The Distribution of Spontaneous Mutations on Quantitative Traits and Fitness in *Drosophila melanogaster*

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ABSTRACT

Starting from a completely homozygous population of *Drosophila melanogaster*, two groups of 100 inbred lines each were established and maintained for 46 generations, by a single brother-sister mating and two double first cousin matings, respectively. Sternopleural bristle number, wing length and wing width were simultaneously scored in all lines every 4–5 generations. The means of four lines in each group departed significantly from the overall mean and, in each case, this was attributed to a single mutation of relatively large effect on at least one trait (0.3–1.4 environmental standard deviations in absolute value). Further analyses revealed widespread pleiotropy, similar gene action of a given mutation for all traits affected, and predominant additive action. No apparent association was found between the magnitudes of mutational effects on the traits and fitness. However, all recessive mutations were deleterious. The distribution of mutant effects was asymmetrical (positive for bristles and negative for wing measurements). Moreover, these distributions had a high variance and may be leptokurtic, due to the presence of major genes. Estimates of the ratio of new mutational variance to environmental variance ranged within $(0.7–3.4) \times 10^{-3}$, those for wing measurements being generally larger. In agreement with theory, the rate of between-line differentiation was independent of population size.

THE maintenance of genetic variation within populations, as well as the cause of genetic divergence between them, are among the most important and debated problems in evolutionary biology. In the last years, our understanding of these aspects of morphological microevolution has been substantially improved by theoretical studies that take into account the joint action of mutation, selection and drift on polygenic variation.

Starting from a genetically homogeneous population, the within-population genetic variance of a neutral additive trait increases with time until an equilibrium value $2N\sigma_m^2$ is attained, N being the effective population size and σ_m^2 the mutational input of genetic variance per generation (LANDE 1976; CHAKRABORTY and NEI 1982; LYNCH and HILL 1986; COCKERHAM and TACHIDA 1987). The between-population variance also increases with time but its long-term behavior depends on the model of mutational effects considered. It eventually approaches a constant rate $2\sigma_m^2$ per generation (LYNCH and HILL 1986) or converges asymptotically towards an equilibrium value (COCKERHAM and TACHIDA 1987).

Different approaches have been used to include the action of direct stabilizing selection in drift-mutation models [see reviews by HILL (1989) and HOULE (1989)]. The equilibrium variance has been shown to be highly dependent on the shape of the distribution of mutant effects on the trait. It is smaller when this distribution is asymmetrical and/or leptokurtic, since new mutations are more strongly selected against in these situations (KEIGHTLEY and HILL 1988). Pleiotropy can be taken into account by summarizing the effects of mutations on other traits in a net effect on fitness, *i.e.*, by considering the bivariate distribution of mutant effects both on the trait of interest and on fitness (KEIGHTLEY and HILL 1988, 1990a). The behavior of the equilibrium variance depends on the value of the correlation ρ between the absolute values of mutant effects on the trait and fitness. For a perfect correlation, the genetic variance maintained quickly approaches the prediction for pure stabilizing selection on single traits. Otherwise ($\rho < 1$), the genetic variance continuously increases with N but at a smaller rate than predicted from the neutral model.

Experimental information on relevant parameters is scarce. LYNCH (1988a) analyzing previously obtained data, gave estimates of spontaneous mutational heritabilities (σ_m^2 scaled by the environmental variance σ_E^2) within a range of 5×10^{-2} to 10^{-4} for a variety of traits and organisms. More recent estimates from Tribolium (ENFIELD and BRASKERUD 1989), mice (KEIGHTLEY and HILL 1990b) and Drosophila (WEBER and DIGGINS 1990; CABALLERO, TORO and LOPEZ-

FANJUL 1991; MACKAY *et al.* 1992) are within this range.

Indirect evidence on the effect of mutations on quantitative traits, mainly referring to abdominal and sternopleural bristle number in Drosophila, has been summarized by MACKAY (1990) and LOPEZ-FANJUL and CABALLERO (1990). On the whole, the data suggest that the distribution of spontaneous mutant effects on these traits and fitness are asymmetric and leptokurtic. Direct data for abdominal bristle number (CABALLERO, TORO and LOPEZ-FANJUL, 1991) also conform with that view. However, it must be borne in mind that the majority of the new variance observed could be attributed to genes of large effects.

Spontaneous mutations affecting quantitative traits are best studied by experiments based upon an initially homozygous population divided into lines (inbred or artificially selected), and waiting for mutations to accumulate. Thus, a permanent divergence of the performance of a line from the overall mean can be unambiguously ascribed to the increase in frequency of a new mutation. Moreover, its effect and type of gene action can be subsequently evaluated for the quantitative characters studied, as well as its pleiotropic effect on fitness. In addition, the data provide estimates of mutational variances and covariances and can also be used to check specific predictions on the temporal change of the shape of the distribution of the mean of the lines. In the present experiment, the inbreeding approach was used to study three traits in lines started from an isogenic *Drosophila melanogaster* base population.

MATERIALS AND METHODS

Base population: The *D. melanogaster* isogenic line for all chromosomes obtained by CABALLERO, TORO and LOPEZ-FANJUL (1991) was used as the base population. This line carried the recessive eye-color marker sepia (*se*) in chromosome *III*, as an indicator of possible contamination with exogenous flies. It was classified as Q (weak P) or M′ (pseudo-M) for the P-M system of hybrid dysgenesis. No significant response after eight generations of divergent selection for abdominal bristle number was detected in lines started from this base population. This result confirmed that the procedure used to obtain the isogenic line was fully effective in producing complete homozygosity.

The isogenic line was maintained thereafter as a control line in eight bottles. A circular mating scheme was employed to ensure a sufficiently large population (about 800 parents per generation). Given the duration of this experiment (46 generations), this procedure implies negligible drift and ample opportunities for natural selection to act against deleterious mutations.

Inbred lines: Starting from the isogenic line after five generations of multiplication, two groups (*B* and *C*) of 100 inbred lines each were established (generation 0). Each of them was maintained either by a single brother-sister mating (lines *B*) or by two double first cousin matings (lines *C*) per generation. Thus, all individuals within the same group and generation had the same regular pedigree.

