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The effects of stabilizing selection on the time of development in *Drosophila melanogaster**

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INTRODUCTION

Schmalhausen (1949) defined stabilizing selection as '. . . a selective advantage, particularly in fluctuating environments, of a certain norm over deviations from this norm'. He further states, 'It leads to elimination of most of the deviations, and to the establishment of more stable mechanisms of normal morphogenesis'. The importance of stabilizing selection and its consequences has been emphasized by Waddington (1957) and by Lerner (1954, 1958). Wright (1935), Robertson (1956) and Kojima (1959) have worked on the mathematical theory of stabilizing selection. Experimental investigation of the phenomenon has, however, lagged behind the theory. Falconer & Robertson (1956) working with body-weight in mice and Falconer (1957) with abdominal bristles in *Drosophila* both reported no response to stabilizing selection. On the other hand, Thoday (1958, 1959), using sternopleural bristles in *Drosophila*, and Rendel (1960), using scutellar bristles in *Drosophila*, reported positive results. It is evident that more experimental work must be done on different characters and different organisms in order to secure an understanding of this mode of selection. The experiments to be reported below concern the effects of stabilizing selection on the time of development from oviposition to emergence of *Drosophila melanogaster*.

The experiments were designed to discover whether this particular trait can be made to respond to stabilizing selection, and, if so, to investigate the changes in gene pool organization underlying this response.

MATERIAL AND METHODS

The foundation stock used for this study was derived from a wild population inhabiting a Southern California citrus grove.

For any given line and generation eggs were collected over a 12-hour interval at random from about 15 to 20 pairs of selected parents. The eggs were divided among 8 replicate shell vials, with 20 eggs per vial and standard Cream of Wheat-Molasses medium containing a dead yeast supplement. The replicate vials of the different lines were arranged in Latin squares and incubated at 25° C. for the first 13 generations and at 22° C. thereafter. Approximately 9 days later emergence commenced. Flies were collected, sexed, counted and stored at 8-hour intervals for about 4 days, when the emergence was usually completed.

* This paper is dedicated to Prof. L. C. Dunn on the occasion of his retirement.

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Distribution histograms were constructed from the accumulated counts, decisions were made as to which flies were to be selected, and then the parents of the next generation were chosen from among the stored flies and mated. After 3 days the eggs of the next generation were collected and distributed in the vials.

The experiment was started with 8 lines. Two lines were subjected to stabilizing selection where the flies which emerged close to the mean time of emergence (usually the modal class) were selected as parents. Two lines were selected in a manner converse to the stabilizing lines where the first flies to emerge were saved and mated to the last flies to emerge. For this purpose reciprocal matings were made each generation in separate containers and then eggs were collected from a mixed population of inseminated females. This latter mode of selection conforms to Mather's (1955) 'Disruptive selection' with disassortative mating. Finally, four control lines were carried in each of which the flies were mated at random with respect to their time of emergence. Each control line was identified with a specific selection line by having the same numbers of parents as that line.

In the latter part of the experiment from certain lines stocks were derived and subjected to ordinary directional selection both in the 'slow' and 'fast' directions for one generation. In so far as selecting, mating and culturing were concerned, these directional selection experiments were handled in the same way as described above, except that Latin squares were used in such a way that an element in the square constituted a pair of vials where one contained eggs from fast developing parents and the other from slow developing parents.

RESULTS

The experiment continued for 40 generations. At generation 20 four lines had to be discontinued, comprising one stabilizing line, one disruptive line, and their two controls. Only the results of the four remaining lines will be presented, except in one instance where it will be shown that before termination the discarded lines were behaving in the same fashion as those saved. At generation 35 the two remaining control lines were dropped. The lines will be designated 'S' for the stabilizing line, 'D' for the disruptive line and 'CS' and 'CD' for the two respective controls. Since S and D turned out to have roughly the same effective population sizes, CS and CD will be treated simply as two random control lines.

Table 1 gives certain overall statistics pertinent to the experiment. The Table of variance components (1A) shows that 26% of the total variance was due to vial differences so that even though the distributions on which selection was practised were constructed without regard to differences in vial means, most of this selection operated on the within-vial variance. In the same Table the contribution to the total variance in emergence time by the 12-hour collecting interval was computed assuming that the rate of egg laying was constant during the interval (uniform distribution). The additive genetic variance is a crude overall estimate arising from the directional selection tests done late in the experiment and to be described below. The remainder component was obtained by simple subtraction. From

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Table 1B some notion of the intensity of selection may be obtained by comparing the mean variance of the selected parents with the mean variance of the populations from which they came. Unfortunately, there was some mild directional selection in opposite directions in the S and D lines. Sample emergence distributions and the selection imposed on them are shown in Fig. 1. These distributions were combined

Table 1. *Some general statistics of the experiment*

A. Sources of variation in control lines (hr^2)

Variance among replicate vials		37 (26%)
Variance within vials		
Additive genetic	27 (26%)	
Egg collecting time	12 (11%)	
Remainder	67 (63%)	
	—	106 (74%)
	Grand total	143 (100%)

B. Selection pressures

(1) Stabilizing line	
Mean total variance	107 hr^2
Mean variance of selected parents	12 hr^2
Mean directional differential	$-2 \cdot 2$ hr
(2) Disruptive line	
Mean total variance	176 hr^2
Mean variance of selected parents	304 hr^2
Mean directional differential	$+3 \cdot 3$ hr

C. Miscellaneous statistics

(1) Mean of control lines	
Females	239·7 hr
Males	246·4 hr
(2) Mean coefficient of variation within vials of control lines	4·1%
(3) Viability of control lines	67·0%
(4) Effective number of selected parents	
Stabilized line	39·2
Disruptive line	30·9
(5) Mean number of flies raised per generation per line	96·8

from a late period in the experiment simply because during that period emergence was checked at 4-hour intervals thus giving better visual information on the form of the distributions.

