THE EFFECTS OF DISRUPTIVE AND STABILIZING SELECTION ON BODY SIZE IN *DROSOPHILA MELANOGASTER*. III. GENETIC ANALYSIS OF TWO LINES WITH DIFFERENT REACTIONS TO DISRUPTIVE SELECTION WITH MATING OF OPPOSITE EXTREMES

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A genetic analysis was made of two lines which when subjected to disruptive selection with compulsory mating of opposite extremes (D^-) showed a different response viz. one, D^-1 , showing predominantly an increase of environmental variance and possibly interaction variance, the other, D^-2 , showing an increase of genetic variance. Crosses between extreme flies within lines revealed that D^-1 genomes from large flies are dominant to genomes from small individuals. In D^-2 the genetic variation is predominantly additive variance. Tests for dominant chromosome effect in crosses with an inbred stock with recessive markers showed clear third chromosome differences in D^-2 and not in D^-1 . Chromosome exchange between extreme flies corroborated the importance of genetic differences in D^-2 . A factor or complex of factors with large effect decreasing body size is located on third chromosomes from small flies in D^-2 . Interaction between chromosomes has a similar magnitude in the two lines. Crowding and temperature experiments did not reveal an increased general sensitivity to environmental factors in D^-1 , which was suggested by the enlarged environmental variance of this line.

Introduction

Disruptive selection with mating of opposite extremes, practised on body size, caused a large increase of the phenotypic variance (Bos & SCHARLOO, 1973a), but the causes of this increase were different in two replicate lines (Bos & SCHARLOO, 1973b). In one line (D^-2) only the additive genetic variance increased, in the other line (D^-1) the environmental and residual components contributed most of the increase.

To elucidate the mechanisms which are responsible for the differences between the two lines several tests were performed.

A further analysis of the genetic variance in the lines was made by crosses of extreme flies, by introduction of their chromosomes into a stock with recessive markers and by interchange of their chromosomes.

The sensitivity to environmental factors was tested by rearing the flies of the different lines at a large range of temperatures and at various

population densities. The stability of the genetic changes was investigated by relaxation of both D^- -lines and by applying stabilizing selection in these two lines.

Materials and methods

All tests were performed on flies from the disruptive selection lines with compulsory mating of opposite extremes (D^- -1, D^- -2), from the control lines (C-1, C-2), or from the Groningen 1967 base population (Bos & SCHARLOO, 1973a).

CROSSES

In Generation 37 (G37) and G38 progenies from the $L \times S$ and $S \times L$ crosses (L indicates large and S small flies) in the D^- -lines were measured. In G43 of D^- -selection crosses have been performed (1) Crosses between extreme flies within the lines ($L \times L$ and $S \times S$), (2) Crosses between these extremes and flies from the base population ($B \times L$ and $B \times S$) and (3) backcrosses. Per cross two to five single-pair matings were made. From each of these cultures thorax lengths of ten ♀♀ and ten ♂♂ were measured. In the backcrosses of F_1 females with small or large males one or two ♀♀ with one ♂ were used as parents.

TESTS FOR DOMINANT CHROMOSOME EFFECTS

In the tests for dominant chromosome effects on thorax length five extreme, small and five extreme, large males from each D^- -selection line (G35) have been crossed individually to three females of an inbred stock with recessive markers on the three large chromosomes ($y; bw; st, p^p$). Five F_1 males from each cross were backcrossed individually to three $y; bw; st, p^p$ females. From each of the four genotypic classes of the F_2 progenies ($y; bw; st, p^p$ and $y; bw/+; st, p^p$ and $y; bw; st, p^p/+$ and $y; bw/+; st, p^p/+$) five ♀♀ and five ♂♂ have been measured. From these data dominant second and third chromosome effects were estimated (cf. THODAY (1959) and GIBSON & THODAY (1963)). Flies were cultured in vials with 10 ml standard medium (Bos & SCHARLOO, 1973a).

