EFFECTS OF DISRUPTIVE SELECTION

VIII. IMPOSED QUASI-RANDOM MATING

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1. INTRODUCTION

THODAY and Boam (1961) described a small pilot experiment in which a population of *Drosophila melanogaster* kept under quasi-random mating and exposed to disruptive selection for sternopleural chaeta number, maintained very high variance even though the selection pressure was relatively low. They suggested a modification of their breeding system which would make it correspond more closely to random mating. The present paper describes the results of an experiment using their suggested system together with appropriate controls.

We designate the mating system as " imposed quasi-random mating "; it is imposed because the flies have no choice of mates and all matings provide equal numbers of progeny for assay and selection; it is *quasi*-random, not random, because, though the different classes of mating occur in the relative frequencies expected under random mating, no deviation around these frequencies is allowed.

2. MATERIALS AND METHODS

The experiments were initiated with a new wild stock " Southacre " of *D. melanogaster* which originated from the combined progeny of four fertilised females captured together near Cambridge in the summer of 1961.

Four four-pair bottle cultures were set up from " Southacre " two generations after it was established. Two assays each of twenty flies of each sex from each bottle were made of the progeny. From the combined progeny of the first assay the eight flies of each sex with the highest, and the eight with the lowest chaeta number were selected. The resulting four groups of flies were each divided at random into two lots of four flies each. They were then set up for 24 hours in four 3 in. vials. The first (HH) contained four high females with four high males. The second (HL) contained four high females with four low males. The third (LH) contained four low females with four high males and the last (LL) contained four low females with four low males.

Twenty-four hours later the flies were removed from the vials and the males were discarded. The four groups of females were placed in four separate bottles. The progeny were collected as virgins over four days and twenty flies of each sex were assayed from each bottle. The selection procedure was repeated on the combined progeny of the four cultures. When one or two of several flies with the same chaeta number had to be selected, random numbers were used to ensure against bias towards choosing flies from particular cultures. Records were kept of the cultures from which the selected flies came so as to provide a measure of the opportunity for gene flow between the HH and LL " sub-lines ". This line will be referred to as the DR line.

A control line (C) was set up using four four-pair bottle cultures, the parents of which were taken at random from the first assay of the " Southacre " cultures.

This control line was maintained in the same way as the DR line and assayed in each generation up to and including generation 10 and every fourth generation thereafter, but the parents for each generation were chosen at random from the flies collected in the previous generation.

A further line (S), maintained in the same way as the others, was set up from the second assay. In this line females having nineteen chaetæ and males with eighteen were selected in each generation to give four four-pair bottle cultures in a line under stabilising selection.

Flies were also selected from the second assay to initiate a line (DM) under disruptive selection with mating choice, which was run concurrently with the three other lines. A preliminary report of some of the results obtained with the DM line has already been published (Thoday and Gibson, 1962), and it will not be discussed in this paper.

In some generations the selected males from the DR line were mated (after they had been used to maintain the line) in single pair cultures to homozygous *y bw st* virgin females from our standard genome-assay stock, in order to estimate the effects on chaeta number of single second and third chromosomes from these flies (see Thoday and Boam, 1959; Gibson and Thoday, 1962). From the progeny of each cross twelve wild-type males were testcrossed in single pair cultures to *y bw st* ♀♀. On emergence of the progeny five flies of each sex and marker genotype from each culture were assayed for chaeta number.

As these assays had given no clue as to the causes of the high chaeta number flies that were produced in this line, this type of assay at generation 15 was also taken to F_2 to provide a rough assessment of the homozygous effects of the chaeta factors in the DR line.

3. RESULTS

(a) *The natural selection control and the stabilising selection line*

The variances and means of the chaeta numbers in the three lines are given in fig. 1. The mean chaeta number of the control line fell slightly until generation 10, but when it was next assayed at generation 14 it had risen to 18·8 per fly. The control line mean (17·85) has tended to be lower than that of the S line (18·28) which was selected around a mean of 18 5 chaetæ per fly. During the culturing of generation 10 electricity cuts affected the constant temperature room and it is clear that this affected the chaeta number of all the lines, the S line mean falling to 17·1 chaetæ per fly.

