STABILIZING AND DISRUPTIVE SELECTION ON A MUTANT CHARACTER IN DROSOPHILA. I. THE PHENOTYPIC VARIANCE AND ITS COMPONENTS

W. SCHARLOO, M. S. HOOGMOED AND A. TER KUILE

Genetisch Instituut der Rijksuniversiteit, Groningen, Genetisch Laboratorium der Rijksuniversiteit, Leiden, The Netherlands

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IN an earlier paper (SCHARLOO 1964b) it was shown that artificial stabilizing selection on the expression of a cubitus interruptus mutant causes a decrease in phenotypic variance and that artificial disruptive selection with random mating of the selected individuals causes an increase in phenotypic variance. Further analysis showed that under disruptive selection the increase in phenotypic variance was a consequence of an increase in additive genetic variance. The decrease of phenotypic variance in the stabilizing line, however, seemed to be caused by a decrease of both the genetic and the environmental components. The within-fly variance in both lines was unaffected.

KAMSHILOV (1939), WADDINGTON (1960), WADDINGTON and ROBERTSON (1966), and RENDEL and SHELDON (1959) succeeded in diminishing the sensitivity of mutant expression to environmental factors but these authors did not apply simple stabilizing selection. KAMSHILOV and WADDINGTON practised selection directly against environmental effects, and RENDEL and SHELDON performed selection for low variability within families.

PROUT (1962) emphasized that although it is assumed in theoretical discussions (SCHMALHAUSEN 1949; WADDINGTON 1942, 1957) that stabilizing selection would decrease the sensitivity of developmental pathways to both genetical and environmental differences, there was no experimental evidence to support this view. In his experiments in which stabilizing and disruptive selection was practised on the duration of larval development in *Drosophila*, he found no change of environmental variance under the influence of stabilizing selection and an increase in environmental variance in his disruptive line in which—instead of random mating—compulsory mating of opposite extremes occurred. He suggested that the latter kind of selection was particularly suited to change the environmental variance.

In THODAY's extensive experiments (see THODAY 1965) on the effect of disruptive and stabilizing selection on chaeta number in *Drosophila*, attention was almost exclusively focussed on the consequences for the genetical variance.

In view of the considerable importance in the theory of evolution of the influence of stabilizing and disruptive selection on sensitivity to environmental and genetical differences, it was decided to compare the effect of stabilizing selection and both types of disruptive selection.

FIGURE 1.—Diagram of a ci^{D-G} wing. The expression of the fourth-vein interruption was measured as the percentage ratio of the third vein, both measured from the first crossvein. The sum of the ratios of the two wings of an individual is used as phenotypic value.

MATERIALS AND METHODS

The mutant used in these experiments, cubitus interruptus dominant of Gloor (ci^{D-G}), is a recessive lethal on chromosome 4, which causes in single dose a terminal interruption of the fourth longitudinal wing-vein (SCHARLOO 1963). The expression of ci^{D-G} is measured as the percentage ratio of the part of the fourth vein present to the length of the third vein, both measured from the first crossvein (Figure 1). Both wings of each individual were measured and the sum of the two expression ratios was used as the character selected for. The phenotypic variance (V_p) and its components, the genetic variance (V_G), the additive genetic variance (V_A), the within-fly contribution (V_{WF}) and the environmental variance (V_E), are variances of this value, computed within cultures.

In contrast to the original ci^D mutant (see SCHARLOO 1962) the expression of ci^{D-G} has an approximately linear scale (SCHARLOO 1964a,b) so that the normal statistical methods of quantitative genetics can be used.

The wing veins were measured on etherized live flies placed on an adjustable platform attached to the moving stage of a normal microscope (see ROBERTSON and REEVE 1952).

With the help of suitably marked stocks, one chromosome 4 with the mutants ci^{D-G} and spa^{pol} (sparkling poliart, STURTEVANT 1961) was introduced into the background of the Pacific cage population (see ROBERTSON 1960). In order to have a large reservoir of genetic variability, a large number of flies was used for each introduction. At the start of each selection experiment and for the progeny tests on the base population, separate introductions were made.

Because ci^{D-G} is a homozygous lethal, it had to be held in heterozygous condition over a normal chromosome so that wild-type individuals segregate each generation.

The flies were reared in $\frac{1}{8}$ liter (Leiden) or $\frac{1}{4}$ liter (Groningen) creamers at a temperature of 25°C. The food was composed of water (1000 ml), agar (10g), cornmeal (15g), sugar (10g), dried yeast (1.5g) and Nipagine (1.5g) while live yeast was added. After transfer of the second experiment to Groningen (after generation 11) the flies were reared on a medium consisting of water (1000 ml), agar (19g), sugar (54g), dried yeast (32g) and propionic acid (5 ml).

In all experiments 20 males and 20 females were measured from each bottle.