CHROMOSOME INTERCHANGE

Interchange of chromosomes was performed in G44 (1) between opposite extremes within D^- -lines and (2) between both extremes of a D^- -line and base population flies (B). The scheme of the interchange used in this study is according to SCHARLOO (1970a, table 1). The present analyses were performed with the same SM5/Bla; TM3/Sb stock. For detailed description of the mutations in the genome of this stock, see

LINDSLEY & GRELL (1967). Eight pairs of large flies and eight pairs of small flies were selected from each of the two D^{-} -lines to start the interchange programs. The flies from the D^{-} -1 selection line were at least 119.0 (♀♀) or 101.1 (♂♂) units, the maximum size of the small flies was 101.9 in ♀♀ and 92.9 in ♂♂. Large flies from D^{-} -2 were at least 115.7 (♀♀) or 97.8 (♂♂) units, the largest small flies were 96.2 or 92.7 units respectively. To increase the number of small and large flies, one generation of linear selection within a D^{-} -line was applied ($S \times S$ and $L \times L$ crosses). All types of crosses of the interchange program have been performed with 8 pairs of parents in each of two duplicate bottles, except in phase (a) in which 15 pairs of flies were used in each of three bottles per crossing type. Only occasionally was a smaller number of parents used. Flies from the selection lines used in phase (c) of the exchange were sibs (cultured at 17° C) of the flies used in phase (a). In the chromosome test flies were reared in a climate room (25° C and 70% relative humidity). Culture bottles (50 ml, ϕ 44 mm) contained 15 ml standard medium. Each chromosome combination was assayed by rearing 4 cultures, each with 100 eggs. These eggs had been collected from two groups of 20 parents, except in one of the L/S exchanges (assay 1). In the latter test each culture contained 4 pairs of parents, which were allowed to produce eggs (with a maximum of 150 eggs/culture).

TEMPERATURE AND CROWDING TESTS

In G29 of the D^{-} -selection a temperature experiment was performed on the C-lines, the D^{-} -lines and the base population (B). From each of four groups of parents one sample of eggs (60 eggs/culture) was cultured at each of the following temperatures: 15°, 17.5°, 20°, 22.5°, 25°, 27.5° and 30° C. A group of parents consisted of 10 pairs. Culture vials contained 10 ml of the usual standard food. From each culture 10 females were measured. A crowding experiment was run in G33 with samples of different size of the same parents, in vials with 8 ml medium at 25° C, 70% relative humidity. The range of densities was: 25, 40, 60, 80, 120, 160, 240 and 320 eggs/culture. At every density four cultures per line were used. Emerged flies were collected, sexed and counted daily. Thorax length was measured of all the emerged females.

RELAXATION OF SELECTION

In G25 or G26 duplicates of the selection lines, described by Bos & SCHARLOO (1973a) were maintained without selection by shaking hatched flies into fresh bottles. At certain intervals during relaxation samples of

eggs were raised under uncrowded conditions and body size was measured in the usual way.

STABILIZING SELECTION WITHIN D^- -LINES

In G62 a stabilizing selection experiment was started on both D^- -lines (SD^- -1 and SD^- -2). From a sample of 20 ♀♀ (resp. ♂♂) the four ♀♀ (resp. ♂♂) nearest to the mean value of thorax length were selected as parents of the next generation. Each line consisted of four culture bottles (after G4, three bottles) and was maintained by applying a cyclical mating system.

GENETIC VARIATION IN OTHER CHARACTERS

At the start of the selection lines in 1967 and in G65 progeny tests for sternopleural bristle number with random mating were performed. In GO 51 single pair matings of the base population were used. In G65 tests were performed in D^- -1 (75 pairs), D^- -2 (36 pairs) and in C-1 (77 pairs) (for details of progeny tests, see BOS & SCHARLOO, 1973b). Furthermore genetic variation for alcohol dehydrogenase isoenzymes was studied. Enzyme assays have been performed on agar gel (method: WIEME, 1965).

