STABILIZING AND DISRUPTIVE SELECTION ON A MUTANT CHARACTER IN DROSOPHILA. III. POLYMORPHISM CAUSED BY A DEVELOPMENTAL SWITCH MECHANISM

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ARTIFICIAL disruptive selection practiced on the expression of the mutant cubitus interruptus dominant of Gloor (ci^{D-g}) produces polymorphism (SCHARLOO, HOOGMOED and TER KUILE 1967). Under two different systems of disruptive selection the frequency distributions of the length of the fourth wing vein became bimodal. In the line under disruptive selection with random mating (D^R) this was caused by a genetical switch mechanism (SCHARLOO 1970). In the line under disruptive selection with compulsory mating of opposite extremes (D^-) the mechanism was different. Genetic variance, environmental variance, and within-fly variance increased simultaneously. This was explained in terms of a model for the development of the fourth vein (SCHARLOO, HOOGMOED and TER KUILE 1967). But without invoking a genetical switch mechanism the discontinuity in the phenotypic frequency distribution can only be explained by a threshold mechanism in the development of the fourth vein. The presence of such a mechanism was suggested by the occurrence in the D^- line of many flies which had in one wing a very long vein and in the other a very short vein.

In this paper it will be shown that such a threshold mechanism is present, and an analysis is made of its genetic basis.

MATERIALS AND METHODS

The selection line used for the analysis was the D^- 2 line described in SCHARLOO, HOOGMOED and TER KUILE (1967). The character measured was the relative length of the fourth wing vein in the mutant ci^{D-g} , expressed as the ratio to the complete third vein in percent (expression ratio). Both wings of the flies were measured and the sum of the ratios of the two wings was used as the quantitative character. Within-fly variances were calculated as the means of the squares of the differences between the two wings. Frequency distributions, however, are based on the measurements of the left wing only. The flies were reared at 25°C if not mentioned otherwise. In temperature experiments flies were kept at 17.5, 20, 22.5, 25, 27.5, and 30°C. Temperatures were maintained with an accuracy of $\pm 0.5^\circ\text{C}$.

The methods used in the genetic analysis and other methodological details can be found in the accompanying paper (SCHARLOO 1970).

RESULTS

The effect of individual chromosomes: The analysis was started by crossing

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FIGURE 1.—The effects of individual second and third chromosomes (+) extracted from four males of the D^- line and introduced into the inbred stock $\gamma; bw; st p^p$. Mean relative vein lengths of these males:

●	48.8
○	51.6
×	185.1
+	168.5

The effects of second chromosomes were estimated as the mean of the difference between $\gamma; bw; st p^p$ and $\gamma; bw/+; st p^p$, and the difference between $\gamma; bw; st p^p/+$ and $\gamma; bw/+; st p^p/+$. The effects of third chromosomes were estimated as the mean of the difference between $\gamma; bw; st p^p$ and $\gamma; bw; st p^p/+$ and the difference between $\gamma; bw/+; st p^p$ and $\gamma; bw/+; st p^p/+$ flies. For these comparisons 20 ♀♀ and 20 ♂♂ of each genotype were measured.

two low males and two high males individually to females of the $\gamma; bw; st p^p$ tester stock. From the F_1 progeny of each male several males were crossed again individually to females of the tester stock. In their progeny the effects of the separate chromosomes could be estimated. Figure 1 shows that there are still genetic differences on both autosomes in the D^- line. But there is no large discontinuity as in the D^a line (SCHARLOO 1970). Moreover, chromosomes with high effects are found in low flies and *vice versa*. The genetic differences do not determine whether a fly will have a low or high expression ratio: the polymorphism is not caused by a genetical switch mechanism on the second or third chromosome.

Also the X chromosomes do not carry large genetic differences. Reciprocal crosses between progeny of extreme flies of the D^- line in G6 did not reveal sex-linked effects (Table 1). In G18 reciprocal crosses were made between low and high extremes in four single-pair matings. In the progeny of each pair low and high flies appeared again in both sexes. From the progeny in each culture again four single-pair matings were made in the same way. In the second generation progeny extreme flies still appeared in both sexes.

TABLE 1

Mean values of the F_1 of reciprocal crosses between progeny of two extreme pairs in the progeny test of the D⁻ line in G6†

Midparent value of the low pair 55.9, midparent value of the high pair 155.0.

	Females	Males
High parents	137.4	147.2
Low parents	91.5	84.7
F_1 H $\times$ L	110.4 $\pm$ 3.4	103.4 $\pm$ 4.4
F_1 L $\times$ H	110.7 $\pm$ 4.9	107.6 $\pm$ 5.1

† cf. SCHARLOO, HOOGMOED and TER KUILE (1967).