Four selection methods were used: (1) *Divergent directional selection*. Selection for high and low expression ratio was performed in separate lines by selecting from each sample the four flies with the highest or the lowest expression ratio respectively. In all lines, three cultures were present in each generation. (2) *Stabilizing selection* (S). From each sample the four flies with values nearest to the mean values of their sample were used as parents of the next generation. (3) *Disruptive selection with random mating* (D^R). From each sample the two animals with the lowest and the two animals with the highest values were selected. High and low females were placed together with high and low males in a single bottle so that random mating between the selected individuals could occur. (4) *Disruptive selection with disassortative mating* (D^-). Flies were selected in the same way as in D^R but high females were mated with low males and low females with high males in separate vials. After 24 hours the males were discarded and both high and low females were transferred to one bottle.

When a culture failed, extra flies were measured from the other cultures of the line. Only in rare cases in the later part of Experiment 2 was it necessary to resort to mass culture.

Stabilizing (S1) and disruptive selection with disassortative mating (D-1) were practised during 13 generations. In these lines three cultures were present in each generation. In the second experiment (E2), stabilizing selection (S2), disruptive selection with disassortative mating (D-2) and disruptive selection with random mating (D^R2) were compared. From generation 0 (G0) to G14 in all lines four culture bottles were used in each generation. After G14 in D^R2 and S2 only three cultures were reared, but after G30 D^R2 was expanded again to four bottles per generation.

RESULTS

Divergent directional selection: From Figure 2 it can be seen that directional selection immediately causes large changes of the mean value. In both experi-

FIGURE 2.—Divergent directional selection. Mean value, phenotypic variance and the within-fly contribution in the course of generations.

ments which are separated by an interval of about one year, already in G4 and G5 respectively, individuals occurred in the lines selected for a long fourth vein who had one complete fourth vein. Because overlap of ci^{D-G} with wild type would make it impossible to obtain a proper estimate of the mean value and to maintain the full selection pressure, these lines were not continued.

The phenotypic variance V_P shows large differences in the high lines. In both low lines V_P drops already considerably in the first line, which was maintained under selection until G13. The decrease of V_P could be caused by a decrease of its genetic components which leads at last to a cessation of selection response. But probably scaling problems are involved too in this low range of the expression ratio. In both low lines V_{WF} decreases appreciably after G4, when the mean value falls below 80. This suggests that in this range the scale is not linear and that change of the expression ratio becomes more difficult here.

In G10 of the low line, of the non- ci^{G-D} individuals which segregate each generation about 8% had a nonterminal fourth-vein interruption, halfway between the wing tip and the second crossvein. In G11 and G13 similar percentages of non- ci^{D-G} individuals with a fourth-vein interruption occurred, but in G12 such flies were not found.

FIGURE 3.—Stabilizing selection (S1) and disruptive selection with disassortative mating (D-1). Mean value, phenotypic variance and within-fly contribution in the course of generations. Broken lines represent the stabilizing line and full lines the disruptive line.

Disruptive and stabilizing selection: Mean values. In both experiments the mean values (Figures 3, 4) are relatively constant, especially when compared with the change under divergent directional selection. The mean value of the disruptive lines fluctuates more than the mean of the stabilizing lines, but this must be expected in view of the higher phenotypic variances in the disruptive lines.

FIGURE 4.—Stabilizing selection (S_2 and resumed selection on S_1 , see text), disruptive selection with disassortative mating (D_2^2) and disruptive selection with random mating (D_2^R). Phenotypic variance, within-fly contribution and mean value in the course of generations.

Phenotypic variances: The phenotypic variance (V_p) of the base population differed appreciably in the two experiments. The causes of these differences are not known. In both stabilizing lines V_p decreased. The temporary increase in variance in S1 G11 was caused by a short period of low temperature which increased the expression ratio in some flies. In S2, progress halts temporarily at values of about 150.0. Only after G15 does a gradual decrease in V_p occur. After about one year of mass culture of S1, selection was resumed (Figure 4). During this mass culture, V_p has increased somewhat, but after one generation of selection it fluctuated at the lower side of 50, i.e. at about the same level as S2. In both D⁻ lines, V_p decreased somewhat in G1. This could be expected when opposite extremes are crossed. But after G1 V_p increased gradually. In D^R2 V_p also increased rapidly. In all disruptive lines V_p fluctuated violently in the later stages of the selection.

In order to obtain a better insight into the meaning of the changes in variance, frequency distributions were drawn for E2 (Figure 5). The base population has an unimodal frequency distribution. In the S lines (for which no frequency distributions are given here) the decrease of V_p occurs through a concentration of the distributions around the mean value. In G6 in both D-2 and D^R2 the frequency distribution has become flat and the range has increased at both sides of the mean. In later generations the distributions become bimodal with only a few values between the peaks. In D-2 the intermediate values often belong to asymmetric individuals with one wing having a very high and the other wing having a very low value. The relative size of the two peaks differs, but the lower one is in general the larger one.