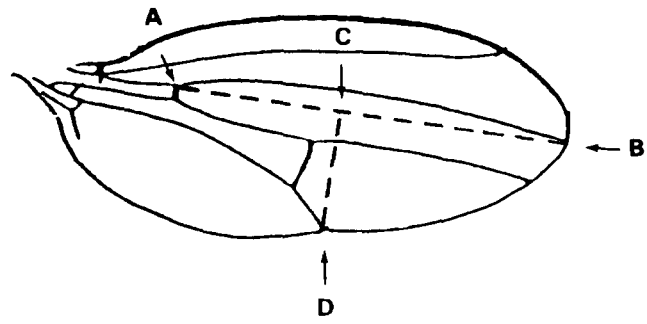

FIGURE 1.—Wing dimensions (*A–B, C–D*) measured.

To minimize losses of lines, two (lines *B*) or four (lines *C*) single pair matings were established per line and generation, but only one (lines *B*) or two (lines *C*) contributed offspring, both for evaluation and as parents of the next generation. In the case of the *C* lines, we utilized offspring of each pair of parents equally.

To confirm the homozygosity of the base population, the insertion pattern of the *copia*-like mobile element 412, visualized by *in situ* hybridization on salivary gland chromosomes, was compared across 45 randomly chosen *B* lines at generation 62 (R. VILLA and J. ALBORNOZ, unpublished). Although the spontaneous rate of mutation of this element is very low (HARADA, YUKUHIRO and MUKAI 1990), natural populations are highly polymorphic for copy number (MONTGOMERY, CHARLESWORTH and LANGLEY 1987). The same fourteen insertions were observed in all lines.

Culture conditions and traits scored: Flies were reared on the standard medium formula of this laboratory (brewer's yeast-agar-sucrose). All cultures were incubated at 24° ± 1°. In the inbred lines, each mating was kept in a separate vial (20-mm diameter, 100-mm height) with 15 ml medium added (one or two vials per line and generation in the *B* and *C* lines, respectively). The isogenic line was kept in 250-ml bottles with 50 ml medium added, but flies reared in vials were used for evaluation.

Traits considered were: (1) the total number of bristles on the right and left sternopleural plates, (2) the length of the right wing from *A* to *B* (Figure 1), and (3) the width of the same wing from *C* to *D* (Figure 1). From generation 0, the performance of all lines for these three traits was simultaneously evaluated at 10 intervals (usually every 4–5 generations) during a period of 46 generations. Each trait was scored on ten females per line and generation of evaluation, but different sets of flies were scored for bristles and wing measurements. Therefore, covariances could only be calculated for the latter.

Measurements were obtained on wings removed and mounted on slides. Using a projection system consisting of a microscope and two mirrors, the enlarged image (1 unit = 1.22×10^{-3} mm) was reflected upon a digital data tablet where distances between reference points (*A–B, C–D*) were measured. The tablet was linked to a computer where data was stored and processed.

Mutant analysis: This analysis refers only to inbred lines showing a significant deviation from the overall performance of its group in at least one trait. In each case, crosses were made between males taken from the line at generations 48–50 and control females. The resulting F_1 females were likewise crossed to males either from the control (B_0 backcross) or from the inbred line (B_1 backcross). Each backcross was kept under crowded conditions and with a large number of parents in three independent bottles during two generations. At the third generation, six males and six virgin females were taken from each bottle and, from these, 18

single pair matings were randomly established in vials. In parallel, single random pair matings were also established between individuals of the line under study (10 pairs), the control (30 pairs), and the parents of a new F_1 (10 pairs). In the following generation (4th), the performance for the pertinent traits of ten female offspring per mating was simultaneously estimated in the inbred line (P_1, 100 females scored per line), the control line (P_0, 300 females scored), its F_1 (100 females scored) and the two backcrosses (180 females scored per backcross). This procedure was adopted in order to standardize culture density in all lines considered.

Assuming that the observed divergence can be attributed to a single fixed mutation per line, estimators of the additive ($a = (\bar{P}_1 - \bar{P}_0)/2$ and dominance ($d = \bar{F}_1 - (\bar{P}_0 + \bar{P}_1)/2$) effects can be obtained. In addition, the performance of the two backcrosses (after three generations maintained at large population size) can be compared with their expected value at Hardy-Weinberg equilibrium ($\bar{B}_0'$ $\bar{B}_1'$) (*e.g.*,

$$\bar{B}_0' = (3/4)^2 \times \bar{P}_0 + 2 \times (3/4) \times (1/4) \times \bar{F}_1 + (1/4)^2 \times \bar{P}_1).$$

Significant differences between observations and expectations ($D_0 = \bar{B}_0 - \bar{B}_0'$, $D_1 = \bar{B}_1 - \bar{B}_1'$) were interpreted as an indication of the corresponding mutation having an effect on fitness. Of course, the power of this test is inversely related to the degree of dominance of the mutation involved.

Estimation of mutational variances and covariances: For each group of lines, generation of evaluation and trait, a hierarchical analysis of variance was performed on the data. The variance was partitioned into sources arising from variation between individuals within vials (V_w), between vials within lines (V_v) (only in the *C* lines), and between lines (V_b).

Starting from a completely homozygous population and assuming that all mutations are neutral, additive and of small effect, σ_m^2 can be estimated over a *t*-generation period by the linear regression coefficient of V_b on twice the cumulated inbreeding coefficient of the lines (F) calculated from pedigrees, as indicated by the equation

$$V_{bt} = V_{be} + 2\sigma_m^2 \sum_{1}^{t-1} F_t$$

(LYNCH and HILL 1986), where the intercept of the regression line (V_{be}) is a pooled estimate of the fraction of the between-line variance attributable to nongenetic factors. However, the sampling variance of the regression coefficient, as obtained from standard theory, is an underestimate, due to autocorrelation between the values of V_b in different generations (LYNCH 1988b).