Temperature experiments: The expression of ci^{D-g} is dependent on temperature. At lower temperatures the fourth vein becomes complete. The relation between temperature and the relative length of the fourth vein and the shape of the frequency distributions reveal the properties of the scale of our quantitative character. In the base population and in the stabilizing lines the scale of the relative vein length (expression ratio) is linear over a large range of the possible values (SCHARLOO 1964 a, 1964 b; SCHARLOO, HOOGMOED and TER KUILE 1967; SCHARLOO, ZWEEP, SCHUITEMA and WIJNSTRA, in preparation).

In the first experiment a stabilizing line (S2, see SCHARLOO *et al.* 1967) and the D⁻ line were compared; in a second experiment the S line and an inbred line obtained from the D⁻ line by full-sib mating during 15 generations were compared. Prior to these tests in all stocks the ci^{D-g} fourth chromosome was balanced over spa^{cat} to avoid difficulties of discriminating ci^{D-g} from wild-type flies when the fourth vein becomes complete at lower temperatures. No genetic material other than the spa^{cat} chromosome was introduced (for crossing scheme see SCHARLOO 1962).

In both experiments the response curve of the S line is almost linear: the frequency distributions are unimodal and have a relatively low variability (Figure 2), and the within-fly contribution is low and almost constant at all temperatures used (Figure 3). The D⁻ line and the D⁻ inbred are more sensitive to temperature than the S line. At high (27.5°) and at low temperatures (17.5°, 20°) the frequency distributions are unimodal with low variability. But at intermediate temperatures (22.5°, 25°) the distributions show large variability or are clear-cut bimodal, in particular in the D⁻ inbred. In the S line the mode of the unimodal distribution coincides in several cases with the 70-value, while in both D⁻ stocks values in this region occur only rarely.

The within-fly variance in the D⁻ stocks is high when variability is high, that is when bimodal distributions are present. At 22.5° and 25° the within-fly variance of the D⁻ inbred is more than ten times higher than at lower and higher temperatures. When the within-fly variance is high, extremely asymmetrical flies which have one wing with a very long fourth vein and the other with a very short vein are numerous.

It must be concluded that in the D⁻ line the development of the fourth vein is

FIGURE 2.—Frequency distributions of the relative length of left wing vein of S and D⁻ inbred (left), and of S and D⁻ (right) at different temperatures (in °C). Unbroken lines indicate the D⁻ stocks, broken lines the S stock. Mean values indicated on the abscissa by ○ (S) and ● (D⁻). Each distribution in the left half of the figure is based on 160 flies, in the right half on 80 flies.

WITHIN-FLY VARIANCE

FIGURE 3.—The within-fly variance at different temperatures (°C) in the S line (two experiments), and in the D⁻ inbred and D⁻ lines.

changed. The initially linear relation between factors that affect vein length and the relative vein length is now nonlinear. A threshold-like situation had arisen in which small genetic or nongenetic differences can determine whether the vein length becomes low or high. Intermediate values are improbable.

Genetic analysis: The genetic basis of the threshold-like change in the development of the fourth vein in the D⁻ line was investigated by interchange of the large chromosomes between the D⁻ inbred and the S line (crossing scheme in SCHARLOO 1970). The ensuing lines were reared at different temperatures. Frequency distributions of the measurements on one wing are given for the females in Figure 4. The same variability pattern was found in males. Comparison of these distributions with the distributions of the D⁻ inbred and the S line reveals that only the combination S S D⁻ shows no bimodality. All other combinations show more or less bimodal distributions at one or more temperatures. But bimodality is not found at the same temperatures in the different genotypes. If present, the saddle of the distribution is always found around the expression ratio of 70 (measured on one wing). Bimodality is found in S D⁻ S at 20° and D⁻ S D⁻ at 27.5°C.

The within-fly variances of the combinations are high when bimodality occurs (Figure 5). Only the combination S D⁻ S shows a rather low value, although

FIGURES 4A, 4B, and 4C.—Frequency distributions of the relative length of the left wing vein in homozygous combinations of chromosomes 1, 2, and 3 from the S line and D⁻ inbred line. Each distribution based on 60 ♀ ♀ with the exception of the D⁻ S D⁻ distribution at 22.5°C where only 30 ♀ ♀ could be measured.

even here the highest asymmetry is found at 20° when bimodality occurs.

Both the first and the second chromosome of the D⁻ inbred contribute to the threshold-like change in the development of the fourth vein. In fact they cause bimodality even when acting alone. In S S D⁻, in which no bimodality was revealed, the within-fly variance is higher than in the S line at most temperatures. Probably the third chromosome too has some effect on the developmental system of the fourth vein.