Results

CROSSES

The results in the comparisons of crosses between extreme flies within D^- -lines and crosses between these extremes and flies from the base population are shown in Table 1 and Fig. 1.

In both D^- -lines there were differences in mean thorax length between large $\times$ large (L) and small $\times$ small (S) crosses and this was largest in the D^- -2 line. The value of the base population (B) was intermediate. Values of crosses and backcrosses in the D^- -1 line did not differ much. They were all grouped around the value of the L $\times$ L cross and suggested dominance of the L genomes over S genomes. In D^- -2 there was no clear dominance. All crosses (with the exception of the backcross S $\times$ (B $\times$ S)) did have intermediate values, as could be expected with additivity of effects.

TESTS FOR DOMINANT CHROMOSOME EFFECTS

In Table 2 mean values of the y ; bw ; st , p^p females, of the extreme D^- -males and of their F_1 values are given. The F_1 values exceeded mid-parent values in all cases. The excess is larger in the progenies of small

TABLE 1

MEAN THORAX LENGTH IN CROSSES BETWEEN EXTREME FLIES (L = LARGE, S = SMALL) WITHIN D⁻-SELECTION LINES AND CROSSES BETWEEN THESE EXTREMES AND FLIES FROM THE GRONINGEN 1967 BASE POPULATION (B) IN G43, AND IN G37 AND 38 (L × S AND S × L). [Sexes averaged. s.e. = mean standard error of females and males; n = number of flies. Large and small flies differed at least 14.7 (♀♀) or 8.2 (♂♂) units in D⁻-1 and 14.2 (♀♀) or 9.8 (♂♂) units in D⁻-2. 1 unit = 0.01 mm.]

		L × L	S × S	L × S	S × L	B × B	B × L	B × S	S × (B × S)	(B × S) × S
D ⁻ -1	mean	102.4	97.8	103.1	101.8	99.4	101.1	100.7	101.5	100.4
	s.e.	±1.3	±1.8	±0.9	±1.0	±0.7	±1.0	±1.0	±1.1	±1.4
	n	60	40	117	96	100	100	60	105	139
D ⁻ -2	mean	104.2	94.5	97.8	98.4	99.4	102.7	98.6	97.8	96.7
	s.e.	±1.3	±1.0	±1.2	±0.8	±0.7	±0.7	±0.9	±1.2	±0.9
	n	80	20	153	97	100	80	60	132	92

Fig. 1. Mean thorax length in crosses between extreme flies within D^- -lines and crosses between these extremes and flies from the base population (cf. also Table 1). L = Large, S = Small, B = base stock.

males. Almost all individual chromosomes did have positive effects independent of the type of males used (Fig. 2). This is reflected in the highly significant main effects for marked chromosomes in the analysis of variance (Table 3). This phenomenon is a consequence of heterosis which will occur in these crosses with the inbred mutant stock.

The effects of third chromosomes of the D^- -2 males were clearly separated into two groups (Fig. 2): the effects of chromosomes from small males (open symbols) fell in general far below the values of the large males (black symbols). In the analysis of variance (Table 3) this is shown by the significant effect of the class of male giving genomes (M) and the specific

TABLE 2
MEAN THORAX LENGTH VALUES OF THE PARENTS AND THEIR F_1 PROGENIES ($\pm S_x$) IN THE TESTS FOR DOMINANT CHROMOSOME EFFECTS (0.01 mm). [Between brackets the number of flies measured. M.P. = mid-parent value]

Male parents*	Origin	Thorax length	M.P. Thorax length	F_1 thorax length		Average
				♀♀	♂♂	
D^- -1	Large	104.0 ± 0.61 (5)	101.3	109.7 ± 0.41 (50)	94.5 ± 0.30 (50)	102.1
	Small	90.6 ± 0.98 (5)	94.6	107.3 ± 0.37 (50)	92.7 ± 0.33 (49)	100.1
D^- -2	Large	100.1 ± 1.11 (5)	99.3	108.8 ± 0.31 (50)	92.2 ± 0.25 (50)	100.5
	Small	84.8 ± 1.55 (5)	91.7	103.8 ± 0.32 (49)	88.5 ± 0.32 (50)	96.2

