Quantitative Genetics of Ovariole Number in *Drosophila melanogaster*. II. Mutational Variation and Genotype-Environment Interaction

Marta L. Wayne and Trudy F. C. Mackay

Department of Genetics, North Carolina State University, Raleigh, North Carolina, 27695-7614

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ABSTRACT

The rare alleles model of mutation-selection balance (MSB) hypothesis for the maintenance of genetic variation was evaluated for two quantitative traits, ovariole number and body size. Mutational variances (V_M) for these traits, estimated from mutation accumulation lines, were 4.75 and 1.97×10^{-4} times the environmental variance (V_E), respectively. The mutation accumulation lines were studied in three environments to test for genotype × environment interaction (GEI) of new mutations; significant mutational GEI was found for both traits. Mutations for ovariole number have a quadratic relationship with competitive fitness, suggesting stabilizing selection for the trait; there is no significant correlation between mutations for body size and competitive fitness. Under MSB, the ratio of segregating genetic variance, V_G, to mutational variance, V_M, estimates the inverse of the selection coefficient against a heterozygote for a new mutation. Estimates of V_G/V_M for ovariole number and body size were both approximately 1.1×10^4. Thus, MSB can explain the level of variation, if mutations affecting these traits are under very weak selection, which is inconsistent with the empirical observation of stabilizing selection, or if the estimate of V_M is biased downward by two orders of magnitude. GEI is a possible alternative explanation.

THERE are two ubiquitous—and contradictory—observations about natural populations: the presence of genetic variation and the operation of selection. Genetic variation in natural populations has been observed for virtually every quantitative trait examined (Roff and Mousseau 1987; Falconer and Mackay 1996). However, we also consistently observe stabilizing and/or directional selection on quantitative traits (Endler 1986). The contradiction arises in that we expect selection to eliminate genetic variation, yet we see the two phenomena side by side. How then is this variation, the raw material of adaptive evolution, maintained in natural populations? Three main hypotheses exist: mutation-selection balance (MSB) (Barton and Turelli 1989; Barton 1990), balancing selection (Gillespie 1984; Barton 1990), and genotype × environment interaction (GEI) (Levene 1953; Hedrick 1986; Gillespie and Turelli 1989). MSB proposes that the processes of mutation and selection are in equilibrium, such that the rate of input of new mutations affecting a quantitative trait is exactly counterbalanced by selective elimination of deleterious mutations. Under this hypothesis, genetic variation is caused by evolutionary "noise," *i.e.*, neutral or slightly deleterious mutations. In contrast, balancing selection, whether by overdominance and/or frequency-dependent selection, involves selection actively maintaining genetic variation. For the case of overdominance, only a small fraction of loci (as few as 100) may maintain large amounts of genetic variation (Barton 1990). Frequency-dependent selection may also maintain large amounts of genetic variation. However, both balancing selection models are theoretically difficult to reconcile with observations of stabilizing selection as a result of genetic load constraints (Barton 1990). One may consider GEI as a special case of balancing selection. It also involves selection actively maintaining variation: if one genotype is not the "most fit" in all environments and if the environment of an organism is variable either in space or time, then selection on different genotypes in the different environments will promote genetic variation (Gillespie and Turelli 1989).

MSB models make simple predictions about the relationship between mutational variation (V_M) and segregating genetic variation (V_G). The exact relationship between segregating and mutational variance differs according to whether one assumes pure stabilizing selection acting on the quantitative trait, high per-locus mutation rates, and weak selection (Lande 1975); low per-locus mutation rates and strong selection (Turelli 1984); or selection acting on the deleterious side effects of mutations affecting a quantitative trait, which can give the appearance of stabilizing selection (Barton 1990; Keightley and Hill 1990; Kondrashov and Turelli 1992). The latter class of pleiotropic models, developed for the "rare-alleles" model of mutation,

Corresponding author: Marta L. Wayne, Box 7614, Department of Genetics, North Carolina State University, Raleigh, NC 27695-7614.
E-mail: mlwayne@tosca.gnets.ncsu.edu

is appealing since it captures the essential genetic features of the mutation process: per-locus mutation rates are low, mutational effects can be large relative to segregating allelic variation, and mutations are neutral or deleterious with respect to fitness (Kondrashov and Turelli 1992). Under this class of models, $V_M/V_G \approx s$, the strength of selection against a new mutant heterozygote (Barton 1990; Kondrashov and Turelli 1992; Caballero and Keightley 1994; Keightley 1994) and $V_G/V_M = t$, the mean persistence time in generations of a deleterious mutation (Crow 1979, 1993).