In D^R2 G18 the frequency distribution is bimodal. In G36 both peaks have moved to still more extreme values. In the high region there is some overlap with wild type. The first ci^{D-G} individuals with one complete vein were found in G18, and progeny tests on later generations revealed that a few ci^{D-G} individuals had full fourth veins. This must have led to an underestimate of V_p in the later generations of the D^R line and will have prevented classification and selection of the ci^{D-G} individuals with the highest expression ratios.

In G35 from D^R a double series of samples was measured. Parallel to G36, which was reared according to the normal D^R mating system, the flies selected from the extra series were mated according to a quasi-random system. Matings low $\times$ low, low $\times$ high, high $\times$ low and high $\times$ high were made. The frequency distribution, based on equal numbers (40 of each sex) of the progeny from each mating, is strikingly similar to the frequency distribution of G36 of the D^R line. This indicates that mating between the parent flies in the D^R line occurred still at random.

The phenotypes in the S and the D⁻ line were of the normal ci^{D-G} type in both experiments. Besides a change of fourth-vein length, correlated responses of the fifth vein and the position of the second crossvein, no other phenotypic changes were apparent.

But in the D^R2 line already in G6 the extreme low flies lack the second crossvein. At the same time non- ci^{D-G} flies were found without the second crossvein

FIGURE 5.—Frequency distributions of the base population and both disruptive lines (random mating D^R , disassortative mating D^-) of Experiment 2. In the D^R line the frequency distribution of G36 (2) is formed by combination of equal numbers of flies from cultures obtained from matings low $\times$ low, high $\times$ high, low $\times$ high and high $\times$ low. The frequency distributions are based on 120 individuals in D^R G24, 160 in the base population and in D^- G25, on 240 in D^- G25 and on 320 in D^R G36.

and often with an interruption of the fourth vein halfway between the wing tip and the second crossvein. Further in G18 individuals appear with a high expression ratio and with plexus-like venation. Both their frequency and the plexus expression increase in later generations. The cl^{D-g} flies with a complete fourth vein were of this type. Further analysis of these phenotypes will be given elsewhere.

Within-fly contribution: The within-fly contribution (V_{WF}) to V_P is computed as the mean square difference between the two wings of an individual. V_{WF} seems to drop slightly in both S experiments. In both D^- lines V_{WF} increases considerably. In D^{R2} , V_{WF} has a tendency to be slightly higher than V_{WF} in S2. After G25 when V_P rises again, far larger V_{WF} values occur.

Analysis of phenotypic variance: A better insight into the causes of the changes

TABLE 1

Phenotypic variances and their composition in the base population (B), and in the disruptive (D-1) and stabilizing (S1) selection lines in Experiment 1. Further explanation in text

	Population			
	B	D-	S	S
Generation	5	5	5	reselction
Phenotypic variance	264.5	369.7	76.6	38.3
Heritability	0.71 ± 0.05	0.63 ± 0.08	0.48 ± 0.09	0.24 ± 0.08
Additive genetic variance	187.5	232.9	36.8	9.3
Within-fly variance	24.7	51.1	25.5	19.2
Residual variance	52.3	85.7	14.3	9.8
Number of pairs	36	47	20	31

TABLE 2

Phenotypic variances and their composition in the base population (B) and in the disruptive (D_{E2} and D-2) and stabilizing (S1) selection lines in Experiment 2. Further explanation in text.

	Population											
	B	B	D _{E2}	D _{E2}	D _{E2}	D-2	D-2	D-2	D-2	S2	S2	S2
Generation	6	18	6	18	37	6	18	35	6	19	36	36
Phenotypic variance	200.6	231.0	823.0	1226.7	1643.5	505.1	895.5	2132.7	149.0	53.2	37.2	37.2
Heritability	0.62	0.68	0.90	0.94	0.85	0.74	0.54	0.73	0.51	0.47	0.30	0.30
	± 0.11	± 0.08	± 0.04	± 0.03	± 0.05	± 0.07	± 0.06	± 0.06	± 0.04	± 0.06	± 0.08	± 0.08
Additive genetic variance	124.4	157.1	740.7	1153.6	1398.4	378.8	483.6	1556.9	76.0	25.0	11.1	11.1
Within-fly variance	23.9	25.8	27.3	28.4	32.4	34.0	82.0	99.8	26.3	18.7	18.6	18.6
Residual variance	52.3	48.1	55.0	44.7	212.7	92.3	329.9	476.0	46.7	37.7	9.5	7.5
Number of pairs	35	40	34	34	23	40	46	19	28	36	31	31

in phenotypic variability, could be obtained if its components were known. Therefore progeny tests were performed at several stages of the selection lines. In all tests positive assortative mating was used, as recommended by REEVE (1953). Heritabilities were computed as the regression of the mean values of progenies on the midparent values. In E1 the tests were performed on G6, in E2 and G6 or G7, on G18 or G19 and on G35, G36 or G37.

