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

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THE occurrence of two or more distinct phenotypes within a population is an important device for adaptation to a heterogeneous environment. These differences can be found on different phenotypic levels, e.g., enzymes or morphological characters. Morphological characters are under the control of many genes. Discontinuity in the variability of a morphological character can only be brought about by some kind of switch mechanism that steers development into one of alternative pathways. Much attention has been given to genetical switch mechanisms (FORD 1965) involving genes (or supergenes) with large effects on some character. In addition, the possibility exists that a switch mechanism is a property of the developmental system of the character. A threshold mechanism can determine that minor genetical or environmental differences are sufficient to cause the appearance of alternative phenotypes.

MATHER (1955) suggested that disruptive selection could generate polymorphism. In the first paper of this series (SCHARLOO, HOOGMOED and TER KUILE 1967) it was shown that artificial disruptive selection causes polymorphism. Under this type of selection the initially approximately normal distribution of the length of the fourth longitudinal wing vein of the mutant cubitus interruptus dominant of GLOOR became changed into a distribution with two modes which were clearly separated. But the mechanisms by which this happened were different under the two systems of disruptive selection, viz., disruptive selection with random mating of the selected flies (D^R) versus disruptive selection with compulsory mating of opposite extremes (D^-). In the D^- line the increase in phenotypic variance was caused by an increase of all variance components, viz., genetic variance, environmental variance, and within-fly variance. In the accompanying paper (SCHARLOO 1970) it is shown that the polymorphism in the D^- line was produced by a developmental switch mechanism.

In contrast, the increase in phenotypic variance in the D^R selection line was caused by an increase in genetic variance only. The difference between the extreme flies of the D^R line must therefore be a genetic difference. The genetic analysis presented here shows that the polymorphism was governed by a genetical switch mechanism located on the second chromosome.

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TABLE 1
Whole-chromosome interchanges¹ among *D. melanogaster* previously subjected to disruptive selection² for extreme expressions of a quantitative trait³

	Chromosomes							
	1	2	3	4	1	2	3	4
a.	L/L	L/L	L/L	Ci/L	X/Y	SM5/Bla	TM3/Sb	+/+
b1.	L/L	L/L	L/L	Ci/L	L/Y	L/SM5	L/Sb	Ci/+
b2.	L/L	L/L	L/L	Ci/L	L/Y	L/Bla	L/TM3	Ci/+
b3.	L/L	L/L	L/L	Ci/L	L/Y	L/Bla	L/Sb	Ci/+
b4.	L/L	L/L	L/L	Ci/L	L/Y	L/SM5	L/TM3	Ci/+
c1.	L/L	L/SM5	L/Sb	Ci/+	X	H/H	H/H	Ci/H
c2.	L/L	L/Bla	L/TM3	Ci/+	X	H/H	H/H	Ci/H
c3.	L/L	L/Bla	L/Sb	Ci/+	X	H/H	H/H	Ci/H
c4.	L/L	L/L	L/L	Ci/L	X	L/SM5	L/TM3	Ci/+
d1.	L/L	L/L	L/TM3	Ci/+	X	SM5/H	Sb/H	Ci/+
d2.	L/L	L/SM5	L/L	Ci/+	X	Bla/H	TM3/H	Ci/+
d3.	L/L	L/SM5	L/TM3	Ci/+	X	Bla/H	Sb/H	Ci/+
e1.	L/L	L/SM5	TM3/H	Ci/+	inter se →	L/L	H/H	
e2.	L/L	SM5/H	L/TM3	Ci/+	inter se →	H/H	L/L	
e3.	L/L	SM5/H	TM3/H	Ci/+	inter se →	H/H	H/H	

¹ Trait—relative length of both fourth longitudinal wing veins.

² Selection lines heterozygous for the mutation cubitus interruptus dominant of Gloor (here abbreviated to Ci).

³ Following an analogous scheme the other three new homozygous combinations (HHL, HLH, and HLL) can be obtained.