The estimate of s for heterozygous mutations for viability in Drosophila is around 0.02 (Mukai 1979); thus, we expect $V_G/V_M \approx 200$ if variation for viability is under MSB equilibrium. This expectation seems to hold; V_G for egg to adult viability is 1×10^{-2} and $V_M = 1.3 \times 10^{-4}$ (Crow and Simmons 1983). A review of the mutational variability literature by Houle *et al.* (1996) suggests that the relationship may hold for most life history, growth, and morphological traits. However, the conclusions of Houle *et al.* (1996) rely on extrapolating estimates of V_G from various measurements in the literature. Further, these estimates are frequently not generated by the same investigators and are averaged across disparate populations, times, and methods of measurement (Houle *et al.* 1996). Our most precise information to date on both V_G and V_M comes from Drosophila bristle number, for which $V_G \approx V_E$ while typically mutational heritability is $V_M/V_E \approx 10^{-3}$ (Falconer and Mackay 1996). Thus, for bristle number, $V_G/V_M \approx 1/s \approx 1000$. Segregating genetic variation for bristle number can be maintained by a balance between mutation and selection against deleterious pleiotropic fitness effects of bristle mutations if selection coefficients against bristle mutations are of the order 10^{-3}, *i.e.*, if mutations affecting bristles are under weak selection. This is inconsistent with direct estimates of viability effects of *P*-element insertions affecting bristle number (Mackay *et al.* 1992a; Lyman *et al.* 1996) and with observations of moderate stabilizing selection on bristle number (*e.g.*, Kearsey and Barnes 1970; Nuzhdin *et al.* 1995). This inconsistency has spurred investigations as to the possible sources and magnitude of bias affecting estimates of V_M (Keightley *et al.* 1993; Houle *et al.* 1996). Clearly, reliable estimates of V_M and V_G for other traits are necessary to further evaluate the generality of the MSB hypothesis.

Here we report estimates of mutational variance for ovariole number and body size in *Drosophila melanogaster.* Ovariole number is a fitness-related trait in *D. melanogaster* because of its relationship to fecundity; the maximum daily rate of egg production, occurring between days 4 and 10 of the fly's life, is significantly associated with ovariole number (David 1970; Boulétreau-Merle *et al.* 1982). Body size, like ovariole number, is a morphological trait that may be closely related to fitness because it is correlated with male reproductive success in *D. melanogaster* (Partridge and Farquhar 1983; Partridge *et al.* 1987a, 1987b). Body size is correlated with ovariole number across populations (Lemeunier *et al.* 1986), although not within populations (Wayne *et al.* 1997). We compare our estimates of mutational variation for ovariole number and body size with our previous estimates of genetic variation for these traits (Wayne *et al.* 1997) and evaluate the adequacy of the MSB hypothesis to explain genetic variation for the two traits in light of these comparisons. We also tested the new mutations affecting the two traits for GEI, as GEI has been reported for both ovariole number (Delpuech *et al.* 1995; Wayne *et al.* 1997) and body size (David *et al.* 1994; Wayne *et al.* 1997) in natural populations and because GEI is a possible mechanism for the maintenance of genetic variation.

MATERIALS AND METHODS

Fly lines: The mutation accumulation lines used were those described by Mackay *et al.* (1992b) and Mackay *et al.* (1995). In brief, a subline of the Harwich strain was subjected to full-sibling inbreeding for 41 generations and then subdivided into replicate sublines. The sublines were maintained at a constant population size of 10 males and 10 females for 227 and 230 generations until the measurement of ovariole number and body size, respectively. The small population size reduces the efficacy of selection relative to that of genetic drift, enabling the fixation of deleterious mutations that would be eliminated in a larger population. The effective population size is taken to be 14 individuals ($0.7N$; Mackay *et al.* 1992b). Mutations whose selective effects are less than or equal to $1/(2\ N_e)$ or less than 3.6×10^{-2} will approximate neutrality (Ohta 1973).

The chromosome *3* substitution lines used to generate estimates of standing genetic variation (V_G) are derived from the Raleigh, NC, farmers' market population (for details see Mackay *et al.* 1996; Wayne *et al.* 1997). These lines consist of wild chromosome *3*s substituted into a standard inbred background (*Samarkand*), such that the lines differ from one another only by their third chromosomes.

Environments: Three environments were considered in this study. The 18° and 25° environments were walk-in constant temperature rooms; the 28° environment was a Percival incubator. Incubators and constant temperature rooms vary from one another in many ways besides temperature, including light and humidity conditions; for the sake of brevity, and as temperature is the controlled environmental variable, we refer to the three environments as "temperature" in the text and in the analysis of variance (ANOVA), but it is worth noting that there is other, uncontrolled environmental variation that may be fixed between environments as well.

Quantitative traits in mutation accumulation lines: Ovariole number and body size were measured on the same 20 mutation accumulation lines examined for abdominal and sternopleural bristle number by Mackay *et al.* (1995). Females for ovary dissection were collected from vials set up at a constant density of 10 females and 10 males; parents were removed from vials after 5 days. Ovarioles were dissected from nonvirgin females aged 5–10 days and stained in a saturated solution of potassium dichromate for approximately 4 min before dissection in a droplet of Ringer's solution (Coyne *et al.* 1991). Ovarioles of both ovaries from three females per vial, for each

of two vials in each of four blocks in each of three temperature environments (18°, 25°, and 28°) for the 20 lines, were counted ($n = 960$ ovaries).