The results are given in Tables 1 and 2. The additive genetic variances were computed by multiplication of the heritabilities and the phenotypic variances. By subtraction of the additive genetic variance V_A and the within-fly variance V_{WF} , from the phenotypic variance V_P a residual component (V_R) is obtained. This residual variance sets an upper limit to the environmental variance. Arguments can be found in the next paragraph that the V_R represents the common environmental variance, at least in the base population.

Base population: Simultaneously with most progeny tests on the selection lines progeny tests were performed on the base population. For each test ci^{D-g} was introduced anew into the background of the Pacific cage population. The agreement of the different tests is reasonable, the value of V_{WF} is virtually constant and the estimates of V_R are remarkably similar to another.

Stabilizing lines: Under stabilizing selection the additive genetic variance decreases immediately. The environmental variance decreased to about a fifth of the original value in both lines, but in S2 this decrease started only after G6.

Disruptive lines: The heritability in the D- lines does not differ much from the values of the base population. The increase of V_P is caused by an increase of all three variance components simultaneously.

In D^R until G18, the increase of V_P is caused exclusively by increase of V_A . In the last progeny test, however, V_R has also increased, and although in the test generation V_{WF} is not much higher, far higher V_{WF} values were found in the preceding generations.

Variance in inbred lines and crosses: Base population. With the purpose of obtaining an independent estimate of the environmental variance V_E (common environmental variance, FALCONER 1961) and the total genetic variance V_G , inbred lines were started from the base population. They were maintained by full-sib mating. One series was started simultaneously with E1. From the 13th to the 18th generation of inbreeding, when flies were available, mass-cultures were branched off from the inbred lines, and crosses were arranged. The combined results are in Table 3. A more accurate test, in which all cultures were

TABLE 3

Variability in inbred lines and crosses from the base population. Explanation in text.

	N	V_P	V_{WF}	V_E
Inbred lines (4)	847	64.8	26.2	38.6
Crosses (3)	479	56.1	25.6	30.5
Homogeneous stocks	1326	61.9	25.9	36.0

TABLE 4

Mean expression ratio, phenotypic variance and its components in inbred lines and crosses from the base population

Line or cross	N	Mean(♀♀)	V_P	V_{WF}	V_E
2	120	88.5	110.8	44.3	66.5
3	241	121.5	80.3	29.9	50.4
4	240	129.5	66.8	22.6	44.2
7	240	121.3	57.8	17.9	39.9
2 × 4	120	117.7	87.3	45.5	41.8
4 × 3	120	129.2	110.9	20.9	90.0
7 × 2	122	105.9	103.9	26.5	77.4
7 × 3	120	125.2	103.3	23.9	79.4
4 × 7	120	124.9	44.5	20.7	23.8
7 × 4	120	121.9	49.7	15.5	34.2
Inbred lines	841		83.8 (78.9)	25.4 (28.7)	58.4 (50.2)
Crosses	722		83.8 (90.5)	25.5 (26.9)	58.3 (59.6)
Total	1561		83.8 (85.4)	25.5 (27.7)	58.3 (57.7)

reared in a short period, was done on a new series of inbred lines (inbred during 18 generations) simultaneously with the progeny test on G18 and G19 of E2. The full results are in Table 4.

Both within inbred lines and within crosses, V_P shows a marked heterogeneity (according to Bartlett's test P for homogeneity < 0.001). Nevertheless, mean values for the variances of inbred lines and crosses were computed. When individual V_P values were weighed according to the number of individuals on which they are based, the figures for inbreds and crosses are almost identical. Also when unweighed mean values are computed (figures in brackets) from the V_P 's of the individual lines and crosses (the reciprocal crosses 7×4 and 4×7 are reckoned as the same cross) the differences are only small. If we assume that our inbred lines are homozygous, the values of inbred lines and crosses can be combined in one estimate for genetically homogeneous cultures. If we further assume that these values are representative for the nongenetic variance components in the base population, an estimate can be obtained of the total genetic variance in the base population.

Such an analysis is given in Table 5. In the first test the residual variance in the progeny-test is larger than the estimate of the environmental variance from the homogeneous cultures. But in the second experiment the situation is reversed.

The genetical variance in the base population seems to be almost completely additive. When a nonadditive genetic variance component is present, it is small.