MATERIALS AND METHODS

The analysis presented here was performed on the D^R2 line (SCHARLOO, HOOGMOED and TER KUILE 1967) at various stages in the selection process. This line was heterozygous for the mutant cubitus interruptus dominant of GLOOR (*ci^{D-G}* abbreviated to *Ci* in Table 1). One effect of *ci^{D-G}* is an interruption of the normally continuous fourth longitudinal wing vein (cf. SCHARLOO, HOOGMOED and TER KUILE 1967). The length of the interrupted fourth longitudinal vein was measured relative to the length of the complete third longitudinal vein on the same wing. The sum of relative lengths for both wings of a fly, expressed in percent, is called the *expression ratio*. Expression ratios were used as the quantitative character subjected to artificial selection of various sorts (D^R, D⁻, and S). Mean expression ratios and their frequency distributions were used as measures of response to selection. The purpose of the analysis presented here was to localize the genetic basis for response.

Interchange of chromosomes was performed according to the scheme outlined in Table 1 in which L and H indicate chromosomes from flies with low and high expression ratios. In the analyzer stock one inverted chromosome is *SM5* (Second Multiple 5) with the dominant marker Curly (*Cy*); its homologue carries Blade (*Bla*). One inverted third chromosome is *TM3* (Third Multiple 3) with the dominant marker Serrate (*Se*); its homologue contains dominant Stubble (*Sb*). For more detailed description of the above mutations and others to be mentioned below, see LINDSLEY and GRELL (1968). Both *SM5* and *TM3* are reliable suppressors of recombination when present alone. But when inversions are present in other chromosomes, *TM3* is not completely reliable. In Table 1, only the (e) crosses, where *SM5* and *TM3* are both present in the same female, would allow exchange of genetic material of the marked analyzer chromosomes with that of the unmarked test chromosomes (H,L) from the D^R selection line. The scheme in Table 1 avoids use of unreliable crossover suppressors for the X chromosome. The small fourth chromosome was ignored.

The flies were reared in 1/8 liter creamers on a medium composed of water (1000 ml), agar (19 g), sugar (54 g), dried yeast (32 g) and Nipagine (1.5 g) at a temperature of 25 ± 0.5°C.

RESULTS

Genetic analysis—Chromosome substitution and crosses between extreme flies: Extreme low females (L) and extreme high females (H) of the D^R line were crossed to males from a stock with the dominant markers *Pm* and *Sb*. F₁ males with both dominants were backcrossed to the appropriate extreme females of the next generation. At the same time when extreme females were crossed to males with dominant markers, L males and H males were crossed to a stock with recessive markers on the three large chromosomes (*γ*; *bw*; *st p^p*). F₁ males were backcrossed to the *γ*; *bw*; *st p^p* females. The chromosome combinations generated by these crosses and their mean relative vein lengths are given in Figure 1.

The first experiment was started with flies from generation 6 (G6) of the D^R line. At this time bimodality had not yet occurred, but the phenotypic variance was already three times the original value and the heritability had increased from 0.62 to 0.90 (see SCHARLOO, HOOGMOED and TER KUILE 1967).

In the test for recessive effects, introduction of marked second chromosomes (*Pm*) had a considerable effect in both H and L genotypes. Introduction of marked third chromosomes (*Sb*) had a significant effect in high genotypes only. This was confirmed in a test for dominant effects by the introduction of chromosomes from extreme flies in the stock with recessive markers. Both large autosomes were involved in generating extreme high flies, but second chromosomes

FIGURE 1.—Mean relative length of both fourth wing veins (expression ratio) for chromosomal combinations obtained by substituting chromosomes with dominant markers into extreme (H vs. L) genotypes of the D^R selection line, by substituting H and L chromosomes into an inbred stock with recessive markers, and by reciprocal crosses to yield unmarked heterozygotes for extreme genotypes. The columns indicate the deviation of each chromosomal combination from the original genotype.

only were involved in the extreme low phenotypes. Recessive and dominant effects had approximately the same magnitude. This agrees with the results of reciprocal crosses between H and L flies: the F_1 mean values were approximately half way between the parental means.

The absence of a difference between the means of the F_1 males shows that sex-linked factors are not involved.