In Table 1C the coefficient of variation was computed using the within-vial variance since it is this kind of variability which is of immediate interest here. 'Viability' is the percentage emerging per vial out of the 20 eggs introduced. The effective population sizes were computed by taking the harmonic means of the approximate effective sizes over all generations.

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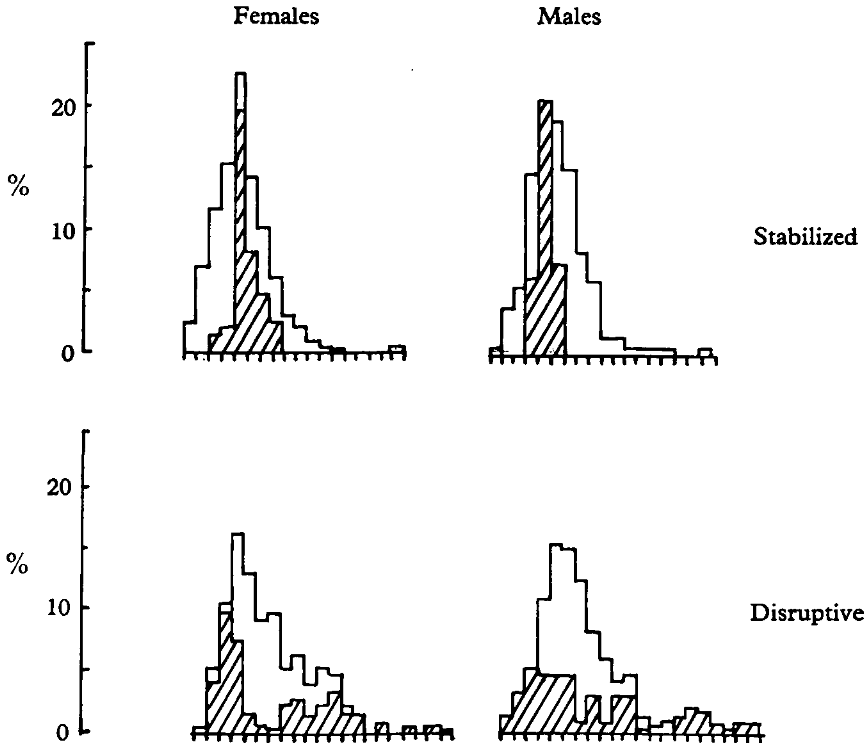

FIG. 1. Emergence distributions for females and for males in the two selection lines combined over generations 21–25 inclusive when emergence was checked every 4 hours. Shaded portions indicate the selected parents.

Variance

A convenient measure of response to the selection pressure applied in the two selected lines is the variance, which is expected to decrease in the S line and to increase in the D line. The variance to be discussed now is the variance within vials. This statistic showed large erratic fluctuations from generation to generation. For this reason as well as for economy of space 5-generation mean variances are reported in Table 2 and Fig. 2. An inspection of Fig. 2 reveals an upward trend, which occurred both in experimental and in the control lines. This general increase in variance might possibly have some significance for the theory under investigation, but it seems more likely that it was due to changes in the environmental condition of the entire experiment. The first 13 generations were grown at 25° C., while the later generations were grown at 22° C. This temperature change of course affected the mean as well (see Table 4). Also less tangible factors must have exerted some influence, such as the transfer of the entire experiment into a new building and the turnover of assistants preparing the medium and handling flies. Therefore, interpretation of the data must depend completely on the changes of the selection lines relative to one another and to the controls. Accordingly, the variances of the selection lines were expressed as deviations from the mean variance of the two

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Table 2. *Five-generation means of variances* (hr^2)

Generation	S	D	CS	CD	d_S	d_D
0–5	64 (397)	106 (297)	84 (330)	67 (466)	−12	+31
6–10	67 (620)	88 (524)	99 (369)	91 (464)	−28	+7
11–15	68 (499)	112 (531)	114 (368)	81 (572)	−30	+15
16–20	46 (531)	91 (508)	80 (436)	73 (591)	−31	+15
21–25	77 (577)	195 (446)	94 (605)	111 (603)	−26	+93
26–30	51 (705)	138 (488)	120 (637)	93 (655)	−56	+32
31–35	136 (783)	288 (362)	233 (617)	137 (677)	−49	+103
36–40	128 (662)	315 (425)			−60*	+127*

S, stabilizing line; D, disruptive line; CS and CD, two control lines; d_S, deviation of the S line from the mean of the two controls; d_D, deviation of the D line from the mean of the two controls. In parentheses are given the number of flies counted.

* Obtained by apportioning the difference between D and S as it was apportioned above and below control in the generation 31–35 interval.

control lines. Table 2 (columns d_S and d_D) and Fig. 3 show the 5-generation means of these deviations. Since the controls were terminated at generation 35, the final point in Fig. 3 is a guess arrived at by taking the total difference between the S and D lines and assigning the same fractions above and below the control that were observed for the generation 31–35 point.