Estimates of the mutational heritability (h_m^2) were obtained dividing σ_m^2 by the environmental variance (σ_E^2). In the *C* lines, σ_E^2 can be estimated by V_w, thus excluding between-vial environmental differences. In the *S* lines, environmental differences between individuals and vials are confounded in V_w and, therefore, h_m^2 will be slightly underestimated.

The mutational variance has also been estimated by restricted maximum likelihood (REML) using an animal model, as implemented by MEYER (1989). We assumed the initial genetic variance to be insignificant (an arbitrary value of 10^{-8} was used for all traits). Subsequently, additive mutations arise with effects symmetrically distributed around zero. Consequently, the genetic variance increases by σ_m^2 units each generation which is allocated to each individual.

For each trait, the model adjusted was

$$y_{ijk} = g_i + v_{ij} + a_{ijk} + e_{ijk},$$

where g_i is a fixed generation effect ($i = 1, 10$), v_{ij} is a random vial effect nested to generation ($j = 1, 100$ in the *B* lines, $j = 1, 200$ in the *C* lines), y_{ijk}, a_{ijk} and e_{ijk} are, respectively, the phenotypic, additive genetic and environmental values corresponding to the ijkth individual ($k = 1, 10$ in the *B* lines, $k = 1, 5$ in the *C* lines). The a_{ijk} values present a variance-covariance structure described by the "numerator relationship matrix," that can account for the increased covariance between relatives by mutation (WRAY 1990).

ANOVA estimates of the mutational covariance (cov_m) between the two wing measurements were obtained by calculating the mutational variance of a composite trait (T), defined as the sum of wing length (L) and wing width (W). Thus,

$$\text{cov}_m = (\sigma^2_{m(T)} - (\sigma^2_{m(L)} + \sigma^2_{m(W)}))/2.$$

REML estimates of cov_m were obtained by using the bivariate algorithm proposed by THOMPSON and HILL (1990). This procedure also allows estimation of environmental and between-vial covariance components.

Confidence intervals for REML estimates of h_m^2 were based on their profile likelihood (MEYER and HILL 1992). Confidence limits are defined such that values with a difference in log likelihood from the global maximum likelihood of greater than two are outside the interval (*i.e.*, roughly equivalent to 95% confidence limits). Estimates of σ_m^2 were obtained from univariate analyses and, therefore, confidence intervals for mutational genetic correlations could not be calculated.

RESULTS

Individual lines: At certain times in the course of the experiment, the performance of several inbred lines departed from their corresponding group average. For each line and trait, the average deviation over the last three evaluations was computed and tested for significance (Duncan multiple range tests were used, $P < 0.05$). Four lines in each group (B60, B64, B78, B96, C20, C58, C59 and C84) diverged significantly from the overall mean in at least one trait.

The evolution of these line means (deviated from their group average) is presented in Figure 2 for all traits in σ_E units (calculated as the square root of the average REML estimate of the environmental variance for the *B* and *C* lines, given in Table 4 below). Two lines departed significantly for only one trait (C59 for bristle number and C58 for wing length), the remaining six differing in at least two traits. All lines showed a rapid change in the mean occurring between two successive evaluations (4–5 generations), subsequently maintaining this new level. In general, deviations for more than one trait in the same line appeared simultaneously. No directional change over time of the coefficient of variation of each deviant line was detected for any trait. This observation indicates that the mutations involved were already fixed when first detected, implying that fixation was attained quickly (4–5 generations at most). In the *B* lines, all mutations appeared before generation 12, contrasting with the *C* lines where they were mostly detected in later evaluations.