Stabilizing line. Simultaneously with the second series of inbreds from the base population, inbred lines were started from S1, five generations after stopping the

TABLE 5

*Composition of the variability of the base population based on two independent experiments.
Explanation in text*

Variance component	Experiment 1		Experiment 2		Total percent	Computation
		Percent		Percent		
Additive genetic	187.5	70.9	156.6	67.8	69.4	$h^2 \times V_P$ base population
Nonadditive genetic	15.1	5.7	−9.4	−4.0	0.8	V_P base population less V_P inbreds and crosses less additive genetic
Within-fly	25.9	9.8	25.5	11.0	10.4	V_{WF} inbreds and crosses
Environmental	36.6	13.6	58.1	25.2	19.4	V_P inbreds and crosses less V_{WF}

selection in this line. Tests on three lines and their crosses were made simultaneously with the test on the inbreds of the base population. The values in Table 6 show that there is also in these lines marked heterogeneity of V_P (P for homogeneity < 0.01). The values for inbreds and crosses do not differ very much. The V_P for all genetically homogeneous stocks together is only half the value for inbreds and crosses from the base population, and V_E is reduced still more, while V_{WF} does not differ much.

Temperature experiments: The influence of temperature differences on the expression ratio was investigated in selection lines which showed a change in environmental variance. The results of these experiments which were not done simultaneously are combined in Figure 6.

The D- lines seem to be more sensitive to temperature differences than the S lines and the base population.

DISCUSSION

From these experiments it is clear that stabilizing and disruptive selection cause large changes in phenotypic variance. However, the underlying causes

TABLE 6

Mean expression ratio, phenotypic variance and its components in inbred lines and crosses from the stabilizing line S1

Line or cross	N	Mean($\bar{y}$)	V_P	V_{WF}	V_E
2	240	142.9	65.0	37.9	27.1
3	239	132.4	31.8	18.8	13.0
9	240	128.1	26.1	15.8	10.3
2 $\times$ 9	247	134.7	49.0	21.7	27.3
3 $\times$ 2	240	130.6	33.3	14.4	18.9
9 $\times$ 3	247	132.3	32.7	13.5	19.2
Inbred lines	719		41.0	24.1	16.9
Crosses	734		42.3	16.6	24.7
Total	1453		41.7	20.4	21.3

FIGURE 6.—The influence of temperature on the mean value in the base population (B), the stabilizing lines (S1 and S2) and on the lines under disruptive selection with disassortative mating (D-1 and D-2). The experiments on S1, D-1 and B were performed on G6 of Experiment 1, and on G30 and G32 of S2 and D-2 respectively.

seem to be quite different. But before discussing these changes in detail, it seems relevant to have a closer look on the phenotypic variance of the base population and the properties of the character used in these experiments.

The phenotypic variance is higher in the later determinations. The causes of this increase are not known, but they are relatively small when compared with the changes in the selection lines. Heritability values are obtained by three methods—from directional selection (two experiments), from progeny tests (three tests), and from a comparison of genetically homogeneous stocks with the base population (two experiments). Although the heritabilities obtained from the selection lines are somewhat lower than from the other tests, all results are in reasonable agreement. Genetical variance seems to make up more than 60% of the phenotypic variance, and of the nongenetic variance about 60% comes from real environmental variance and one third is from differences within flies. Comparison with homogeneous stocks indicates that the genetic variance of the base population is almost exclusively additive genetic variance. This shows that the residual variance in the progeny tests is—at least in the tests on the base population—a reliable estimate of the common environmental variance. Comparison of the values of inbred lines and crosses makes it clear that the heterozygosity does not affect the expression nor its variance. This is in agreement with earlier experiments on *ci^D* (SCHARLOO 1963) and with results of SHELDON *et al.* (1964) on scute.

These results have a wider significance in relation to theories which connect the present composition of the phenotypic variance of a character to selection pressures in the past (ROBERTSON 1955, 1966). He argued that a high heritability and a small nonadditive variance component must be expected in characters which were not subjected to selection. He gave convincing evidence for selective neutrality of abdominal and sternopleural chaeta number in *Drosophila* (CLAYTON *et al.* 1967; ROBERTSON 1966), characters in which the variability composition is very similar to the variability composition of *ci^{D-G}*.

However, MATHER (1966) maintains that sternopleural and abdominal bristle number in *Drosophila* obtained its genetical architecture under the influence of

stabilizing selection, although experimental evidence is not available. If this were true, one would expect a low additive genetic variance and perhaps canalization, which would express itself on a nonlinear scale.

The interruption of the fourth wing-vein which was used as a measurable character in our experiments, does not exist in normal flies. Because the progeny tests were performed immediately after the introduction of the mutant into the Pacific background, the genetical architecture of the character cannot be affected by a history of natural selection acting on this character. The large additive genetic variance component and the lack of a nonadditive component is precisely what one would expect on the basis of ROBERTSON's theory.