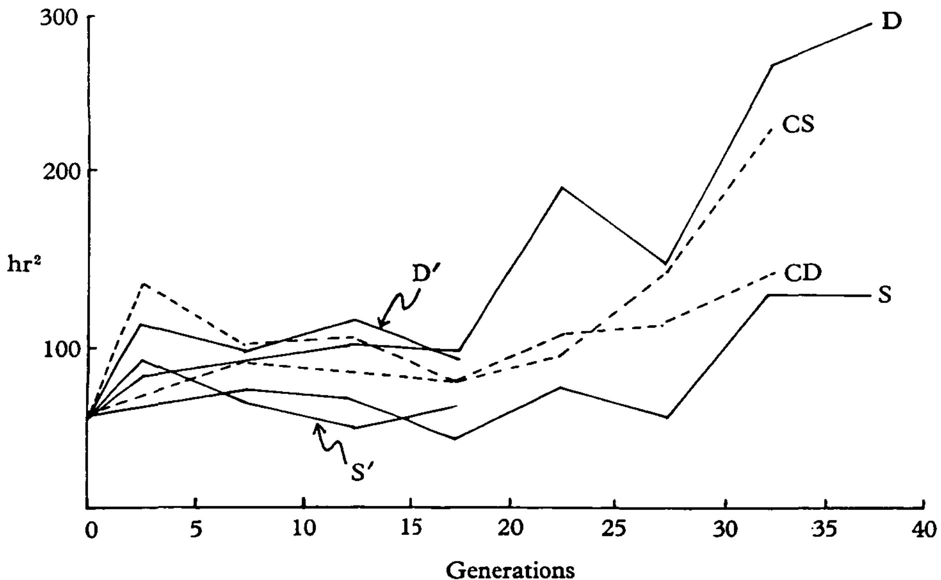

FIG. 2. Response of within-vial variance to different modes of selection. Mean variances of successive 5-generation intervals are shown. CS, Control for S; CD, Control for D; S, Stabilized line; D, Disruptive line; S′, early replicate of stabilized line; D′, early replicate of disruptive line.

Examination of Fig. 3 (and Table 2) reveals that generally the variances changed in the expected directions. In the figure S′ and D′ are the replicate stabilizing and disruptive lines which were terminated at generation 20. The stabilized line, S, differentiated from the rest early and exhibited a steady decline throughout the

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experiment. The S′ line behaved in the same way as far as it went. The two disruptive lines, D and D′, showed little differentiation from controls during the first 20 generations, but the remaining D line showed a distinct, though erratic, increase from generation 20 to 40.

Some statistical corroboration of these latter conclusions were obtained by computing certain regression lines (Table 3). For this purpose the variances were converted to standard deviations which are expected to be more nearly normally distributed than the variances. The regressions were performed on the values for each generation rather than the 5-generation means.

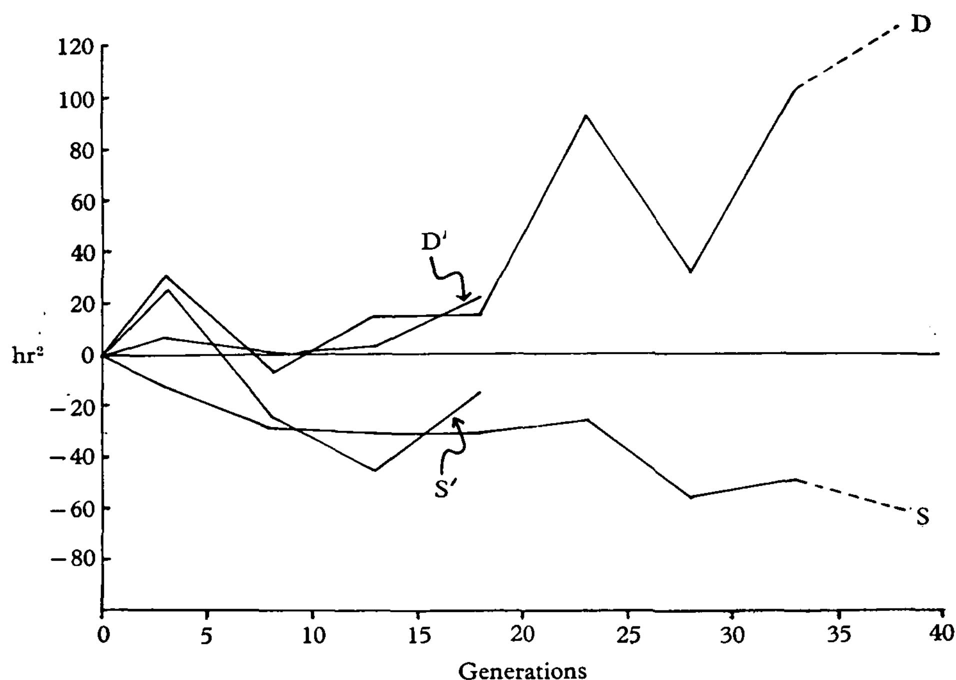

Fig. 3. Variance of selected lines expressed as deviation from mean of two control lines. 5-generation intervals; S, S′, D and D′ as in Fig. 2. Last points estimated as explained in text.

The regression of the difference between the two experimental lines (standard deviation of D minus the standard deviation of S) on generation number was computed assuming the line passed through the origin. A slope of 0·17 standard deviation/generation was obtained which differs significantly from zero ($t = 9{\cdot}39$, d.f. = 38). Thus the selection lines diverged from one another. However, the two control lines also diverged from one another with the CS developing more variability than the CD control particularly toward the end of the experiment (see Table 2). The regression of CS–CD on generation gave a slope of 0·06 standard deviation/generation which also differs significantly from zero ($t = 2{\cdot}75$, d.f. = 32). The difference between the two rates of divergence (0·17–0·06) differs significantly from

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zero ($t = 4{\cdot}22$, D.F. $= 70$). This last comparison indicates that the selection lines diverged faster than these particular two controls, at least. Whether or not the two selection lines could be considered random samples from a population of diverging control lines is another question. However, the selection lines did diverge in the direction expected, *a priori*. It seems likely that the two modes of selection were the causes of the observed changes in variability.

Table 3. *Regressions through the origin of difference between standard deviations on generation number*

Comparison	$b \pm$ S.E.	t	D.F.	Probability (P)
D–S	0·169 ± 0·018	9·39	38	$P < 0{\cdot}001$
CS–CD	0·055 ± 0·020	2·75	32	$P < 0{\cdot}01$
S–M_c	−0·091 ± 0·012	−7·58	32	$P < 0{\cdot}001$
D–M_c	0·097 ± 0·022	4·41	32	$P < 0{\cdot}001$
Difference between				
b's: (D–S) – CS–CD)	0·114 ± 0·027	4·22	70	$P < 0{\cdot}001$

$b \pm$ S.E., regression coefficient ± standard error; t, for the hypothesis: $b = 0$; D–S, regression on generation no. of the difference between the standard deviations of D and S lines; CS–CD regression on generation no. of the difference between standard deviation of the two control lines; S–M_c, regression on generation no. of the difference between standard deviations of the S and mean standard deviation of the two control lines; D–M_c, regression on generation no. of the difference between the standard deviations of the D and the mean standard deviation of the two control lines.

