STABILISING SELECTION IN CONSTANT AND FLUCTUATING ENVIRONMENTS

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SUMMARY

Selection in both constant and fluctuating temperature environments for a constant bristle number in *Drosophila melanogaster* resulted in the maintenance of that number and in a decrease of both genetic and environmental variance. Although mean bristle number in the control lines increased over the early generations there was a significant net decrease. Phenotypic variance decreased significantly, especially in later generations. In both control lines and in the line selected at 20-29° C. there was a significant decrease in asymmetry of bristle number. There was also a decrease in environmental variance in these three lines. Transplant experiments showed that when the lines were cultured in alien environments phenotypic variance tended to increase in the fluctuating and decrease in the constant temperature environment. However at generation 39, the phenotypic variance of lines selected in the fluctuating environment increased when samples of the lines were cultured in the constant temperature environment. The results indicate that stabilising selection can be effective in both fluctuating and constant temperature environments.

1. INTRODUCTION

NATURAL selection is thought to be mainly conservative. Its consequences are more often maintenance of the status quo than systematic changes in gene frequencies. There is evidence that the modal phenotypes in a population have the highest reproductive fitness (Rendel, 1943; Karn and Penrose, 1951; Jayant, 1966). The optimum phenotype itself changes with shifts in gene frequency or in the environment and it is assumed that the modal phenotype is a result of natural selection.

In a population inhabiting a relatively stable environment, the fittest genotypes would be those tending to produce intermediate phenotypes. When the fitness of a phenotype decreases with its deviation from the modal phenotypes, selection is said to be stabilising. Such selection has been investigated theoretically (Wright, 1935; Robertson, 1956; Latter, 1960; Curnow, 1964; Singh and Lewontin, 1966; Gale and Kearsey, 1968) and experimentally (Falconer, 1957; Thoday, 1959; Prout, 1962; Scharloo, 1964; Barnes, 1968). All of these studies assumed or were carried out in a constant environment.

The present experiments were started with the view of seeing whether stabilising selection in an environment with marked fluctuations in temperature could bring about a reduction in additive genetic variance and whether natural selection under the same conditions would have similar effects.

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2. METHODS

Flies from the second generation progeny of a *Drosophila melanogaster* female taken on an apple dump near Cambridge were used to set up four bottle cultures in both a constant 25° C. incubator and in a fluctuating incubator in which the temperature was 29° C. for 12 hours followed by 20° C. for 12 hours. The time required to change from one temperature to the other in the fluctuating incubator was about half an hour.

Twenty virgin flies of each sex from each of the four cultures at 25° C. were assayed for sternopleural bristle number. Sixteen males and 16 females were taken at random from the combined progenies to set up four control line cultures at 25° C. each with four pairs of parents. The 16 males and 16 females nearest the mean bristle number (17 for females, 16 for males) were selected from the remaining flies and used as parents for the four cultures of the stabilising selection lines maintained at 25° C. In subsequent generations 20 flies of each sex were assayed from each of the four cultures in a line. The parents of the next generation were selected from the combined progeny for the stabilised lines and taken at random for the control lines. The lines cultured in the fluctuating environment were established and maintained in a similar way except that in the selected line females with 18 bristles and males with 17 bristles were selected as there was a significant difference in mean bristle number between the flies cultured in the two environments (see below).

Changes in additive genetic variance were estimated by heritability tests in the base population and in all the lines at generations S_{11}, S_{19} and S_{39}. At these generations replicate samples of all the lines were grown in both the fluctuating and constant temperature environments to provide estimates of phenotypic variance in the two environments and to provide parents for the heritability tests. In the heritability tests single-pair matings were set up in which the parents were positively assortatively mated for bristle number and their progenies grown in the same temperature environment as the parents. Five flies of each sex were assayed from the progeny of each pair of flies and the regression of offspring mean bristle number on mid-parent mean was calculated.

