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EFFECTS OF DISRUPTIVE SELECTION

VI. A SECOND CHROMOSOME POLYMORPHISM

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1. INTRODUCTION

THODAY AND BOAM (1959) demonstrated that a population " D+ " of *Drosophila melanogaster*, which they had exposed to disruptive selection for sternopleural chaeta number, was polymorphic. Their analyses showed that the high chaeta number flies in the population were heterozygous for third chromosomes increasing chaeta number and third chromosomes with an intermediate effect on chaeta number, and that the low chaeta number flies were heterozygous for low and intermediate second chromosomes. The second chromosomes of the high chaeta number flies and the third chromosomes of the low chaeta number flies had intermediate effects on chaeta number.

We have investigated the genetic factors distinguishing these " Intermediate " and " Low " second chromosomes with a view to elucidating the nature and explaining the origin of this second chromosome polymorphism. Some of the results have been summarised in a preliminary report (Gibson and Thoday, 1959).

2. MATERIAL AND METHODS

Assays of chaeta number have mostly been made in 4×1 in. vial cultures owing to the large numbers of cultures required. Normal oatmeal agar medium has been used, and the flies have been cultured at $25 \pm 1°$ C., except on one occasion when temperature control failed in the culture room. Most assays have involved test-cross procedures which will be described with the results.

We have studied second chromosomes extracted from the D+ population at generation 42 when the mean chaeta numbers of the high and low halves of that population differed by 2·1 chaetæ. The extraction procedure and the identification of low and intermediate second chromosomes are described below.

In addition we have used the same homozygous *y bw st* inbred stock as was used by Thoday and Boam (1959) in their assay, a *dp cn bw* stock and a *Cy L/Pm* inbred-Oregon-background stock, both made for the purpose by Mr Boam.

Some experiments were also done on second chromosomes extracted from the " Dronfield " wild stock from which the D+ population originated. Some of these second chromosomes were taken from Dronfield in May 1959, four years after the D+ population was itself started. Others were taken in July 1959.

3. EXTRACTION OF CHROMOSOMES

Thoday and Boam (1959) used four single pair cultures each generation to maintain the D+ population. Each of the four represented a separate male line. The males of cultures H_1 and H_2 were

* Work done while holding a D.S.I.R. studentship, and accepted for the Ph.D. of Sheffield University.

TABLE 1—*Classification of extracted second-ch*

(For description see text)

Original fly	Genome	Class of second chromosome	Test with A		Test with B			
			Mean	Mean square	Mean	Mean square		
$L_3♀_1$	1	I	...	...	...	...	.	
	2	I	...	...	...	...	...	
	3	L	18·132	5·167	18·001	4·987	−−	
	4	I	...	...	...	...	...	
	5	I	18·764	1·964	18·250	3·897	+−	+− / −+
	6	L	...	...	...	...	−−	
	7	I	18·991	1·875	17·893	3·965	+−	
	8	I	...	...	...	...	...	
	9	I	...	...	...	...	...	
	10	I	...	...	...	...	...	
$L_2♀_6$	1	L	18·031	4·998	18·069	5·139	−−	
	2	I	...	...	...	...	...	
	3	I	...	...	...	...	...	
	4	I	...	...	...	...	...	
	5	I	...	...	...	...	...	+− / −+
	6	I	19·367	2·021	18·432	4·033	+−	
	7	I	...	...	...	...	...	
	8	I	18·443	3·628	19·409	1·874	−+	
	9	I	19·302	1·802	18·529	3·761	+−	
	10	I	...	...	...	...	...	
$L_2♂_1$	1	L	18·261	4·997	18·371	5·029	−−	
	2	L	...	...	...	...	−−	
	3	I	...	...	...	...	...	
	4	L	...	...	...	...	−−	
	5	I	18·998	1·875	18·379	3·876	+−	+− / −−
	6	L	18·014	5·067	18·137	5·219	−−	
	7	I	...	...	...	...	...	
	8	I	19·637	2·376	18·817	3·998	+−	
	9	I	19·459	2·296	18·098	4·049	+−	
	10	I	19·593	1·835	18·327	4·231	+−	
$L_2♂_2$	1	I	...	...	...	...	...	
	2	I	19·031	1·897	18·021	3·989	+−	
	3	L	...	...	...	...	−−	
	4	L	...	...	...	...	−−	
	5*	I	19·287	2·283	18·302	4·096	+−	+− / −−
	6	I	...	...	...	...	...	
	7	L	...	...	...	...	−−	
	8	I	19·658	2·296	18·459	4·198	+−	
	9	I	19·003	2·310	18·039	4·185	+−	
	10	L	18·091	5·062	18·173	5·191	−−	
$L_4♀_1$	1	I	18·383	4·686	19·776	1·983	−+	
	2	L	...	...	...	...	−−	
	3	I	...	...	...	...	...	
	4	L	...	...	...	...	−−	
	5	L	...	...	...	...	−−	−+ / −−
	6	I	...	...	...	...	...	
	7	I	18·157	3·852	19·629	2·256	−+	
	8	L	18·037	5·234	18·201	5·496	−−	
	9	L	...	...	...	...	−−	
	10	L	...	...	...	...	−−	
$L_4♀_2$	1	L	...	...	...	...	−−	
	2	I	...	...	...	...	...	
	3	L	...	...	...	...	−−	
	4	L	...	...	...	...	−−	
	5	L	18·201	5·476	18·187	5·181	−−	+− / −−
	6	I	18·974	2·463	18·687	4·009	+−	
	7	L	...	...	...	...	−−	
	8	L	...	...	...	...	−−	
	9	I	18·497	2·201	17·843	4·157	+−	
	10	I	18·604	2·203	17·953	4·037	+−	
$L_4♂_1$	1	I	...	...	...	...	...	
	2	L	...	...	...	...	−−	
	3	I	18·446	3·899	19·046	2·499	−+	
	4	L	...	...	...	...	−−	
	5	L	...	...	...	...	−−	−+ / −−
	6	L	...	...	...	...	−−	
	7	L	...	...	...	...	−−	
	8	I	18·616	3·934	19·451	1·687	−+	
	9	I	18·185	3·528	19·657	2·007	−+	
	10	I	...	...	...	...	...	
$L_4♂_2$	1	I	18·357	4·139	18·876	2·047	−+	
	2**	I	18·264	5·07	18·891	2·253	−+	
	3	L	...	...	...	...	−−	
	4	I	18·251	3·979	19·773	1·764	−+	
	5	L	...	...	...	...	−−	−+ / −−
	6	L	...	...	...	...	−−	
	7	I	...	...	...	...	...	
	8	L	...	...	...	...	−−	
	9	I	18·369	4·032	19·078	2·016	−+	
	10	L	18·024	5·067	18·319	4·985	−−	

* Chromosome A ** Chromosome B

Where there is no entry in columns 4 to 8 appropriate tests were not made.

selected for high chaeta number, and mated to females of cultures L_3 and L_4 that were likewise selected for high chaeta number. The males of cultures L_3 and L_4 were selected for low chaeta number and mated to low chaeta number females from H_1 and H_2.

Most of the results described below were obtained from chromosomes extracted from eight low chaeta number flies selected from cultures L_3 and L_4. Of these flies two females and two males were from L_3 and two females and two males were from L_4. Comparable extractions from high chaeta number flies of cultures H_1 and H_2 were made at the same time by Mr Wolstenholme, and we have in some tests used the second chromosomes from his stocks.

To insure against loss of male lines, Thoday and Boam set up several single pair cultures for each male line in each generation, but used only that with the most extreme parental chaeta-number provided it was fertile. In the generation when our extraction was made there were not sufficient flies in the most extreme L_3 culture, and the extraction was therefore made from the second culture of this male line.

These eight " original " flies were mated singly to the *y bw st* stock and from the progeny of each of them 10 F_1 males were taken and placed separately in cultures with 3 *y bw st* females each. The resulting 80 cultures were all successful and provided genomes that could thereafter be preserved by repeated test crossing to *y bw st* females, 6 males and 6 females being used in each culture in each generation. The first two columns of table 1 give the designation of the eight original flies, and the 80 resulting genome stocks.