The question as to whether the divergence between the two selection lines is due to both lines or only one line may be answered by considering the regression on generation number of the difference between each experimental line and the mean of the two controls. The rate of divergence of both lines from controls differ significantly from zero as shown in Table 4. Thus the divergence is due both to an increase in the D and a decrease in the S line.

Table 4. *Five-generation averages of mean times of development in hours. Based on unweighted means of males and females*

Generation interval	S	D	CS	CD
0–5	220·3	219·5	222·3	218·2
6–10	231·0	229·8	231·2	231·7
11–15	233·9	231·5	239·2	234·4
16–20	249·1	248·3	250·9	250·3
21–25	250·6	258·9	256·8	253·3
26–30	251·6	257·4	258·3	258·1
31–35	250·2	261·3	257·5	250·9
	261·9	279·4		

S, D, CS, CD as in Table 2.

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Mean

The mean development time is reported in abbreviated form in Table 4. The development times were grouped by taking averages over 5-generation intervals and over males and females. Inspection of the Table shows a general upward trend such as is also exhibited by the variances. Figure 4 shows these values for the two experimental lines expressed as deviations from the mean of the two control lines.

Although no statistical tests were performed, probably all of the lines differentiated from one another (including the controls). The principal objective of this

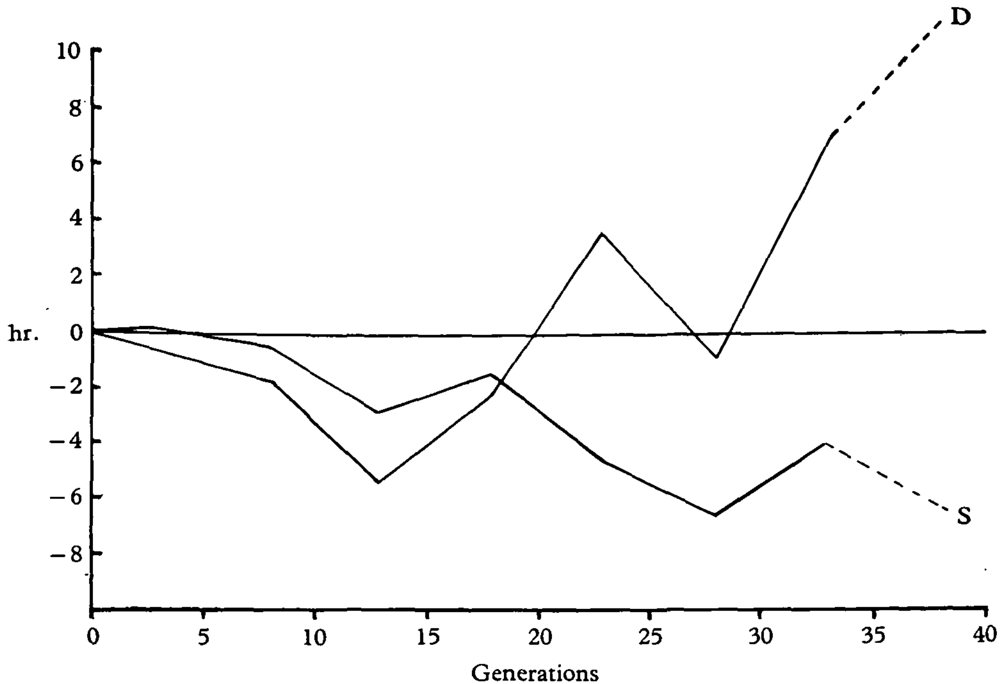

Fig. 4. Mean development time of selected lines expressed as deviations from mean of two control lines. 5-generation intervals; S and D as in Fig. 2. Last points estimated in same manner as last points in Fig. 3.

experiment is to explain the changes in variability, and to this end it is of importance to note that after generation 20 the D line developed a higher mean and the S line a lower mean relative to each other and to the controls. In order to assess the changes in variability with the effects of the mean removed, it therefore appeared necessary to examine the behaviour of the coefficients of variation.

Coefficients of variation

The 5-generation means of the coefficients of variation (CV) are shown in Table 5. Figure 5 shows the selection lines expressed as deviations from the mean CV's of the two control lines. Examination of Fig. 5 reveals the same pattern of changes

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Table 5. *Five-generation means of coefficients of variation (in %) based on unweighted mean CV's of males and females*

Generation interval	S	D	CS	CD
0–5	3·7	4·5	3·9	3·7
6–10	3·4	4·1	4·3	4·2
11–15	3·5	4·5	4·3	3·9
16–20	2·7	3·8	3·5	3·4
21–25	3·4	5·3	3·8	4·0
26–30	2·7	4·5	4·4	3·8
31–35	4·4	6·3	5·7	4·7
36–40	4·3	6·2		

S, D, CS, CD as in Table 2.

as described for the variances. In Table 6 are set forth the same set of regressions analyses as were performed on the standard deviations and all of the slopes so computed differ statistically from zero.