3. RESULTS

(i) *The base population*

At the time the selection and control lines were initiated replicate samples of the base population were cultured at 25° C. and in the fluctuating temperature environment. From the progenies heritability tests were set up in

TABLE 1

Base population. Heritability tests in both constant and fluctuating environments

$$20°C.-29°C. \qquad b = 0.35 \pm 0.084$$
$$25°C. \qquad b = 0.59 \pm 0.077$$

Analysis of variance

Source	d.f.	Mean square	Probability
Joint regression	1	8828·4	<0·001
Difference between regressions	1	5961·1	<0·001
Difference between means	1	9281·3	<0·001
Error	55	137·9	—

TABLE 2

Components of variance on the base population cultured in both constant and fluctuating environments

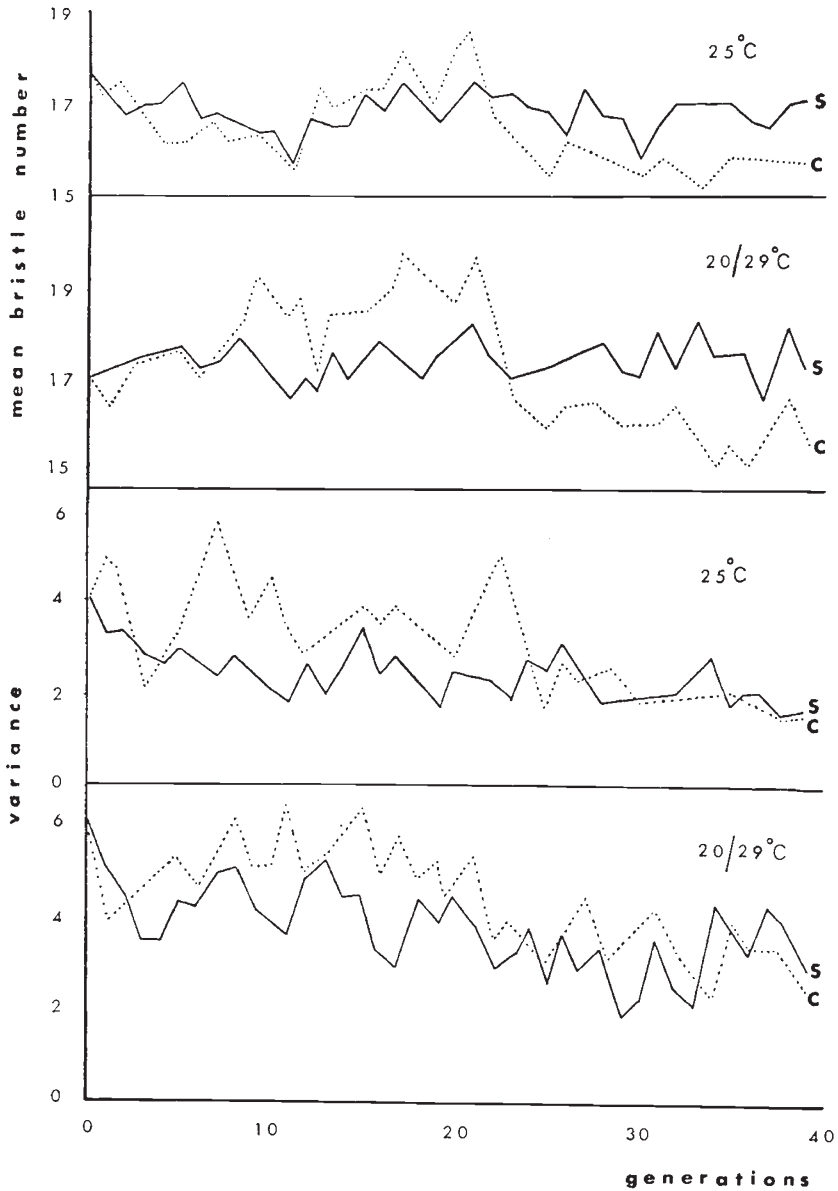| Temperature	VP	VA	VE
25°C.	4·2	2·5	1·7
20°C.-29°C.	6·2	2·2	4·0

FIG. 1.—Mean bristle number and variance in the control (*C*) and selected (*S*) lines.

the two environments.   The regressions of progeny mean bristle number on mid-parent mean in the two environments were significantly different (table 1).   The heritability estimates were used to partition the phenotypic variance in the samples of the base population cultured in the two environments (table 2).   As might have been expected, phenotypic variance was higher in the fluctuating temperature environment than in the constant environment and this difference was largely due to the higher environmental and/or non-additive genetic variance in the fluctuating environment.