The mean total variance of the S line (3·034) does not differ significantly from that of the control line (3·442) and there is no significant difference between the overall within sex and culture variances of the two lines. Judging by these comparisons of phenotypic variance it would appear that stabilising selection was less effective in this experiment than in that described by Thoday (1959). There is of course a directional element and, since the environment fluctuates, there is also a disruptive element of the selection under which the S line was maintained. Nevertheless it seemed surprising that so little evident change of variance occurred.

When it was decided that this line should be terminated, heritability tests of the stabilising and control lines were set up to see what differences there might prove to be. Flies were mated assortatively and as far as possible equal numbers of flies throughout the whole range

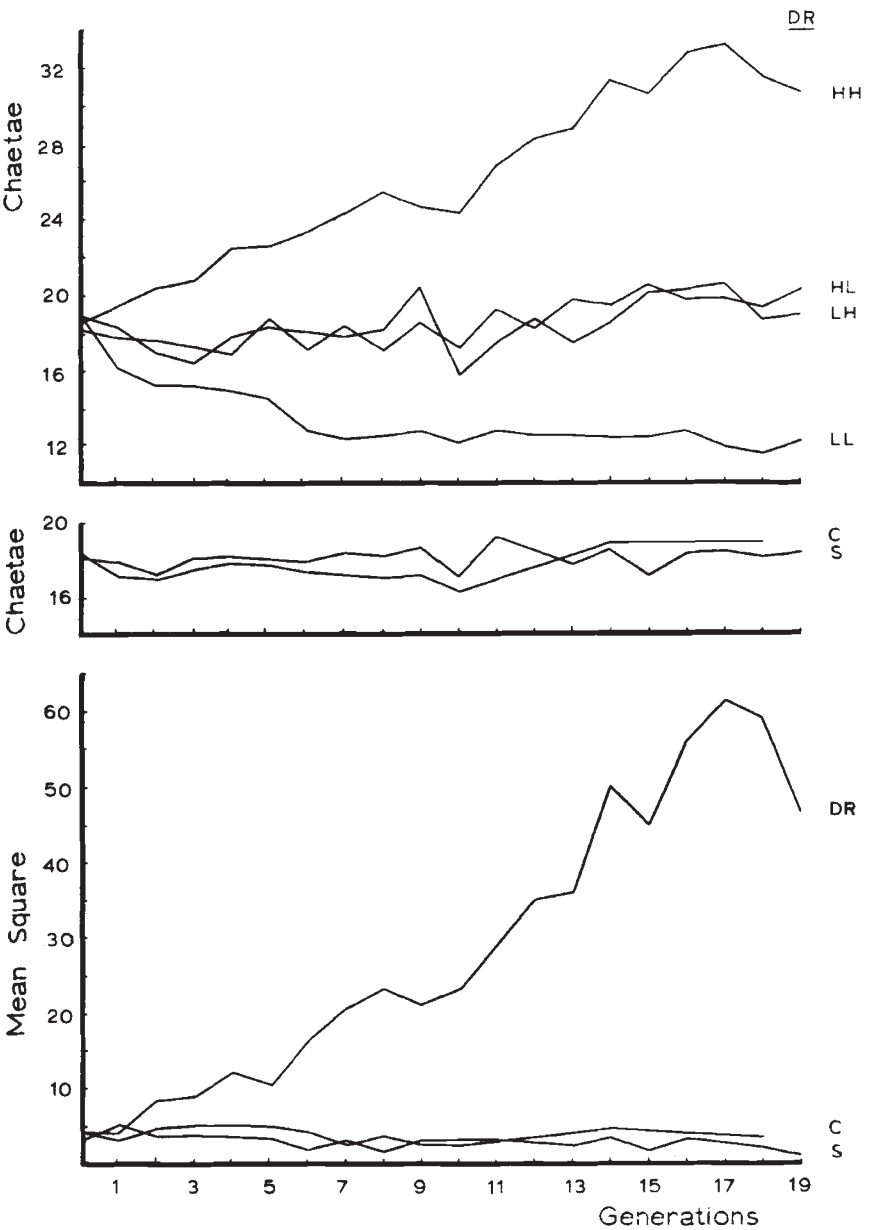

FIG. 1.—The means and overall variances of chaeta number in the three lines. In the figure for the means in the line DR, the four cultures are represented separately to show the divergence of the HH and LL means from those of the cultures HL and LH.

of bristle numbers of the lines were tested. The results of these tests are shown in fig. 2. The regression of offspring mean on mid-parent chaeta number was 0·2 for the control line and to our surprise almost 0·7 for the stabilising line. We of course expected the stabilising line to have less rather than more genetic variance than the control line.