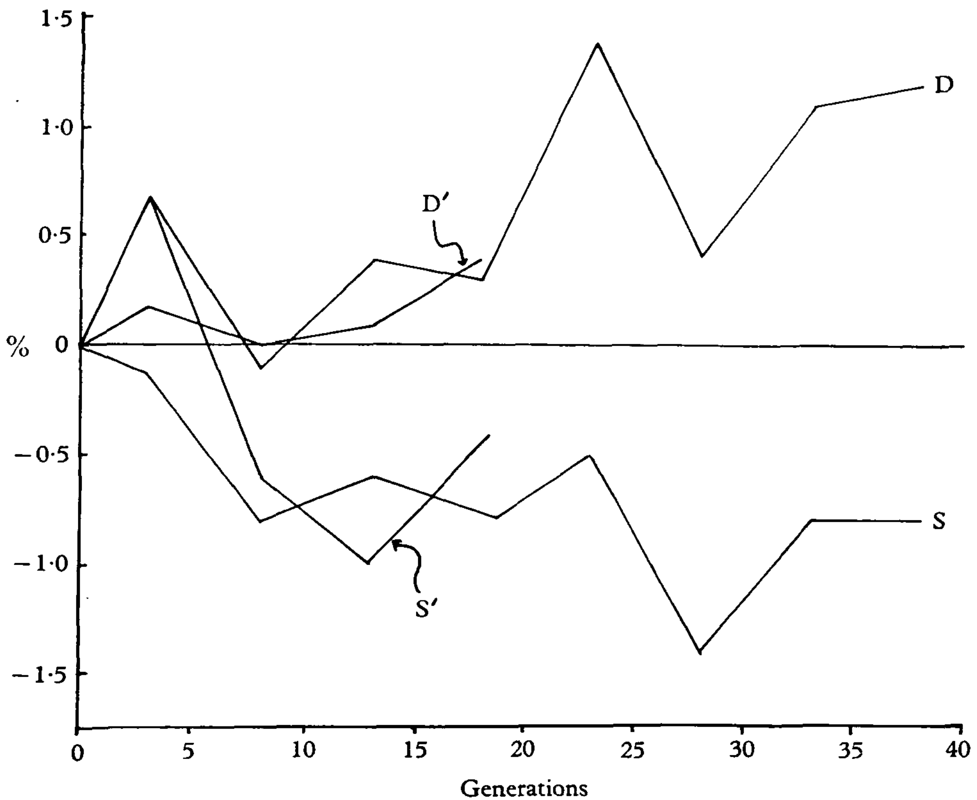

FIG. 5. Coefficients of variation (CV) in per cent of selected lines expressed as deviations from mean CV of two controls. S, S′, D and D′ as in Fig. 2. Last points estimated in same manner as last points in Fig. 3.

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The use of the CV to remove scaling effects can be justified in this case since probit-log plots of emergence distributions give reasonably good straight lines and the CV is directly proportional to the standard deviation of a log-normal distribution.

Table 6. *Regressions through the origins of difference between coefficients of variation on generation number*

Comparison	$b \pm$ S.E.	t	DF	Probability (P)
D–S	0·06 ± 0·007	8·6	37	$P < 0{\cdot}001$
CS–CD	0·02 ± 0·008	2·5	31	$0{\cdot}01 < P < 0{\cdot}02$
S–M_c	0·04 ± 0·006	6·7	31	$P < 0{\cdot}001$
D–M_c	0·03 ± 0·009	3·3	31	$0{\cdot}001 < P < 0{\cdot}01$
Difference between b's: (D–S) – (CS–CD)	0·04 ± 0·01	4·0	68	$P < 0{\cdot}001$

Conventions as in Table 3. b, in CV (%) per generation.

Therefore it seems reasonable to conclude that the changes in variability of the selection lines arose at least in part as the result of causes more significant and interesting than simply a scaling effect operating through shifts in the means.

Viability

One component of fitness can be assessed from the number of flies emerging from a vial into which 20 eggs were introduced. The number of such flies was expressed as a fraction of 20, and the means over the eight replicate vials for each line and for each generation were recorded. This fraction will be termed 'viability'.

In Fig. 6 are plotted the mean viabilities for 5-generation intervals. After generation 20 the viability of the D line clearly falls below that of the other lines. During the last half of the experiment (generations 21–40) the mean difference between the viabilities of the S and D lines was 0·3197. The probability of obtaining such a deviation or greater from a true difference of zero is less than 0·001. However, this result must be interpreted with caution since for some reason the two control lines showed almost as much differentiation early in the experiment and subsequently came together. The controls showed almost the same amount of change (of the difference) as the experimental lines. Therefore, it will only be suggested that the drop in viability of the D line has been produced by disruptive selection.

Response to directional selection

During the latter half of the experiment a number of tests were performed in order to determine the responsiveness of each line to simple directional selection. The object of these tests was to learn something about the nature of the gene pool changes which underlie the observed changes in phenotypic variance.

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Between generations 20 and 36 six tests were performed on each of the four lines. Each test on a given line consisted of selecting directionally in both directions from a population one generation removed from the selection line. The selection was performed for one generation only.

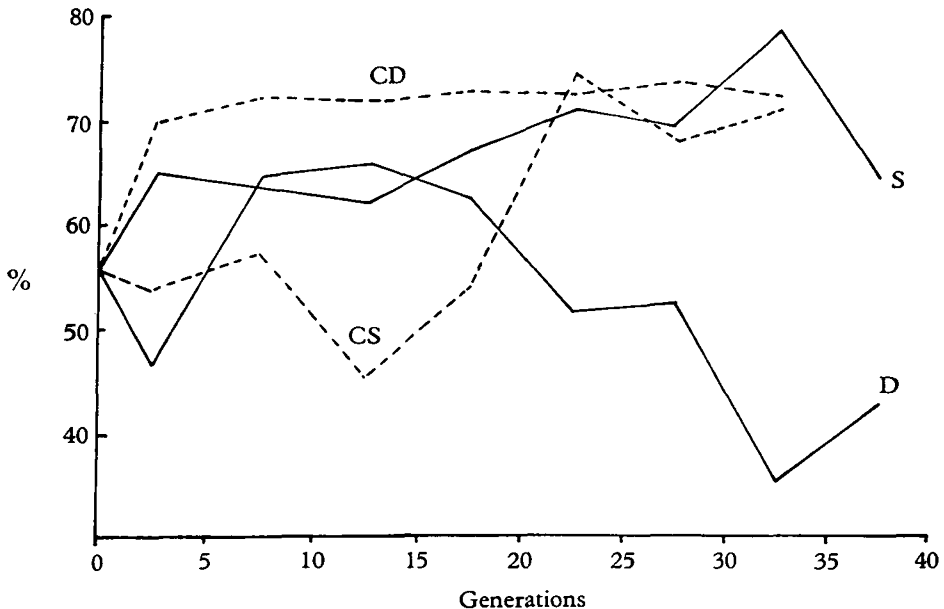

FIG. 6. Viability in selected and control lines; CS, CD, S, and D as in Fig. 2. Viability = per cent emerging from 20 eggs.