(ii) Generations S_1 to S_{19}

As heritability tests were carried out at S_{19} it is useful to consider first changes in mean bristle number and variance (within sex and culture) which occurred in the lines over these early generations (fig. 1).   The regression of mean bristle number in the 25° C. stabilised line on generation

TABLE 3

Regressions of mean bristle number and variance over the first nineteen generations of selection

Character	Temperature	Line	Linear regression	Probability of difference between regressions
Bristle number	25°C.	S	-0.005 ± 0.017	N.S.
	25°C.	Control	0.048 ± 0.025	
	20-29°C.	S	0.043 ± 0.022	<0.05
	20-29°C.	Control	0.128 ± 0.020	
Variance	25°C.	S	-0.029 ± 0.009	N.S.
	25°C.	Control	-0.049 ± 0.037	
	20-29°C.	S	-0.093 ± 0.019	<0.05
	20-29°C.	Control*	0.057 ± 0.025	

* Quadratic term negative and significant $(P < 0.05)$.

was not significant whereas the regression of phenotypic variance on generation was negative and significant (table 3).   The mean bristle number in the 25° C. control line increased, but not significantly, and there was no significant change in phenotypic variance.

In the fluctuating environment the mean bristle number of the control line increased significantly while that of the stabilised line did not.   Phenotypic variance had decreased significantly in the stabilised line but not in the control line in the fluctuating environment by generation 19 (table 3).

Comparison of regression coefficients in selected and control lines indicated significant difference in regressions of both mean and variance on generations in the fluctuating environment but of neither in the 25° C. environment (table 3).   There was only one significant deviation from linearity as shown by the addition of quadratic and higher terms to each regression equation.

Heritability estimates based on tests carried out at S_{19} (table 4) were used to partition phenotypic variance in the lines.   These data (table 5) indicate that, compared to the controls, there was less additive genetic variance in both selected lines.   Phenotypic variance in both 25° C. control and selected lines increased when the lines were cultured in the fluctuating environment, but there was more additive genetic variance in the control lines than in the

stabilised line. In contrast, phenotypic variance in both fluctuating temperature lines decreased when the lines were cultured at 25° C.; the control line showed a decrease in additive genetic variance and the stabilised line a decrease in environmental variance. The difference in phenotypic variance between samples of the base population cultured in the two environments were earlier shown to be largely due to changes in environmental variance.

TABLE 4

Heritability tests at S_{19}

Line temperature	Line	Culture temperature of test	Heritability
20-29°C.	S	20-29°C.	0.32 ± 0.12
	Control	20-29°C.	0.62 ± 0.17
	S	25°C.	0.45 ± 0.12
	Control	25°C.	0.38 ± 0.10
25°C.	S	25°C.	0.46 ± 0.17
	Control	25°C	0.84 ± 0.20
	S	20-29°C.	0.62 ± 0.17
	Control	20-29°C.	0.72 ± 0.18

These data suggest that during the first 19 generations artificial stabilising selection was more effective in reducing additive genetic variance than any natural stabilising selection that the control lines might have been subjected to. There is evidence that environmental variance was reduced in the control lines. The apparent effectiveness of artificial selection is corroborated by the heritability tests at S_{11}, where the variance components in the selected lines were intermediate between the estimates for S_0 and S_{19}. In the control lines the only clear trend is a reduction in environmental variance.

TABLE 5

Components of variance in the lines at S_{19}

Temperature	Line	Culture temperature of test					
		20/29°C.			25°C.		
		VP	VA	VE	VP	VA	VE
20-29°C.	S	3.9	1.3	2.6	3.2	1.4	1.8
	Control	4.9	3.0	1.9	3.4	1.3	2.1
25°C.	S	3.9	2.4	1.5	1.9	0.9	1.0
	Control	4.5	3.2	1.3	3.1	2.6	0.5

(iii) *Generations S_{19} to S_{39}*

The selection regime was continued for a further 20 generations after generation S_{19}. Mean bristle number decreased significantly in both control lines over the 39 generations in contrast to the increase in bristle number in these lines over the first 19 generations. The quadratic terms in the regression equations for the means of both control lines are significant.

The regressions of mean bristle number on generations were not significant in either of the stabilised lines (table 6).