A second test involved flies of generation 25 (G_{25}) of the D^R selection line. At this time bimodality had been present for ten generations. The differences between extreme flies were larger than in G_6 . Introduction of dominant markers showed that the effect of second chromosomes had increased in both H and L genotypes. Third chromosome effects were now (G_{25}) of minor importance. Introducing H and L chromosomes into the recessive marker stock confirmed this result. Almost the entire difference between low and high expressions seemed to be caused by genes on the second chromosome. The recessive effects of high second chromosomes were larger than the dominant effects. The dominant effects of low second chromosomes seemed to have increased relatively more than the recessive effects. This agrees with the mean expression ratios of the cross between L and H which had shifted to lower values.

Exchange of chromosomes: A further test was made by exchanging H and L second chromosomes from the D^R line and by exchanging these with second chromosomes from the stabilizing selection line S2 (SCHARLOO, HOOGMOED and TER KUILE 1967). The results are given in Figure 2. A pair of L second chro-

FIGURE 2.—Expression ratios for homozygous combinations of chromosomes 1, 2, and 3 from H and L genotypes of the D^R and S selection lines. Each mean is based on measurement of both wing veins of at least 40 females and 40 males.

somes is sufficient to obtain full L expression in both H and S backgrounds ($HLH \approx SLS$). A pair of H second chromosomes gives normal H expression in an L genetic background (LHL). Moreover a pair of S second chromosomes causes the same expression in both H and L backgrounds ($HSH \approx LSL$). Therefore expression differences within the D^R line are determined almost completely by second chromosome differences.

Frequency distribution of expression ratios: During the first ten generations of D^R selection, the range of expressions increased and the distribution became flatter. In G6, high and low extremes were true breeding. The intermediate flies were at least partly heterozygous, segregating for high and low expression ratios.

After G15 the frequency distributions were clear-cut bimodal. This means that heterozygotes had a phenotype similar to either high or low homozygotes or that no heterozygotes were formed. In G36 extra samples were measured from

FIGURE 3.—Frequency distributions of expression ratios of the D^R line in G36. The upper distribution is obtained by the mating system which is normal for this line (L and H in the same bottle). The lower figure is obtained by combining results of separate $L \times L$, $L \times H$, $H \times L$, and $H \times H$ crosses. The contribution of $H \times H$ is indicated in black; the contribution of $L \times L$ is hatched and the joint contributions from $L \times H$ and $H \times L$ are white. Each distribution is based on measuring 320 flies.

four cultures of the D^R line. Extreme flies were selected in the normal way. But instead of mating all selected flies together in one bottle, matings $L \times L$, $L \times H$, $H \times L$ and $H \times H$ were performed in separate bottles. All crosses were duplicated. From each bottle 40 females and 40 males were measured. In Figure 3 all the measurements were used to construct a single frequency distribution but the contributions from the three different types of mating are indicated as hatched, white, or black, respectively. ($L \times H$ and $H \times L$ reckoned as the same type of mating.) The form of the combined frequency distribution of expressions is almost identical to that obtained for the D^R selection line, which shows that mating was still at random in that line. Descendants of $L \times L$ matings contribute only to the peak of the distribution at low values and descendants of $H \times H$ matings contribute mainly to the high peak. The parents and their progeny are obviously homozygous for the "low" or "high" second chromosomes, respectively, which were revealed in the substitution and exchange experiments.

The majority of descendants of $H \times L$ and $L \times H$ matings contributed to the mode at low values. But a minority of the progeny of these matings contributed to the mode at high values. The exceptional progeny of these matings could be a consequence of increasing dominance of L second chromosomes. In G36 dominance probably had increased to such an extent that some of the selected low flies were heterozygous L/H instead of L/L on chromosome 2.

In later generations of the D^R line the same kind of threshold mechanism for fourth vein development occurred as in the D^- line (SCHARLOO 1970). This was revealed by the large increase in within-fly variance in the D^R line after G28 and the occurrence of asymmetrical flies having a short fourth vein in one wing and a long vein in the other wing. Small nongenetic differences could be sufficient to push heterozygous L/H flies over the threshold to far higher values than would be expected on a linear scale. Some homozygous H/H flies could then fall to relatively low values below the threshold.