Thorax length was measured using an ocular micrometer on flies at least 24 hr post-eclosion and raised under the same conditions as for ovariole number. Ten males and ten females were measured for each of the 20 lines, for two vials in each of four blocks in each of the three temperature treatments ($n = 9600$ flies).

The bristle number data reported here were obtained from the same lines, sample sizes, *etc*., as those reported in Mackay *et al.* (1995), except that the measurements here are from generation 230 of mutation accumulation, the time most directly comparable to that at which ovariole number was measured.

Quantitative traits in chromosome *3* substitution lines: Flies scored for ovariole number and body size were reared in vials set up at a constant density of five females and five males (for details of estimation of genetic variation for both ovariole number and body size, see Wayne *et al.* 1997). For ovariole number, ovarioles from both ovaries of each of three females from one vial from each of four blocks for 48 lines were counted ($n = 576$ flies). For body size, 10 males and 10 females from one vial from two blocks for each of 15 lines (a subset of the lines used to measure ovariole number) were measured ($n = 600$ flies).

For bristle number, flies were reared at a constant density of 10 females and 10 males. Abdominal and sternopleural bristles were counted on 10 males and 10 females from one vial from each of two blocks from a larger sample of 63 chromosome *3* substitution lines from the same population for a total of 2620 flies.

As measurements for standing genetic variation for all the above traits were conducted on homozygous lines, we made the simplifying assumption of complete additivity, under which the line component of variance equals twice the genetic variance ($\sigma^2_L = 2V_G$; Falconer and Mackay 1996). However, this estimate includes both additive and dominance variation.

Quantification of fitness: Line means for competitive fitness were generously provided for the mutation accumulation lines at 25° by J. D. Fry (Fry *et al.* 1996). Fitness was estimated by a one-generation competitive test, placing mated females of the mutation accumulation line in a vial with mated marked (*yellow*) females of a standard tester stock in a fixed ratio, permitting them to lay eggs for 5 days, and then scoring the frequency of wild-type flies that emerged (for further details see Fry *et al.* 1996).

Statistical analysis: Statistical analyses were conducted using the SAS system for Macintosh version 6.10 (licensed to North Carolina State University by SAS Institute, Inc.). Procedures used include GLM, VARCOMP, REG, and CORR.

RESULTS

Results of a factorial ANOVA for ovariole number are presented in Table 1. Variation was partitioned into the cross-classified main effects of line (random), block (random), and temperature (fixed) and their two- and three-way interactions. Replicate vial was a random effect nested within line × block × temperature. We found a highly significant effect for among-line variance (V_L), demonstrating the presence of mutational variation ($P < 0.001$). Temperature also had a significant effect ($P < 0.016$), but the main effect of block was not significant. The line by temperature interaction, which indicates GEI, was not significant ($P < 0.178$). However, the effect of temperature differed significantly among blocks ($P < 0.001$), perhaps because of temporal fluctuations in relative humidity, which was uncontrolled. The line × block × temperature interaction term was also highly significant, indicating genotype × uncontrolled environment interaction for ovariole number. The component of variance attributable to GEI was 0.63 times the among-line component.

Thorax length was measured on both female and male flies of the 20 mutation accumulation lines in the three temperature environments. The ANOVA thus included the additional cross-classified fixed effect of sex and two-, three-, and four-way interactions including sex (Table 2). The main effects of line ($P < 0.001$), temperature ($P < 0.001$), and sex ($P < 0.001$) were all highly significant, but the main effect of block was not. The GEI terms of line × block, line × temperature, line × block × temperature, and line × block × temperature × sex were all significant. Therefore the total variance attributable to GEI accounted for 9.6% of the total variance of thorax length, compared to only 5.4%

TABLE 1

Variance components for number of ovarioles

Source	d.f.	Mean square	Var. comp.	% total var.
Line	18	154.180	0.920***	9.0
Block	3	279.257	0.186	1.8
Temperature	2	1393.010	Fixed*	
Line × block	54	16.094	0.000[a]	0.0
Line × temperature	36	27.084	0.118	1.1
Block × temperature	6	157.434	0.597***	5.9
Line × block × temperature	108	21.406	0.583**	5.7
Vial (line × block × temperature)	228	14.409	0.894***	8.8
Fly (vial)	912	9.047	2.152***	21.1
Error	1368	4.742	4.742	46.5

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

[a] The actual value was −0.148, the biological interpretation of which is 0.000.