An assay was made of all the genomes in the first testcross generation with a view to classifying the second chromosomes into the " Intermediate " and " Low " classes found by Thoday and Boam (1959). Chaeta numbers of 5 flies of each sex of each of the four eye colours were counted.

The results of this assay are presented in fig. 1, in the form that Thoday and Boam used. The figure illustrates for each genome the difference of chaeta number distinguishing red+brown eyed from scarlet+white eyed flies, which measures the effect of third chromosomes relative to that of the *st* chromosomes of the *y bw st* stock, and the difference of chaeta number distinguishing red+scarlet-eyed from brown+white-eyed flies which measures the effect of second chromosomes relative to that of the *bw* chromosomes from the *y bw st* stock.

The extent to which the differences illustrated in fig. 1 can be attributed to variation of the chromosomes extracted from the polymorphic population depends, of course, on the homogeneity of the chromosomes in the *y bw st* stock. This can be assessed by investigating the variation of the *bw st* homozygotes segregating from the testcrosses. The *bw st* homozygotes also permit assessment of the variance of the X chromosomes produced by the original flies, because the original male extractions only give genomes with *y* X chromosomes,

whereas the female extraction testcrosses contain extracted wild-type X chromosomes. The relevant analysis of variance is summarised in table 2. The only significant item is the overall sex difference, from which it can be concluded that the *y bw st* stock is homogeneous, that between-culture variance is negligible, and that there is no evidence of variety of X chromosomes, or difference between the effects on

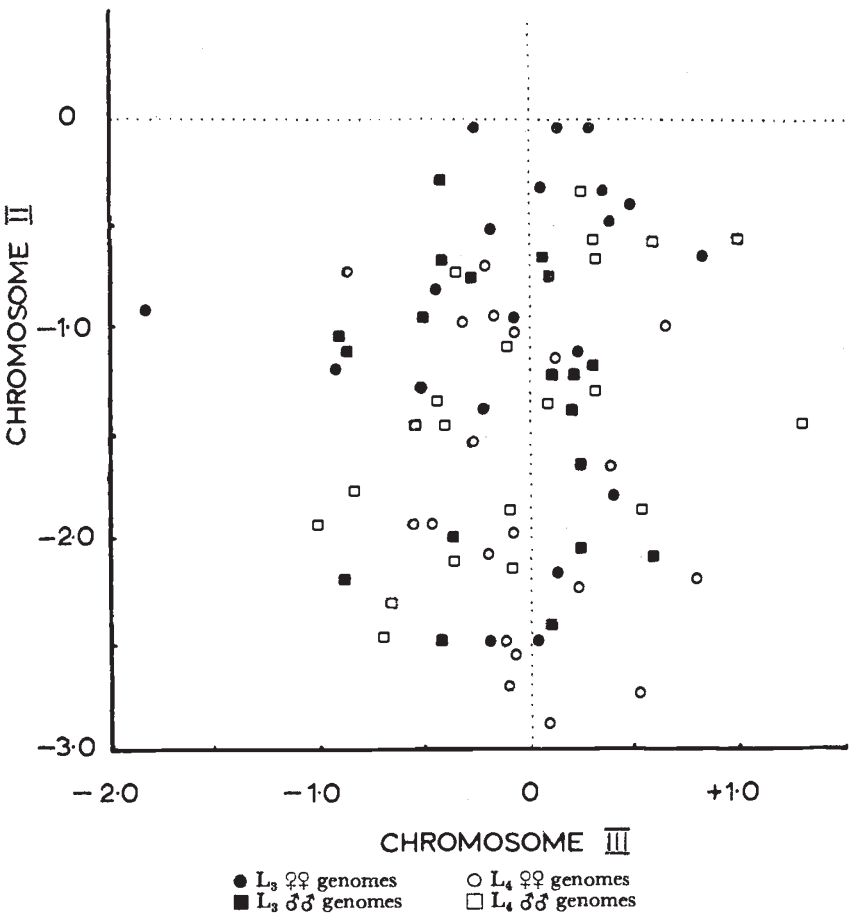


FIG. 1.—Deviations from homozygous *y bw st* standards in chaetæ per fly produced by chromosomes of the 80 genomes extracted from the D+ polymorphic population.

chaeta number of the extracted X and the *y* X chromosomes. Thoday and Boam (1959) came to the same conclusion.

It is therefore legitimate to interpret the data in fig. 1 in terms of variation of the chromosomes originally extracted from the polymorphic population. It is clear from the figure that there is a wide variety of second chromosomes. The differences distinguishing the means for $+/bw$ from the means for bw/bw flies vary from 0 to -3 chaetæ, and there is a strong suggestion of bimodality. The results are not, however, as clear cut as those of Thoday and Boam for in their

TABLE 2

Summarised analysis of variance of chaeta numbers of the bw/bw, st/st *segregants from the first assay*

Source	*n*	MS	P
Sex	1	122·461	<0·001
Extraction sex *	1	0·661	...
Sex × extraction sex	1	0·362	...
Cultures and residual genomes	78	1·940	>0·05
Other interactions	78	1·945	>0·05
Between flies within sexes (error)	640	1·508	
Total	799		

* Genomes extracted from females versus genomes extracted from males.

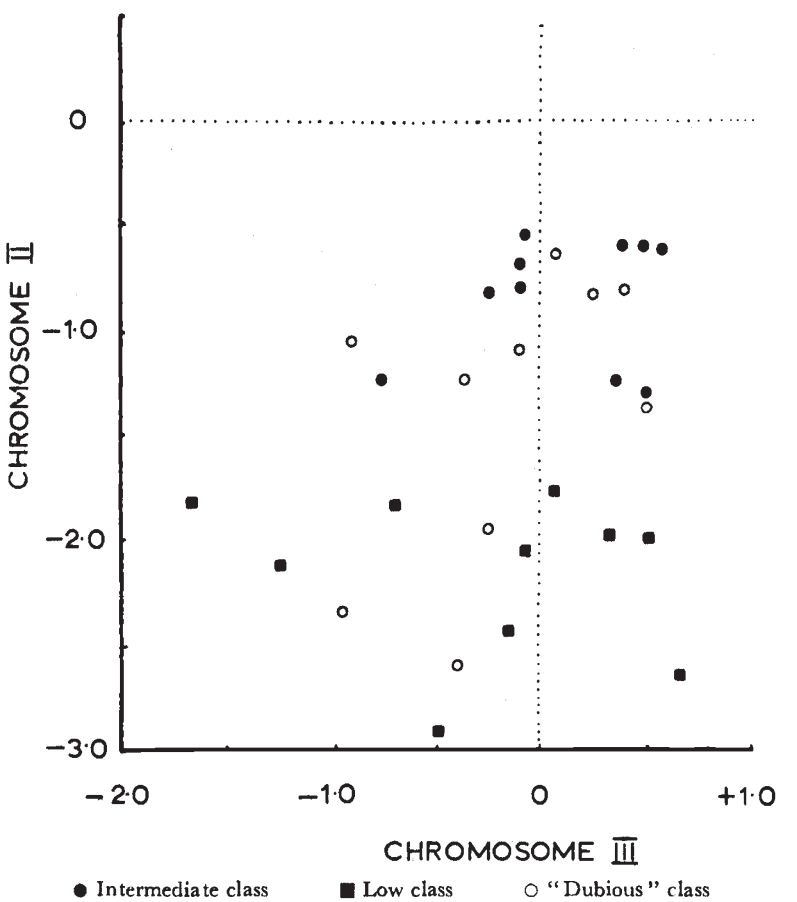


FIG. 2.—The results of the reassay of dubious genomes. The figure shows the results of reassaying 10 genomes previously classified as having low second chromosomes, 10 previously classified as having intermediate second chromosomes, and 10 previously dubious but now falling clearly into one or other of the two classes.

assay the second chromosomes fell into two quite obvious groups, their "intermediate" chromosomes (0·5 to −1·5) and their "low" chromosomes (−2·2 to −3·7). We therefore sought more precise information about the chromosomes that could not readily be assigned to one or other of these groups. There were 10 such chromosomes, with differences of −1·2 to −1·6, and these were re-assayed in a further testcross generation. At the same time 10 of the chromosomes provisionally assigned to the intermediate class and 10 of those provisionally assigned to the low class were chosen at random from those that fell into these classes and re-assayed to test the consistency of the classification. As a result of this re-assay of the dubious chromosomes (fig. 2), we were able to assign all the second chromosomes to one or other of the classes, intermediate or low (table 1, column 3). The results agree well with those of Thoday and Boam (1959) except that the difference distinguishing the two classes was not so great as they obtained, and that there are now low second chromosomes in the sub-line L_3 as well as in L_4.