The regressions of variance (within sex and culture) for the 39 generations were negative and significant for the control and selected lines in both

environments (table 6). Thus the change in phenotypic variance in both of the control lines occurred mainly over the last 20 generations (fig. 1). Only the regression of variance for the control line in the fluctuating environment was significantly non-linear.

Phenotypic variance in the four lines cultured in both environments was partitioned as before using heritability tests carried out at S_{39} (tables 7 and 8).

TABLE 6

Regression of mean bristle number and variance over 39 generations

Character	Temperature	Line	Linear regression	Probability of difference between regressions
Mean bristle number	25°C.	S	-0.003 ± 0.041	<0.05
	25°C.	Control*	-0.031 ± 0.012	
	20-29°C.	S	$+0.005 \pm 0.044$	<0.05
	20-20°C.	Control*	-0.074 ± 0.016	
Variance	25°C.	S	-0.022 ± 0.006	<0.05
	25°C.	Control	-0.081 ± 0.015	
	20-29°C.	S	-0.031 ± 0.012	N.S.
	20-29°C.	Control*	-0.065 ± 0.012	

* Quadratic term in these equations negative and significant $(P < 0.01)$.

TABLE 7

Heritability tests at S_{39}

Line temperature	Line	Culture temperature of test	Heritability
20-29°C.	S	20-29°C.	0.48 ± 0.08
	Control	20-29°C.	0.35 ± 0.06
	S	25°C.	0.35 ± 0.09
	Control	25°C.	0.38 ± 0.07
25°C.	S	25°C.	0.28 ± 0.08
	Control	25°C.	0.40 ± 0.10
	S	20-29°C.	0.22 ± 0.09
	Control	20-29°C.	0.25 ± 0.12

TABLE 8

Components of Variance at S_{39}

		Culture temperature of test					
		20-29°C.			25°C.		
Temperature	Line	VP	VA	VE	VP	VA	VE
20-29°C.	S	2·7	1·3	1·4	5·7	2·0	3·7
	Control	2·6	0·9	1·6	3·4	1·3	2·1
25°C.	S	4·1	0·9	3·2	2·1	0·6	1·5
	Control	3·2	0·8	2·4	1·5	0·6	0·9

The results are in marked contrast to those obtained at S_{19}. The 25° C. control line had a similar amount of additive genetic variance but less environmental variance than the 25° C. stabilised line when the lines were cultured in either environment, although phenotypic variance was higher in the foreign environment. The control line in the fluctuating temperature environment had less additive genetic variance than the stabilised line but a similar amount of environmental variance. When these two lines were cultured in the constant 25° C. environment phenotypic variance increased and there was more additive genetic variance and environmental variance in the stabilised line than in the control. The data in tables 2 to 8 on the components of variance are summarised in table 9.

TABLE 9

Summary of components of variance at S_0, S_{19} and S_{39}

		Line temperature	
		20-29°C.	25°C.
Generation	Line	VA+VE	VA+VE
S_0	Base population	2·2+4·0	2·5+1·7
S_{19}	C	3·0+1·9 (1·3+2·1)	2·6+0·5 (3·2+1·3)
	S	1·3+2·6 (1·4+1·8)	0·9+1·0 (2·4+1·5)
S_{39}	C	0·9+1·6 (1·3+2·1)	0·6+0·9 (0·8+2·4)
	S	1·3+1·4 (2·0+3·7)	0·6+1·5 (0·9+3·2)

Variances in brackets were obtained in the alien environment.

Analyses of variance were performed for each line in each generation, including culture and sex as independent variables and mean and variance of bristle number as dependents. No clear trend was observed in the proportion of total variance explained by culture differences. However, between-cultures variance was only rarely significant from generation 23 onwards, having been significant in at least two lines in every generation except one prior to that time.

Analyses of variance including line showed inter-line variance to be significant in 10 of the first 19 generations and in 15 of the last 20.

Thus the downward trend in variance observed within sex and culture is repeated in the variance between cultures (within lines), presumably largely environmental in origin. This reduction in inter-culture variance is partly responsible for the line differences observed in later generations.

6. DISCUSSION

Of particular interest in the present experiments are the comparisons between the responses of the stabilised and control lines that occurred over the first 19 generations of selection and those that occurred later. Phenotypic variance decreased steadily over the 39 generations in both stabilised

lines but the mean bristle numbers of these lines did not change significantly. In contrast, the mean bristle numbers of both control lines increased between S_0 and S_{19}, although over 39 generations there was a significant decrease in mean bristle number. Phenotypic variance showed little change in either of the control lines over the early generations but then it decreased significantly to values slightly lower than those in the stabilised lines.