TABLE 2

Variance components for body size

Source	d.f.	Mean square	Var. comp.	% total var.
Line	18	38.120	0.054***	5.4
Block	3	65.779	0.015	1.5
Temperature	2	4312.209	Fixed***	—
Sex	1	22,114.943	Fixed***	—
Line × block	54	8.022	0.026*	2.6
Line × temperature	36	7.380	0.019*	1.86
Line × sex	18	2.781	0.005	0.5
Block × temperature	6	29.340	0.034**	3.1
Block × sex	3	2.380	0.000[a]	0.0
Temperature × sex	2	17.330	Fixed*	—
Line × block × temperature	108	4.487	0.031*	3.2
Line × block × sex	54	1.719	0.007	0.7
Block × sex × temperature	6	2.914	0.004*	0.4
Line × temperature × sex	36	1.221	0.000[a]	0.0
Line × block × temperature × sex	108	1.288	0.018*	1.9
Vial (line × block × temperature)	228	2.861	0.097***	9.7
Sex × vial (line × block × temperature)	228	0.919	0.025***	2.5
Error	8208	0.0667	0.667	66.8

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.
[a] The actual value was <-0.001, the biological interpretation of which is 0.000.

for the line component. Clearly, mutational variation for body size is enormously sensitive to both controlled (temperature, sex) and uncontrolled (block) environmental variation. The line × sex term, which indicates mutational variation for sexual dimorphism for body size, was not significant ($P < 0.115$), but the effects of temperature were different in males and females.

Line means of the traits within the three environments are shown in Figure 1. As documented previously, ovariole number has a curvilinear relationship to temperature, with the maximum number of ovarioles at the intermediate temperature (Delpuech *et al.* 1995), while body size varies linearly and negatively with temperature. Line means for body size were correlated with those for ovariole number at 18° ($R^2 = 0.324$, $P < 0.054$), but not at 25° or 28° (data not shown; SAS procedure CORR, Kendall statistic).

The mutational variance, V_M, was estimated from the among-line variance, assuming an additive neutral model, by $V_L = 2V_M[t - 2N_e(1 - \exp(-t/2N_e))] + 2V_o[1 - \exp(-t/2N_e)]$ (Lynch and Hill 1986). V_o, the genetic variance of the full-sib inbred Harwich base population, is approximated by $5V_M$ (Mackay *et al.* 1992b). t is the number of generations of mutation accumulation (227 and 230, ovariole number and body size, respectively). N_e, the effective population size of the mutation accumulation lines, was taken to be 14 (*i.e.*, $0.7N$; Mackay *et al.* 1992b). V_M was calculated for the two traits both across and within environments (see Table 3). As might be expected, given the smaller amount of GEI for ovariole number, the values of the mutational heritability (V_M/V_E) are more similar for ovariole number across environments than for body size, as follows from the greater proportion of variance accounted for by GEI for this trait. The coefficients of mutational variance,

$$CV_M = \frac{100\sqrt{V_M}}{\bar{x}}$$

(Houle 1996), were also calculated both across and within environments, and again the values were more similar across environments for ovariole number than for body size.

As both ovariole number and body size are considered to be fitness-related traits because of their association with reproductive success in females and males, respectively (David 1970; Boulétreau-Merle *et al.* 1982; Partridge and Farquhar 1983), we examined whether or not mutations affecting these traits were correlated with a competitive measure of fitness at 25° (Fry *et al.* 1996). While body size had neither a linear nor a quadratic relationship with this measure of fitness for either sex separately or for sexes combined (data not shown), ovariole number had a significant quadratic, but not linear, correlation with fitness (quadratic, $R^2 = 0.344$, $P = 0.01$; linear, $R^2 = 0.008$, $P = 0.66$) (Figure 2). This suggests that stabilizing selection acts on new mutations affecting ovariole number. If stabilizing selection is direct, acting on ovariole number *per se*, its strength (V_S) can be estimated from the regression (b) of fitness (w) on the squares of the deviation of line means from the optimum ovariole number

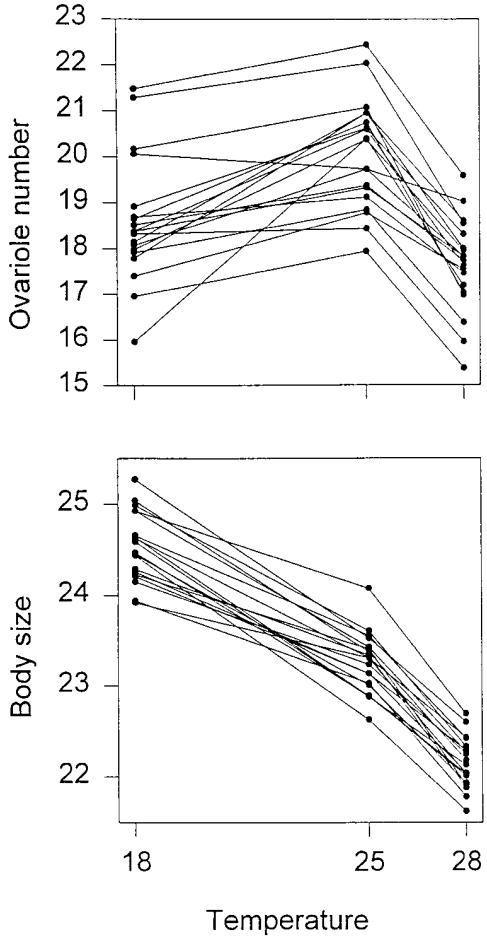

Figure 1.—GEI for ovariole number and body size. The line means for the traits in the three temperature environments are shown; each genotype is represented by a line connecting its three means. Crossing of reaction norms suggests GEI, which was significant for the line × temperature term for body size ($P < 0.009$) but not for ovariole number ($P < 0.166$). Both traits had significant GEI for uncontrolled environmental variation (see Tables 1 and 2).

