DEVELOPMENTAL STABILITY OF *DROSOPHILA MELANOGASTER* UNDER ARTIFICIAL AND NATURAL SELECTION IN CONSTANT AND FLUCTUATING ENVIRONMENTS

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ABSTRACT

Populations of *Drosophila melanogaster* in constant 25° and fluctuating 20/29° environments showed increases in developmental stability, indicated by decreases in bilateral asymmetry of sterno-pleural chaeta number. In both environments, rates of decrease in asymmetry were greater under natural selection (control lines) than under artificial stabilizing selection. Overall mean asymmetry was greater in the fluctuating environment.——There was no evidence that decreased asymmetry was due to heterozygosity, and the decline in asymmetry was not explained by the decline in chaeta number in the lines under only natural selection. However, the decline was consistent with changes in total phenotypic variance and environmental variance.—— The divergence between lines after 39 generations of selection was seen in differences in asymmetry and also in the genotype-environment interaction expressed in cross-culturing experiments.

IN an earlier paper, GIBSON and BRADLEY (1974) reported trends in means and variances of sternopleural chaeta numbers under artificial stabilizing selection and in populations under only natural selection. In both constant and fluctuating temperatures, there were declines in both genetic and environmental variances. In the present paper, trends in asymmetry under both selection regimes in the two environments are examined, and evidence is presented for divergence between lines that persists even when flies are grown in the opposite environment.

Bilateral asymmetry of development in *Drosophila melanogaster* has been used by MATHER (1953), THODAY (1958) and his colleagues, BEARDMORE (1960), VAN VALEN (1962) in Drosophila and other genera, MASON, ERLICH and EMMEL (1967) in butterflies, and by SOULÉ (1967, 1979) in a lizard, as a measure of developmental homeostasis or of buffering capacity against environmental variation. The use of asymmetry as a measure of homeostasis has been questioned by REEVE (1960) based on his work and that of MATHER (1953) showing response to selection for symmetry. However, VAN VALEN (1962) pointed out that MATHER (1953) was selecting for *antisymmetry* (asymmetry due to negative interaction between sides) or for *directional asymmetry* (systematic bias in one direction) and not for *fluctuating asymmetry* (without negative interaction

between sides). SOULÉ (1967) disagreed with REEVE's (1960) hypothesis that wild stocks are at or near their maximum levels of homeostasis and should not be responsive to selection.

In spite of the controversy, asymmetry (or *fluctuating asymmetry*) has been widely used as a monitor of developmental stability, and I have used it in this paper to measure changes in four lines of Drosophila.

<div align="center">METHODS</div>

Four bottle cultures derived from a single wild-caught *Drosophila melanogaster* female were set up in each of 2 environments, one with constant temperature (25°) and the other with a fluctuating temperature (29° for 12 hr and 20° for 12 hr). Each temperature switch was completed in about 30 min. The diagnostic character was sternopleural chaeta number, the 2 sides of each fly being counted separately. As previously described in GIBSON and BRADLEY (1974), 4 pairs of flies were selected *randomly* as parents in each of 4 control (C) line cultures in each environment, whereas the 16 pairs of flies nearest the mean were selected as parents for the 4 cultures of the stabilized (S) lines in each environment. The progeny from all 4 cultures of the stabilized (S) lines in each environment. The progeny from all 4 cultures in each line were treated as one population in selecting parents for the next generation. Since mean chaeta number differed between environment and between sexes, the chaeta numbers used were 17 and 16 for females and males, respectively, in the constant temperature and 18 and 17 for females and males, respectively, in the fluctuating temperature. Selection in the stabilized lines was toward these values throughout the experiment. Twenty flies of each sex were chosen randomly and counted in each culture of each line in each environment. Thus, 160 flies were assayed each generation in each line, 32 being used as parents.