The cause of this quantitative difference between the two assays, and of the relative difficulty of classifying the second chromosomes in the present assay appears to be related to differences between the results of culture in $\frac{1}{2}$-pint milk bottles and in 4 × 1 in. vials (see below).

4. GENOTYPE-ENVIRONMENT INTERACTIONS

That distinction of chromosome classes was less clear cut, and that the effects on chaeta number of the low chromosomes was of smaller magnitude in the present assays than in that of Thoday and Boam led us to suspect that the 6 pair vial cultures, which we used, might give different results from the single pair bottle cultures, which they used.

We therefore ran a replicated experiment using single pair cultures and 6 pair cultures in bottles and in vials to test this. Six low chromosomes were used.

The results and the analysis of variance of the results are summarised in table 3. There is some evidence that the number of parents influences the results, and the difference due to bottles and vials is highly significant both overall and in the interaction terms concerning second chromosomes × vessels. The difference between Low/*bw* and *bw*/*bw* flies is −2·96 chaetæ in bottles and −2·31 in vials.

We thus see why our first assay was less clear cut than that of Thoday and Boam.

5. THE SECOND CHROMOSOMES

(i) *Recombination tests*

Thoday and Boam (1959) argued that the low second and high third chromosomes in their line were probably produced by recombination rather than having been present in the Dronfield wild stock or having originated by mutation in their line.

TABLE 3

Effect of culture vessels and number of parents on the mean chaeta-number of bw/bw *and* Low/bw *flies*

Tubes		Bottles		Single pair of parents		Six pairs of parents	
bw	bw^+	*bw*	bw^+	*bw*	bw^+	*bw*	bw^+
19·33	17·02	20·14	17·17	19·80	17·23	19·67	16·97

Analysis of variance

Source	*n*	MS	P
$+/bw$	1	1669·538	v. small
$+/st$	1	1·350	...
$+/bw \times +/st$	1	26·004	< 0·001
Genotypes	3	565·630	< 0·001
Culture vessels V	1	55·104	< 0·001
Number of parents P	1	8·817	< 0·05 > 0·01
V × P	1	2·604	> 0·05
$+/bw \times$ V	1	25·350	< 0·001
$+/bw \times$ P	1	0·937	...
$+/bw \times$ V × P	1	0·417	...
$+/st \times$ V	1	0·205	...
$+/st \times$ P	1	1·350	...
$+/st \times$ V × P	1	2·204	> 0·05
$+/bw \times +/st \times$ V	1	0·263	...
$+/bw \times +/st \times$ P	1	0·338	...
Sex	1	92·504	< 0·001
Genomes	5	4·856	< 0·05 > 0·01
Genomes × genotypes	15	1·963	> 0·05
Genomes × environments	15	1·558	...
Genomes × genotypes × environments	45	2·173	> 0·05
Other interactions	90	1·616	> 0·05
Total experimental	185	11·931	< 0·001
Error (individuals)	774	1·599	
Total	959		

The results of our assays provide evidence consonant with this view (table 1, column 3) for, though the original L_4 flies and the L_3 males produced equal numbers of intermediate and low second chromosomes among their progeny, both the L_3 females produced a

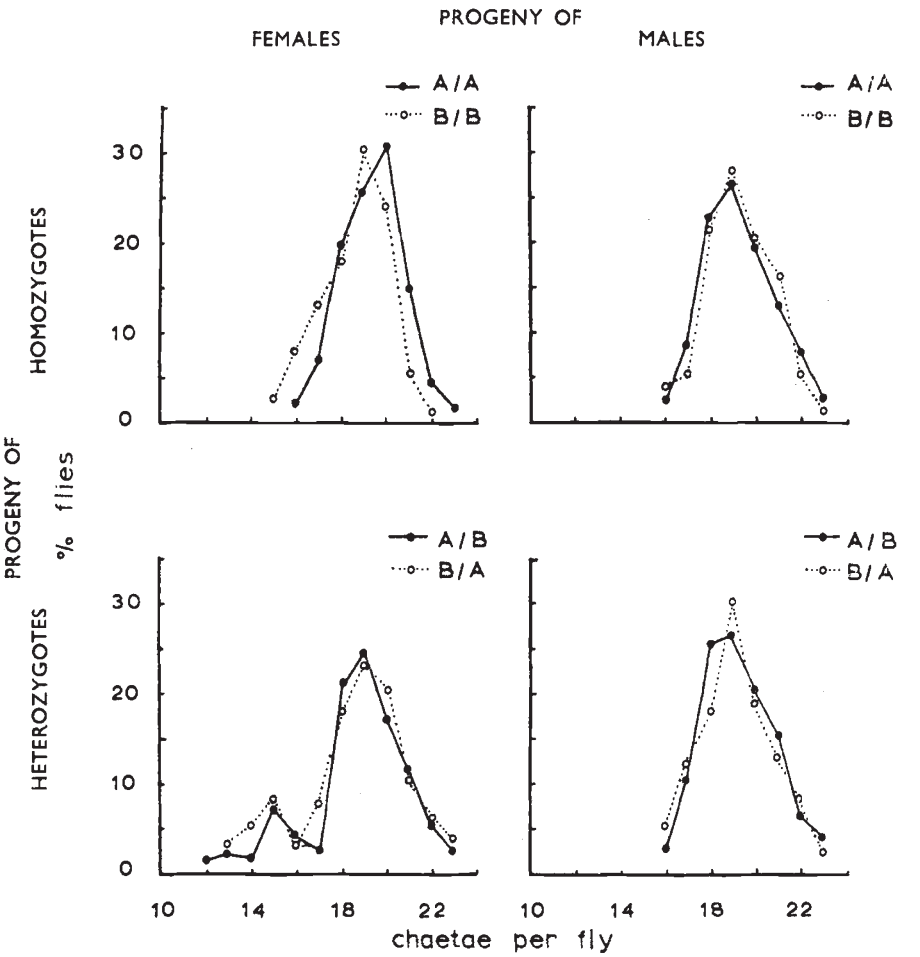


FIG. 3.—Distributions of chaeta number in the progeny of flies homozygous for chromosomes A and B, or heterozygous A/B, when these flies are mated to *y bw st* homozygotes.

significant deficiency of low chromosomes ($\chi^2_{(1)} = 9{\cdot}80$) the results for these two females being homogeneous ($\chi^2_{(1)} = 0{\cdot}20$). This could be explained if it were supposed that neither of these two females themselves contained a low chromosome, but that they were both heterozygous for two different intermediate chromosomes that could produce about 15 per cent. low chromosomes by recombination. It may be relevant here that the L_3 flies were not extracted from the most extreme L_3 culture (see p. 3).

To test this possibility, and at the same time to test whether flies homozygous for the various chromosomes were viable, the chromosomes were put through a breeding programme designed to put them in various heterozygous and homozygous combinations.

+/*bw st*/*st* males containing the chromosomes to be tested were mated to *Cy L*/*Pm* females and the *Cy L* progeny were back crossed to the +/*bw st*/*st* males. Of the resulting progenies one half should contain no *bw* flies. From the progenies lacking *bw* flies, 16 *st*/*st* females were obtained for each chromosome combination, and mated to *y bw st* males. Of the resulting cultures, those that produced only bw^+, and, hence, came from bw^+/bw^+ females, were assayed for chaeta number, 40 of each sex being counted.