These data suggest that up to S_{19} artificial stabilising selection was more effective in both the constant and fluctuating temperature environments than any natural stabilising selection that the control lines might have been subjected to. Heritability tests at S_{19} provided consonant results, for there was less additive genetic variance in both stabilised lines than in the control lines. Thereafter all of the lines show evidence of effective stabilising selection, although the optimum phenotype was probably lower in the control lines than the phenotype artificially selected in the stabilised lines. Barnes (1968) showed that sternopleural bristle number was subjected to stabilising selection in cage populations maintained at two temperatures, the optimum phenotype depending on the temperature. Evidence for the adaptive significance of sternopleural bristle number in populations maintained at different temperatures was previously obtained by Beardmore (in Thoday, 1959). However, it is not obvious why the mean bristle number in both of the present control lines first increased and then decreased. In fact, the sudden decrease in mean bristle number (and to a lesser extent the decrease in variance) which occurred in both control lines around S_{23} accounts for most of the changes over the last 19 generations. This sudden decrease does not seem to be related to changes in either temperature or relative humidity, both of which were recorded and show no marked variation during this period. In any case the sudden decrease in mean did not occur in the stabilised lines cultured in the same incubator. The population size in the control line at 25° C. did fall 20 per cent. below that required for a complete assay and the deficiency of progeny in this line persisted for several generations. However, there was no such deficiency in numbers in the control line in the fluctuating environment. The reduction in mean observed around S_{23} occurred in all four cultures in both control lines.

Inbreeding cannot be excluded as a relevant factor in the changes described in any of these lines (Robertson, 1952, 1956). But it seems unlikely to be the sole factor as neither of the control lines showed the increase in phenotypic variance which frequently accompanies inbreeding when quantitative characters are scored (Reeve and Robertson, 1953). Each of the four cultures of each line was maintained by four pairs of parents to minimise inbreeding effects.

The base population of the present experiments had been maintained at 25° C. in the laboratory for only two generations prior to the start of the experiments. Of course, the environmental conditions to which the natural population was adapted are unknown, but they are unlikely to have included constant temperature. In the transplant tests carried out at S_{19} phenotypic variance in both the fluctuating temperature lines decreased when these lines were cultured at 25° C. However, in similar tests at S_{39} the phenotypic variance increased when the lines were cultured at 25° C. That this increase in phenotypic variance resulted from developmentally unstable genotypes at 25° C. is suggested by comparisons of asymmetry of bristle number of flies cultured in the two environments.

Asymmetry, measured as the unsigned difference between the sides of the flies divided by total bristle number (A/T, see Thoday, 1958) was lower in flies cultured in the fluctuating environment than in flies cultured in the foreign 25° C. environment at S_{39}. This difference in asymmetry in the two environments was not apparent in the tests carried out on these two lines at S_{19}. Thus is seems likely that adaptation to the fluctuating temperature conditions resulted in a loss of flexibility to adjust to a more constant temperature. Over the 39 generations of selection, mean asymmetry did not change significantly in the line selected at 25° C. In the two control lines and in the line selected at 20-29° C. there was a significant linear reduction in asymmetry.

The decreases in phenotypic variance which occurred in all four lines were not solely due to changes in additive genetic variance. Compared to the base population, environmental variance was less at S_{39} in both selected and control lines in the fluctuating environment and it was also less in the 25° C. control line.

Not all experiments with artificial stabilising selection result in decreased phenotypic variance, for Falconer (1957) reported little effect from selection of intermediate sternopleural bristle numbers in *Drosophila melanogaster*. The present results, however, are consonant with those of Thoday (1959), Prout (1962) and Scharloo (1964) in showing that artificial stabilising selection can bring about a reduction in phenotypic variance. The results differ from those of Prout and Thoday in that the reduction in some lines was not only in additive genetic variance but also in environmental variance.

In addition, the present experiments indicate that stabilising selection could be effective in the fluctuating environment that natural populations of *Drosophila* are more likely to be subjected to than the constant temperature conditions usually employed in laboratory experiments.

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