Two measures of asymmetry were used. One was simply the average unsigned difference between numbers of left and right sternopleurals $|L-R|$, referred to as mean asymmetry. The other was the mean squared difference between sides $(\Sigma L-R)^2/n$, referred to as variance asymmetry, which it closely approximates since the mean signed difference between L and R is very small. The former measure was used by TEBB and THODAY (1954), THODAY (1958) and BEARD-MORE (1960). The latter measure was used by MATHER (1953). REEVE (1960) has shown that mean square asymmetry (variance asymmetry) is a measure of independent variance in the 2 groups of sternopleurals and thus a measure of what VAN VALEN (1962) termed fluctuating asymmetry. While these measures are very similar, their relationship depends on the nature of the asymmetry. When the correlation between chaeta numbers on the 2 sides is negative so that antisymmetry predominates, the value of $\Sigma(L-R)^2/n$ tends toward the mean $(L-R)$ squared. When the asymmetry is mainly fluctuating, $(L-R)$, the signed difference, is normally distributed, and the value of $\Sigma(L-R)^2/n$ exceeds the mean $(L-R)$ squared. As indicated in the RESULTS, the asymmetry in the present case was mainly fluctuating.

A third measure of asymmetry is the linear correlation between sides, less useful since considerable asymmetry could exist even with a high correlation. Correlations are reported as evidence for the absence of antisymmetry.

Another criterion of selection is change in total phenotypic variance, reported by GIBSON and BRADLEY (1974). Total variance in chaeta count is made up of between- and within-fly components or, as REEVE (1960) gave it, $4 \sigma_c^2 + 2 \sigma_i^2$. The variance σ_c^2 is that common to both segments, and σ_i^2 is the independent variance in each segment. The term $2 \sigma_i^2$ is equivalent to variance asymmetry, or $\Sigma(L-R)^2/n$. Changes in both σ_c^2 and σ_i^2 are reported. The component σ_c^2 includes genetic and what have been referred to as common environmental effects (FAL-CONER 1960). The component σ_i^2 is due primarily, if not entirely, to internal environmental effects.

Phenotypic variance can also be partitioned into genetic and environmental variance components. Genetic variance, in particular additive genetic variance, was estimated from off-spring–parent regression in assortatively mated samples of flies from each environment, as

described in GIBSON and BRADLEY (1974). Progeny were also grown in the opposite environment to assess the extent of environmental influences on observed divergence between lines.

<div align="center">RESULTS</div>

Nature of asymmetry: The asymmetry measured appeared to be *fluctuating* asymmetry, as defined in METHODS, and hence a measure of developmental instability. If sufficient antisymmetry, the only reasonable alternative form of asymmetry, were present, the distribution of $(L-R)$ would have been bimodal. There was no evidence of bimodality or even platykurtosis in the data. The measure $(L-R)$ fitted a normal distribution with a range of ± 3 or ± 4, in most cases ± 3 chaetae. The similarity, throughout the experiment, between mean and variance asymmetry, as defined in METHODS, is further indication that the asymmetry was fluctuating since the former measures all kinds of asymmetry and the latter, as stated in METHODS, is a measure of the variance due to independent effects on the two sides.

Changes in asymmetry under selection: Trends in asymmetry, measured as mean and variance asymmetry, are shown in Figure 1 and as linear regressions in Table 1. In no instance was there a significant second- or third-order trend. In both environments, the reduction in asymmetry over the 39 generations was greater in the control (C) lines under natural selection only, with significant divergence between stabilizing (S) and C lines in variance asymmetry in both environments, and in mean asymmetry in the 25° environment. The reduction in asymmetry measured either way was not significant in the S line at 25°. All other decreases in asymmetry were significant (Table 1).

As stated earlier, the correlation between right and left sides is an alternative measure of asymmetry (or of symmetry). Average correlations over all generations were consistent with the changes in asymmetry reported in Table 1. The overall correlation was 0.44 in the C line at 20/29°, 0.33 in the S line at 20/29°, 0.32 in the C line at 25° and at 0.21 was the lowest in the S line at 25°. The fact that the correlations were all positive further indicates that the asymmetry was fluctuating.

The degree to which some unknown secular trends might explain the change in asymmetry was investigated using the correlation between line means in the same environment. These correlations are shown in Table 2 for each of four periods during the experiment and over the entire experiment. The heterogeneity among the correlations and the low overall correlations suggest that external environmental trends within incubators were not important in causing asymmetry. However, there was a surprisingly high correlation ($P < 0.01$) between generation means of the two C lines, but no correlation between means of the S lines.