Table 7. *Some statistics of the directional selection tests. Each value in the table is a mean of six values from six independent tests*

Line	N_p	i	N_o	r	$h^2 \pm$ S.E.
CS	75	2·68	302	0·652	26·5 ± 8·0
CD	73	2·60	294	0·621	24·3 ± 2·7
S	66	3·02	293	0·348	11·2 ± 2·7
D	73	2·48	235	0·382	14·1 ± 4·2

N_p = total number of parents selected per test;
N_o = total number of offspring raised per test;
i = mean selection intensity in both directions in standard units;
r = mean selection response in both directions in standard units;
h^2 = mean $(r/i) \times 100$ = heritability in per cent.

Table 7 gives some general statistics emerging from these experiments. Each value in the Table is a mean of six values taken from the six independent tests; i is the mean selection intensity (standard units), computed by taking the difference between the means of fast and of slow selected parents and dividing by the standard deviation. r is the selection response in standard units computed from the two

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progenies in an analogous way. h^2 is the mean of the six ratios $100\,(r/i)$, and therefore is the mean of six estimates of heritability (%). The standard error of h^2 (S.E.) was determined from the variance of the six estimates of h^2.

It will be noted that the two control lines show about the same heritability of about 25%; this is slightly higher than the heritability in both experimental lines, whose values are close to 13%. This relationship can be made more meaningful by an examination of the theoretical components of variance whose relative values h^2 measures.

Table 8. *Estimates of theoretical components of variance*

Generation when test was conducted	CS line				CD line			
	h^2	T	G	R	h^2	T	G	R
20	2·3	74	2	72	19·4	46	15	61
24	45·0	108	49	59	21·7	138	30	108
27	25·7	110	26	84	21·6	82	18	64
30	28·8	230	66	164	29·6	131	39	92
33	51·7	189	98	91	29·8	122	36	86
36	7·7	195	15	180	23·4	142	33	109
Mean	26·5	151·0	42·7	108·3	24·3	115·2	28·5	86·7
standard error	8·0	—	14·5	20·7	1·7	—	4·0	8·5

Generation when test was conducted	S line				D line			
20	3·9	50	2	48	14·4	140	20	120
24	3·8	92	3	89	−2·5	222	−6	228
27	10·7	47	5	42	12·9	99	13	86
30	18·6	134	25	109	29·7	298	89	209
33	12·9	106	14	92	17·7	224	40	184
36	17·4	102	18	84	12·5	375	47	328
Mean	11·2	88·5	11·2	77·3	14·1	226·3	33·8	192·5
standard error	2·7	—	3·8	11·0	4·2	—	13·5	34·9

h^2 = heritability in per cent.
T = means of the total phenotypic variance in the selection line taken over 3 values before, during and after the generation when test was performed.
G = additive genetic variance estimated as $h^2 \times T$.
R = 'remainder' component estimated as $T - G$.

In Table 8 certain data pertaining to the individual tests for each of the four lines are shown. The first column for each of the four lines shows the individual estimates of h^2, the means of which were given in Table 7. The column headed 'T' is the total phenotypic variance in the main selection line just before, during, and just after the time when the test was performed. By multiplying T by h^2 the total variance is resolved into two components: G, the additive genetic component, and, by simple subtraction ($T - G$), R, which can most simply be termed the 'remainder' component.

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The means of these values for each of the four lines suggest the following relationship: the additive genetic component (G) tends to have the same value in the two controls and in the D line, while the S line is low. The remainder component (R) tends to have the same value in the two control lines and the S line, while in the D line it is high.

These relationships between the controls and selected lines may be seen in Fig. 7 which is a diagrammatic presentation of the data of Table 8.

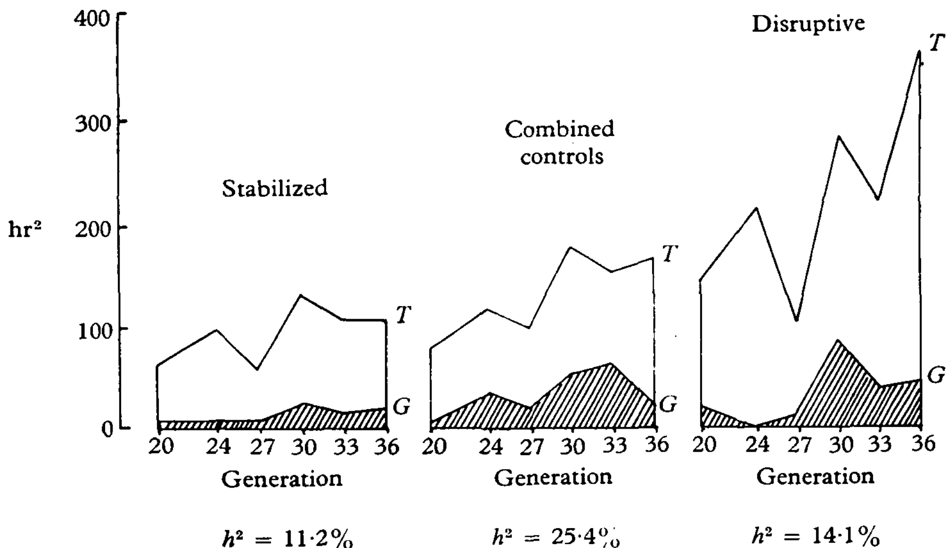

FIG. 7. Diagrammatic presentation of theoretical variance components derived from directional selection tests. Data from Table 8. For each test the height of the upper line (T) represents the total phenotypic variance in the main experiment around the time the test was performed. The height of the lower line (G), demarking the shaded area, indicates the amount of additive genetic variance obtained from the product $h^2 \times T$.