So high a heritability in the S line suggested that maternal or cytoplasmic inheritance might be involved. To test this possibility we used the progeny of the heritability test. We set up the four matings within and between the two extremes classes of culture and found that the high × low progeny had chaeta numbers indistinguishable from the high × high, and the low × high were indistinguishable from the low × low.

From the progeny of these matings we set up the eight kinds of mating (female given first) HH × HH, HH × LL, HL × HH, HL × LL, LH × HH, LH × LL, LL × HH, and LL × LL, where HL for example refers to a fly with a high mother and a low father. Four cultures of

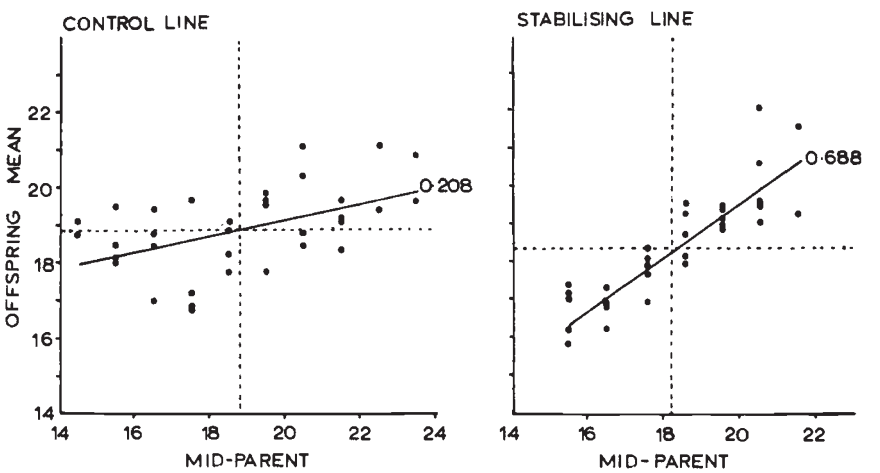

FIG. 2.—The relations of offspring mean to mid-parent chaeta number obtained in the heritability tests of the C and S lines made at the end of the selection experiment. The regression lines are drawn and the regression coefficients given.

each kind of cross were obtained and five flies of each sex from each progeny were assayed for chaeta number. The progeny of these matings gave means (table 1) showing no significant effect of fathers but a considerable difference arising from both maternal grandmothers and maternal grandfathers. The evidence therefore seems to demonstrate almost complete influence of maternal nuclear genotype on offspring's phenotype in the stabilising line, the total difference arising from this maternal effect being about 3·5 chaetæ (see abstract report, Gibson and Thoday, 1963). The chaeta number difference distinguishing the extreme flies from the S line is therefore inherited exactly as is coiling in snails (Boycott and Diver, 1923; Sturtevant, 1923).

The genetic variety independent of maternal effects was therefore tested by investigating second and third chromosomes extracted from males of the two lines S and C. Six male flies from each of three chaeta number classes (15 or 16, 18 or 19 and 21 or 22) were taken from each line and mated in single pair cultures to *y bw st* females. An F_1 male fly from each culture was mated to a single *y bw st* female and five flies

of each sex and genotype from each progeny were assayed for chaeta number. The results are given in table 2. The mean square associated with parental class × marker genotypes in the S line is not significant whereas in the C line it is. This significant interaction in the C line arises from heterogeneity of third chromosomes. There is therefore no

TABLE 1

Maternal inheritance of chaeta number difference in the S line

Mean chaeta numbers

♀ Parent	♂ Parent	
	HH	LL
HH	22·6	22·1
HL	20·0	19·8
LH	20·5	20·2
LL	18·6	19·0

Analysis of variance of culture totals (sexes separate)

Source	MS	*n*	P
Total	53·3	63	...
Between cultures within sexes+sex culture interactions (error)	8·6	48	...
Sex	144·0	1	< 0·001
Sex × parental classes	14·6	7	> 0·05
Parental classes	385·4	7	small
Fathers	14·1	1	≏0·2
Fathers × mothers	13·9	3	≏0·2
Total mothers	880·7	3	small
Mother's mother	976·6	1	small
Mother's father	1580·1	1	small
Interaction	85·6	1	< 0·01

evidence of additive genetic variance in the S line data, but such variance occurs in the C line.. The S line has less ordinary genetic variety than the control.