Asymmetry may be related to total chaeta number (MATHER 1953) and may change with the trends in chaeta number reported earlier by GIBSON and BRADLEY (1974). The correlations between generation means of chaeta number $(L+R)$ and asymmetry $|L-R|$ are shown in Table 3, together with the regressions of chaeta number on generation. The correlations were generally positive
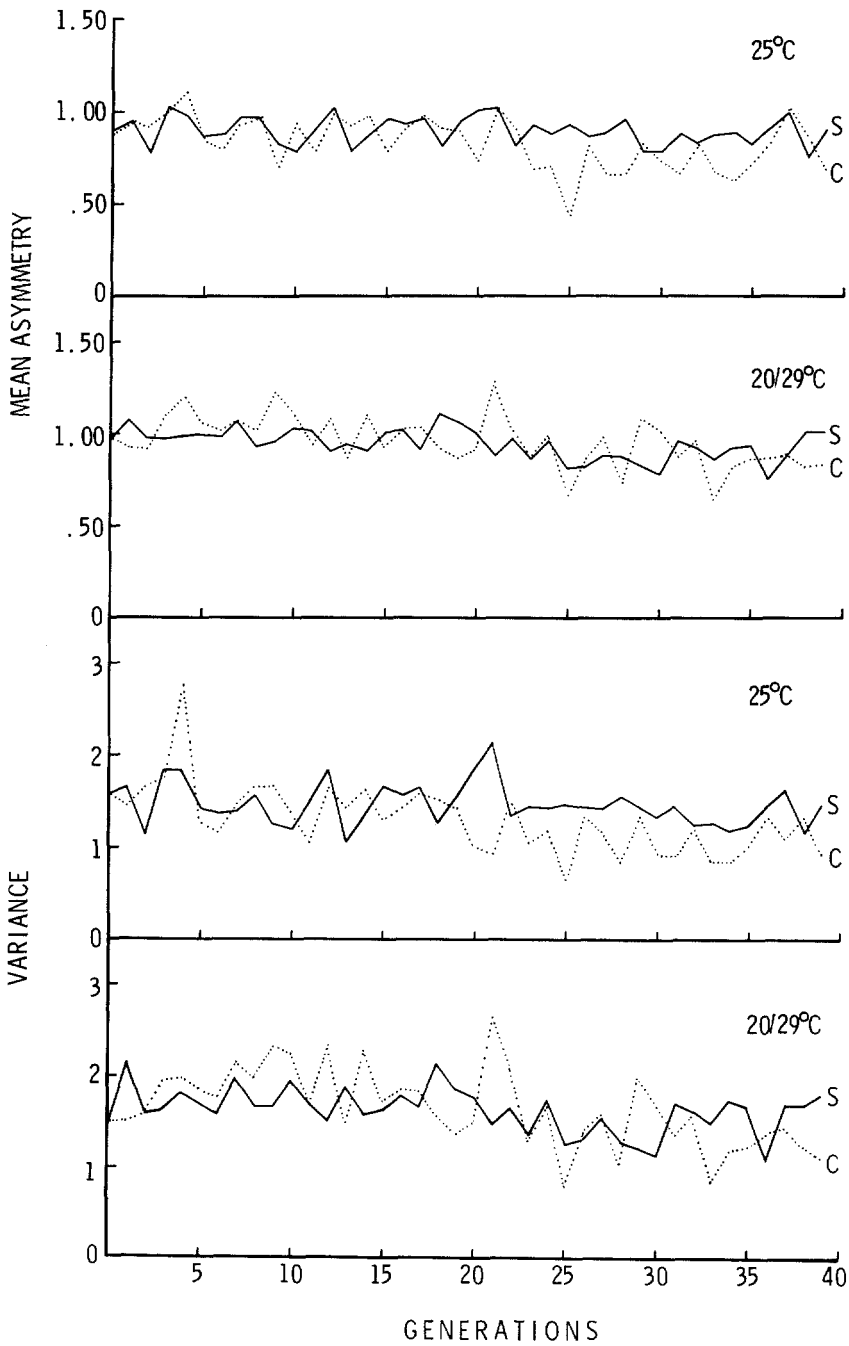
FIGURE 1.—Mean and variance asymmetry in lines under artificial stabilizing (S) and natural (C) selection in two temperature regimes.