Effects of individual chromosomes: Chromosomes were extracted from both peaks of the distribution of D^R at G36 by crossing individual males to several females of the γ ; bw ; $st p^p$ stock. From the progeny of each male several males were crossed individually to γ ; bw ; $st p^p$ females. From each of the four classes of progeny 20 females and 20 males were measured. Dominant effects of individual second and third chromosomes (+) from the D^R selection line could then be estimated. The effects of second chromosomes were estimated as the mean of the difference between γ ; bw ; $st p^p$ and γ ; $bw/+$; $st p^p$ and the difference between γ ; bw ; $st p^p/+$ and γ ; $bw/+$; $st p^p/+$. Estimates of third chromosome effects were made by taking the mean of the difference between γ ; bw ; $st p^p$ and γ ; bw ; $st p^p/+$ and the difference between γ ; $bw/+$; $st p^p$ and γ ; $bw/+$; $st p^p/+$ (Figure 4).

The second chromosome effects are neatly separated into two groups. The flies from the low peak in the distribution were of two easily distinguishable types. The two lowest flies (measurements 36.0 and 39.6) had a very short fourth wing vein and no second crossvein. These flies had two second chromosomes which shorten the fourth vein in γ ; bw ; $st p^p$. They were homozygous for a factor on the second chromosome which causes interruption of the fourth vein and absence

FIGURE 4.—Each symbol indicates the effects of introducing a second and third chromosome from six males of the D^R line into the γ ; bw ; $st\ p^p$ inbred line. Expression ratios of these males:

●	36.0
+	39.6
□	48.2
×	52.8
△	177.5
○	178.0

of the second crossvein, even in the absence of ci^{D-g} (SCHARLOO 1968 and unpublished). This factor is allelic to crossveinless-2 ($cv-2$, 2—96.2). The other two low flies (relative vein length 48.2 and 52.8) were heterozygous for a chromosome of this type and a chromosome which increases vein length in the γ ; bw ; $st\ p^p$ stock. The two high flies (175.5, 178.0) possessed two second chromosomes which increase vein length and cause extra venation. This effect is allelic to plexus (px , 2—100.5). These results confirm the interpretation of the forced “random” mating test: In the D^R selection line also, flies which possessed one or two L second chromosomes contribute to the low peak of the distribution while flies from the high peak were homozygous for H second chromosomes.

DISCUSSION

The polymorphism in the D^R line is governed by a genetical switch mechanism on the second chromosome. Large differences on the second chromosome were present quite early (G6) in the selection experiment. But at that time differences on the third chromosome were still important. In later generations the differences between the extreme flies were concentrated completely in the second chromosome. At the same time the effect of the second chromosome differences became larger and dominance of the low chromosomes developed. Interchange

of the other D^R chromosomes for chromosomes of the stabilizing line did not influence the effect of the second chromosome differences. This suggests that the increased effect and development of dominance are a consequence of changes internal to the second chromosome and not changes in the genetic background. It seems that the third chromosome effects were lost when the differences on the second chromosome could do the job alone.

In the later stages of selection a change in the development of the fourth wing vein occurred. This is revealed by the rising asymmetry after G28 and the increase of the environmental variance in the progeny test at G36 (SCHARLOO, HOOGMOED and TER KUILE 1967). A threshold mechanism in the fourth vein, similar to that in the D^- line (SCHARLOO 1970), seems to be fixed in the D^R line. Together with the genetical switch mechanism it contributes to the establishment of an extreme polymorphism. This cooperation of a genetical and developmental switch mechanism is, of course, what will be often present in natural polymorphisms, e.g., sex and mimicry.

Genetic differences on the second chromosome were also responsible for the increased variation of our first D^R line (SCHARLOO 1964). In that rather short experiment (selection during 13 generations) bimodality was not obtained, but the frequency distributions in G7 and G13 showed a remarkable similarity with the distribution in the earlier generations of D^{R2} . This suggests that the results obtained with this type of selection have a more general significance. It can be concluded that disruptive selection with random mating causes in the first place an increase of the additive genetic variance. When the genetic differences available for this purpose are exhausted, other possibilities for increasing phenotypic variance are used—in our D^{R2} line fixation of the genes causing a developmental switch mechanism.