In both the analyses of variance the mean square associated with cultures is larger than that associated with cultures × marker genotypes, although it is only in the S line that culture-totals vary significantly. The reason for this significant culture-total variance is obscure but it may be that increased homozygosity in the S line has led to poor developmental homeostasis and thus greater environmental variance. However, whether or not this is a reasonable explanation does not detract from the demonstration that, apart from that arising from maternal inheritance, there is more genetic variance in the C than in

TABLE 2

Analyses of variance of results of assaying genomes from males of the S and C lines against y bw st

Stabilising Line

Source	*df*	M^2	P
Marked chromosomes			
II	1	153·125	>0.05 e_1; $<0.05>0.01$ e_2
III	1	210·125	>0.05 e_1; <0.01 e_2
II × III	1	105·125	>0.05 e_1; >0.05 e_2
Total chromosomes (C)	3	156·1250	>0.05 e_1; <0.01 e_2
Class of male giving genomes * (P)	2	39·1805	...
Interactions (P × C)			
II	2	9·292	...
III	2	55·042	... e_1; >0.05 e_2
II × III	2	37·041	... e_1; >0.05 e_2
Total P × C	6	33·7918	... e_1; >0.05 e_2
Culture totals (error 1)	15	91·7139	<0.01 e_2
Cultures × Genotypes (error 2)	45	28·6694	...
Total	71	...	...

Control Line.

Sources	*df*	M^2	P
Marked chromosomes			
II	1	767·018	<0.01 e_1; <0.01 e_2
III	2	17·018	...
II × III	1	8·677	...
Total chromosomes (C)	3	264·237	<0.01 e_1; <0.01 e_2
Class of male giving genomes * (P)	2	129·127	$<0.05>0.01$ e_1; <0.01 e_2
Interactions (P × C)			
II	2	4·178	...
III	2	173·345	$<0.05>0.01$ e_1; <0.01 e_2
II × III	2	13·347	...
Total P × C	6	63·624	>0.05 e_1; $<0.05>0.01$ e_2
Culture totals (error 1)	15	34·525	>0.05 e_2
Cultures × genotypes (error 2)	45	23·873	...
Total	71		

* 15 or 16 *versus* 18 or 19 *versus* 21 or 22 chaetæ.

the S line. Thus stabilising selection would appear to have reduced additive variance despite its failure to reduce phenotypic variance.

The maintenance of the maternal effect genetic variance in the S line is of interest. The flies selected in this line were clearly heterozygous at the nuclear locus (loci) controlling the chaeta number of the progeny through action in their mother. This selection must have acted effectively by eliminating the progeny of both types of homozygous mother. It must however have acted one generation in retrospect for these mothers themselves are indistinguishable from their heterozygous sibs.

(b) *The disruptive selection line*

(i) *Description of the line.* The range of chaeta numbers for each generation for the four cultures comprising the DR line are shown

