% of that observed in ST). Nevertheless, the depression found in each case must be ascribed to different causes. Because of medium hardening, larval burrowing and, therefore feeding, was more difficult in HT and HSC than in D, but this latter, more accessible environment had a much lower nutritional value. However, once the third instar is reached, larvae will continue development even in the absence of food (Robertson 1963). This was reflected in comparable pupa-to-adult viabilities being observed in all envi-

ronments considered, although small significant differences were detected.

In general, the coefficient of variation of the distribution of line means was larger in stressful environments. All distributions were asymmetric, positive for fecundity and negative for both viability components. This is consistent with previous data on fecundity (Fernández and López-Fanjul 1996) and egg-to-adult viability (Mukai 1985; Houle et al. 1994). For each trait, the sign of the coefficient of asymmetry was generally the same in all four environments, although some values did not reach significance.

All flies scored were *sepia* homozygotes, indicating that no genetic contamination from external sources occurred in any of the mutation accumulation lines.

Mutational Variances and Covariances

The variance (covariance) components from the analyses of variance (covariance), which were used to estimate the mutational variances (covariances) and the environmental variances, are given in Tables 2 and 3 for all traits (pairs of traits) and environments.

Significant between-line variances and covariances were found in most cases, excepting those involving pupa-to-adult

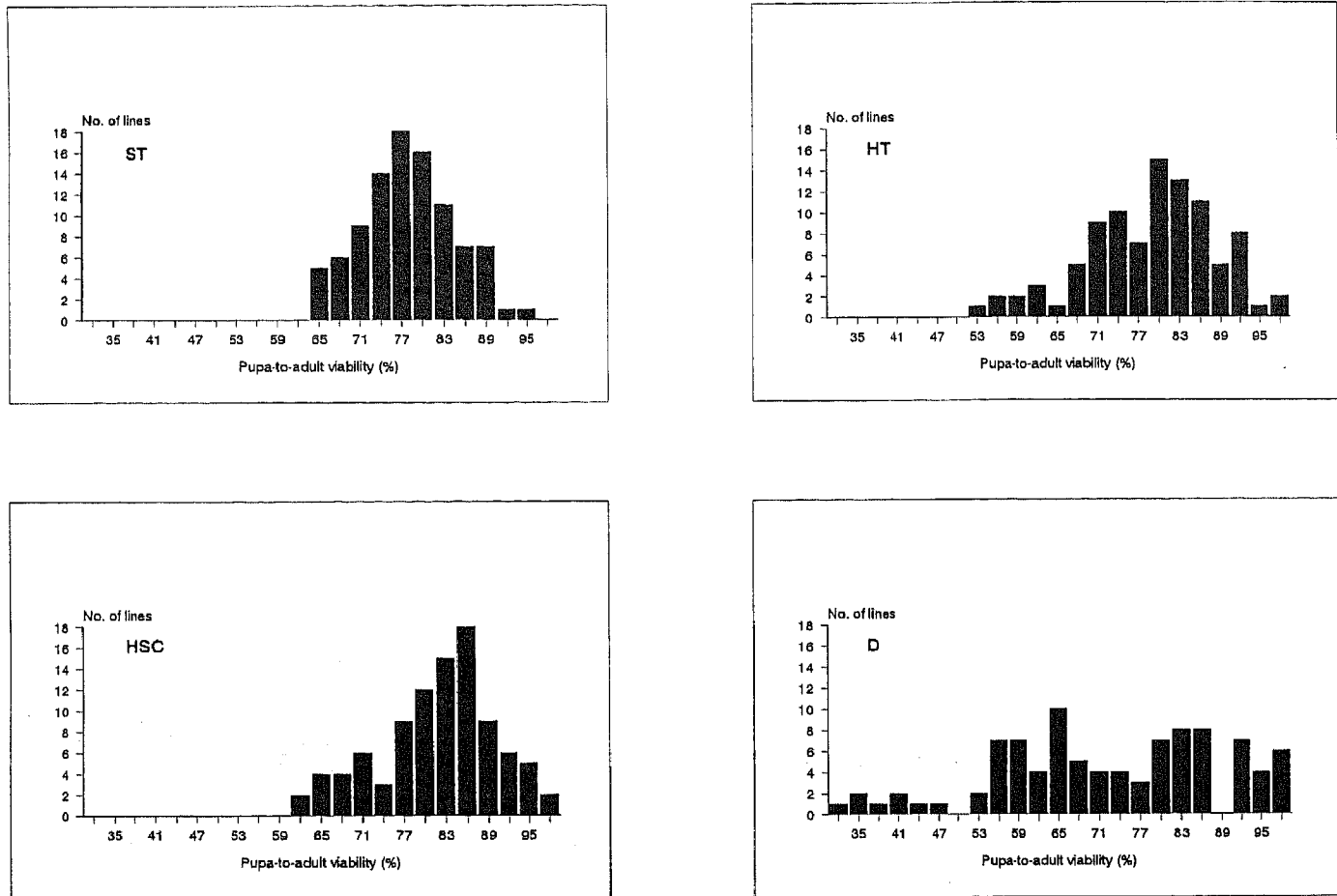


FIG. 3. Distribution of the mean pupa-to-adult viability of the mutation accumulation lines in different environments (ST, HT, HSC, and D). See text for environmental characteristics.

viability in HSC. The results indicate real differences between lines due to fixation of different mutations, some of them having pleiotropic effects on more than one trait.

In general, between-generation and line-generation interaction variance components were also significant. The first