To make combinations of chromosomes the procedure was altered by mating F_1 *Cy L* females from the progeny of one chromosome stock to +/*bw st*/*st* males from another chromosome stock. The procedure thereafter was the same.

No cultures free of *bw*/*bw* flies were produced in the programmes designed to produce females homozygous for any of the low chromosomes. It was concluded that the low chromosomes probably contained recessive lethals. Apart from intermediate $L_3♂1(5)$, which also appeared to be recessive lethal, *bw*/*bw* free cultures were obtained in all the breeding programmes designed to produce females homozygous for intermediate chromosomes. Likewise combinations of intermediate chromosomes and of intermediate and low chromosomes were successfully produced, but no combinations of separately extracted low chromosomes were. The combinations of intermediate $L_3♂1(5)$ with six different low chromosomes were all viable indicating that if $L_3♂1(5)$ was lethal, it and the low chromosomes were lethal for genetically different reasons. We do not consider the lethality of this intermediate chromosome relevant to our investigation.

The assay results we shall describe in detail were obtained from the progeny of females homozygous for Intermediate chromosomes $L_3♂2(5)$ and $L_4♂2(2)$, and females heterozygous for these two intermediate chromosomes. *These two chromosomes will henceforth be referred to as intermediate chromosomes A and B.* The means and variances of these progenies are given in table 4.

It is clear from the variances that the heterozygous females produced a greater variety of progeny than the homozygotes, and therefore that the two intermediate chromosomes are different. The two chromosomes when homozygous, however, produced progeny of similar means, so that the greater variance in the progeny of the heterozygotes must be brought about by recombination not simple segregation.

The mean of the progeny of the heterozygous females is lower than that for the progeny of homozygotes so that the heterozygotes clearly produced low chaeta number chromosomes by recombination. Similar results were obtained with the intermediate chromosomes $L_3♂1(5)$

and $L_4\sigma 1(8)$. Fig. 3 shows the distribution curves obtained with chromosomes A and B. There is clear evidence of a new class of low chromosomes in the progeny of females heterozygous for these intermediate chromosomes.

There are therefore at least two classes of intermediate chromosome, differing at at least two loci, which can produce low chromosomes by recombination. One of these is genetically $\underline{+\ -}$ and the other $\underline{-\ +}$, the " recombinant low " chromosomes being $\underline{-\ -}$. There is no sign of the reciprocal recombinant class, $\underline{+\ +}$, a matter to be considered later.

TABLE 4

Means and variances of chaeta number in the progeny of males or females of known second chromosome constitution mated to y bw st *females or males respectively*

Combinations of second chromosomes		Variance and mean chaeta number of progeny of females	Variance and mean chaeta number of progeny of males
Homozygous for an intermediate second chromosome	A/A	$V = 1{\cdot}687$ $\bar{x} = 19{\cdot}450$	$V = 2{\cdot}138$ $\bar{x} = 19{\cdot}125$
	B/B	$V = 2{\cdot}200$ $\bar{x} = 19{\cdot}300$	$V = 2{\cdot}283$ $\bar{x} = 19{\cdot}287$
Heterozygous for separately extracted intermediate second chromosomes	A/B*	$V = 4{\cdot}691$ $\bar{x} = 18{\cdot}680$	$V = 2{\cdot}207$ $\bar{x} = 19{\cdot}364$
	B/A*	$V = 3{\cdot}934$ $\bar{x} = 18{\cdot}610$	$V = 2{\cdot}324$ $\bar{x} = 19{\cdot}530$

* A/B and B/A were synthesised by reciprocal crosses.

In view of these results combinations were made in the manner described above between the intermediate chromosomes A and B, and all the other intermediate second chromosomes that had been maintained after the original assay. The results are given in table 1 (columns 4 to 7). It will be seen that some of the chromosomes produced variable progeny of lower mean when combined with A and less variable progeny of higher mean with B, others give the reverse result. None produced variable progeny with neither A nor B and none with both. Wherever the progeny is variable the mean is lower, wherever it is less variable the mean is higher. It seems clear that all the intermediate chromosomes fall into one or other of the two classes that A and B define.

A final experiment was done, at the suggestion of Professor Mather, to test further the hypothesis that recombination is the origin of the increased variance of the progeny of females heterozygous for the two classes of intermediate chromosome. This was to combine chromosomes A and B in males instead of females and assay the

progeny of such males. The results of this test are also given in Table 4 and illustrated in fig. 3. There is no increase of variance in the progeny of such males in which, of course, recombination cannot occur. Other tests of other intermediates gave the same results.

Comparable assays of the progeny of females or males heterozygous for intermediate and low chromosomes give the expected results,

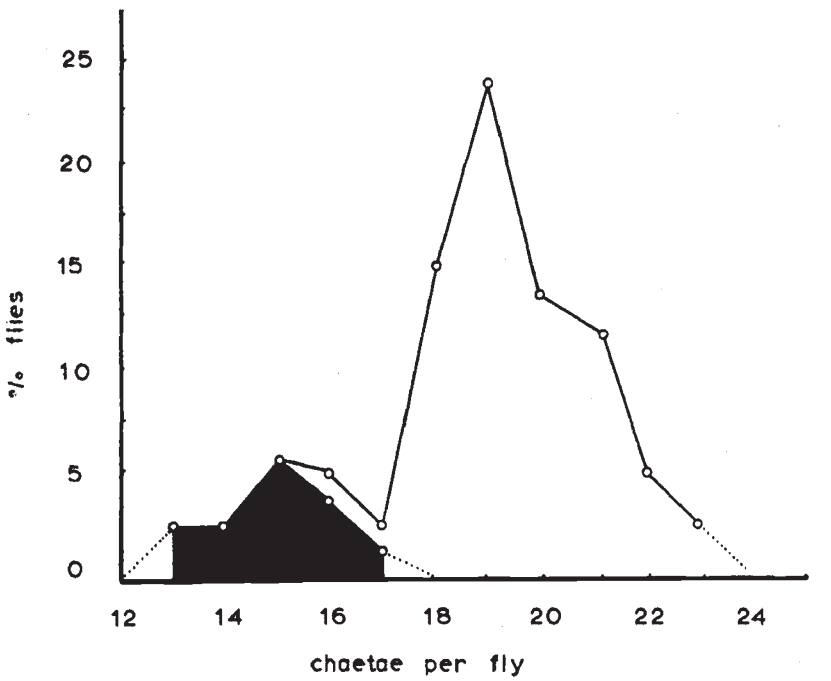


FIG. 4.—Distribution of chaeta-number in the combined progeny of two females heterozygous for chromosomes A and B, mated to *y bw st* males. The black area represents the second chromosomes derived from these progenies that proved to be homozygous lethal.

which are also listed in table 1 (columns 4 to 7). The distributions for these data are bimodal, with the two modal classes equally frequent.

(ii) *Lethality tests*

Our failure to obtain evidence of flies homozygous for the low second chromosomes in the tests described in the previous section was explained by supposing the extracted low chromosomes (and the intermediate chromosome $L_{3}\delta 1(5)$) to be homozygous lethal. This was confirmed with proper *Cy L/Pm* lethal tests.

These were carried out by mating *y* +/*bw st*/*st* flies from the appropriate genome stock to *Cy L/Pm* females. F_1 *Cy L* males were then double mated, first to the *y bw st* stock and then to *Cy L/Pm* females. Those that produced *bw*/*bw* progeny in the first mating were discarded. *Cy L*/+ males and females from the progeny of the remainder were collected. These were mated together to test for homozygous lethality.

They were also cross mated to test whether differently extracted low chromosomes combined to give lethality. Fifteen extracted low chromosomes were tested homozygous, and 8 were tested in all the 28 possible combinations each in 12 replicates, and none of the cultures produced wild-type flies.