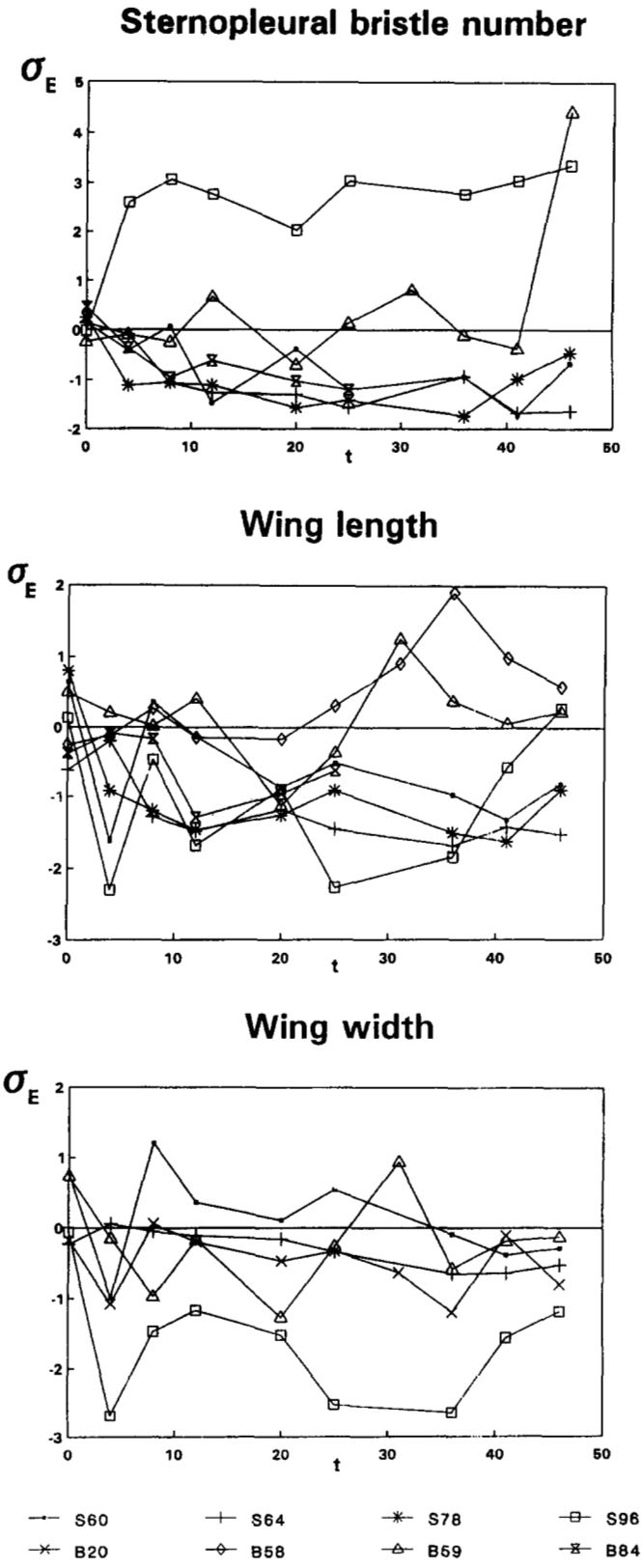

FIGURE 2.—Mean of inbred lines significantly departing from their corresponding group average plotted against generation number.

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

Effects and gene action of mutations: The performance of the lines significantly departing from their group average, the control line and the corresponding F_1's, were simultaneously estimated for all traits and are shown in Table 1 (in the scale of measurement). From these values, estimators of additive (a) and dominance (d) effects were obtained and are also presented in Table 1 (in σ_E units). In a given line, d was only calculated when a was significantly different from zero. These effects were tested for significance ($P < 0.05$), independently for each trait over all lines, using the Bonferroni sequential comparison method (RICE 1989). Line C84 showed a markedly reduced fertility and was lost at generation 25. At that moment, its mean significantly deviated from the overall mean (in σ_E units) for bristle number (-0.95 ± 0.19) and wing length (-0.76 ± 0.22), but not for wing width (-0.23 ± 0.20). Most mutations affecting wing measurements decreased the trait values, but those affecting bristle number acted in both directions.

The type of gene action was unambiguously established in most instances. However, additivity could not be distinguished from complete dominance in four cases. This is to be expected for mutations of relatively small effects, such as those affecting wing width. In the remaining instances, the procedure followed may introduce a bias in favor of additive action, but only to a very limited extent, *i.e.*, for incomplete recessives (or incomplete dominants) when their additive effect is small. Two mutations, one additive (C58) and the other complete recessive (C20), affected a single trait. Another mutation (B78) affected two traits additively. The remaining four mutations affected all three traits studied. Of these, B96 was recessive in all cases, B60 and C59 were additive for two traits and additive or dominant for the other, and B64 was incomplete dominant in one case and additive or dominant for the others. No obvious association was found between the effect and gene action of the mutations and the type of line (B or C) in which they occurred.

Correlations between mutant effects on two traits were -0.30 ± 0.42 (bristle number-wing length), -0.64 ± 0.34 (bristle number-wing width) and 0.90 ± 0.19 (wing length-wing width).

Effect of mutations on fitness: All F_1's were backcrossed to their inbred (B_1 backcross) and control (B_0 backcross) parental lines. For all traits the differences ($D_0 = \bar{B}_0 - \bar{B}_0'$, $D_1 = \bar{B}_1 - \bar{B}_1'$) between the backcross performance and its Hardy-Weinberg expectation ($\bar{B}_0'$ and $\bar{B}_1'$) are shown in Table 2 and each was tested for significance using the Bonferroni method within each trait over all lines ($P < 0.05$). Only data from those lines previously shown to have an effect on a given trait are presented.

In four cases (B64, B78, C58 and C59), both D_0 and D_1 were not significantly different from zero for

TABLE 1

Contemporary performance of control (P_0) and inbred (P_1) lines and their corresponding F_1 (scale of measurement), and additive (a) and dominance (d) effects (in environmental standard deviation units)

Line	P_0	P_1	F_1	a[a]	d[a]	Gene action
Sternopleural bristle number:						
B60	21.92 ± 0.12	19.76 ± 0.20	20.80 ± 0.20	−0.58 ± 0.06*	−0.02 ± 0.12	Additive
B64	21.92 ± 0.12	20.32 ± 0.18	20.54 ± 0.20	−0.43 ± 0.05*	−0.31 ± 0.11	Additive-Dominant[b]
B78	21.92 ± 0.12	20.12 ± 0.20	21.18 ± 0.18	−0.48 ± 0.06*	0.08 ± 0.11	Additive
B96	22.32 ± 0.08	27.38 ± 0.18	22.56 ± 0.18	1.36 ± 0.05*	−1.23 ± 0.10*	Recessive[c]
C20	22.74 ± 0.12	22.18 ± 0.18	23.00 ± 0.18	−0.15 ± 0.05		
C58	22.74 ± 0.12	22.58 ± 0.26	22.42 ± 0.20	−0.04 ± 0.07		
C59	22.74 ± 0.12	27.92 ± 0.66	24.20 ± 0.36	1.39 ± 0.19*	−0.60 ± 0.27	Additive
Wing length:						
B60	1241.9 ± 1.8	1200.8 ± 2.7	1215.7 ± 3.5	−0.60 ± 0.04*	−0.17 ± 0.11	Additive
B64	1224.4 ± 1.9	1165.5 ± 3.2	1182.1 ± 3.1	−0.86 ± 0.06*	−0.38 ± 0.10*	Incomplete dominance[d]
B78	1224.4 ± 1.9	1194.8 ± 2.6	1214.8 ± 3.0	−0.43 ± 0.04*	0.16 ± 0.10	Additive
B96	1233.1 ± 1.3	1186.1 ± 3.4	1220.0 ± 2.8	−1.37 ± 0.06*	0.30 ± 0.10*	Incomplete recessive[d]
C20	1234.3 ± 1.7	1229.5 ± 2.5	1242.3 ± 2.0	−0.07 ± 0.04		
C58	1234.3 ± 1.7	1249.3 ± 2.2	1243.9 ± 2.8	0.22 ± 0.04*	0.06 ± 0.09	Additive
C59	1241.9 ± 1.8	1222.3 ± 3.7	1232.1 ± 2.9	−0.29 ± 0.06*	0.01 ± 0.11	Additive
Wing width:						
B60	479.1 ± 0.8	473.1 ± 1.3	472.7 ± 1.6	−0.19 ± 0.04*	−0.21 ± 0.12	Additive-Dominant[b]
B64	472.5 ± 0.8	459.6 ± 1.7	461.5 ± 1.3	−0.41 ± 0.06*	−0.29 ± 0.11	Additive-Dominant[b]
B78	472.5 ± 0.8	468.9 ± 1.2	468.1 ± 1.3	−0.12 ± 0.04		
B96	476.2 ± 0.6	444.6 ± 1.2	469.1 ± 1.2	−1.01 ± 0.04*	−0.55 ± 0.08*	Incomplete recessive[d]
C20	470.7 ± 0.8	466.4 ± 1.0	473.1 ± 1.0	−0.14 ± 0.04*	0.29 ± 0.08*	Recessive[c]
C58	470.7 ± 0.8	472.5 ± 1.1	470.9 ± 1.2	0.05 ± 0.04		
C59	480.0 ± 0.8	469.9 ± 1.6	471.4 ± 1.7	−0.32 ± 0.05*	−0.22 ± 0.13	Additive-Dominant[b]