show the importance of environmental temporal fluctuations affecting all lines, due to unidentified agents. The second includes vial effects, as well as the true mutational line-generation interaction. Obviously, this interaction is different from the genotype-environment interaction analyzed in this

TABLE 1. Mean (\bar{x}) and coefficients of variation (CV) and asymmetry (g_1) of the distributions of the means of the mutation accumulation lines for different traits and environments. Standard (ST), high temperature (HT), high saline concentration (HSC), diluted (D).

Trait and parameter	Environment			
	ST	HT	HSC	D
Fecundity (No. eggs):				
\bar{x}	17.01 ± 0.22	20.27 ± 0.47	7.08 ± 0.20	5.51 ± 0.19
CV (%)	21.76	28.82	31.49	37.55
g_1	1.09*	0.29	0.88*	1.21*
Egg-to-pupa viability (%):				
\bar{x}	73.83 ± 0.74	45.15 ± 1.34	57.65 ± 1.13	47.96 ± 1.29
CV (%)	16.91	36.87	22.41	28.71
g_1	-1.58*	-0.07	-0.07	0.20
Pupa-to-adult viability (%):				
\bar{x}	76.27 ± 0.46	79.25 ± 0.84	81.89 ± 0.81	72.85 ± 1.58
CV (%)	10.35	13.07	11.30	22.19
g_1	-0.39*	-0.88*	-1.07*	-0.45*

* $P < 0.05$.

TABLE 2. Estimates of between-line (V_L), between-generation (V_G), line-generation interaction (V_{LG}), and within-line (V_W) components of variance for different traits and environments. Standard (ST), high temperature (HT), high saline concentration (HSC), diluted (D). Respective degrees of freedom in environments ST, HT, HSC, and D were: (94, 94, 94, 94) for V_L (all traits); (2, 1, 2, 1) for V_G (all traits); (188, 94, 188, 94) for V_{LG} (all traits); (855, 760, 1031, 653) for V_W (fecundity and egg-to-pupa viability); and (770, 583, 834, 425) for V_W (pupa-to-adult viability).

Trait and variance component	Environment			
	ST	HT	HSC	D
Fecundity:				
V_L	5.73*	25.28*	2.91*	1.63*
V_G	5.60*	32.97*	4.26*	9.42*
V_{LG}	5.50*	7.14*	2.25*	2.67*
V_W	47.92	79.07	22.81	9.77
Egg-to-pupa viability:				
V_L	107.74*	164.07*	47.69*	47.81*
V_G	-1.54	31.19*	19.81*	175.31*
V_{LG}	18.33	97.70*	142.62*	30.70
V_W	717.66	815.21	949.51	1095.00
Pupa-to-adult viability:				
V_L	19.94*	33.79*	-13.69	155.24*
V_G	38.69*	3.70*	7.60*	79.74*
V_{LG}	12.68*	23.46*	77.25*	11.81
V_W	197.05	339.11	507.01	613.64

* $P < 0.05$.

study. The significance of the interaction component was not altered when the analyses were performed on log-transformed (fecundity) or arcsine-transformed (viability) data (not shown).