TABLE 1

Regression of mean and variance asymmetry on generation

Character	Temperature	Line	Linear regression	Probability of difference between regressions in S and C lines
	25°	S	—0.0014 ± 0.0011	$P < 0.05$
Mean asymmetry	25°	C	—0.0070 ± 0.0018	
	20/29°	S	—0.0037 ± 0.0010	N.S.
	20/29°	C	—0.0072 ± 0.0017	
	25°	S	—0.0037 ± 0.0051	$P < 0.05$
Variance asymmetry	25°	C	0.0193 ± 0.0030	
	20/29°	S	—0.0089 ± 0.0033	$P < 0.05$
	20/29°	C	—0.0210 ± 0.0050	

S lines were under artificial stabilizing selection; C lines were under natural selection only.

Adding second- and third-order terms to the regression equations did not remove significant additional variance.

and were higher in lines where asymmetry decreased most. In these lines (C lines at both temperatures), $(L + R)$ also decreased significantly, especially over the last 20 generations. It is in this latter half of the experiment that the correlations were greater. Thus, it appears that part of the decrease in asymmetry in the C lines (under only natural selection) was related to the decrease in the mean chaeta number. However, in the S line at 20/29°, asymmetry decreased (Table 1) without a decrease in chaeta number, so that the decline in asymmetry cannot be entirely attributed to change in chaeta number.

Changes in variance components under selection: Change in asymmetry has been used as one criterion for response to stabilizing selection. Another criterion is the decline in variance of chaeta number $(L + R)$ noted by GIBSON and BRAD-LEY (1974). This variance can be partitioned into components due to common

TABLE 2

Correlations between mean asymmetries in lines grown at 25° and at 20/29°

Generations	Correlations between S and C lines in each environment	
	at 25°	at 20/29°
1–10	0.48	—0.22
11–20	—0.17	—0.65
21–30	0.10	0.26
31–39	0.22	0.11
1–39	0.19	0.20

	Correlations between S lines and between C lines	
	S lines	C lines
1–10	0.15	0.51
11–29	—0.33	0.63
21–30	0.02	0.90
31–39	—0.39	0.47
1–39	—0.03	0.82

TABLE 3

Correlations (r) between mean chaeta number and asymmetry and regressions (b) of mean chaeta number on generation in the four lines; variances between generations

Generations	S at 25°		C at 25°		S at 20/29°		C at 20/29°	
	r	b	r	b	r	b	r	b
1–20	0.25	—0.005	—0.03	0.048	—0.13	0.043	—0.10	0.128*
21–39	0.24	—0.003	0.64*	—0.120*	0.26	0.034	0.48*	—0.178*
1–39	0.22	—0.003	0.49*	—0.031*	0.16	0.005	0.32	—0.074*
			Between generation variances					
L—R	0.007		0.022		0.007		0.078	
(L+R)	0.308		0.842		0.266		2.016	

* $P < 0.05$.

effects (genetic and common environmental) and independent effects (internal environmental), as explained in METHODS. Trends in the common and independent (asymmetry) variance and also in total variance are shown in Table 4. There was a significant decline in all four lines in total variance, in three of four lines in each of the two components. In the S line at 20/20°, $\sigma_c{}^2$ did not decline significantly, and in the S line at 25°, $\sigma_i{}^2$ did not decline significantly. The latter was noted earlier in Table 1. The decreases in the two components were lower in the S lines than in the C lines.