[a] Values in environmental standard deviation units. (1.86, 34.21 and 15.68 for sternopleural bristle number, wing length and wing width, respectively).

[b] d not significantly different from zero and a.

[c] d not significantly different from $-a$.

[d] d significantly different from zero and $|a|$.

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

TABLE 2

Differences ($D_0 = \bar{B}_0 - \bar{B}_0'$, $D_1 = \bar{B}_1 - \bar{B}_1'$) between the observed performance of backcrosses after three generations ($\bar{B}_0$ and $\bar{B}_1$) and their Hardy-Weinberg expectations ($\bar{B}_0'$ and $\bar{B}_1'$) (subscripts 0 and 1 refer to the control line and the corresponding inbred line, respectively)

	Bristle No.		Wing length		Wing width	
Line	D_0	D_1	D_0	D_1	D_0	D_1
B60	0.42 ± 0.11*	0.29 ± 0.13	0.09 ± 0.11	0.33 ± 0.12	0.02 ± 0.10	0.12 ± 0.11
B64	0.16 ± 0.06	−0.04 ± 0.14	0.08 ± 0.11	0.32 ± 0.11	−0.11 ± 0.08	−0.07 ± 0.11
B78	−0.07 ± 0.13	0.37 ± 0.13	−0.15 ± 0.10	−0.06 ± 0.11		
B96	−0.40 ± 0.13*	−1.36 ± 0.16*	−0.43 ± 0.09*	0.67 ± 0.14*	−0.34 ± 0.13	1.05 ± 0.13*
C20					−0.13 ± 0.08	0.22 ± 0.06*
C58			0.17 ± 0.08	0.03 ± 0.11		
C59	0.44 ± 0.23	−0.55 ± 0.38	−0.29 ± 0.11	−0.05 ± 0.12	−0.18 ± 0.11	0.08 ± 0.13

In environmental standard deviation units (1.86, 34.21 and 15.68 for sternopleural bristle number, wing length and wing width, respectively).

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

any trait. This indicates that any effects of these mutations on reproductive fitness were not large enough to be detected. Significant values of D_0 and D_1 were detected in line B96 for all traits, and in lines B60 and C20 for bristles (D_0) and wing width (D_1), respectively. Mutation B60 affected all traits, although significance was easier to establish on bristles where its effect was largest. Mutation C20 only affected wing width. In all instances, $\bar{B}_0$ and $\bar{B}_1$ were closer to $\bar{P}_0$ than expected. Thus it can be assumed that the mutant allele was the less fit. Consequently, D_0 and D_1 will both be expected to be negative for mutations with positive effect and vice versa. A single exception to this rule was found (D_0 for wing length in line B96). In this respect, it should be pointed out that the behavior of D_0 is less informative than that of D_1, as $\bar{B}_0$ is closer than $\bar{B}_1$ to $\bar{P}_0$.

Temporal changes of the distribution of the means of the lines: The evolution of the mean and the coefficients of variation, asymmetry and kurtosis

of the distribution of the means of all lines are shown in Figure 3 for each trait and group of lines. Linear regression coefficients of the mean and the coefficient of variation on generation of evaluation are given in Table 3. In all cases, the overall mean oscillated in a similar manner, with a maximum at generation 20 and a minimum at generation 36. This common behavior probably reflects parallel changes in body size attributable to between-generation environmental differences. On the whole no directional trend was detected.

At generation 0, the distribution of mean bristle score did not depart significantly from normality in both types of lines. In the *B* lines, the distribution became progressively more variable, asymmetric (positive) and leptokurtic, although no further changes were observed from generation 12 onwards. In the *C* lines, however, the values of the three coefficients remained stable until they suddenly experienced a drastic increase in the last evaluation (generation 46), equalling or surpassing those of the *B* lines. These changes can be attributed to mutations of large effect occurring earlier in four *B* lines and later in two *C* lines. Removal of these lines from the analysis resulted in similar temporally stable values of the above mentioned coefficients for both sets of lines.

For wing measurements, the coefficient of variation also increased with time (not significantly), but no differences could be established between the *B* and *C* lines. The distributions of the means of the lines were generally asymmetric (negative) and leptokurtic. There was a tendency for the values of the coefficients of asymmetry and kurtosis, corresponding to the two groups of lines, to approach each other in the second half of the experiment. These results could be assigned to a similar number of mutations of large effect being fixed in both groups of lines at different moments (early in the *B* lines and later on in the *C* lines). However, eliminating those lines known to carry a mutation affecting wing measurements from the analyses, did not appreciably change the values of the three coefficients for the two traits considered.