Estimates of mutational variances, heritabilities, and coefficients of variation are given in Table 4. For fecundity and pupa-to-adult viability comparable mutational heritabilities were obtained in all media. For egg-to-pupa viability, however, estimates obtained in harsh media (HSC and D) were significantly smaller than those calculated in more benign environments. This distinction blurred when mutational coefficients of variation were considered.

Mutational correlations between pairs of traits scored in the same environment (Table 5) were generally low, excepting a very odd high value between fecundity and pupa-to-adult viability in D. Those between fecundity and egg-to-pupa viability were positive in all media, even if competition between immatures may induce a negative correlation between these two traits, as less viable cultures tend to produce large-sized offspring, which will correspondingly lay more eggs (Sang 1949; Prout and McChesney 1985). Our cultures were all raised at low densities and, therefore, important effects on adult weight resulting in fecundity changes can in principle be disregarded. Mutational correlations involving pupa-to-adult viability were positive in HSC and negative in ST and D.

Mutational correlations between the same trait scored in different environments (Table 6) were generally small and either positive or nonsignificant, excepting a large negative value of that between fecundity in ST and HSC. Thus, the effect of mutations on a trait substantially differed across

TABLE 3. Estimates of between-line (cov_L), between-generation (cov_G), line-generation interaction (cov_{LG}), and within-line (cov_W) components of covariance for different pairs of traits and environments. Standard (ST), high temperature (HT), high saline concentration (HSC), diluted (D). Respective degrees of freedom in environments ST, HT, HSC, and D were: (94, 94, 94, 94) for cov_L (all pairs of traits); (2, 1, 2, 1) for cov_G (all pairs of traits); (188, 94, 188, 94) for cov_{LG} (all pairs of traits); (855, 760, 1031, 653) for cov_W (fecundity/egg-to-pupa viability); and (770, 583, 834, 425) for cov_W (fecundity/pupa-to-adult viability and egg-to-pupa viability/pupa-to-adult viability).

Trait and covariance component	Environment			
	ST	HT	HSC	D
Fecundity/Egg-to-pupa viability:				
cov_L	12.20*	3.71*	3.21*	2.22*
cov_G	1.47*	33.35*	0.80*	41.04*
cov_{LG}	-7.44	6.62*	9.30*	1.85*
cov_W	44.38	68.67	25.81	12.57
Fecundity/Pupa-to-adult viability:				
cov_L	-4.21*	6.32*	-0.02	-17.65*
cov_G	-14.28*	13.52*	6.00*	-28.66*
cov_{LG}	-3.91*	2.69	-2.30*	3.01
cov_W	-6.48	-15.38	3.01	-21.64
Egg-to-pupa/Pupa-to-adult viability:				
cov_L	-1.60*	12.86*	0.20	-11.50*
cov_G	-0.62*	10.69*	-5.06*	-174.72*
cov_{LG}	-0.44	5.93	-12.47*	18.88*
cov_W	11.03	-29.64	14.40	-17.81

* $P < 0.05$.

environments, implying the existence of important genotype-environment interactions. As the between-line variance component of pupa-to-adult viability in HSC was negative, the corresponding correlations could not be calculated.

DISCUSSION

Mutational Variances and Covariances

In *D. melanogaster*, the rate at which new variation (covariation) due to spontaneous mutation affecting life-history traits arises, has been measured using two different experimental designs. In both methods, mutations are allowed to accumulate for a number of generations in a set of independently maintained lines, all originating from a common stock, and estimates of mutational variances (covariances) are obtained from the between-line divergence (codivergence) for the trait(s) of interest. In one method, a lethal-free second chromosome is extracted from a natural population, and replicates are preserved in the heterozygous state (and protected from recombination) by maintaining them against a marked balancer chromosome (Mukai 1964). As the second chromosome represents about 40% of the genome, estimates may be extrapolated to the whole by multiplying by 2.5. The alternative method starts from a homozygous base (a line made isogenic for all chromosomes) from which replicate lines are derived and maintained by a pair of parents per line and generation (Santiago et al. 1992). Thus, mutations accumulate over the whole genome.