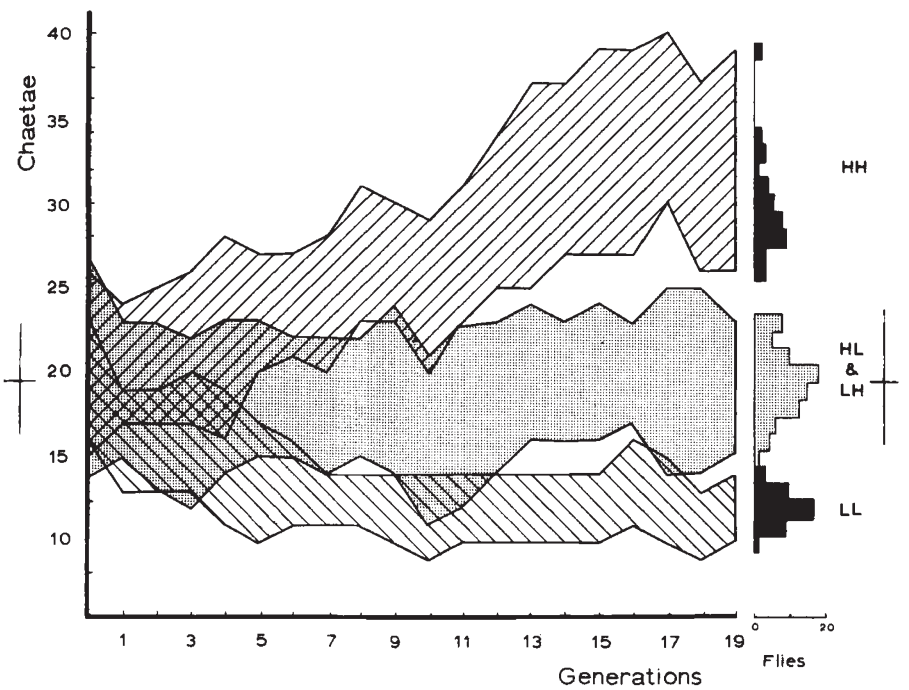

FIG. 3.—The ranges of chaeta number in the three classes of culture in each generation of the DR line. The cross hatched region shows the overlap between the distributions for the HH and LL cultures in the earlier generations. The HL and LH cultures are not distinguished from one another. At the right of the figure the distributions of chaeta number obtained in the last generation are given. It will be noted that there is little overlap in later generations between the HH or the LL cultures and the hybrid cultures HL and LH.

in fig. 3. It is clear that disruptive selection has been strikingly successful. The HH and LL sub-lines diverged with remarkable rapidity (see fig. 1).

The mean chaeta number per fly of the H×H culture rose from 18 to 33 in seventeen generations. At this level fertility and viability troubles became serious and in the next two generations the H×H

cultures lost three chaetæ in mean and died out, thus terminating the whole line. The mean chaeta number per fly of the L×L culture soon reached a plateau at fifteen chaetæ per fly but in generation six there was an accelerated response taking it to a mean of 12·9 chaetæ per fly. The line made very little progress for the next thirteen generations.

The cultures produced by the H×L and L×H matings fluctuated around a mean chaeta number of eighteen chaetæ per fly and showed no significant divergence. It is notable that these "hybridised" cultures provide no evidence of the maternal inheritance found in the S line nor of sex-linked inheritance of chaeta number differences. The H×L culture did not have a consistently higher mean chaeta number for either sex than the L×H culture (fig. 1).

TABLE 3

Cultures of origin of the selected flies in the D.R. line

(There was no gene flow from S_6-S_9 and from S_{11} to the end of the experiment.)

		S_1		S_2		S_3		S_4		S_5		S_6		S_{10}	
		♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
H×H .	H	5	6	6	6	7	8	7	8	6	8	8	8	8	8
	L	...	...	...	...	...	...	...	...	...	...	...	...	...	...
H×L .	H	1	...	2	2	...	...	...	...	...	...	...	...	...	...
	L	...	...	...	...	...	...	2	...	...	...	...	...	1	...
L×H .	H	2	2	...	...	1	...	1	...	2	...	...	...	...	...
	L	2	...	...	1	2	4	1	1	...	...	...	...	...	1
L×L .	H	...	...	...	...	...	...	...	...	...	...	...	...	...	...
	L	6	8	8	7	6	4	5	7	8	8	8	8	7	7

The contribution of each culture to the flies selected in each generation was recorded (table 3). Apart from generation 10, which, as already mentioned, was subject to unusual envrionmental conditions, gene flow between the H×H and L×L sub-lines ceased by generation 6. Thus the H×H and L×L lines rapidly became isolated. The total variance of the DR line reached 16·1 after six generations, reached 62·3 at generation 17, and was 45·9 when the line was terminated, far greater than that in the C or S lines (fig. 1). In the experiment of Thoday and Boam (1961) the mean variance maintained was 41. Their experiment demonstrated that disruptive selection could maintain great variety despite quasi-random mating. The present experiment shows that it can also promote such variety in these conditions.