In earlier experiments (SCHARLOO 1964a b) it was found that the scale of expression of ci^{D-g} was approximately linear in a large part of its possible range. In our experiments with divergent directional selection this conclusion is vindicated, but in the range of lower expression ratio, change seems to become more difficult.

The base population and the stabilizing line have their frequency distributions completely in the linear range of the scale. In both disruptive lines the non-linearity of the scale probably had some influence in the later selection generations. Moreover, in the D^R2 line in its later generations, some of the ci^{D-g} flies possessed complete fourth veins. This will also lead to an underestimate of the phenotypic variance and will have prevented recognition and selection of the ci^{D-g} flies with the highest expression ratios. In both stabilizing lines the result is the same at the end of the experiment. The phenotypic variance decreased, and this is a consequence of a reduction of the genetic variance and the environmental variance to one quarter or one sixth of their original values, while the within-fly variance decreased only slightly. But S2 did not change its environmental variance until after G15. Additional support for the conclusion that stabilizing selection decreases environmental variability was obtained by comparing the phenotypic variance of three inbred lines from S1 and their crosses with the variance of inbred lines and crosses from our base population. The fact that similar values are found for inbreds and crosses emphasizes that the level of heterozygosity is not involved in the decrease of sensitivity to environmental factors.

The temperature experiments on the stabilizing lines suggest that the decrease of the environmental variance is not a consequence of a decrease of the sensitivity of development to environmental factors in general but to a decrease of sensitivity to specific factors in the environment during the selection experiment. This is in agreement with our earlier results (SCHARLOO 1964b).

Experiments with similar selection methods were done by THODAY (1959) and PROUT (1962), who found that stabilizing selection caused a decrease in genetic variance only.

Experiments on disruptive selection with disassortative mating were done by FALCONER and ROBERTSON (1956) on body weight in mice, by THODAY (1959) on sternopleural bristle number and by PROUT (1962) on duration of larval life in *Drosophila*. While the results of FALCONER and ROBERTSON were inconclusive,

THODAY found indications for increase of the genetical variance and the within-fly variance. PROUT reported an increase of the environmental variance.

In both our experiments, but most obviously in D-2, all variance components increased simultaneously. Moreover, the temperature experiment suggests that here, in contrast with the situation in the stabilizing line, the change of the environmental variance is an increase in the sensitivity of development to environmental factors in general.

Now the question arises how this difference between the stabilizing lines and the disruptive (D-) lines can be understood. These phenomena can be explained in terms of a model similar to that used to explain irregular changes in expression ratio and its variability in selection experiments on *ci^D* and Hairless (SCHARLOO 1962; SCHARLOO, HOOGMOED and VREEZEN 1966). This model implied that the developmental system of the fourth wing vein of *ci^D* consists of two components. One of the components is the concentration of a vein-inducing or vein-forming substance which is supposed to vary in a population according to a normal distribution. The second component is a gradient of competence of the wing-cells to react to the presence of the vein-forming substance with formation of vein material. This gradient of competence along the track of the fourth vein would decrease from wing base to wing tip. In *ci^D* the decrease is not linear and this would be responsible for the irregular advance and irregular variability patterns when selection is applied. In *ci^{D-G}* the relation between factors that change expression and the expression ratio is approximately linear over the larger part of the scale. Therefore it can be supposed that in *ci^{D-G}* the gradient of competence is linear (Figure 7).

According to this model change of phenotypic variance can occur in two ways:
(a) By a change of the variability of the concentration of the vein forming substance in the population. This variability could be changed by a decrease of the

FIGURE 7.—Model of the development of the fourth vein in *ci^{D-G}* and possible mechanisms for changing variability in expression. Further explanation in text.

environmental sensitivity of the processes forming the substance, by a change of the sensitivity of these processes for differences acting within flies, or by a decrease of the genetic variability of these processes. This could result in independent changes of the corresponding variance components. (b) By a change of the slope of the gradient of competence. This would change the effect of both kinds of nongenetic differences and of genetic differences simultaneously so that all three variance components would change.

It was shown in the S lines that the change in phenotypic variance was caused by a change of both genetic and environmental variance but that the within-fly variance did not change much. Therefore it seems probable that these changes in variance occurred independently. In both D⁻ lines, however, all variance components change simultaneously. This suggests that in this case the slope of the competence gradient has changed. If this were so, one would perhaps expect that all variance components would change by the same factor. But in view of the uncertainty inherent in the estimate of variance components and the possibility that the slope of the gradient changed only locally, this requirement cannot be taken too strictly.

Support for this hypothesis is found in the result of the temperature experiment and in the occurrence of extreme asymmetric individuals having one wing with a very high value and the other with a very low value. Further, in some inbred lines from the D⁻ line variability is still very large, with bimodal frequency distributions and the same extreme asymmetric flies. This points to non-linearity of the scale in this lines, a matter which will be pursued further.