Mutational variances and covariances: ANOVA and REML estimates of the between-vial and the environmental variances, as well as those of the mutational heritability are listed in Table 4 for all traits and types of lines. For each trait, both estimation procedures resulted in similar values of these three parameters. REML estimates of h_m^2 were all significantly different from zero. However, significance could not be shown for ANOVA estimates. Mutational heritabilities of wing measurements were consistently higher than that of sternopleural bristle number. REML estimates were also obtained excluding the data from the eight lines in which mutations were individually detected. Their values were always smaller. Nevertheless, those for wing measurements remained significant. The procedure did not appreciably change the estimations of the between-vial and environmental variances for any trait.

Considerable between-vial variances were observed, of the order of 10% or 20% of the environmental variance for bristle number or wing measurements, respectively. ANOVA estimates of the between-vial variance do not include between-line environmental differences ascribable to vial effects (confounded with genetic differences in the total between-line variance). Therefore, REML estimates are preferable and mutant effects are given in REML environmental standard deviation units.

Estimates of the mutational genetic correlation between wing measurements were moderately high and positive. They became smaller but remained positive after excluding data from the lines known to carry mutations. Strong between-vial effects were detected, of the order of 40% of the environmental covariance.

DISCUSSION

Analysis of mutant effects on metric traits: The mean of eight inbred lines significantly departed from the corresponding group average for at least one trait in a small number of generations, stabilizing afterwards. When one line diverged for more than one trait, all responses appeared simultaneously. Given the rarity of mutational events, such that multiple mutations in one individual are extremely unlikely, the results indicate that a single mutation had occurred in each line and attained fixation during that interval.

For wing measurements, all mutations but one (C58 for wing length) decreased the expression of their carriers. For sternopleural bristle number, three mutations decreased and two increased expression, the latter having much larger effects. Both results are based upon a small sample of mutations and, therefore, inferences on the asymmetry of the distribution of mutant effects on bristle number may be biased. However, our observations are in agreement with the response to artificial selection in the upward direction being usually larger for bristle number (LATTER and ROBERTSON 1962; ROBERTSON 1966; MCPHEE and ROBERTSON 1970) and smaller for wing length (TANTAWY 1961; TANTAWY, MALLAH and TEWFIK 1964; PREVOSTI 1967).

Most mutations negatively affected all traits, the only exceptions being B96 and C59, which increased bristle number and decreased wing measurements. These two mutations had the largest effects detected in the experiment. On the whole, the data revealed widespread pleiotropy, only two mutations (C20 and C58) affecting a single trait. Furthermore, four (B64, B96, C58 and C59) out of the seven mutations studied in detail also had an effect on abdominal bristle num-

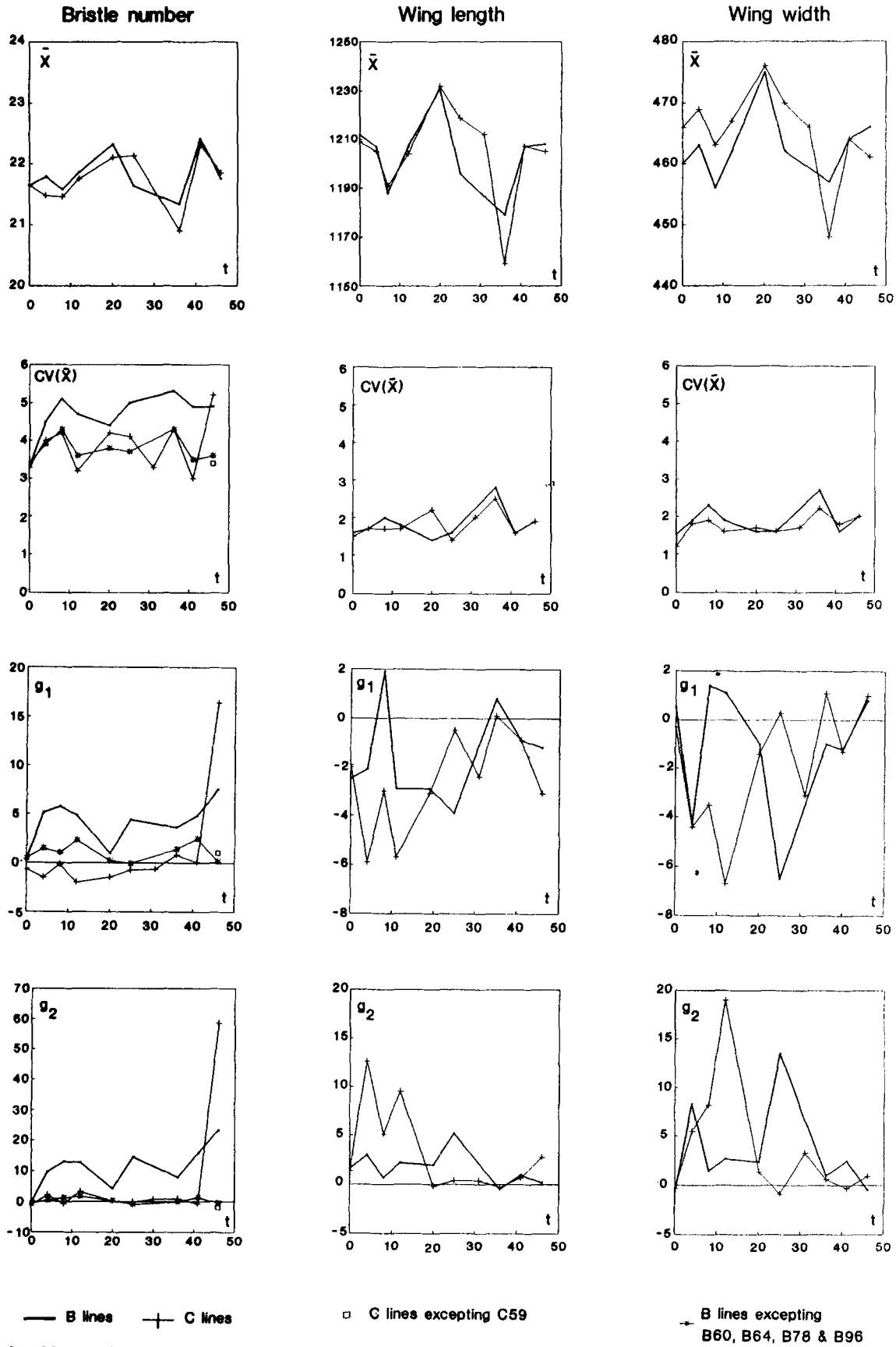

FIGURE 3.—Mean and coefficients of variation, asymmetry and kurtosis of the distribution of the means of the inbred lines plotted against generation number.