* Mean value of ♀♀ ($y; bw; st, p^p$) = 98.5 ± 0.14 ($n = 855$).

involvement of chromosome III is reflected in the highly significant Male $\times$ Chromosome III interaction. One of the small $\sigma\sigma$ (Fig. 2, symbol $\odot$) was clearly heterozygous for a "large" and a "small" third chromosome: two of the F_2 progenies showed a high value and two showed a very low value. There are no differences in second chromosome effects between chromosomes from small and chromosomes from large flies.

The effects of the D^{-1} chromosomes are not separated into two groups (Fig. 2) and in the analysis of variance (Table 3) there is no effect of the class of male giving genomes. However, the average effect of genomes of small males is somewhat lower than the effect of genomes of larger males in particular the effects of the third chromosome (Fig. 3). This specific third chromosome effect is reflected in the significant $M \times III$ interaction

Fig. 2. The effects of introducing a second and a third chromosome from five small and five large males of the D^{-1} (left) and the D^{-2} (right) line into the $y; bw; st, p^p$ mutant stock. Thorax length of these males was:

Small $\sigma\sigma$		Large $\sigma\sigma$	
D^{-1}	D^{-2}	D^{-1}	D^{-2}
$\odot$ 88.0	79.9	$\bullet$ 102.7	97.8
$\diamond$ 89.7	83.1	$\blacklozenge$ 102.7	97.8
$\circ$ 89.7	84.8	$\times$ 104.3	99.4
$\triangle$ 92.9	88.0	$\blacktriangle$ 104.3	102.7
$\square$ 92.9	88.0	$\blacksquare$ 106.0	102.7

On the abscissa effects of second chromosomes are presented, on the ordinate the effects of third chromosomes. Each symbol indicates the value calculated from one backcross progeny of an $F_1 \sigma$ and represents the effect of introducing one haploid genome from one extreme σ from a D^{-} -line.

TABLE 3

ANALYSIS OF VARIANCE OF THE DOMINANT CHROMOSOME EFFECTS, CALCULATED FROM THE CROSSES OF LARGE AND SMALL MALES FROM THE TWO D^{-} -SELECTION LINES WITH FEMALES OF THE $y; bw; st, p^p$ MUTANT STOCK. [P values were determined by testing the mean squares of the effects against the mean square for culture totals.]

Source		$y; bw; st, p^p \times D^{-1}$			$y; bw; st, p^p \times D^{-2}$		
		d.f.	M.S.	P	d.f.	M.S.	P
Marked chromosomes	II	1	331.9	$\ll 0.01$	1	99.8	$\ll 0.01$
	III	1	366.9	$\ll 0.01$	1	179.2	$\ll 0.01$
	II $\times$ III	1	0.1	n.s.	1	1.0	n.s.
Total chromosomes (C)		3	233.0	$\ll 0.01$	3	93.3	$\ll 0.01$
Class of male giving genomes (M)*		1	0.8	n.s.	1	31.1	$\ll 0.01$
Interactions (M $\times$ C)	II	1	1.7	n.s.	1	1.6	n.s.
	III	1	14.8	< 0.01	1	55.0	$\ll 0.01$
	II $\times$ III	1	1.2	n.s.	1	0.4	n.s.
Total (M $\times$ C)		3	5.9	< 0.05	3	19.0	$\ll 0.01$
Culture totals		42	1.8	—	37	3.1	—
Cultures $\times$ genotypes		126	0.5	—	111	0.4	—
Total		175			155		

* L versus S.

in the analysis of variance (Table 3). But the third chromosome effect is far smaller than in D^{-2} .