The systems of selection that come closest to ours are those used by THODAY and GIBSON (1962), SCHARLOO, DEN BOER and HOOGMOED (1967), CHABORA (1968), and BARKER and CUMMINS (1969) selecting for sternopleural chaetae number. Low and high females had equal opportunity to mate with low and high males, but after elimination of the males, they were transferred to separate bottles. Although only THODAY and GIBSON obtained sexual isolation, the other authors established divergence between the low and high parts of their experiments. But in these cases no genetic analysis was performed.

An accurate genetic analysis was done in THODAY's experiments (THODAY and BOAM 1959; GIBSON and THODAY 1962; WOLSTENHOLME and THODAY 1963) again with selection on sternopleural chaetae number. But the selection system differed in important respects. Not only were there separate low and high parts, but they were not formed by random mating of the extreme flies selected from the experiment as a whole. Low females selected from the low part were mated to low males from the high part to form the low part in the next generation. High females selected from the high part were mated to high males from the low part to form the high part. There is 50% gene flow between the high and low parts, but it is a *selected* gene flow. In this D^+ line no phenotypic discontinuity arose. But THODAY and BOAM claim that their line was

formally polymorphic because the genetic factors responsible for the difference in mean chaetae number between the low and high parts are maintained in high frequency by selection. In contrast to both our D^R lines, their factors were located on the two large autosomes. Because the effects of separate factors were quite small and the second chromosome factors were arranged in repulsion as a consequence of their extreme viability interactions, these factors could have been present unnoticed in the base population of THODAY's experiments.

The genetical switch mechanism present in our D^R line could not have been present as such in the base population. Besides large effects on vein length in *ci^{D-g}*, the low chromosome causes interruption of the crossvein and the fourth vein, and the high chromosome causes extensive extra venation even in the absence of *ci^{D-g}*. The high chromosome effect is allelic to *px* and the low chromosome effect to *cv-2*. These factors are four map units apart. There are strong indications that the factor on the low chromosomes is complex: the fourth vein effect is separable from the crossvein effect.

However, a preliminary analysis indicates that at least some of the wild-type generating second chromosomes carry both the factor which interrupts the fourth vein and a plexus allele. The plexus allele suppresses the vein defects of the mutant interrupting the fourth vein which in turn suppresses the extra venation caused by plexus.

It seems relevant that in four experiments from different base populations selected for long fourth vein in *ci^D*, plexus alleles did show up (SCHARLOO 1962 and unpublished). Similar mechanisms could be involved in other selection experiments in which there appeared major mutants which affected the selected character (WADDINGTON 1956; FRASER 1963, 1967).

The concentration of genes involved in the polymorphism into a small part of one chromosome segment suggests a comparison with the supergenes involved in the well known cases of polymorphism in snails and butterflies (reviewed by FORD 1965). CLARKE and SHEPPARD (1960) argued that when a polymorphism is governed by a genetical switch mechanism, disruptive selection will bring about dominance of one of the factors involved.

In our experiments such a situation was created artificially. Disruptive selection first generated gene combinations (supergenes) having a large effect and then the selection pressure produced dominance of the complex factors.

SUMMARY

Disruptive selection on the relative length of the fourth wing vein in the mutant cubitus interruptus dominant of GLOOR in *Drosophila melanogaster* caused a clear-cut morphological polymorphism. This polymorphism was governed by a genetical switch mechanism. Disruptive selection first built up gene combinations with large effects in a part of the second chromosome four map units long. Alleles of *cv-2* and *px* were involved. In later generations the second chromosome effects became larger and one of the complex factors became dominant. This caused the appearance of a bimodal frequency distribution of wing

vein length. In the last stage this genetical switch mechanism was reinforced by a developmental switch mechanism, viz., a threshold mechanism affecting the development of the fourth vein.

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