(y^2) where the optimum is assumed to be the overall mean, since

$$V_s = -\frac{1}{2b_{wy^2}}$$

(Keightley and Hill 1990). For mutations affecting ovariole number, $b_{wy^2} = -0.028$, and $V_S = 18$. This estimate is similar to the average intensity of stabilizing selection observed in natural populations ($V_S = 20$; Turelli 1984). However, stabilizing selection may be apparent rather than direct, caused by pleiotropic deleterious effects on fitness by mutations affecting ovariole number. The strength of apparent stabilizing selection via pleiotropy, ρ, is the correlation between fitness and the absolute value of the deviations of line means from the overall mean (Keightley and Hill 1990). For ovariole number, $\rho = -0.532$ (see Figure 2).

DISCUSSION

Mutational heritabilities of ovariole number and body size are small: The mean mutational heritability, averaged over many traits and species, is frequently quoted as $V_M/V_E = 10^{-3}$ (Barton 1990; Lynch 1988). Our estimates of mutational heritability (see Table 3) for ovariole number (4.75×10^{-4}) and for body size (1.97×10^{-4}) are smaller than this average by a factor of 10. These low values suggest either that there is a very small mutational target size for the two traits or that mutations affecting these traits are extremely deleterious such that they are eliminated from our lines, despite the lines being maintained at a small population size. A small mutational target size for ovariole number is an interesting possibility, as there are few if any candidate genes known that specifically alter ovariole number, despite the multitude of loci that affect female fecundity (Lindsley and Zimm 1992). For body size, however, we know of many candidate genes affecting the trait (Lindsley and Zimm 1992), so the small mutational target explanation seems unlikely for body size.

Both ovariole number and body size are morphological traits thought to be closely related to fitness. Ovariole number is correlated with female reproductive success via a simple relationship between the number of ovarioles and the rate at which eggs are produced by the female (David 1970; Boulétreau-Merle *et al.* 1982); body size in *D. melanogaster* is particularly important with regard to male mating success (Partridge *et al.* 1985, 1987a,b). Houle *et al.* (1996) find that traits have different patterns of values of mutational parameters based on their classification as life history or morphological traits. In general, they find that life history traits have lower mutational heritabilities (V_M/V_E) but higher mutational and environmental coefficients of variation (CV_M and CV_E) than morphological traits. The very low mutational heritabilities (V_M/V_E) for ovariole number and body size are more consistent with life history traits, but their low CV_Ms are more consistent with morphological traits (Houle *et al.* 1996). The CV_E for ovariole number is at the upper bound for morphological traits, but for body size lies within this range. As substantial environmental variation has been demonstrated previously for ovariole number (Robertson 1956; Wayne *et al.* 1997), the large value of CV_E is not surprising and possibly sheds light on why the mutational heritability of ovariole number is atypical for a morphological trait.

The quadratic relationship of ovariole number to fitness is suggestive of its role as a life history-related trait and is consistent with previous results describing a relationship between ovariole number and fecundity (David 1970; Boulétreau-Merle *et al.* 1982). However, a study relating standing genetic variation for ovariole number to a different competitive measure of fitness yielded no significant relationship between the two traits (Wayne

TABLE 3

Mutational variances for ovariole number and body size

Trait	V_L	V_E	$V_M/V_E \times 10^4$	CV_M	CV_E
Ovariole number					
Across temperatures	0.919***	4.742	4.75	0.2531	11.608
18°	1.224***	5.066	5.92	0.2948	12.114
25°	1.191***	3.998	7.30	0.2691	9.957
28°	0.698***	5.162	3.31	0.2346	12.887
GEI	0.583**	4.742	3.01	0.2015	11.608
Body size (thorax length)					
Across temperatures	0.054***	0.667	1.97	0.0491	3.502
18°	0.093**	0.713	3.16	0.0612	3.442
25°	0.090***	0.616	3.53	0.0634	3.376
28°	0.035*	0.671	1.26	0.0415	3.697
GEI	0.089*	0.667	3.22	0.0629	3.502

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; see text for explanation of calculation of V_M. Estimate of V_G for ovariole number and body size from Wayne *et al.* (1997). GEI for ovariole number is the line × block × temperature interaction; for body size, GEI is the sum of four interaction terms (line × block + line × temperature + line × block × temperature + line × block × temperature × sex).