CHROMOSOME INTERCHANGE

With each of the two D^{-} -lines three types of chromosome interchanges have been performed: (1) between genomes of large (L) and small (S) individuals within the same line (L/S). (2) between genomes of the base population (B) and genomes of small individuals (S) of a D^{-} -line (B/S) and (3) between genomes of large (L) flies and genomes from the base population (L/B).

From each interchange program eight homozygous chromosome combinations were obtained (e.g. from exchange 1: LLL, LLS, LSL, LSS, SLL, SLS, SSL and SSS).

In four different assays the average effects of chromosomes in homozygous state on thorax length have been calculated according to Table 4 (see also MATHER & HARRISON, 1949). The average chromosome effects on thorax length and their interactions are given in Figs. 4a, b, c and d. The significance of the effects was computed in an analysis of variance. When a first order interaction was significant the analysis was performed separately for the alternative states of the chromosomes involved in the interaction. For instance, when the interaction between chromosome I and chromosome III was significant, in the L $\times$ S exchange separate analysis of

Fig. 3. Average effects and interaction of chromosomes II and III on thorax length in a $y; bw; st; p^p$ genome. The presented values are the average of five mean chromosome effects, calculated from the different F_2 progenies of each of the small and large parental males. The chromosomes are selected from two D^- -lines. Mean thorax length of the involved lines is: $y; bw; st; p^p$ 91.8, D^-1 105.1 and D^-2 99.4 (sexes averaged).

variance was performed on the figures obtained when chromosome I was in its L and in its S state; the same procedure was then repeated for chromosome III. When the second order interaction was significant, the calculation of chromosome effects was performed separately for all possible genetic backgrounds.

The results of repeated assays (assay 1 and 2) did not show many differences. Effects calculated from male or female data were much the same (Fig. 4). When S chromosomes were introduced into L-genomes (assay 1 and 2) or into B-genomes (assay 3), the following effects were consistently present:

In D^-1

(1) the average (A) effect of a second S-chromosome was—contrary to

TABLE 4

SCHEME FOR THE CALCULATION OF THE AVERAGE EFFECTS OF CHROMOSOMES IN HOMOZYGOUS CONDITIONS. [The effects in the column headings are computed by adding the mean values of the genotypes, marked + in the relevant column and subtracting the mean values indicated with - . (cf. MATHER & HARRISON, 1949).]

	Main effects			Interactions			
	X	II	III	X-II	X-III	II-III	X-II-III
1. LLL	-	-	-	+	+	+	-
2. LLS	-	-	+	+	-	-	+
3. LSL	-	+	-	-	+	-	+
4. LSS	-	+	+	-	-	+	-
5. SLL	+	-	-	-	-	+	+
6. SLS	+	-	+	-	+	-	-
7. SSL	+	+	-	+	-	-	-
8. SSS	+	+	+	+	+	+	+

Fig. 4a. Chromosome interchange between genomes of large (L) and small (S) individuals within D⁻1 or D⁻2 (L/S). Main effects (chromosome I, II and III) and interactions (1 × 2, 1 × 3, 2 × 3, and 1 × 2 × 3) in different genetic backgrounds (1 unit = 0.01 mm). Black columns ♀♀, white columns ♂♂. Indications above columns: A = Average effect; +1 (or +2, or +3) = Chromosome I (or II, or III) was in its + state (in this assay chromosomes from L genotypes, - represents chromosomes of S genotypes); +1 - 3 = Chromosome I was in its + state and Chromosome III was in its - state; x = the effect was significant with $P < 0.05$ and † = the effect was significant with $P < 0.01$.

expectation—always positive and significant. In assays 1 and 3 the effect was positive in almost all types of genetic background, in assay 2 only when chromosome I or III were also in their S form.

(2) the (II × III) first order interaction was positive in all three assays and significant in assay 2 and 3.

(3) the (I × III) first order interaction was predominantly negative; and significant in assay 1 (♀) and 2 (♀ and ♂).

In D⁻2

(1) the third S-chromosome effect was large, negative and significant in all exchange programs and in all genetic backgrounds, but the effect is smaller in the base population genomes than in the “large” genomes.