The declines in the common and independent components of chaeta variance are consistent with the results reported by GIBSON and BRADLEY (1974) on changes in genetic and environmental variances. These results are repeated in Table 5 in a different format. The least reduction in environmental variance was in the S line at 25°, the only line in which $\sigma_i{}^2$ (asymmetry or independent variance) did not decline. The data also suggest that the lines in different environments had diverged considerably in additive genetic (V_A) and environmental (V_E) variance. This can be seen by comparing components measured in the same environment. For example, at generation 39, V_A and V_E were 1.3 and 1.4, respectively, for progeny of $S_{20/29}$ grown in the 20/29° regime and 0.9 and 3.2 respectively, for S_{25} progeny grown in the 20/29° regime. Finally, at generation 39, the environmental components were higher when the progeny were grown

TABLE 4

Regressions of total chaeta variance and the two components of chaeta variance on generation in the four lines

	S at 25°	C at 25°	Lines S at 20/29°	C at 20/29°
Variance due to common effects $(4\sigma_c^2)$	—0.021 ± 0.007	—0.062 ± 0.015	—0.022 ± 0.014	—0.044 ± 0.011
Variance due to asymmetry $(2\sigma_i^2)$	—0.004 ± 0.005	—0.019 ± 0.003	—0.009 ± 0.003	—0.021 ± 0.005
Total variance	—0.022 ± 0.006	—0.081 ± 0.015	—0.31 ± 0.012	—0.065 ± 0.012

TABLE 5

Genetic (V_A) and environmental (V_E) components of variance in total chaeta number at 0, 19 and 39 generations in progeny from each line cultured in both environments

| Generation | 0 | | Our environment | | | | Alien environment | | | |
| | | | 19 | | 39 | | 19 | | 39 | |
Component	V_A	V_E	V_A	V_E	V_A	V_E	V_A	V_E	V_A	V_E
Line										
S_{25}	2.5	1.7	0.9	1.0	0.6	1.5	2.4	1.5	0.9	3.2
C_{25}	2.5	1.7	2.6	0.5	0.6	0.9	3.2	1.3	0.8	2.4
$S_{20/29}$	2.2	4.0	1.3	2.6	1.3	1.4	1.4	1.8	2.0	3.7
$C_{20/29}$	2.2	4.0	3.0	1.9	0.9	1.6	1.3	2.1	1.3	2.1

in the alternative environment, indicating different selection pressures in the two environments.

Distinction between lines: Data just described suggest that the lines in different environments had diverged by the end of the experiment. Examination of the asymmetry data when the progeny from the lines were grown in the environment other than that of their parents provides other evidence of divergence between lines.

In general, asymmetry was greater in the 20/29° environment. In 33 generations, the average asymmetry (S and C lines) was greater in the fluctuating environment, in four generations it was greater in the constant 25° environment and in two generations the asymmetries were equal. However, as can be seen from Table 6, when mean and variance asymmetries were measured in the native and alien environment of each line, the distinction between environments had disappeared or rather had been subsumed in the lines. This is indicated by

TABLE 6

Asymmetries at generation 39 in progeny of each line grown in the two environments

Line	25° Mean	25° Variance	20/29° Mean	20/29° Variance
S at 25°	0.967	1.633	0.964	1.614
C at 25°	0.924	1.176	0.882	1.297
S at 20/29°	1.087	2.026	1.040	1.968
C at 20/29°	0.989	1.568	0.889	1.419

| | Analysis of variance of mean asymmetry | |
	d.f.	MS
Lines	3	0.0150**
25° lines *vs.* 20/29° lines	1	0.0169*
S at 25° *vs.* C at 25°	1	0.0127*
S at 20/29° *vs.* C at 20/29°	1	0.0155*
Environments	1	0.0011
Lines × Environments	3	0.0023
Within	2252	0.0027
Total	2259	

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

the significant difference between lines (assayed in the two environments) and the lack of significance of the mean differences between environments. It should be noted that the differences between lines within environments were also significant, as indicated earlier by the trends in asymmetry (Table 1).

DISCUSSION

While considering the results of this experiment, it should be recalled that the four lines were derived from a single female, which makes them more alike initially but also less representative of D. melanogaster in general. Hence, comparisons between lines and environments can be made with greater assurance, but between experiments with less assurance, than in those cases where populations were derived from representative samples of flies.

According to the measure used, developmental stability increased in three of the four lines, the exception being the line under artificial stabilizing selection at 25°. The right-left asymmetry was considered a suitable measure (of instability) since it was shown to be fluctuating asymmetry. The increase in stability (indicated by a decrease in asymmetry) was greater in the lines under natural selection only, suggesting that, at least with respect to asymmetry, the artificial selection for mean chaeta member was counter to natural selection, in degree if not direction.