et al. 1997). Several interpretations are possible: first, that the competitive measure of fitness used here (Fry *et al.* 1996) was a more sensitive estimator of female fitness than the one used by Wayne *et al.* (1997) as it eliminated the possible swamping effects of male mating success; second, that the selective values of the mutations accumulated in these lines for ovariole number are small enough to be maintained in these lines in the laboratory, but large enough to be eliminated in natural populations; and third, that segregating variation for ovariole number is caused by very few loci relative to the number of loci at which there is segregating variation for fitness. This last explanation is consistent with the hypothesis of a small mutational target size for ovariole number. These possibilities can be distinguished by exploring the possible role of ovariole number as a determinant of fitness in more detail and by explicitly considering the number of loci that cause variation in ovariole number. Correlations of ovariole number with various fitness components may also help to illuminate the source of pleiotropy (apparent stabilizing selection), which is suggested by the large value of ρ (see results), in the maintenance of genetic variation for the trait.

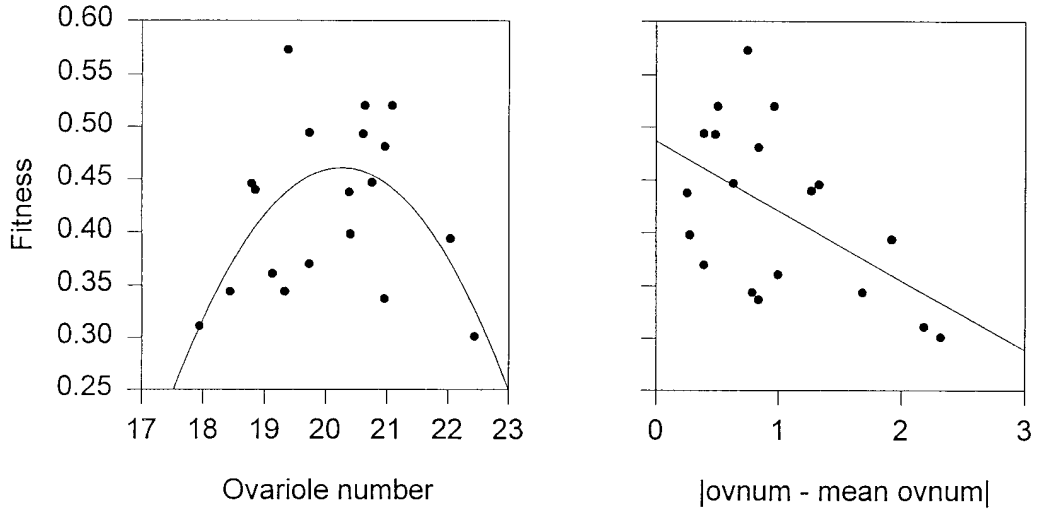

Figure 2.—Stabilizing selection for ovariole number. The left plot shows the relationship between ovariole number and fitness ($R^2 = 0.346$, $P = 0.027$), which suggests stabilizing selection on ovariole number. There is no significant linear relationship between ovariole number and fitness. The right plot illustrates apparent stabilizing selection: the correlation, ρ, of the absolute deviation of the line mean ovariole numbers from the sample mean ovariole number on fitness is an estimate of the strength of pleiotropy.

Why was mutational variation for body size, strongly associated with male mating success in *D. melanogaster* both in the laboratory and in the field (Partridge *et al.* 1985, 1987a,b), not correlated with mutational variation for fitness? The fitness assay used here did not incorporate female choice or male competition, and therefore we might not expect to see such a correlation. We also do not see any mutational variation for genotype × sex interaction, *i.e.*, sexual dimorphism, for body size, although there is standing variation for sexual dimorphism in body size in *D. melanogaster* (Wayne *et al.* 1997). We are left to speculate either that such mutations are very rare or that they are very deleterious and were selectively eliminated from the lines. It is worth pointing out that the regime under which the mutations were accumulated would permit selection for male mating success; however, a final conclusion must await quantitative trait locus mapping and/or a mutagenesis study to determine the genetic potential for genotype × sex mutations for body size.

Maintenance of genetic variation: We have estimates of V_M for the same set of mutation accumulation lines, at the same point in time, for ovariole number, body size, and bristle number. We also have estimates of V_G for these traits from a set of chromosome *3* substitution lines derived from a natural population collected in Raleigh, NC (Wayne *et al.* 1997; T. F. C. Mackay, unpublished data). This provides us with a rare opportunity to compare mutational and segregating variation for these traits and hence to evaluate the adequacy of MSB as an explanation for the maintenance of quantitative genetic variation.