(2) the effect of first S-chromosomes was positive in all genetic combinations in assay 1 and 2, and mostly significant; in assay 3 the effect is positive on the average, but varying in size and sign dependent on the genetic background.

Interaction effects in the two D^- -programs were in general not notably different in sign, size or significance and are far smaller than some of the main effects.

B-chromosomes caused rather small negative effects in L genomes (assay 4); nor in D^-1 , nor in D^-2 there are genetic factors with large effects increasing body size in the large flies.

Scoring of scutellar bristles in the genotypes obtained in some of the interchange experiments, confirmed the positive correlation between body size and the number of extra scutellars (BOS & SCHARLOO, 1973a). In both

Fig. 4b. Cf. legend of Fig. 4a.

Fig. 4c. Chromosome interchange between genomes of the base population (B) individuals and small (S) individuals from D^{-1} or D^{-2} (B/S). Cf. legend of Fig. 4a. In this assay B chromosomes are considered to be in the + and S chromosomes in the - state.

D^{-} -lines introduction of a chromosome I from small flies, which had a positive effect on body size, increased scutellar bristle number (Table 5). The third chromosome of small flies in D^{-2} caused a decrease of thorax length and of scutellar bristle number.

RELAXATION OF SELECTION AND STABILIZING SELECTION WITHIN D^{-} -LINES

In Table 6 the effect of relaxation of D^{-} -selection on variance (c.v.²) is demonstrated in four replicates of each D^{-} -line. The variance decreased gradually with duration of relaxation.

Stabilizing selection applied to the D^- -lines caused a decrease of the enlarged variance as in the relaxation lines (Fig. 5). In SD^- -1 this occurred with violent fluctuations during the first eight generations. In SD^- -2 variance decreased very smoothly to the same level of variance as in SD^- -1.

GENETIC VARIATION IN OTHER CHARACTERS

Heritability of sternopleural bristle number was estimated in GO (B) and G65 (C-1, D^- -1 and D^- -2). Heritability remained constant in C-1 and D^- -1, and decreased in D^- -2 (Table 7). Therefore and because total variability decreased in C-1, D^- -1 and D^- -2, both additive genetic (V_A) and residual (V_R) variance components decreased in the experimental lines. Thus, there was no relation between the changes of variance in

Fig. 4d. Chromosome interchange between genomes of large (L) individuals from D^- -1 or D^- -2 and base population (B) individuals. Cf. legend of Fig. 4a. In this assay + represents L chromosomes and - B chromosomes.

TABLE 5

THE AVERAGE MAIN EFFECTS OF THE INTRODUCTION OF CHROMOSOMES FROM SMALL EXTREME FLIES (S) INTO GENOMES OF LARGE EXTREME FLIES (L) ON MEAN SCUTELLAR BRISTLE NUMBER. [Calculated according to Table 4 (♀♀).]

	Chromosome		
	I	II	III
D ⁻ -1 Assay 1	+0.18	-0.20	+0.05
Assay 2	+0.28	-0.22	+0.10
D ⁻ -2 Assay 1	+0.25	-0.08	-0.16
Assay 2	+0.21	-0.21	-0.28

Fig. 5. The effects of stabilizing selection on mean value and c.v.² of thorax length in the two D⁻-selection lines (sexes averaged).

TABLE 6

THE EFFECT OF RELAXATION OF SELECTION ON THE PHENOTYPIC VARIANCE (c.v.²) OF THORAX LENGTH (SEXES AVERAGED) IN THE D⁻-LINES. [In each line four independent replicates were maintained.]