DISCUSSION

In the D⁻ line the polymorphism is controlled by a threshold mechanism in the development of the fourth wing vein. There is still a fairly large amount of

FIGURE 4B.

genetic variability (SCHARLOO, HOOGMOED and TER KUILE 1967). This certainly contributes to the phenotypic variability. The variability in the D^- inbred is smaller than in the D^- selection line. This can be explained as a consequence of inbreeding. Moreover, the peaks of the D^- inbred are closer together than in the D^- selection line (compare Figure 2 in the present paper with Figure 5 in SCHARLOO *et al.* 1967). This seems puzzling. It could be that in the D^- selection line a dominant gene is present which contributes to the extent of the threshold region. This gene may not have been fixed in the selection line when inbreeding started and could have been eliminated in the particular inbred line used in our analysis.

The analysis of reciprocal crosses and of the effect of individual chromosomes from extreme flies shows that the genetic differences cannot be separated into two groups as in the D^R line. Moreover, they are not decisive in the determination of vein length. When such a threshold-like development is fixed in the

FIGURE 4C.

genotype, polymorphism is possible even without the presence of genetic variability. When the mean relative vein length is located in the threshold region small environmental differences will be sufficient to tip the balance either way, i.e., high or low.

A similar threshold-like development of the fourth wing vein is present in the original ci^D (cubitus interruptus dominant) mutant without practicing disruptive selection (SCHARLOO 1962). It is perhaps relevant that there too the saddle of the bimodal distribution was always found around the 70-value (measured on one wing). In ci^D it was shown conclusively that the threshold process was connected with a definite position in the pattern of wing venation (SCHARLOO 1962). When the chromosome combinations of the D^- inbred and the S line were reared at different temperatures the combinations did not show bimodal distributions at the same temperatures. But the saddle of the distribution was always around the 70-value. This suggests that in ci^{D-g} in the D^- inbred the

FIGURE 5.—The within-fly variance in homozygous combinations of the large chromosomes from S and D⁻ inbred lines.

threshold process is bound to a fixed region in the fourth vein. In *ci^{D-g}* it was generated from an originally linear scale when the D⁻ selection accumulated the appropriate genes which were located on several chromosomes. But the similarity with *ci^D* suggests perhaps that properties of the development of the fourth vein predetermine the region in which the threshold process appears in *ci^{D-g}*. Therefore it will be possibly more difficult to obtain the same result in other characters or even at other positions along the fourth wing vein.

Nevertheless, what is achieved in the D⁻ line is a real change of the general canalization of the character. The effects of all kinds of factors affecting expression—genetic factors, environmental factors and factors acting within flies—are changed by this type of disruptive selection. This is in contrast to the results of WADDINGTON (1960), KINDRED (1965), DRUGER (1967) and to the situation in our stabilizing lines (SCHARLOO 1964 b; SCHARLOO, HOOGMOED and TER KUILE 1967) where only a change of sensitivity towards specific factors was obtained. Only RENDEL and his associates (RENDEL and SHELDON 1960; RENDEL, SHELDON and FINLAY 1966) obtained a change in sensitivity to temperature differences and a change of variability at the same time. They applied simultaneously selection against variability within families and selection for two scutellar bristles. This result was explained according to a model for the determination of scutellar

bristle number (RENDEL, SHELDON and FINLAY 1965; RENDEL 1968). They assume that the scute locus is the key gene (major gene in RENDEL's terminology) for the development of scutellar bristles. A main argument seems to be that differences in bristle number caused by differences at the scute locus are larger than variability caused by the modifiers which happened to be present in the stock in which the scute mutant was introduced. This idea seems to repeat the error of classical genetics in equating mutant genes with characters.

Canalization of scutellar bristle number would be brought about by control of the major gene on the scute locus. Control would be caused by the developmental system as a whole, i.e., the potential total bristle number. The model is based on the model of JACOB and MONOD for regulation of gene action in unicellular organisms. However, it is not clear what is meant by control of the action of a gene in a multicellular animal, when the character involved is formed by the cooperation of many cells, each with its own set of genes. It is not only the number of bristles that is highly canalized but the normal number of four is present in a constant pattern. This aspect is emphasized in the work of STERN (1954; 1965) in his analysis of the formation of bristle patterns in mutants with the help of genetic mosaics. He concluded that the formation of a bristle depends on the presence of a prepatter that specifies the bristle position and the presence of cells in the hypoderm competent to react to the prepatter by bristle formation. SCOWCROFT, GREEN and LATTER (1968) have shown that the pattern cannot be ignored when analyzing canalization of scutellar bristle number. A satisfactory model for the development of scutellar bristles has to take in account both aspects of number and pattern. This was done by MAYNARD SMITH and SONDHI (MAYNARD SMITH 1960; MAYNARD SMITH and SONDHI 1961) in their work on the pattern of ocelli and chaetae on the head in the mutant ocelliless.