TABLE 3

Linear regression coefficients of the mean ($b_{\bar{x}t}$) and the coefficient of variation (b_{CVt}) of the distribution of the means of the inbred lines on generation of evaluation (t)

Regression coefficient	Type of line	Bristle No. ($\times 10^{-3}$)	Wing length	Wing width
$b_{\bar{x}t}$	B	3.96 ± 7.71	−0.14 ± 0.33	0.07 ± 0.12
	C	6.94 ± 8.92	−0.19 ± 0.42	−0.19 ± 0.15
b_{CVt}	B	21.00 ± 10.65	7.27 ± 8.80	4.81 ± 8.69
	C	12.26 ± 14.18	7.11 ± 6.91	9.89 ± 4.73

ber (line C84 died before it could be scored for this trait) (M. A. LOPEZ and N. ARENZANA, unpublished). However, it is not easy to extrapolate from these four traits to others, as the former are all related to body size.

In *D. melanogaster*, previously reported estimates of the genetic correlation between wing length and width were high and positive (WILKINSON, FOWLER and PARTRIDGE 1990). However, those between wing measurements and sternopleural bristle number were of varying sign and small or nonsignificant (WILKINSON, FOWLER and PARTRIDGE 1990; SCHEINER, CAPLAN and LYMAN 1991). This is in agreement with our estimates of the correlation between mutant effects for those traits. Notwithstanding, all mutations affecting bristle number also had an effect on wing length and, of these, two-thirds also influenced wing width. Therefore, the mutational covariances between wing measurements and bristle number appear to be different from standing genetic covariances in natural populations.

For bristle number and wing length, all mutations had the same action and were predominantly additive. Values of d smaller than 0.6 σ_E cannot be discerned from zero and, therefore, our conclusion of a mutation acting additively may be biased. Nevertheless, complete recessivity (or dominance) was ruled out in most cases and it can at least be safely stated that the heterozygote differed in value from the two homozygotes. In natural populations, the genetic variance of bristle score has been shown to be largely due to the segregation of additive alleles (ROBERTSON 1967). For mutations affecting wing width, additivity and complete dominance cannot be distinguished in this experiment. Unfortunately, previous information from natural populations is unavailable, although the heritability of related traits appears to be large (WEBER 1990).

Pleiotropic effects of mutations on fitness: The description of the joint distribution of spontaneous mutant effects on a quantitative trait and fitness obtained is highly dependent on the technique used to isolate mutations. With artificial divergent selection (or accumulation of mutations in replicated independent chromosome lines), the fixation probability of mutations affecting the selected trait will be highest, whatever their pleiotropic effect on fitness. Therefore, this is the best procedure to characterize the original distribution of effects. On the other hand, the distribution of mutations isolated by inbreeding will be closer to that found in wild populations, after natural selection.

Using the accumulation technique, *P* element-induced mutations affecting abdominal and sternopleural bristle number in *D. melanogaster* have been studied by MACKAY, LYMAN and JACKSON (1992). They found that *P* element insertions were generally deleterious and the distribution of their effects on the metric traits were negatively skewed and highly leptokurtic. Moreover, those mutations with an effect large enough to allow individual study (>0.3 σ_E), were mostly recessive and drastically reduced viability. For abdominal bristle number, these results are compatible with those for spontaneous mutations obtained by CABALLERO, TORO and LOPEZ-FANJUL (1991) using the selection approach. In contrast, our mutations affecting sternopleural bristle number were predominantly additive and did not appreciably affect fitness.

In natural populations of *D. melanogaster*, standing genetic variation for sternopleural bristle number and wing length possesses some common properties. Both are additive traits, showing intermediate to high heritabilities [see ROFF and MOUSSEAU (1987) for references] and little or no inbreeding depression (ROBERTSON 1967; ROBERTSON and REEVE 1952). Sternopleural bristle number does not appear to be causally related to fitness, *i.e.*, it is a "peripheral" trait (ROBERTSON 1967; GARCIA-DORADO, MARTIN and GARCIA 1991), but pleiotropic fitness effects of alleles affecting bristle number have been demonstrated in many selection experiments. Wing length shows clinal variation with temperature [see COYNE and BEECHAM (1987) for a review]. Furthermore, a fraction of the response achieved by artificial divergent selection carried out at different temperatures becomes lost after a subsequent period of relaxation (TANTAWY, MALLAH and TEWFIK 1964). These results can be tentatively interpreted as pleiotropic effects of natural selection acting on a different character (BARTON 1990). The data obtained in this experiment is consistent with those observations and provide a more detailed description of the loci underlying the genetic variation of the traits studied.

Our results have implications on the magnitude of the genetic variance for metric traits that can be maintained with mutation-selection balance. These can be discussed within the theoretical framework developed by KEIGHTLEY and HILL (1990a). In this situation, metric traits appear to be under stabilizing selection, but its apparent strength is dependent on

TABLE 4

Estimates of between-vial and environmental components of variance and covariance, and mutational heritabilities (h_m^2) and genetic correlations (r_A)