Generations of relaxation	D ⁻ -1					D ⁻ -2				
	A	B	C	D	total	A	B	C	D	total
0					31.5					20.0
3	18.8	35.9	18.4	18.8	22.8	24.0	23.0	14.4	16.2	19.1
5	8.4	10.6	21.1	11.8	13.6	18.9	22.2	15.0	8.9	16.3
10	5.7	14.4	8.3	6.1	8.6	17.0	17.6	8.4	13.1	14.1
17	12.3	9.7	7.9	6.1	8.9	10.4	16.0	16.8	14.4	14.6
34	6.4	3.2	3.9	6.9	5.3	11.5	—	7.0	11.9	9.8

body size and sternopleural bristle number. When frequencies of alcohol dehydrogenase were determined (on agar gel) in G50 it was revealed that the *Adh^s* allele (frequency in the base population was about 0.55) was fixed in both control lines and almost so in D⁻-1 (frequency = 0.98). In D⁻-2 the frequency of *Adh^s* was 0.84 in G50 and 0.80 in G75, which suggested constant frequency.

TEMPERATURE EXPERIMENTS

In terms of means the D⁻-lines were less sensitive to temperature than the base population and the controls (Fig. 6). However, the variances (c.v.²) were fluctuating strongly at different temperatures in the D⁻-lines. There was a tendency for the D⁻-1 line to have the highest variance at the extreme temperatures and for the D⁻-2 line having maximum variability at intermediate temperatures. An earlier test in G13 (not presented here) showed essentially similar results.

CROWDING EXPERIMENT

The reaction of all lines is rather similar (Fig. 7). With increasing egg-densities mean thorax length is decreasing and c.v.² is increasing. Only

TABLE 7

HERITABILITY (h^2), TOTAL VARIANCE (V_p), ADDITIVE GENETIC VARIANCE (V_A) AND RESIDUAL VARIANCE (V_R) OF STERNOPLEURAL BRISTLE NUMBER IN GO (B) OR G65 OF SELECTION.

	B	C-1	D ⁻ -1	D ⁻ -2
Sternopleurals h^2	0.42 ± 0.06	0.47 ± 0.07	0.42 ± 0.07	0.26 ± 0.07
V_p	5.59	3.69	2.93	2.82
V_A	2.35	1.86	1.23	0.73
V_R	3.24	1.83	1.70	2.19

Fig. 6. The effect of temperature on the mean value ($\bar{x}$) and c.v.² of thorax length in D⁻-selection lines, control lines (C) and the Groningen 1967 base population (B) in G29.

at low and intermediate densities is the variance of the D⁻-lines higher than in the controls.

Discussion

Our analysis confirms that the increase in variance in the two D⁻-lines is caused by different mechanisms. The crosses and backcrosses with extremes of the D⁻-2 line result in progenies showing intermediate values for thorax length, which reveals the predominance of additive gene effects. In D⁻-1 genetic differences are smaller and "large size" is dominant over "small size", which is also found in earlier work on body size (ROBERTSON & REEVE, 1955a,b).

The effects of third chromosomes of D⁻-2 large males in the recessive marker stock are significantly different from those of small ♂♂ (Fig. 2). A similar separation is not found for D⁻-1. In these tests even chromosomes of small D⁻-individuals caused a large increase of body size in the inbred mutant stock. This is caused by heterosis and the covering of recessive genes in the marker stock. Heterotic effects on body size were often found in crosses between inbred lines or in crosses between inbreds and base

populations (ROBERTSON & REEVE, 1955; BOS & SCHARLOO, 1973b). Robertson and Reeve found that heterosis was largest in crosses in which stocks with small body size were involved. This differential effect should tend to diminish the expression of size differences in the progenies of extreme D^- -flies when crossed to the marker stock. Nevertheless, there is a significant difference between the progenies of large and small flies that is caused by a difference on the third chromosome.

In our chromosome exchange assays homozygous chromosome combinations are compared. The main difference between extreme, large and extreme, small flies in D^-2 is caused by a factor or complex of factors on the third chromosome. The origin of this difference seems to be recombination. This can be concluded from the large difference between S and L chromosomes while the difference between S and B chromosome is smaller and B chromosomes also have a negative effect compared with the L chromosomes. Therefore, the difference between third chromosomes from S and L is not a single gene difference.