The decline observed was not due to common environmental effects within an incubator. Indeed, there was a surprisingly high correlation between generation means of the C lines, which were grown in different incubators. The reason for this high correlation can also be guessed at, but, if real, it suggests that selection pressures in the C lines (under natural selection only) were similar in the constant and fluctuating environments. This is borne out by the similarity in reduction of asymmetry in the two lines.

The decline in asymmetry was partly, but not entirely associated with a decline in total chaeta number $(L + R)$. In earlier studies, SOULÉ (1976) and MATHER (1953) also found positive correlations between mean and asymmetry in various characters in lizards and in chaeta number in Drosophila, respectively. However, after adjustment for changes in total chaeta count, the regressions in Table 1 changed relatively little from -0.0070 to -0.0066 in the C line at 25° and from -0.0072 to -0.0064 in the C line at 20/29°.

A predictable consequence of stabilizing selection is a decline in both phenotypic and additive genetic variance (ROBERTSON 1956). This was noted earlier (GIBSON and BRADLEY 1974). KAUFMAN, ENFIELD and COMSTOCK (1977) pointed out that other models lead to conservation of genetic variance. The results reported in the present paper are consistent with the ROBERTSON model, also described earlier by WRIGHT (1935), in that the decline in phenotypic variance was partly a result of decline in common variance (σ_c^2) (Table 4), and this latter variance contains the genetic variance in chaeta number, as well as environmental variance common to both sides of the fly. It should be noted that the independent or internal environmental variance (σ_i^2) also declined in three of the four lines.

It is clear that the consequences of stabilizing selection depend on whether mean phenotypes are exhibited by the more heterozygous individuals and, in turn, whether asymmetry and heterozygosity are related. The relationship between fitness and heterozygosity has lately received renewed attention. Soulé (1979) used data on asymmetry in lizards to address the question, assuming asymmetry to be a criterion of fitness. In 15 populations, he found asymmetry in four characters to be negatively correlated with heterozygosity.

In contrast, Mather (1953) showed that asymmetry in Drosophila decreased at the same time lines were being inbred, indicating a positive correlation with heterozygosity. On the other hand, Beardmore (1961) and Beardmore and Levine (1963) concluded that asymmetry and heterozygosity in Drosophila were negatively related, based on his finding that two more genetically variable lines showed lower average asymmetry than two less genetically variable lines. Soulé (1979) suggested that intra-individual variability (such as variance due to asymmetry) could be lower in more heterozygous individuals, while overall morphological variability was actually higher. Hence, the environmental variability one is relating to heterozygosity needs to be defined. In the present experiment, there is evidence against greater buffering capacity (lower asymmetry) due to heterozygosity. This evidence is found in the decrease in asymmetry in three of the four lines (Table 1), accompanied by decrease in additive genetic variance (Table 5) and also by a decline in variance due to common effects (Table 4), which variance is at least partly genetic.

The consequences of natural and artificial stabilizing selection in the two environments have been demonstrated not only in the decline in variance components, as already discussed, but also in the divergence between lines. This divergence was particularly demonstrated by growing progeny from one environment in the other. It appears from Table 5 that adaptation to the alien environment decreased with time, as illustrated by the difference between the environmental variances in progeny cross-cultured in generations 19 and 20. On the other hand, environmental variance decreased in lines in their own environment. It might also be noted that the environmental components of variance in the two environments are comparable at generation 39, whereas at generation 19, and even more so initially, the variation in the fluctuating temperature was greater.

The divergence among lines is illustrated in a different way by the data in Table 6. In this case, the criteria are mean and variance of asymmetry. The latter is a portion of the total environmental variance. While the lines are clearly distinct in asymmetry, as confirmed by analysis of variance, the mean and variance asymmetries of each line are quite similar in the two environments. The significant differences between S and C lines in both environments confirm the earlier inference that asymmetry was decreased more in the lines under only natural selection. The conclusion from this is that stabilizing selection around a fixed chaeta number (or at least around the inital mean number) was less effective in increasing homeostasis than was natural selection. In other words, the optimum chaeta number in the laboratory environments was probably lower than the initial mean of the population.

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