Trait(s)	Estimation procedure	Type of line[a]	Between-vial	Environmental	$h_m^2 \times 10^{-3}$ or r_A	Confidence interval ($\times 10^{-3}$)
Bristle No.	REML	*B*	0.29	3.46	3.35	2.29–4.73
		B'	0.30	3.47	0.35	0–0.75
		C	0.42	3.45	0.73	0.30–1.39
		C'	0.35	3.43	0.29	0–0.73
	ANOVA	*B*[b]		3.52	0.98	NS[c]
		C	0.20	3.46	0.76	NS
Wing length	REML	*B*	286.99	1081.52	2.66	1.66–4.13
		B'	281.22	1079.33	1.42	0.71–2.50
		C	398.77	1244.50	2.63	1.47–4.57
		C'	409.25	1257.49	2.06	1.07–3.93
	ANOVA	*B*[b]		1162.99	1.60	NS
		C	233.26	1268.92	2.00	NS
Wing width	REML	*B*	46.30	233.41	1.47	0.79–2.49
		B'	45.63	234.39	0.63	0.14–1.30
		C	48.27	258.52	1.30	0.72–2.12
		C'	48.96	260.57	1.20	0.65–2.06
	ANOVA	*B*[b]		245.98	0.89	NS
		C	27.39	259.75	1.46	NS
Length-width	REML	*B*	121.67	289.54	0.57	
		B'	97.36	220.76	0.66	
		C	101.04	224.51	0.45	
		C'	129.62	300.90	0.35	
	ANOVA	*B*[b]		263.19	0.60	
		C	75.55	294.78	0.88	

[a] In *B'* and *C'*, lines significantly departing from the overall mean were excluded from the analyses.
[b] Between-vial and environmental components of variance (covariance) confounded.
[c] NS = not significantly different from zero ($P < 0.05$).

the correlation ρ between the effects of mutations on quantitative traits and fitness. Although ρ cannot be quantified from our data, it will be smaller than one in all cases, as we have found mutations with relatively large effect on the traits and no detectable side-effects on fitness. In this experiment, pleiotropic effects of mutations on fitness were detected in four cases (out of eight). However, both deleterious and quasi-neutral mutations had a similar range of effects on the three metric traits. Gene action was predominantly additive and dominance effects were of variable sign. Furthermore, additive mutations tended to be quasi-neutral. Thus, additive alleles of considerable effect can be found segregating at intermediate frequencies in natural populations, contributing a large fraction of the total variance. This is in agreement with genetic variation for sternopleural bristle number being essentially generated by segregation at a few loci (ROBERTSON 1967; GALLEGO and LOPEZ-FANJUL 1983).

Mutational heritabilities: Under the neutral model (LYNCH and HILL 1986), the rate of genetic differentiation between lines is independent of population size and, therefore, will be the same in the *B* and *C* groups. In agreement with the expectation, the regressions of the coefficient of variation of the means of the lines on generation number (b_{CVt}) were all positive and no significant differences between types of lines were detected for each trait. Nevertheless, none of these regressions were significantly different from zero. Under the neutral model, the between-line genetic variance increases linearly over time. However, most observed changes could be attributed to fixation of major mutations occurring in a nonlinear fashion, *i.e.*, early in *B* lines and later on in *C* lines. This will result in large sampling errors of the regression coefficients. In addition, fluctuations in the environment common to all individuals in a generation have been observed and they will inflate the standard error of the regression over that expected from purely genetic causes. For neutral mutations, the intercept of the regression line provides a pooled estimate of the between-line variance attributable to nongenetic factors. Its value oscillated in the range 50–200 σ_m^2 for all traits and groups of lines. Therefore, it is not surprising that the precision of our estimates of b_{CVt} or ANOVA h_m^2 was low.

Estimates of mutational heritabilities were obtained from the between-line genetic variance. Therefore,

they are independent of, or not greatly affected by, the level of dominance or the linkage disequilibrium of the mutations involved (LYNCH and HILL 1986). Leptokurtosis of the distribution of mutant effects will result in lower estimates, particularly at low effective sizes (KEIGHTLEY and HILL 1988). This phenomenon was more clearly observed for bristle number, where most temporal changes in the shape of the distribution of the mean of the lines could be attributed to those lines carrying major mutations. Furthermore, the mutational heritability of all traits was substantially reduced when data from those lines was excluded from the analyses. Our calculations assume neutrality, however, pleiotropic side effects of mutations on fitness reduce the rate of differentiation of lines and, therefore, lead to underestimates of the mutational heritability. Nevertheless, the importance of selection relative to drift decreases with the magnitude of the effective size, and will be minimal in our case.

Our ANOVA estimates of the between-line variance include both genetic and environmental differences. Thus, the validity of the mutational variance estimates relies on the additional assumption of the between-line environmental variance not following a trend during the interval considered, otherwise the estimate is biased by an unknown amount. However, this problem is accounted for in REML estimates, which were generally higher than those obtained by ANOVA and significantly different from zero in all cases.

Our estimates of the mutational heritability are all relatively close to the "typical" value 10^{-3}. Notwithstanding, those of wing measurements were consistently larger than those of sternopleural bristle number. For morphological traits, previous information is mainly restricted to skeletal measures in mice, the estimates being included in the range $(4.6–31.1) \times 10^{-3}$ (LYNCH 1988a). These values contrast with those corresponding to abdominal and sternopleural bristle number $[(0.3–3.4) \times 10^{-3}]$ (LYNCH 1988a; CABALLERO, TORO and LOPEZ-FANJUL 1991). Thus, in spite of the large standard errors involved, the difference may well be real.

So far we have limited our discussion to those mutations with a considerable effect on at least one trait, but nothing has been said of those with smaller effects. The magnitude of the environmental variance and the number of individuals scored per line set a lower limit to the magnitude of detectable effects ($\sigma_E/3$). Once a line was shown to carry a mutation, more accurate estimates were obtained based on a larger set of data. In this way, significant pleiotropic effects larger than $\sigma_E/6$ were demonstrated. This indicates that isolation of minor mutations requires an experimental effort that would be prohibitive in most cases. Nevertheless, their existence cannot be denied as we have found single mutant effects as small as the power of resolution of the tests permitted. As an alternative, we tried to show the presence of an indefinite number of minor mutations in the set of lines where no major mutations were detected. Exclusion of the eight lines carrying major mutations from the analyses, resulted in a drastic reduction of the mutational heritability of bristle number. However, estimates for wing measurements remained significant. Apparently, minor mutations made a larger contribution to the new variance of these latter traits.

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