Mutations affecting life-history traits include the following classes: (1) deleterious mutations of large effect, generally showing widespread pleiotropy; (2) mildly detrimental mu-

TABLE 4. Estimates of mutational variances (V_m), heritabilities (h_m^2) and coefficients of variation ($CV[V_m]$) (\pm standard error) for different traits and environments. Standard (ST), high temperature (HT), high saline concentration (HSC), diluted (D).

Trait and parameter	Environment			
	ST	HT	HSC	D
Fecundity:				
V_m ($\times 10^{-3}$)	28.95 \pm 8.95*	90.29 \pm 19.85*	9.38 \pm 2.72*	5.25 \pm 2.20*
h_m^2 ($\times 10^{-4}$)	6.04 \pm 1.89*	11.42 \pm 2.58*	4.31 \pm 1.21*	5.38 \pm 2.28*
CV (V_m) (%)	1.00 \pm 0.07*	1.51 \pm 0.11*	1.35 \pm 0.10*	1.31 \pm 0.10*
Egg-to-pupa viability:				
V_m ($\times 10^{-3}$)	544.14 \pm 131.12*	585.97 \pm 166.00*	161.12 \pm 89.40*	154.22 \pm 108.20
h_m^2 ($\times 10^{-4}$)	7.58 \pm 1.86*	7.19 \pm 2.07*	1.70 \pm 0.94*	1.41 \pm 0.99
CV (V_m) (%)	0.99 \pm 0.07*	1.67 \pm 0.12*	0.68 \pm 0.05*	0.81 \pm 0.05*
Pupa-to-adult viability:				
V_m ($\times 10^{-3}$)	100.71 \pm 32.61*	120.67 \pm 52.70*	<0	500.78 \pm 128.00*
h_m^2 ($\times 10^{-4}$)	5.11 \pm 1.68*	3.56 \pm 1.57*	<0	8.16 \pm 2.16*
CV (V_m) (%)	0.40 \pm 0.03*	0.44 \pm 0.03*	<0	1.00 \pm 0.07*

* $P < 0.05$.

tations; and (3) mutations showing antagonistic pleiotropy. Estimates of mutational variances and covariances obtained from mutation accumulation against a balancer will be disproportionately influenced by the first class as, in this case, recessive mutations are virtually sheltered from natural selection (lines carrying lethal mutations were usually excluded from analyses). In inbred lines, however, the fixation probability of that first class of mutations will be small and mutational variances and covariances will be mainly due to the second and third classes of mutations, these having a major role on population survival (Lande 1995). Consequently, estimates of mutational variances obtained from inbred lines are expected to be smaller than those from protected chromosomes, and the mutational covariances will tend to become negative in the first method, since the relative contribution of the third class of mutations will be larger in this case.

Using the balancer chromosome technique, estimates of viability (Mukai 1964; Mukai et al. 1972; Ohnishi 1977), fecundity, longevity, and productivity (Houle et al. 1994) have been reported. Average mutational heritabilities and coefficients of variation were 1.09×10^{-3} (range 0.13–3.44 $\times 10^{-3}$) and 1.45% (range 0.87–2%), respectively. In comparable laboratory conditions (ST), our average estimates were

TABLE 5. Estimates of mutational covariances (cov_m) and correlations (r_m) between pairs of traits in different environments. Standard (ST), high temperature (HT), high saline concentration (HSC), diluted (D).

Traits and parameter	Environment			
	ST	HT	HSC	D
Fecundity/Egg-to-pupa viability:				
cov_m ($\times 10^{-3}$)	61.64*	13.25*	10.85*	7.17*
r_m	0.49*	0.06*	0.27*	0.25*
Fecundity/Pupa-to-adult viability:				
cov_m ($\times 10^{-3}$)	-21.29*	22.58*	-0.06	-56.92*
r_m	-0.39*	0.22*	—	-1.07*
Egg-to-pupa/Pupa-to-adult viability:				
cov_m ($\times 10^{-3}$)	-8.08*	45.94*	0.67	-37.10*
r_m	-0.03*	0.17*	—	-0.13*

* $P < 0.05$.

about 45% smaller (0.62×10^{-3} and 0.8%, respectively). Houle et al. (1994) also reported estimates of mutational correlations between life-history traits averaging 0.67 (range 0.45–0.87). These also were substantially larger than ours in ST medium (range -0.11–0.25). The same phenomenon was observed for abdominal bristle number (López and López-Fanjul 1993b). In the same set of lines used in this experiment, estimates of mutational heritabilities were one order of magnitude smaller than those computed from the response to artificial selection obtained in lines derived from the same base population. In this last case, mutations of relatively large effect were incorporated in spite of deleterious pleiotropic effects on fitness, a great proportion of those being lethals with an effect on bristles in the heterozygote (López and López-Fanjul 1993a; Merchante et al. 1995). 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 has acted.