In the same way 80 chromosomes from the male progeny of two replicate recombination tests of females heterozygous for the two intermediate chromosomes A and B were tested for homozygous lethality. The joint distribution curve for these progenies is given in fig. 4. All the non-*bw* chromosomes extracted from the flies in the black area of the distribution curve in the figure proved homozygous lethal; none of those from the other area did. The " recombinant low " chromosomes are therefore homozygous lethal like the low chromosomes originally extracted from the polymorphic population. The frequency of lethal recombinant lows was 10/80. We therefore estimate the map distance between the loci concerned as about $2 \times 100 \times 10/80 = 25$ cM.

Ten recombinational lows have each been tested in the same way against four of the original low chromosomes. The combinations are lethal demonstrating clearly that, with respect to their lethal effect, the " recombinant lows " and the lows in the polymorphic population are the same.

(iii) *Recombinant low chromosomes and chaeta number*

Seven recombinant lows and seven original lows were then assayed at the same time against *y bw st*, to see whether they had the same effect on chaeta number. Two bottle cultures testing each chromosome were assayed.

The mean difference of chaeta number between $+/bw$ and bw/bw flies was $-2{\cdot}821$ for the original lows, and $-2{\cdot}871$ for the recombinant lows. These two values are not significantly different and we conclude that the recombinant lows are indistinguishable from original lows in their effect on chaeta number as well as their lethality.

6. THE RECIPROCAL RECOMBINANTS

The distribution curves in figs. 3 and 4, and the lethal tests on these chromosomes, show that the two classes of intermediate chromosome produce by recombination a third class of chromosomes that reduce chaeta number when heterozygous and are lethal when homozygous. The recombinations that produce this new class of low chromosomes would, of course, be expected also to produce a reciprocal recombination class of chromosomes giving high chaeta number. Yet there is no sign of such a class in figs. 3 or 4, nor is there sign of the production of such high chromosomes in any of the very extensive data summarised in table 1.

There seem to be three possible explanations. First we might

suppose that the reciprocal recombinant class + + occurs as frequently as – – but has no effect on chaeta number different from that of the intermediate chromosomes + – or – +. Second we might suppose that this class occurs but is gamete lethal or dominant lethal. Third we might suppose that it does not occur, though we would be disinclined to consider this hypothesis unless forced to by positive disproof of both the others.

We have shown that the first hypothesis is highly improbable. The 80 chromosomes whose effects are illustrated in the distribution

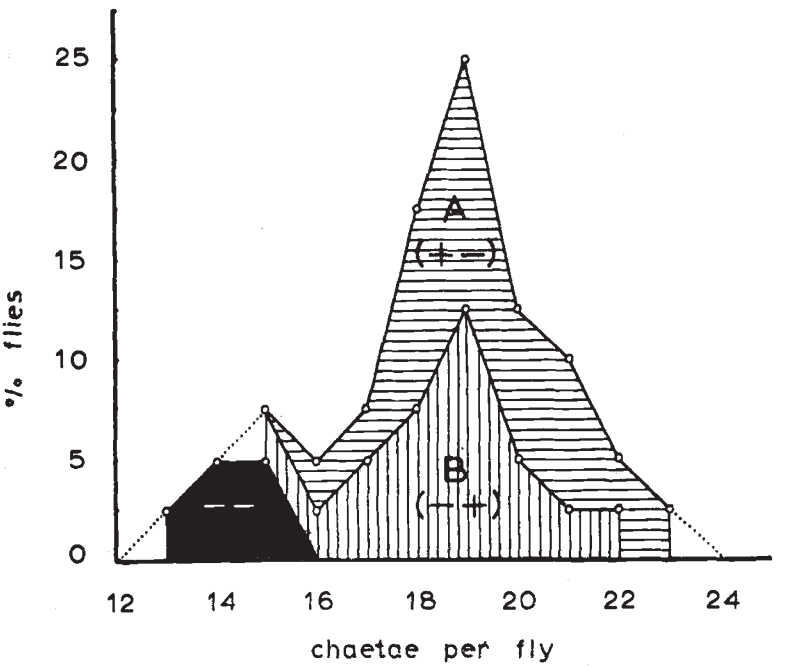


FIG. 5.—Distribution of chaeta number among 40 progeny of A/B heterozygous females, with classification of the second chromosomes concerned (see p. 13).

curve in fig. 4, were obtained in two replicate runs. The 40 chromosomes obtained in one of these runs were tested further. Five of them had been shown to be lethal, that is – –. The remaining 35 might be + +, + – or – +, and on this hypothesis we would expect about 5 of them to be + +. Now if + + produces about the same chaeta number as + – or – +, as the hypothesis requires, females combining + + and + – or + + and – +, should produce progeny no more variable than + – or – + homozygotes, and less variable than – +/+ – heterozygotes. If, therefore, there were about 5 + + chromosomes among these 35, they should be detectable as a class of intermediate chromosomes that do not give variable progeny whether combined with + – or – +. We have therefore combined

each of these 35 non-lethal chromosomes in females both with the A and with the B intermediate chromosomes, and assayed the progeny produced by these females when crossed to *y bw st* males. Nineteen of the chromosomes fell into class A and 16 were class B and there was no evidence of the class + + (fig. 5).

It therefore seems probable that the class + + is dominant lethal. Further evidence supporting this view is given below.

7. MARKER TESTS

We have used the marked chromosome *dp cn bw* to study the positions of the two loci we have uncovered, and also to throw light on the status of the reciprocal recombinant + +.

TABLE 5

Chaeta numbers of the progeny of A/dp cn bw *females mated to males of the* dp cn bw *stock*

Mean chaeta numbers

Types of progeny							
dp cn bw	+ + +	+ + *bw*	*dp cn* +	+ *cn bw*	*dp* + +	*dp* + *bw*	+ *cn* +
20·43	20·39	20·35	20·35	20·45	20·51	20·49	20·40

Analysis of variance

Source	*n*	MS	P
dp regions	1	0·451	...
cn regions	1	0·151	...
bw regions	1	0·061	...
Interactions between regions	4	0·461	...
Total marker genotypes	7	0·358	...
Sex	1	177·661	< 0·001
Sex interactions	7	1·435	...
Total experimental	15	12·681	< 0·001
Error	784	2·9576	...
Total	799		

It was first necessary to determine the genotype of the *dp cn bw* chromosomes with respect to the two chaeta number loci. *dp cn bw* chromosomes were therefore combined with both A and B, and the heterozygous females were backcrossed to *dp cn bw*. Chaeta numbers were obtained in each of the two crosses from 50 flies of each sex of

TABLE 6

Chaeta numbers of the progeny of B/dp cn bw *females mated to males of the* dp cn bw *stock*

Mean chaeta numbers

Types of progeny							
dp cn bw	+++	++ *bw*	*dp cn* +	+ *cn bw*	*dp* ++	*dp* + *bw*	+ *cn* +
20·52	20·53	20·42	20·50	19·06	20·36	20·30	19·39

Analysis of variance

Source	*n*	MS	P
dp regions	1	64·98	<0·001
cn regions	1	57·24	<0·001
bw regions	1	2·88	...
Interactions between regions	4	26·405	<0·001
Total marker genotypes	7	32·96	<0·001
Sex	1	237·62	<0·001
Sex interactions	7	2·171	...
Total experimental	15	32·236	<0·001
Error	784	4·297	
Total	799		

each of the eight marker genotype classes. The results are summarised in tables 5 and 6.

The analyses of variance show no evidence that the A and *dp cn bw* chromosomes differ in effect on chaeta number (table 5). On the other hand B and *dp cn bw* do differ in the *dp* and *cn* regions. Table 6 shows that it is the dp^+ *cn* recombinants from B that stand out, having lower chaeta numbers than other classes. Their distribution is also bimodal (fig. 6). It is clear that the B chromosome has its minus factor in the *dp* region and that the *dp cn bw* chromosome has a minus

factor in the *cn* region. Thus the order of the chaeta loci on the standard linkage map seems to be such that A and *dp cn bw* are + −, and B is − +.