(ii) *The* y bw st *assays.* Table 4 gives the mean effects on chaeta number of second and third chromosomes extracted from DR male flies of the HH and LL cultures, when those chromosomes are heterozygous with our standard *bw* or *st* marked chromosomes. The low chaeta number factors are mostly located on the third chromosome.

The accelerated change on the low side from a mean chaeta number of 14 in generation S_5 to 12 in S_6 was associated with the selected flies becoming homozygous for a low chaeta number third chromosome for which they were heterozygous in S_5.

Before S_{15} these tests provided surprisingly little evidence of high chaeta number factors on either of the two major autosomes. At S_{15} and later, third chromosome effects were evident in genomes taken from the high flies.

TABLE 4

y bw st *assay results : D.R. line*

The table lists the mean effects on chaeta number relative to *bw*/*bw* and *st*/*st* of second and third chromosomes extracted from extreme high (H × H) and extreme low (L × L) chaeta number flies in the D.R. line at various generations, with comparable data for the flies of the original Southacre cultures.

	Culture			
	H × H		L × L	
	II	III	II	III
S_0	−0·65	−0·50	−0·61	−0·55
S_5	−0·36	0	−0·58	−1·18
S_6	−0·30	+0·23	−0·85	−2·38
S_{10}	−0·03	+0·71	−1·14	−1·91
S_{11}	−0·04	+0·59	−1·05	−2·06
S_{15}	−0·30	+1·69	−0·69	−2·41
S_{17}	−0·21	+2·86	−0·89	−2·40

		H *		L *	
		II	III	II	III
Original cultures	A	−0·48	−0·24	−0·72	−0·36
	B	−0·28	−0·23	−0·37	−0·30
	C	−0·31	−0·13	−0·58	−0·23
	D	−0·43	−0·34	−0·53	−0·15

* The highest and the lowest male from each original culture was tested.

It seemed therefore that until S_{15} the high chaeta number genes in the line must have been recessive. To test this an F_2 *y bw st* assay of the high side of the population was made at S_{15}. Twenty male flies of genotype *y*, +/*bw*, +/*st*, were crossed to twenty +/*y*, +/*bw*, +/*st* famale sibs in single pair cultures, and equal numbers of flies of each sex of the eight phenotypes were assayed for chaeta number in numbers proportional to the Mendelian expectations of marker phenotypes. The male flies of the three eye colour phenotypes red, brown and scarlet were progeny tested to *y bw st* females to determine their marker genotypes.

The results are shown in table 5 as differences in mean chaeta number per fly distinguishing various marker genotypes from the *bw/bw*, *st/st* standard. There was no difference in chaeta number distinguishing the *y/y* or *y* yellow-bodied white-eyed flies from the *y/+* or + white-eyed flies and hence no evidence of an additive effect on chaeta number of X chromosomes in the absence of autosomes from the line. In the absence of chromosome III, second chromosomes extracted from the line have little effect on chaeta number but the heterozygous effect of the extracted third chromosomes (in the absence of I or II) is 1·3 chaetæ per fly. This value is the same order as that

TABLE 5

F_2 y bw st *assay results of D.R. at S_{15}*

		III/III	III/*st*	*st/st*
$\frac{y}{+}$	$\frac{II}{II}$	+3·8	+3·6	+0·2
or	$\frac{II}{bw}$	+3·5	+1·83	+0·4
+	$\frac{bw}{bw}$	+3·51	+1·33	0
$\frac{y}{y}$	$\frac{II}{II}$	+3·65	+1·94	+0·45
or	$\frac{II}{bw}$	+3·9	+1·78	+0·30
y	$\frac{bw}{bw}$	+3·6	+1·2	0

obtained in the standard *y bw st* testcross assays at generation 15. However, provided *bw*$^+$ second chromosomes of the line were present homozygous together with a *y*$^+$ chromosome I, the heterozygous effect of the third chromosome is 3·6 chaetæ per fly. The X chromosome and chromosome II seem to have interacted to affect the dominance of the third chromosome. Care must be exercised in interpreting such data as the F_2 assay is crude, there being no control of recombination in the female. These chromosomal interactions nevertheless seem likely to be real and they are most striking. It appears that there must be a factor (or factors) on X which, interacting with a recessive(s) on chromosome II, makes the high chromosome III dominant. Since this F_2 test was made at the same generation as the testcross assays first demonstrated high chaeta number genes, it throws little light on the causes of divergence of the HH sub-line in earlier generations.