TABLE 6. Estimates of mutational correlations between the same trait scored in pairs of different environments. Standard (ST), high temperature (HT), high saline concentration (HSC), diluted (D).

Trait and environment	Environment		
	HT	HSC	D
Fecundity:			
ST	0.23	-0.97*	0.30*
HT	—	-0.09	0.29*
HSC	—	—	-0.15
Egg-to-pupa viability:			
ST	0.05	0.04	0.31*
HT	—	0.29*	0.32*
HSC	—	—	0.76*
Pupa-to-adult viability:			
ST	-0.08	—	0.64*
HT	—	—	-0.16
HSC	—	—	—

* $P < 0.05$, based on the sequential Bonferroni test (Rice 1989).

Mutational Genotype-Environment Interactions

Kondrashov and Houle (1994) proposed that estimates of the genomic deleterious mutation rate obtained in benign conditions may be biased downward, as they would not take into account conditional quasi-neutral mutations (quasi-neutral in good environments and deleterious in bad ones). This kind of mutation will only contribute to the mutational variances of fitness-component traits in harsh media and to the mutational correlations between states of those traits in different harsh media. In parallel, the magnitude of the effects of unconditional deleterious mutations may be intensified with increasing environmental stress. Consequently, the joint presence of both types of mutations will result in larger mutational variances of fitness-component traits in stressful media and in considerable positive correlations between those traits states expressed in different media (irrespective of their quality). In our lines, highly deleterious mutations (unconditional or environment-dependent) will be eliminated and only mildly detrimental mutations will be fixed. Therefore, the data allow Kondrashov and Houle's (1994) proposal to be tested with only one of the possible types of mutational variance present. It is these mildly detrimental mutations that are mainly responsible for the long-term erosion of fitness leading to the eventual extinction of populations (Lande 1995). Our observations consistently showed no increase of the mutational heritabilities of fecundity and viability in poor environments, as well as generally small or nonsignificant mutational correlations between these traits states in different environments. Thus, mutations quasi-neutral in standard laboratory conditions and mildly detrimental in more stressful ones did not, on their own, lead to an increased variance in stressful environments. However, the results provide no information on mutations becoming lethal or semilethal in harsh media.

With reference to the mutational correlations between two traits, those involving pupa-to-adult viability were negative at both productivity extremes (ST and D), but positive at intermediate values (HT). In natural populations of *D. melanogaster*, a shift from negative values at 22°C to positive values at 28°C was reported for the genetic correlation between fecundity and developmental rate or longevity (Giesel et al. 1982). Furthermore, changes in ranking order for viability were observed for temperature sensitive mutants evaluated at different temperatures (Suzuki et al. 1976). This illustrates how the evolution of life-history traits can be a very complex function of environmental conditions, as a perturbation of the mean may change the genetic covariance structure of quantitative traits in populations under selection in temporally or spatially variable environments. In parallel, our estimates of mutational correlations between character states in different environments suggest a high degree of specificity of the mutations involved and, therefore, the potential for a remarkable degree for independent evolution.

Negative mutational covariances involving pupa-to-adult viability suggest antagonistic pleiotropy of some of the mutations involved. However, the efficiency of this mechanism in the maintenance of genetic variation in populations often requires reversal of dominance, which is unlikely to be common (Curtis et al. 1994). In standard laboratory condi-

tions, the additive variance of viability maintained in natural populations of *D. melanogaster* has been shown to be much larger than the dominance variance (Mukai 1985). In parallel, heterozygous effects of our extreme inbred lines on both viability components in ST medium were always found to be nonsignificant (Fernández and López-Fanjul 1996). Moreover, no association was found between the level of additive and nonadditive effects on viability in standard and low nutrition media (Blows and Sokolowski 1995).

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