The genetic background of the D^-2 line seems to contribute to the

Fig. 7. The effect of crowding on the mean value and $c.v.^2$ of thorax length in D^- -selection lines, controls (C) and the base population (B) in G33 (females).

effect of segregation in this line of the complex factors on the third chromosome. In the exchange L/B the third chromosome effects are far largest when L first and second chromosomes are present. In the exchange B/S the effect of the third chromosome is strongly dependent on the genetic background, however, the effect is small both when first and second chromosomes are of the Base stock and when first and second chromosomes are of the S-flies. Thus, in general the third S-chromosome effects are independent of the genetic background in D^{-2} genomes (Figs. 4a and b), but dependent on the background in B-genomes (Fig. 4c). This implicates that in D^{-2} the background is different from the background in the base population. Remarkable is the positive average effect of chromosome I of the D^{-2} small flies, which is also strongly dependent on the genetic background. But this small positive average effect is clearly of minor importance for the determination of fly size in the D^{-2} line compared to the large effects of chromosome III.

Somewhat contrary to expectation too are the rather strong positive effects of chromosomes II from small D^{-1} flies in large D^{-1} flies. This enlarging effect of "small" chromosomes is the only demonstrable main effect in this line, and cannot be responsible by itself for the large differences in body size found in this line. This shows again that in D^{-1} genetic factors play only a minor role in determining differences in body size.

There is no clear difference in genetic interaction between the two lines. The comparison of the residual variance obtained in the progeny tests and the environmental variance of the F_1 's of crosses of inbred lines suggested that dominance and interaction could be an important source of variance in D^{-1} (Bos & SCHARLOO, 1973b). The exchange of chromosomes shows that interchromosomal interaction is not larger than in D^{-2} . The crosses of extreme flies revealed the existence of dominance effects in D^{-1} , which seem to be responsible for the non-environmental part of the residual variance obtained in the progeny tests.

In D^{-2} the increase of the genetic variance is a consequence of the generation of the complex factor on chromosome III. Complex factors of linked loci were produced in earlier experiments on disruptive selection (THODAY, 1965, 1972; SCHARLOO, 1970a). However, in these experiments other types of disruptive selection were used: disruptive selection with positive assortative mating in Thoday's experiments and disruptive selection with random mating in Scharloo's experiments.

In D^{-} -selection PROUT (1962) obtained an increase in phenotypic variance, which was not a consequence of increase of additive genetic variance. But he could not exclude the possibility that genetic interaction had increased.

Scharloo and coworkers (SCHARLOO, HOOGMOED & TER KUILE, 1967;

SCHARLOO, 1970b) obtained by D^{-} -selection an increase in phenotypic variance which was caused by an increase of the genetic component, the environmental component and the within-fly variance. The response of our D^{-} -1 line supports Prout's conclusion that D^{-} -selection is particularly suited to change environmental variance. In the experiments of Scharloo and coworkers the enlarged environmental variance was a consequence of a decrease of the canalization of the character, leading to a general increase of sensitivity to environmental factors affecting it. In the present experiments (D^{-} -1) the increase of environmental variance was not caused by an increase of general sensitivity, since effects of crowding and exposition to different temperatures were not different in D^{-} -1, D^{-} -2 and the base population. Therefore, sensitivity to some specific factor must be responsible for the increased environmental variance in the D^{-} -1 line. Differences in sensitivity to specific components of the food medium were revealed in the work of SANG (1964) and ROBERTSON (1965). In the usual cultures there is plenty of scope for differences in food composition, present or arising during development of a population. This may be a possible origin of environmental variability in D^{-} -1.

Increased genetic variance of body size, caused by the linked complex in D^{-} -2, does not necessarily affect heterogeneity in other parts of the genome. This explains that genetic variability for sternopleural chaetae number did not differ in the two lines whereas heterogeneity on the *Adh*-locus was maintained in D^{-} -2.

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