Both aspects are incorporated in a model for the development of the fourth vein in *ci^D* and *ci^{D-g}* (SCHARLOO 1962; SCHARLOO, HOOGMOED and TER KUILE 1967). In this model (Figure 6, B) it is assumed that formation of the fourth vein is governed by 1) the concentration of a vein-forming substance in the wing, which varies in the population according to a normal distribution. 2) a gradient of competence of the wing cells to react to the vein-forming substance with formation of vein material. Because this gradient along the track of the fourth vein decreases from wing-base to wing-tip it determines 1) the pattern of vein formation, i.e., that the interruption appears always at the wing-tip end of the vein, 2) the variability of the interruption by its slope. The effect of all factors acting on the level of the vein-forming substance will be changed simultaneously when the slope of the gradient is altered.

The changes produced by the different systems of selection described in the first paper of this series (SCHARLOO *et al.* 1967) can now be considered in terms of this model. In the base population the frequency distribution of the vein-forming substance is unimodal and the gradient of competence is linear. In the stabilizing line and in the D^R line the gradient is not changed. In the S line the genetic and environmental variance of the vein-forming substance decreased. In

FIGURE 6.—Model for the changes in the developmental system of the fourth longitudinal wing vein caused by disruptive and stabilizing selection on *D. melanogaster* (see text).

the D^R line the genetical switch mechanism causes a bimodal frequency distribution of the vein-forming substance. In the D⁻ line the simultaneous increase in all components of variance, the increased sensitivity to temperature, and the bimodal frequency distributions can be explained by assuming an unaltered distribution of the vein-forming substance and an increase in the slope of the competence gradient. When this is a strong increase in a limited part of the gradient it can also explain the threshold mechanism in the development of the fourth vein and its association with a particular region of expression values.

The question arises whether the results obtained in our selection experiments (SCHARLOO 1964; SCHARLOO *et al.* 1967) may be generalized. Would we obtain the same type of result with the same types of selection practiced on this or another character?

The three replicates of the stabilizing selection invariably gave the same result: a decrease of the genetic variance and a decrease of the environmental variance. The decrease of the environmental variance reflects only a decrease of sensitivity to the special environmental factors acting during the selection. No change of general canalization was obtained.

In both D^R replicates the increase in phenotypic variance was caused by an increase in genetic variance.

In both D⁻ replicates all variance components increased simultaneously as a consequence of a change of the general canalization of the character.

The results obtained by each mode of selection are consistent for this character. But experiments on body size with the same types of selection (Bos 1969) gave different results.

The phenomena in the D⁻ line have relevance to the problem of the significance of asymmetry as a measure of developmental homeostasis. MATHER (1953) and THODAY (1958) suggested that asymmetry for sternopleural chaetae number reflects the general developmental homeostasis of the individual flies. WADDINGTON (1960), however, showed that successful selection against temperature sensitivity of the expression of the mutant Bar did not affect asymmetry. A similar result was obtained in some of our experiments. In the stabilizing lines the environmental variance decreased without an appreciable change of the within-fly variance.

In our D-lines, however, sensitivity to genetic and environmental differences and to differences acting within flies is changed simultaneously. A change of general canalization of the character has occurred. But only one character is involved and only in a limited range of expression.

Even if such a phenomenon as general developmental homeostasis exists and is reflected in asymmetry, a difference in asymmetry does not necessarily indicate a change of general developmental homeostasis.

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SUMMARY

Disruptive selection with compulsory mating of opposite extremes (D⁻) practiced on the expression of the mutant cubitus interruptus dominant of GLOOR (*ci^{D-g}*) of *Drosophila melanogaster*, caused the appearance of bimodal distributions (polymorphism) of the relative length of the fourth wing vein. Genetic analysis showed that, in contrast to the situation in a line obtained by disruptive selection with random mating, a genetical switch mechanism was not present. Temperature experiments revealed a threshold mechanism in the development of the fourth vein. Interchange of chromosomes between an inbred line derived from the D⁻ selection and a stabilizing selection line showed that the non-linearity of the scale was polygenically determined. The changes obtained by disruptive and stabilizing selection are discussed in terms of a model for the development of the fourth vein in *ci^{D-g}*.

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