Independent increase of the genetic variance component in the D⁻ selection could occur when genes, and especially dominant genes, move to intermediate frequencies. This process and independent increase of the within-fly component and the environmental variance will perhaps have contributed to some extent to the increase of the phenotypic variance. But the genetic situation in the D⁻ line is not a simple one. In the progeny tests even the highest pairs generate some low progeny and vice versa. The possibility cannot be excluded that here the residual variance in the progeny tests contains a component due to interaction and dominance.

In the D^{#2} line the response seems to be a simple one, at least until after the progeny test in G18. Until then the increase in phenotypic variance was a consequence of an increase in additive genetic variance. This is, what can be expected from this type of selection (THODAY 1965; WADDINGTON 1966; SCHARLOO DEN BOER and HOOGMOED 1967). The selected low individuals will probably predominantly descend from $l \times l$ matings and the high individuals from $h \times h$ matings. This type of selection is reported to promote reproductive isolation between the "low" and "high" flies (THODAY and GIBSON 1962). The appearance of bimodal frequency distributions suggested that isolation occurred in our line. But the similarity of the frequency distribution generated by "compulsory random" mating in G35 indicates that mating in the selection line was still at random. This result is in agreement with earlier experiments on *ci^{D-g}* (SCHARLOO 1964), on sternopleural chaetae number (SCHARLOO, DEN BOER and HOOGMOED

1967), on Bar expression (WADDINGTON and ROBERTSON 1966) and on survival on EDTA and normal medium (ROBERTSON 1966), which failed to establish isolation by disruptive selection.

In G6 the result of the progeny test and the shape of the frequency distribution suggest that genetic variation is still largely additive. This is confirmed by the fact that then extreme high and extreme low pairs in the progeny test were true breeding, and by the results of chromosomal substitution (SCHARLOO, unpublished). Although in G18 the frequency distribution is bimodal (which suggests the development of dominance), the residual variance in the progeny test is still at the same level as in the base population. But this has changed in later generations.

The bimodality becomes more extreme, asymmetry becomes very large and in the progeny test on G35 the residual variance has increased considerably. This is perhaps a consequence of a change similar to the change in the D-2 line in which a modification of the gradient of competence was presumed.

Both disruptive lines show bimodal frequency distributions—they are polymorphic. But the mechanisms involved seem to be different. The composition of the phenotypic variance is different. In the D^R line the difference between the two modes seems to be caused by a genetic switch mechanism consisting of one pair of complex effective factors with large effect on expression, localized on the second chromosome (SCHARLOO, unpublished). Preliminary results on the D- line, however, suggest that there the polymorphism is caused by a threshold mechanism in vein development which makes intermediate values improbable.

The model suggested here to explain simultaneous or independent changes of components of variance, has relevance both to problems of canalization and pattern formation (SCHARLOO, HOOGMOED and VREEZEN 1966). The hypothetical gradient of competence governs by its slope the magnitude of the phenotypic variability. This model suggests how stabilizing and disruptive selection can change both the sensitivity to genetic and nongenetic differences by the same mechanism in conformation to the theories of SCHMALHAUSEN (1949) and WADDINGTON (1957). Moreover, the gradient of competence can be compared with a prepattern in the sense of STERN (1954, 1965). The prepattern, which has only one dimension, is responsible for the appearance of the fourth vein interruption in a fixed pattern, i.e. as a terminal interruption. The change of the slope of the competence gradient postulated for the D- line, then, represents a case in which genetic factors have changed a prepattern which governs the level of canalization.

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SUMMARY

Divergent directional selection, stabilizing selection (S), disruptive selection with disassortative mating (D-) and disruptive selection with random mating (D^R) were practised on the expression of the mutant cubitus interruptus domi-

nant of Gloor (ci^{D-g}). Divergent directional selection changes the mean expression rapidly and, in contrast with earlier results on ci^D , overlap with wild type is easily obtained. Response to directional selection, progeny tests, and comparison of genetically homogeneous cultures with the base population showed that in the base population more than 60% of the phenotypic variance of expression is additive genetic variance. Nonadditive genetic variance is, if present, very small. Of the nongenetic variance, about 60% was real environmental variance and the rest was within-fly variance. Stabilizing selection decreases the phenotypic variance, involving a decrease in both the genetic and the environmental variance components. The decrease of the environmental variance seems not to be a consequence of a decreased sensitivity to environmental differences in general but only to differences to which the population was subjected during the selection. Disruptive selection with random mating caused predominantly an increase of the additive genetic variance; only in later generations did an increase of both other components occur. It did not cause reproductive isolation. In both the D^R and the D^- line the frequency distributions are bimodal in later generations. The mechanisms underlying this polymorphism are different. The change of the variance components—simultaneously or independently—were explained in terms of a model for the development of the fourth vein according to which vein formation would be dependent on the concentration of a vein-forming substance and a gradient of competence to react to the vein-forming substance along the tract of the fourth vein.