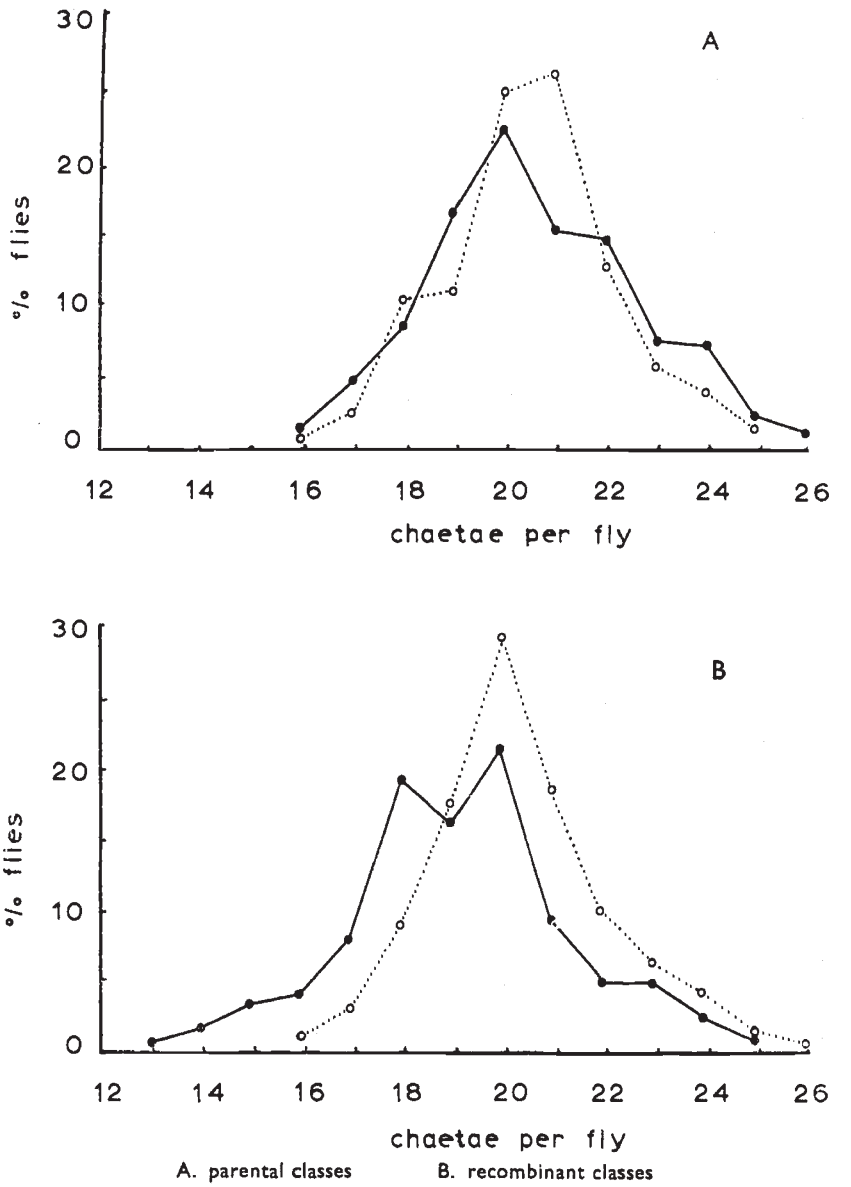


FIG. 6.—Distributions of chaeta number among the testcross progeny of females heterozygous for B and *dp cn bw* chromosomes. The upper figure illustrates the data for the classes parental with respect to the markers *dp* and *cn*. Solid line dp^+ cn^+, broken line *dp cn*. The lower figure illustrates the data for the *dp cn* recombinant classes. Solid line dp^+ *cn*, broken line *dp* cn^+.

Lethal tests of second chromosomes produced by a female heterozygous for B and *dp cn bw* confirm the conclusion. Eight chromosomes

TABLE 7

Lethal tests of chromosomes derived from B/dp cn bw *females*

Marker genotype of chromosome	Number of chromosomes tested	Number homozygous lethal
+ + +	8	0
dp cn bw	8	0
+ *cn bw*	8	5
+ *cn* +	8	2
dp + +	8	0
dp + *bw*	8	0
+ + *bw*	8	0
dp cn +	8	0

TABLE 8

Chaeta numbers of the progeny of the test crosses of two low second chromosomes to dp cn bw

Mean chaeta numbers

Types of progeny							
dp cn bw	+ + +	+ + *bw*	*dp cn* +	+ *cn bw*	*dp* + +	*dp* + *bw*	+ *cn* +
20·55	17·58	17·59	20·61	18·11	19·23	19·42	18·54
20·75	17·44	17·64	20·83	17·87	19·27	19·14	18·71

Analysis of variance

Source	*n*	MS	P
dp regions	1	2330·904	< 0·001
cn regions	1	656·862	< 0·001
bw regions	1	11·572	< 0·05 > 0·01
Interactions between regions	4	27·531	< 0·001
Total marker genotypes	7	444·209	< 0·001
Sex	1	694·201	< 0·001
Sex × genotypes	7	1·763	...
Chromosomes	1	0·012	...
Chromosome interactions	15	2·333	...
Total experimental	31	124·226	< 0·001
Error	2208	2·369	
Total	2239		

of each of the eight marker genotypes were tested, with the results given in table 7. The lethals all occur among the *dp*+ *cn* recombinants.

Similar tests, using *dp cn bw* and $\frac{- -}{}$ low chromosomes permit the study of the left-hand locus since we now know *dp cn bw* to be $\frac{+ -}{}$. The results of chaeta-number assays of seventy flies of each sex and genotype derived from two such tests using two $\frac{- -}{}$ chromosomes are given in table 8. The low chaeta effect proves to be between *dp* and *cn* but nearer *dp* than *cn*. Lethal tests of the derived chromosome were again made (table 9). They put the lethal factor (here the left of the two loci) between *dp* and *cn*, nearer to *dp* than to *cn* and are in general agreement with the results of the tests of *dp cn bw* against A and B.

TABLE 9

Lethal tests of chromosomes derived from females heterozygous for low second chromosomes and dp cn bw

Marker genotype of chromosome	Number of chromosomes tested	Number homozygous lethal
+ + +	8	8
dp cn bw	8	0
+ *cn bw*	8	3
+ *cn* +	8	4
dp + +	8	2
dp + *bw*	8	2
+ + *bw*	8	8
dp cn +	8	0

Having established that *dp cn bw* is $\frac{+ -}{}$ like chromosome A and that B is $\frac{- +}{}$, we may now use the *dp cn bw* chromosome to investigate the problem of the expected reciprocal class $\frac{+ +}{}$. *dp cn bw*/$\frac{+ -}{}$ females do not produce chaeta number variants or lethals by recombination. *dp cn bw*/$\frac{- +}{}$ females do. If the reciprocal recombinant $\frac{+ +}{}$ chromosomes occur but are dominant lethal, they should be detectable in the progeny of *dp cn bw*/$\frac{- +}{}$ females as a deficiency of the *dp cn*+ recombinant class.

Table 10 gives the numbers in the eight marker phenotypes, from test crosses of *dp cn bw*/$\frac{+ -}{}$ and *dp cn bw*/$\frac{- +}{}$ females. There is a pronounced deficiency of *dp cn*+ recombinants in the progeny of the *dp cn bw*/$\frac{- +}{}$ females. We regard this as confirming that the $\frac{+ +}{}$ chromosomes are dominant lethal.

We are now in a position to estimate the positions of the two chaeta number loci in the second linkage group, using the standard map distance of 44·5 units between *dp* and *cn* (Bridges and Brehme, 1946).

Forty *dp*$^+$ *cn bw* chromosomes derived from *dp cn bw*/ $\underline{- +}$ (B) females were tested for lethality and 18 of them were recessive lethal. Of the derivatives from *dp cn bw*/ $\underline{- -}$ females 40 *dp*$^+$ *cn bw* were tested and 26 were lethal, and 40 *dp cn*$^+$ *bw*$^+$ were tested of which 12 were lethal.