In order to gain some statistical support for these relationships certain 't' tests were performed as shown in Table 9. Since G and R are estimates of variances and so are likely to have skewed sample distributions, the tests were performed on the means of the square roots of the G and R values of Table 8. It is assumed that this transformation together with the central limit theorem will cause the sample distributions of these means to be reasonably normal. The pattern of statistical significance seen in Table 9 (last column) suggests that the S line lost all of its variance in its G component, while the D line gained all of its variance in its R component.

DISCUSSION

The directional selection tests indicate that the changes in phenotypic variance in the S and D lines were accomplished by qualitatively different mechanisms. The S line lost variance simply through a reduction of the additive genetic component while the D line acquired variance through an increase in the 'remainder' component.

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Table 9. 't' *tests performed on components of Table* 8. *Square roots of the raw data of Table* 8 *were taken and the* 't' *tests were performed in the means of these transformed values*

Test	Difference ± S.E.	t	D.F.	P
	Additive genetic component (G)			
Difference between two controls	0·64 ± 1·32	0·48	10	$0{\cdot}60 < P < 0{\cdot}70$
Difference between S line and mean of two controls	−2·52 ± 0·87	−2·90	16	$0{\cdot}01 < P < 0{\cdot}02$
Difference between D line and mean of two controls	−0·46 ± 1·46	−0·32	16	$0{\cdot}70 < P < 0{\cdot}80$
	Remainder component (R)			
Difference between two controls	0·94 ± 1·07	0·88	10	$0{\cdot}30 < P < 0{\cdot}40$
Difference between S line and mean of two controls	−1·05 ± 0·84	−1·25	16	$0{\cdot}20 < P < 0{\cdot}30$
Difference between D line and mean of two controls	3·82 ± 1·38	2·77	16	$0{\cdot}01 < P < 0{\cdot}02$

Stabilizing line

The behaviour of the S line is in accordance with the various theoretical investigations of the optimum model (where environmental variance and possible changes in it have not been considered). Most gene action models predict a loss of genetic variance with a tendency towards homozygosis for a genotype with intermediate phenotype (Wright, 1935; Robertson, 1956; Fraser, 1960). The only exception was discovered by Kojima (1959) where a segregating equilibrium is predicted for special combinations of certain levels of intermediate dominance and certain selected values (position of the optimum). It might be expected that these equilibrium conditions of Kojima would hold for only a small fraction of the loci involved in a given trait and that the alleles at the great bulk of the loci would tend to move towards fixation. This is apparently what happened in the S line of this study and this may thus be considered an experimental demonstration of Waddington's (1957) normalizing selection.

However, since the publication of Schmalhausen's book (1949) the response to stabilizing selection which has been given the most attention verbally, but not mathematically, is what Waddington (1957 and earlier) has termed canalization. Such an increase in buffering capacity or developmental homeostasis should have manifested itself here as a reduction in the 'Remainder' component of variance due to a reduction in the environmental part of that component.

It is possible that such a reduction occurred (Table 9) but was so small as to be undetectable statistically. However, when the findings of this work are included together with those of previous experiments on stabilizing selection, which are summarized in Table 10, one is impressed with the inability of stabilizing selection to produce canalization. Either there was no change in variance at all* (Falconer &

* To these negative results the author should add his own unpublished attempt to reduce the variance of wing length with 12 generations of stabilizing selection.

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Table 10. *Summary of experimental findings in stabilizing and disruptive modes selection*

Author	Organism and character	Stabilizing selection		Disruptive selection	
		Response of total variance	Component(s) accounting for response	Response of total variance	Component(s) accounting for response
Falconer & Robertson (1956)	Mouse 6 wk. body-weight	None	—	None	—
Falconer (1957)	*D. melanogaster* Abdominal bristles	None	—	—	—
Thoday (1959)	*D. melanogaster* Sternopleural bristles	Decreased	Genetic	Increase	Genetic
Rendel (1960)	*D. melanogaster* Scutellar bristle number	Decreased*	Unknown, but probably at least environmental	None*	—
Prout (this paper)	*D. melanogaster* Development time	Decreased	Genetic	Increase	Non-additive, possibly environmental

* Rendel did not employ stabilizing and disruptive selection, *sensibus strictis*, but he did select for low and high variance respectively.

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Robertson, 1956; Falconer, 1957) or when variance was reduced it was due to a reduction of the genetic component (this paper and Thoday, 1959). The only exception is the work of Rendel (1960), where his low variance line showed increased canalization against temperature variation. However Rendel's result should be considered an indirect response since selection was carried out under constant temperature conditions so that the selection goal was that of canalization against the array of micro-environments within cultures. Nevertheless, it is a reasonable inference that the latter canalization did occur and the buffering mechanism so produced was sufficiently general to extend to temperature variation.

One reason for the difficulties in producing a canalizing effect could simply be that for many traits and many gene pools there simply is no genetic variability for differences in buffering capacity or that if there is genetic variance, it is non-additive (e.g. overdominant).

Another reason for the inability to produce canalization could be related to the intensity of selection for it. It should be emphasized that stabilizing selection simply calls for a reduction of the phenotypic variance about the selected value. It is not a mode of selection which exclusively favours canalization. It is conceivable that many gene pools are so constructed that the initial response simply involves the reduction of the genetic component of variance, i.e. a move towards homozygosity. Once this happens the total selection intensity diminishes since more of the population falls within the selection limits which are fixed close to the mean, and thus *there remains only a very mild selection for subsequent improved canalization*. One method for counteracting this diminishing intensity might be to deliberately increase the environmental variance by raising the population in a more heterogeneous environment. High selection intensity could then be imposed again and this pressure would be more directly for increased canalization. In fact, it is not unreasonable to suggest that the usual laboratory techniques designed to reduce environmental variance may be in part responsible for the difficulty in the experimental production of a canalization by stabilizing selection. It should be pointed out that in a natural population stabilizing selection normally has much more environmentally induced variance to work on.