Standard *y bw st* testcross assays at S_{17} indicated that the heterozygous effect of the third chromosome, first detected at S_{15}, had increased to 2·86 chaetæ per fly, indicating development of the high chaeta effects of chromosome III beyond that detected in S_{15}.

These assays of genomes from the DR line do not go far to explain the changes produced by selection. Nevertheless, when compared

with the assays of the basic cultures from which the line derived (table 4), they show clearly that some large part of the responses depended on the production of new types of third chromosome in the line, and strongly suggest significant inter-chromosomal interactions were involved.

4. DISCUSSION

Apart from the maternal effect gene(s) in the stabilising selection line, which will be fully considered in another paper, stabilising selection in this experiment had the expected effect of reducing variance relative to that in the control line.

Disruptive selection likewise had the expected effect of increasing variance relative to that of the control line, the increase being very large.

The most notable feature of the results, however, is the rapid development of effective isolation between two halves of the population under disruptive selection. After the sixth generation, with the exception of generation 10 in which environmental conditions were peculiar, all selected high flies came from the H × H culture, and all selected low flies came from the L × L culture. The progeny of the H × L and L × H matings were effectively sterilised by the selection for extreme chaeta number because none of them had extreme enough chaeta numbers to be chosen as parents for the next generation, despite the fact that selection was less intensive than usual in artificial selection experiments. Twenty per cent. of the flies scored were selected in each generation, but, of course, once isolation was approached this proportion was effectively increased until 40 per cent. of the flies of the HH and LL cultures were being selected (see table 3). Thereafter, the HH and LL sub-lines were fully isolated, and the experiment from generation 10 to 17 ceased to involve disruptive selection in any meaningful sense; it became a normal directional selection experiment with one high and one low selection line. The HL and LH cultures merely tested the phenotype of hybrids between the two extreme lines, and contributed nothing to subsequent generations. That there ceased to be at the end any possibility of their doing so is illustrated by the distribution curves for S_{19} given in fig. 3. Not only is there no overlap between the HH and LL cultures, but neither overlap the distributions for the HL or LH cultures. It seems likely therefore that the isolation of the HH and LL sub-lines from one another could have been maintained with even less intense selection than that imposed by selection of 40 per cent. of the flies assayed.

In his paper on disruptive selection, Mather (1955) argued that in appropriate circumstances such selection might be expected to give rise to isolation. The experiment described in this paper was not specifically designed to test this: that was the purpose of the fourth selection system mentioned on p. 514, the results of which have been outlined elsewhere (Thoday and Gibson, 1962). In fact in the DR line described here circumstances were arranged so that half of the flies

assayed had to be hybrids between the two classes of flies selected. Nevertheless the population after generation 6 (or 10) was split into two parts completely isolated by a mechanism analogous with Dobzhansky's (1941, p. 257) mechanism II, " hybrid sterility ". That the hybrids were " sterilised " by selection rather than actually being infertile, renders this analogy somewhat artificial, but the result is in full conformity with Mather's prediction.

5. SUMMARY

1. A disruptive selection (DR) line under a quasi-random mating system has been run using the method suggested by Thoday and Boam (1961). A control (C) and a stabilising (S) selection line were run with it. Selection was for sternopleural chaeta number in a newly-captured wild stock of *D. melanogaster*.

2. The S line did not differ in variance from the control. Nevertheless tests failed to detect ordinary genetic variance in S but did detect it in C. The S line variance was maintained by a large maternal inheritance component.

3. The DR line increased in variance and the extreme classes of culture diverged notably, becoming rapidly isolated from one another for the hybrid cultures ceased to produce any flies with chaeta numbers extreme enough for them to be selected.

4. The results are in conformity with Mather's (1955) prediction that disruptive selection could give rise to isolation.

5. Assays of genomes from the DR line showed something of the location and origin of the genetic factors responsible for the divergence of its component cultures. There are indications of strong interchromosomal interactions.

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