This latter test allows us to estimate the distance of our left-hand locus from *dp* and from *cn*. Let p be the distance from *dp* and q that from *cn*, where $p+q = 0{\cdot}445$ the standard distance between *dp* and

TABLE 10

Number of marker recombination classes in testcrosses progeny of *A*/dp cn bw *and* *B*/dp cn bw *females*

Female tested	Types of progeny								
	dp cn bw	+ + +	+ *cn bw*	*dp* + +	+ + *bw*	*dp cn* +	+ *cn* +	*dp* — *bw*	Total
dp cn bw .	387	382	302	298	338	346	164	160	2377
dp cn bw .	409	382	261	140	345	360	150	80	2127
Total .	796	764	563	438	683	706	314	240	4504

Heterogeneity $\chi^2_{(7)}$ . . . . . = 74·55 P small

2×2 $\chi^2_{(1)}$ testing *dp* and *dp bw* classes versus rest . . = 69·92 P small

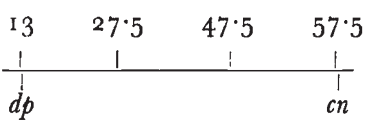
Residual $\chi^2_{(6)}$ . . . . . = 6·63 P<0·5>0·3

cn. Then $p = 12/40 \times 0{\cdot}445$ giving $p = 0{\cdot}134$, $q = 0{\cdot}311$. $q = 26/40 \times 0{\cdot}445$ giving $q = 0{\cdot}289$, $p = 0{\cdot}156$. The two estimates are in reasonable agreement and we take 0·145 and 0·300 as joint estimates of p and q.

Now if r is the distance between the two chaeta loci, we should expect the following proportion of the *dp*$^+$ *cn* chromosomes derived from *dp cn bw*/ $\underline{- +}$ females, to be lethal (ignoring double crossovers):

1. if $r<q$, $r/p+q$;
2. if $r>q$, $q/p+q$.

The observed frequency being 18/40 then either $r = 0{\cdot}200$ or $q = 0{\cdot}200$. 18/40 as an estimate for q is discordant with the data used above to estimate q ($\chi^2_{(1)} = 5{\cdot}625$, $p<0{\cdot}02$) so that r seems likely to be $<q$. The lethal tests (p. 12) also suggest that $r<0{\cdot}3$ hence the map derived is



Similar estimates can be obtained from the data for chaeta number though it is easier and more precise to use the lethal effects. In addition

the estimates based on lethality can be used to calculate the expected frequencies and the means and variances of chaeta numbers of the different classes of progeny from all the various crosses we have done with the *dp cn bw* chromosomes and $\frac{-+}{}$ or $\frac{--}{}$. These calculations have been made, taking double crossing over into account, with the assistance of Dr A. R. G. Owen. The predictions of class frequencies, means and variances agree closely with the results. Their description would involve considerable space here and is not therefore reproduced, for they do not give us different estimates of the location of the chaeta loci, but merely tell us that the map distances given above satisfactorily explain all our data.

TABLE 11

Comparison of the observed numbers of the progeny of a female heterozygous for dp cn bw *and* $\frac{-+}{}$, *with the expected values calculated from the estimated map distance assuming* $\frac{++}{}$ *to be dominant lethal*

	Phenotypes				Total
	dp cn	++	+ *cn*	*dp* +	
Observed . .	769	727	411	220	2127
Expected . .	744	731	439	213	2127

$x^2_{(3)} = 2{\cdot}8779 \qquad P<0{\cdot}5>0{\cdot}3$

The calculations, however, do assume $\frac{++}{}$ to be dominant lethal. This is particularly relevant in the prediction of the frequencies of the classes of progeny of *dp cn bw*/ $\frac{-+}{}$ females test crossed to *dp cn bw*. The resulting expected and observed values are listed in table 11, segregation of *bw* being disregarded. The agreement is excellent.

8. CLASSIFICATION OF THE EXTRACTED CHROMOSOMES

The identification of chromosome A as $\frac{+-}{}$ and B as $\frac{-+}{}$ enables us to assign all the intermediate chromosomes that have been tested against them to these classes.

This has been done for all the intermediate chromosomes in fig. 5, the results being incorporated in that figure. The two classes are about equally frequent and would seem of similar viability.

It has also been done for all the intermediate chromosomes originally extracted from Thoday and Boam's polymorphic population that were kept after the first assay and the results are summarised in table 1 (column 8). This permits us to identify the genotypes of all the eight individual flies from which these chromosomes came and these also are given in table 1 (column 9). We thus see that most of the low

flies selected from the low side of the D^+ population were $--/+-$ or $-+$, though the two L_3 females were $-+/+-$. Of the 16 chromosomes in these eight flies 6 were $--$, 5 were $-+$ and 5 were $+-$. We have also classified 12 of the second chromosomes extracted by Mr Wolstenholme from high chaeta number flies from the high side of the D^+ line. These were 9 $+-$ and 3 $-+$. The polymorphism was therefore maintained by $--$ chromosomes heterozygous in the low chaeta number males, and the probability of loss of such chromosomes was minimised by the occurrence of $+-/-+$ females which could produce $--$ gametes with fairly high frequency. Further, the presence of $+-/-+$ females on the high side of the population would allow the low female migrants selected from these progeny to be sometimes $--$/heterozygotes, thus permitting greater frequency of $--$ chromosomes on the low side. The recessive lethality of $--$ would effectively prevent the occurrence of $--$ homozygotes and the consequent transfer of $--$ chromosomes to the high side of the population.

It should perhaps be pointed out here that we have been unable to detect any cytological abnormalities in these chromosomes in salivary gland preparations.

9. THE ORIGIN OF THESE CHROMOSOMES

Having all these results, we were naturally led to enquire into the origin of the two classes of intermediate chromosome, $+-$ and $-+$. It seemed unlikely that they could have arisen by mutation since the coupling chromosomes are lethal, so that the population when initiated must have been $+-$, $-+$ or both. Two mutations are required to convert one to the other, so that it seemed likely that both were present in the D^+ population at its origin.

The technique for classifying chromosomes into $+-$ and $-+$, is, though exceedingly laborious, straightforward, and it seemed worth while to search for these two chromosomes in the Dronfield wild stock from which D^+ originated.

An assay was therefore made, of 200 genomes, derived from 6 females of the Dronfield stock, against *y bw st*, to determine the variety of second chromosomes in this stock. The results were given in fig. 7 of Millicent and Thoday (1961). It was clear from the results of this assay that all the second chromosomes fall into the range of intermediate chromosomes, and that $--$, if it occurs at all in the Dronfield stock, is rare.

Forty-eight Dronfield second chromosomes were then taken, one from each of 48 Dronfield males, and tested against chromosomes A and B with the results given in table 12. Forty-six of them were $+-$

and 2 were $\underline{-+}$. Appropriate tests have shown that females heterozygous for these two classes of Dronfield intermediates produce homozygous lethal low chaeta number recombinant chromosomes. They too are lethal in combination with the low chromosomes

TABLE 12 (*a*)

The results of testing 48 Dronfield chromosomes against chromosome A (+ −)

Chromosome tested	Mean chaeta number	Variance	Subsequent classification	Chromosome tested	Mean chaeta number	Variance	Subsequent classification
1	19·374	2·076	+ −	25	19·621	1·895	+ −
2	19·658	2·182	+ −	26	18·998	2·378	+ −
3	19·600	2·298	+ −	27	18·798	1·879	+ −
4	19·731	2·401	+ −	28	18·991	2·352	+ −
5	19·429	2·298	+ −	29	19·046	2·223	+ −
6	18·987	2·007	+ −	30	18·385	2·024	+ −
7	19·621	2·321	+ −	31	18·987	1·990	+ −
8	19·398	2·076	+ −	32	19·521	2·468	+ −
9	19·405	2·489	+ −	33	19·489	1·898	+ −
10	19·502	2·255	+ −	34	19·631	1·797	+ −
11	19·619	2·020	+ −	35	19·157	2·460	+ −
12	18·998	1·987	+ −	36	19·700	2·023	+ −
13	19·043	1·873	+ −	37	18·989	1·830	+ −
14	18·976	1·966	+ −	38	19·058	2·300	+ −
15*	18·216	4·328	− +	39	19·032	1·936	+ −
16	19·054	1·879	+ −	40	19·175	1·998	+ −
17	19·321	1·998	+ −	41	19·284	1·873	+ −
18	19·439	2·357	+ −	42	19·355	2·403	+ −
19	19·682	2·339	+ −	43	19·332	2·021	+ −
20	19·371	1·990	+ −	44	19·039	1·989	+ −
21	19·754	1·987	+ −	45	19·368	2·039	+ −
22	19·428	2·049	+ −	46	19·397	2·220	+ −
23	19·491	2·068	+ −	47	19·020	2·019	+ −
24*	18·2163	4·038	− +	48	18·9876	1·986	+ −

* Draws attention to the two − + chromosomes.