Disruptive line

The behaviour of the D line is more difficult to interpret. The directional selection tests indicate that compared to controls all of the increase is attributable to the 'remainder' component and that, therefore, no increase in additive genetic variance was indicated. This latter finding is to be contrasted with Thoday's (1959) results where the increase in variance in his D^- line (selection was conducted in essentially the same way as in the D line of this paper) was accompanied by an increase in additive genetic variance.

In fact, reference to Table 10 will reveal that this result of Thoday's stands alone as the only case where the genetic variance was detectably increased by this mode of selection.

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Theoretical considerations (Moree, 1953; Robertson, A., 1956) predict that there should be an increase in genetic variability due to the movement of alleles initially at frequencies near 0 or 1·00 toward intermediate values which would yield near maximum variance. The difficulty in producing this effect experimentally could be due to the inherent slowness of the process (Falconer & Robertson, 1956, p. 389). However, there is another possibility. If most of the gene frequencies in the foundation population were already at intermediate values then no increase in variance could be produced. Such a circumstance would arise from Lerner's model (1954, p. 86) where gene action is mainly additive in relation to the character under study but is overdominant in relation to fitness. If such a situation is as general as Lerner suggests, then the difficulties encountered by other authors, as well as this one, in experimentally increasing the genetic variance would be expected.

The increase in the 'remainder' component of variance could be due to any one or more than one of its components. The simplest explanation is that there was a loss in buffering as a result of continued selection of extreme phenotypic deviants. This would imply that there was additive genetic variation for buffering capacity, although not enough to be revealed by the less intense stabilizing selection.

The existence of such variation is in great need of experimental verification. The evidence here is meagre because of the ambiguity of the 'remainder' component. To the evidence provided by Rendel's work, already discussed, may be added that of Waddington (1960) who demonstrated genetic variance for buffering of Bar eye size against temperature variation.

More evidence of this sort is needed because the assumption of additive genetic variance for buffering is implicit in much of the current literature on the evolution of buffering mechanisms, yet the ubiquity of such variance has by no means been established. It would appear that although the stabilizing mode of selection is more interesting as a natural process, the disruptive mode is more efficient for demonstrating and studying buffering variance.

Viability

The relative changes in viability in the D and S lines follow what one might expect. If the increase in the 'remainder' component of the D lines represents a loss of buffering then it is not unreasonable that such a disturbance in the control of development would manifest itself as well in lowering some components of fitness. It should be emphasized that this is not simply another case of the concomitant loss of homeostasis and fitness due to inbreeding. The S and D lines should be equally inbred. Furthermore, the S with higher viability has less genetic variance (more homozogous) than the D line with lowered viability. Thus, although inbreeding and the resultant general homozygosity so produced is one way to cause a loss of homeostasis and fitness, in this instance these same effects were evidently produced by the more direct selection against homeostasis itself by picking out and concentrating just those genes which cause a loss of developmental control mechanisms. The implication here is that those loci determining the genotypic value and therefore genetic variance are different from those controlling buffering

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so that it is possible to lose canalization while at the same time retain the genetic variance of the trait.

Stabilizing selection in the S line apparently did not affect viability. This is to be contrasted with Thoday's results where his S line lost fitness. He attributes this to 'homozygosity and consequent imbalance' comparing his S line to an inbred line or newly plateaued selection line. However, the latter two situations are different; an inbred line is generally homozygous while a plateaued line is homozygous only for those loci under selection (assuming homozygosity is the reason for plateauing). Any homozygosity in Thoday's S line which is in excess of that in his D line should be at those loci under selection, i.e. those controlling sterno-pleural bristle number. In order to avoid the extreme assumption that homozygosity at any locus causes a decrement in fitness, it would seem preferable to attribute the loss of fitness to some sort of disturbance of gene pool integration (Wallace & Vetukhiv, 1955) occasioned by selection which may or may not be related to homozygosity. Such a disturbance evidently did not occur in the S line of this paper.

The above considerations are, of course, largely conjectural, but they do serve to point up some of the problems in this area of study. In particular, it would seem that continued study of the effects of 'mass' homozygosity and heterozygosity of inbred lines and their crosses will not reveal much concerning the specificity (if it exists) of loci in their effects on the buffering of different traits. Whereas further studies using stabilizing and disruptive modes of selection (which always involve specific traits) would seem to promise a better analysis of the genetic control of buffering mechanisms.

SUMMARY

The length of time of development, from oviposition to emergence in *Drosophila melanogaster* was subjected to stabilizing selection. In each generation only the individuals emerging close to the mean development time were used as parents of the next generation. This line was designated the 'S' line. In a parallel line disruptive selection was practised; where in each generation the earliest flies to emerge were mated to the flies last to emerge; those emerging at intermediate times were discarded. This line was designated the 'D' line. Two control lines were also carried, where the flies were mated at random with respect to time of emergence. The experiment extended for 40 generations and produced the following results:

(1) The variance of development time decreased in the S line and increased in the D line, relative to the control lines.
(2) The mean development time decreased in the S line and increased in the D line.
(3) The coefficients of variation decreased in the S line and increased in the D line.
(4) The viability, measured as per cent flies emerging, decreased in the D line.

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Toward the end of the experiment the amount of additive genetic variance in the selected lines and in the control lines was estimated from the response to directional selection. The estimates showed that (i) the loss of total variance in the S line can be accounted for completely by a loss in additive genetic variance, and (ii) the increase in the total variance of the D line cannot be ascribed to an increase in the additive genetic variance. It was probably due to an increase in the environmental component of variance, i.e. to a loss of 'buffering capacity'.

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