The mutational heritabilities ($V_M/V_E \times 10^4$) for ovariole number and body size were determined to be 4.75 and 1.97, respectively (Table 3). Similar studies of abdominal and sternopleural bristles on these lines yielded estimates of mutational heritabilities from 230 generations of mutation accumulation of 9.01×10^{-4} and 7.90×10^{-4}, respectively (T. F. C. Mackay, unpublished data), which are close to the "typical" value of 10^{-3} reported for these traits (Mackay *et al.* 1992b; Keightley *et al.* 1993; Houle *et al.* 1996). Mutational variation can be compared with standing genetic variation to make inferences about selection since $V_M/V_G \approx s$, the average selection coefficient against heterozygous effects of new mutations, under the rare-alleles model of MSB (Barton 1990; Kondrashov and Turelli 1992; Caballero and Keightley 1994; Keightley 1994). The reciprocal ratio, $V_G/V_M \approx t$, the average persistence time in generations of new mutations (Crow 1979, 1993). Previously we estimated the standing genetic variation for ovariole number from 43 isogenic chromosome *3* substitution lines and for body size from a subsample of 15 of these lines, under the simplifying assumption of complete additivity (Wayne *et al.* 1997). In addition, estimates of segregating genetic variation for bristle number from a larger sample of 63 chromosome *3* substitution lines from the same population are available. However, while our estimates of V_G are only for chromosome *3*, our estimates of V_M are obtained for the entire genome; thus, we have scaled our estimates of V_G by a factor of 2.5 (Lindsley and Zimm 1992) to make the two values comparable. Estimates of V_G and heritabilities (h^2) for these traits are given in Table 4. Heritabilities of ovariole number, body size, and bristle number deduced from variation among the homozygous third chromosomes are typical for these traits in *D. melanogaster* (Roff and Mousseau 1987), suggesting that the assumptions of strict additivity and the proportion of variance contributed by chromosome *3* did not unduly bias the estimates. Estimates of *s* from these data range from 0.91×10^{-4} (ovariole number) to 5.7×10^{-4} (abdominal bristle number), while estimates of *t* range from 1800 (abdominal bristle number) to 11,000 generations (ovariole number) (Table 4).

Selection coefficients of the order 10^{-3}–10^{-4} and persistence times of 10^3–10^4 indicate that mutations affecting ovariole number, body size, and sensory bristle number are only mildly deleterious. Therefore, MSB can maintain variation for these traits if the variation is caused by alleles that affect the traits, but have very little effect on fitness (Barton 1990; Caballero and Keightley 1994). However, the average heterozygous effect on viability of new mutations is 0.02 (Crow and Simmons, 1983); therefore, this argument implies that there are two classes of mutation: those affecting viability and those affecting other quantitative traits. This seems unreasonable: for example, previous studies of bristle number have shown that new mutations have pleiotropic deleterious fitness effects (Mackay *et al.* 1992a, 1995; Nuzhdin *et al.* 1995; Lyman *et al.* 1996). Further, we have shown here that new mutations for ovariole number are related to fitness, being under moderate-to-strong stabilizing selection ($V_S \approx 18$; $\rho \approx$

TABLE 4

Genetic and mutational variances for ovariole number, body size, and bristle number

Trait	V_G[a]	h^2	$V_M \times 10^{-3}$	$s \times 10^{-4}$[b]	$t \times 10^4$[c]
Ovariole number	24.66	0.63	2.25	0.91	1.09
Body size (thorax length)	1.42	0.61	0.13	0.92	1.05
Abdominal bristles	5.95	0.61	3.37	5.67	0.18
Sternopleural bristles	6.80	0.70	0.95	1.40	0.72

[a] All quantities in the table are whole genome estimates. As V_G was estimated from chromosome *3* substitution lines, these estimates were scaled to the whole genome by multiplying by 2.5 ($V_{GGenome} = 2.5V_{GC3}$; Lindsley and Zimm 1992).
[b] $s = V_M/V_G$ (see text for explanation).
[c] $t = V_G/V_M$ (see text for explanation).

-0.5). From these arguments, it appears that MSB alone is inadequate to maintain variation for these traits.

Given the empirically determined values of the average selection coefficient against new mutations, 0.02 (Crow and Simmons 1983), it is clear that values of V_M/V_G of the order of 10^{-2} or, conversely, persistence times of 100 generations would be consistent with the MSB hypothesis. Therefore, our data may not be inconsistent with models of MSB as the sole force maintaining genetic variation if our estimate of V_G is too large or of V_M too small, both by at least one order of magnitude or else one estimate off by two orders of magnitude. Let us first consider V_G. Our estimate of V_G is based on the assumption that all the genetic variation for ovariole number is additive. If dominance variation were present, this would cause us to overestimate V_G, and, in fact, we know that dominance variation has been observed for ovariole number (Thomas-Orillard 1967). We can address this concern by noting that $V_{GD} = 4q^2V_{GA}$ (Falconer and Mackay 1996), where V_{GD} is the case of complete dominance and V_{GA} is the case of complete additivity. Even in the extreme case in which all the genetic variation is caused by recessive alleles at very high frequencies, V_G would be overestimated only by a factor of less than 4. Further, our estimate of the heritability of ovariole number is similar to other estimates in *D. melanogaster* (*e.g.*, Robertson 1956).