LITERATURE CITED

- CLAYTON, G. A., J. A. MORRIS and A. ROBERTSON, 1957 An experimental check on quantitative genetical theory. I. Short-term responses to selection. *J. Genet.* **55**: 131–151.
- FALCONER, D. S., 1960 *Introduction to Quantitative Genetics*. Oliver and Boyd, Edinburgh.
- FALCONER, D. S., and A. ROBERTSON, 1956 Selection for environmental variability of body size in mice. *Z. Ind. Abst. Vererb.* **87**: 385–391.
- KAMSHILOV, M. M., 1939 Selection as a factor altering the dependence of a character on variations in environmental conditions. *Compt. Rend. (Dokl.) Acad. Sci. U.R.S.S., N.S.* **23**: 326–365.
- MATHER, K., 1966 Variability and selection. *Proc. Royal Soc. London B* **164**: 328–340.
- PROUT, T., 1962 The effects of stabilizing selection on the time of development in *Drosophila melanogaster*. *Genet. Res.* **3**: 364–382. — 1963 The effects of a history of stabilizing selection on sensitivity to foreign environments. (Abstr.) *Proc. 11th Intern. Congr. Genet.* **1**: 165.
- RENDEL, J. M., and B. L. SHELDON, 1959 Selection for canalization of the scute genotype in *Drosophila melanogaster*. *Australian J. Biol. Sci.* **13**: 46–57.
- ROBERTSON, A., 1955 Selection in animals: synthesis. *Cold Spring Harbor Symp. Quant. Biol.* **20**: 225–229. — 1966 Artificial selection in plants and animals. *Proc. Royal Soc. London B* **164**: 341–350.
- ROBERTSON, F. W., 1960 Ecological genetics of growth in *Drosophila*. I. Body size and developmental time on different diets. *Genet. Res.* **1**: 288–304. — 1966 A test of sexual isolation in *Drosophila*. *Genet. Res.* **8**: 181–187.

- ROBERTSON, F. W., and E. C. R. REEVE, 1952 Studies in quantitative inheritance. I. The effects of selection of wing and thorax length in *Drosophila melanogaster*. J. Genet. **50**: 414-448.
- SCHARLOO, W., 1962 The effect of selection and temperature on a mutant character in *Drosophila*. Arch. Neerl. Zool. **14**: 433-511. — 1963 *ci^{D-G}*, cubitus interruptus dominant of Gloor. *Drosophila Inform. Serv.* **38**: 32. — 1963b Mutant expression in inbred lines and crosses. *Experientia* **19**: 405. — 1964a Mutant expression and canalization. *Nature* **203**: 1095-1096. — 1964b The effect of disruptive and stabilizing selection on a cubitus interruptus mutant in *Drosophila*. *Genetics* **50**: 553-562.
- SCHARLOO, W., M. S. HOOGMOED, and W. VREEZEN, 1966 Pattern formation and canalization. *Genen en Phaenen* **11**: 1-15.
- SCHARLOO, W., M. DEN BOER, and M. S. HOOGMOED, 1967 Disruptive selection on sternopleural chaetae number in *Drosophila melanogaster*. *Genet. Res.* **9**: 115-118.
- SCHMALHAUSEN, I. I., 1949 *Factors of Evolution*. Blakiston, Philadelphia.
- SHELDON, B. L., J. M. RENDEL, and D. E. FINLAY, 1964 The effect of homozygosity on developmental stability. *Genetics* **49**: 471-484.
- STERN, C., 1954 Genes and developmental patterns. Proc. 9th Intern. Congr. Genet. (Caryologia Suppl. Vol.) **1**: 355-369. — 1965 Entwicklung und die Genetik von Mustern. *Naturwissenschaften* **52**: 357-365.
- STURTEVANT, A. H., 1961 *spa^{pol}*, sparkling poliart. *Drosophila Inform. Serv.* **35**: 47.
- THODAY, J. M., 1959 Effects of disruptive selection. I. Genetic flexibility. *Heredity* **13**: 178-218. 1965 Effects of selection for genetic diversity. Proc. 11th Intern. Congr. Genet. **3**: 533-540.
- THODAY, J. M., and J. B. GIBSON, 1962 Isolation by disruptive selection. *Nature* **193**: 1164-1166.
- WADDINGTON, C. H., 1942 Canalization of development and the inheritance of acquired characters. *Nature* **150**: 563-565. — 1957 *The Strategy of the Genes*. Allen and Unwin, London. — 1960 Experiments on canalizing selection. *Genet. Res.* **1**: 140-150.
- WADDINGTON, C. H., and E. ROBERTSON, 1966 Selection for developmental canalization. *Genet. Res.* **7**: 303-312.