Our estimate of V_M was obtained assuming neutrality and thus will be biased downward if mutations affecting ovariole number are largely deleterious and so are eliminated by selection despite the small population sizes of the mutation accumulation lines. Keightley *et al.* (1993) estimated the downward bias in V_M for bristle number based on the degree of dominance and pleiotropic fitness effects observed for new *P*-element mutations affecting bristle number (Mackay *et al.* 1992a). The downward bias was of a factor of 4, given the observed correlations between absolute values of mutant effects on fitness, ρ, of -0.4. Our estimate for ovariole number of $\rho = -0.5$, therefore, suggests a similar magnitude of bias in V_M. Even under strong selection it is extremely unlikely that the bias should be greater than a factor of 5 (see Figure 1 in Keightley *et al.* 1993). Thus, assuming the worst case and combining the two possible sources of error, our estimate of t (s) would be too large (small) only by a factor of 20: still inconsistent with MSB.

Our inference that MSB alone cannot be responsible for the level of naturally segregating variation based on our estimates of V_M and V_G is at variance with the conclusion of Houle *et al.* (1996). These authors report median values for V_G/V_M of less than 50 generations for life history traits and 100 generations for morphological traits, which are entirely consistent with MSB. However, the data of Houle *et al.* (1996) are compiled from a variety of researchers, methods, and times. These factors are held constant in our data. Perhaps there is a consistent source of bias in our measurements that is absent in the other dataset. As discussed earlier in this section, we have attempted to correct for two of the most obvious sources of bias, and this was not sufficient to reconcile the datasets; however, obviously an unrecognized source of bias could be present. Another possibility is that the three traits considered here—ovariole number, body size, and bristle number—all happen to be exceptional traits under peculiar selective pressures, whereas most other morphology and life history traits are under MSB. This seems rather unlikely. More precise estimates of V_M and V_G for additional traits, particularly life history traits, need to be obtained to further explore this inconsistency.

GEI has been proposed as an alternative mechanism to MSB for maintaining genetic variation in a trait, where the environment is patchy spatially or temporally (Hedrick 1986; Gillespie and Turelli 1987). The seasonal changes undergone by temperate *D. melanogaster* populations could constitute such a patchy temporal environment. Also, *D. melanogaster* populations show latitudinal clinal variation for ovariole number across three continents (Lemeunier *et al.* 1986; Capy *et al.* 1993; Azevedo *et al.* 1996), suggesting that different numbers of ovarioles are favored in different environments. Significant standing variation for GEI has been reported for ovariole number in both temperate and tropical populations (Delpuech *et al.* 1995). We found significant mutational variation for GEI for ovariole number (significant line $\times$ block $\times$ temperature term; see Table 3). The magnitude of this variance component is large: about 60% of the size of the main genetic term (line). We found even greater significant mutational variance for GEI for body size (see Table 3). For body size, GEI is nearly twice as large as the genetic component (main effect of line). Mutational variation for GEI has also been demonstrated for fitness itself (Fry *et al.* 1996). All these data show the potential of GEI as an important phenomenon for the maintenance of genetic variation. However, for GEI to maintain variation for ovariole number or body size, it is necessary to demonstrate that there is a changing of relative ranking of fitness across environments that coincides with the change in ranking of the trait values. It is unclear how to quantitatively test this hypothesis.

The standing genetic variation, V_G, and the mutational variation, V_M, are critical for providing an understanding of the selective forces acting on a trait in nature. The pairing of a high heritability for ovariole number coupled with a low mutational variance supports our previous conclusion that this trait is under some sort of variation-enhancing selection (Wayne *et al.* 1997). However, the quadratic relationship of mutations for ovariole number to fitness suggests that the trait may be subject to apparent or actual stabilizing se-

lection on the trait, which would serve to reduce V_G, although an analysis of standing genetic variation for ovariole number and a different competitive measure of fitness failed to indicate any relationship (Wayne *et al.* 1997). The rare-alleles models of MSB are not consistent with our data on either ovariole number or body size, first because the estimated values for the ratio of V_G/V_M are too large; and second, in the case of ovariole number, because V_G/V_M estimates $1/s$ and the low value of s so estimated is not consistent with our observations of moderately strong stabilizing selection on the trait. We suggest that GEI, observed in natural populations for both traits and shown here to be a substantial fraction of their mutational variance, may be responsible for maintaining genetic variation for these traits. However, two more prosaic explanations remain to be investigated: that ovariole number has a small mutational target, *i.e.*, only a few loci contribute to the observed mutational variance; or that mutations causing changes in ovariole number are common but are highly deleterious, such that our neutral mutation estimate is biased drastically downward. Clearly, V_G and V_M cannot tell us the entire story. Detailed information about the genetic architecture of these traits, particularly in terms of number and location of loci and their unbiased estimates of per-locus mutation rates and mutational effects, is required to make rigorous inferences about natural selection.

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