TABLE 12 (*b*)

The results of testing some of the above chromosomes against chromosome B (− +)

Chromosome tested	Mean chaeta number	Variance	Subsequent classification
1	18·039	4·3984	+ −
9	18·485	4·0692	+ −
14	18·357	3·8765	+ −
34	18·269	4·1957	+ −
44	18·198	3·7984	+ −
24	19·367	2·0379	− +

originally extracted from the D+ population. The Dronfield wild stock is therefore cryptically polymorphic for chaeta-number genes, and it is this cryptic polymorphism that has been converted into an effective polymorphism by disruptive selection in Thoday and Boam's D+ line.

Two things only remain unknown. The first is the cause of the heterozygosity of the Dronfield stock, for both classes of intermediate chromosome are homozygous viable. Some heterososis maintaining these heterozygous seems likely. The second is the frequency of the two chromosomes in the Dronfield stock when the D^+ population was initiated.

The Dronfield stock was itself established in May 1954 from one fertile female and hence, unless that female had been fertilised by more than one male, the stock cannot have started with the rarest chromosome in a frequency of less than 25 per cent. The D^+ population was started in April 1955, and the assay was made in 1959. It seems likely that the Dronfield female came from a population with a high frequency of both chromosomes, and that adaptation to the uniform environment of a culture room together with inbreeding led to reduction in the frequency of $\underline{-+}$. We know (Beardmore, 1960) that rapid changes can occur in adaptation to constant temperature environments, and it seems likely that the frequency of $\underline{-+}$ will have been significantly reduced by the time that the 8 flies were taken to establish the D^+ population. It seems likely then that the 16 chromosomes in these 8 flies were mostly $\underline{+-}$ only 1 or 2 being $\underline{-+}$.

Now it took 13 generations before disruptive selection produced any consistent effect on the D^+ population. Unequal frequencies of $\underline{+-}$ and $\underline{-+}$ could explain why this should be so, for the initial phase of selection might be needed before the probability of $\underline{+-}/\underline{-+}$ females occurring in the population became sufficient for $\underline{--}$ chromosomes to be produced and established (see Thoday and Boam, 1961).

If $\underline{+-}$ and $\underline{-+}$ had slightly different effects on chaeta number, disruptive selection would of course tend to increase the frequency of the rarer chromosome. Classifying the data from our assays of the D^+ genomes accordingly, we find that $\underline{+-}/bw$ flies average 0·3 chaetæ more than $\underline{-+}/bw$ flies. There is therefore a possibility that such change of frequency might have occurred in those first 13 generations.

10. DISCUSSION

The experiments reported in this paper provide a complete explanation of the second chromosome polymorphism established by Thoday and Boam (1959) by exposing a line taken from their Dronfield wild stock to disruptive selection with positive assortative mating.

Two loci are sufficient for the explanation, and these have been located at about 27·5 cM and 47·5 cM on the second linkage map. The polymorphism is the result of the segregation of two classes of second chromosome in the population, the low and intermediate classes of Thoday and Boam. The low chromosomes carry the alleles giving the lower chaeta number at both loci and these alleles interact

to give a chromosome that is homozygous lethal. The intermediate class of chromosomes proves to comprise two sub-classes, each having the allele giving the higher chaeta-number at one locus and the allele giving the lower chaeta number at the other locus. The two sub-classes are complementary and in heterozygous females produce about 10 per cent. $\underline{- -}$ chromosomes by recombination. The $\underline{+ +}$ reciprocal recombinant does not occur and there is evidence that it is dominant lethal.

The assays of the Dronfield stock, show that it too contains both classes of intermediate chromosome. It is a wild stock heterozygous at two loci linked fairly loosely in the *repulsion phase.*

These experiments have therefore not only enabled us to explain the polymorphism and its origin, but also to demonstrate and locate in a wild stock just such a repulsion linkage balance as Mather (1943) has argued should occur in natural populations of outbreeding species as a result of stabilising selection. Whether the two loci concerned are simple, the chaeta-number and lethality effects being pleiotropic, or are themselves complex effective factors linking chaeta number and fertility genes as in Mather's (1943) models we cannot of course say. These effects, however, are certainly remarkably like those Mather postulated, especially in as much as both recombination products have lethal effects.

The demonstration that a natural population, that from which the Dronfield stock derived, can be heterozygous for such a remarkable pair of loci, underlines the importance that linkage phase and viability interactions must be given in quantitative and in population genetics. It seems most unlikely that extensive experiments designed to give values to the parameters necessary to estimate probable results of selection will have much predictive value until the relevant mathematical models taken into account not only additive, dominance and interaction variance affecting the character under consideration, but also linkage and non-randomness of linkage phase together with additive, dominance and interaction variance in fitness.

Some may feel that the present system is too extreme to base such a generalisation upon. Clearly it is likely to be unusual for both of the reciprocal classes to be lethal. But the very extremity of the system justifies the generalisation. It should be borne in mind that very few lines have been at all fully analysed after selection of a quantitative character. The best analysed are those of Mather (Breese and Mather, 1957) and the strangely neglected *E. coli* line of Cavalli and Maccacaro (1952). *E. coli* is hardly relevant, and Breese and Mather did not search for the relevant genes in the base population. The present experiments provide the first example in which the genes responsible for a selection response have not only been located in the selected population, but also in the unselected population from which the selection line derived. That the first system so fully analysed should prove so extreme, strongly suggests

that systems in which linkage phases are non-randomly distributed because of less extreme viability interactions will prove very common. There is, indeed, evidence that systems involving as extreme viability interactions are not rare, for though ours is the first system sufficiently understood for us to know exactly how a synthetic lethal is produced, others have demonstrated the occurrence of synthetic lethals with some frequency (Dobzhansky, 1946; Misro, 1948).

There is also plenty of evidence that less extreme systems are common. The experiments of Dobzhansky (1946) and of Harrison and Mather (1950) in particular admit of no other conclusions, and even among Dronfield second chromosomes, we already have evidence that other less extreme systems occur (Millicent and Thoday, 1961). We are confident that further analyses along similar lines to those described in this paper, laborious though they are, will prove well worth while in showing how important recombination is in selection experiments, and in throwing light on the genetic structure both of selection lines and of the populations from which they came.

11. SUMMARY

1. The existence of two classes of second chromsome in Thoday and Boam's artificial polymorphic population of *D. melanogaster* has been confirmed.

2. The low chaeta number class has been shown to be homozygous lethal.

3. The intermediate chaeta number class has been shown to comprise two sub-classes differing at two " loci ".

4. The two loci concerned have been located at about 27·5 cM and 47·5 cM in the second linkage group. One class of intermediate chromosomes ($\underline{-+}$) has a low chaeta number factor at the 27·5 position and a high chaeta number factor at the 47·5 chaeta position. The other class ($\underline{+-}$) has the reverse combination of factors. Recombination between them produces the low chaeta number $\underline{--}$ chromosomes which are homozygous lethal.

5. The reciprocal recombinant ($\underline{++}$) is dominant lethal.

6. 48 chromosomes taken at random from the wild stock from which Thoday and Boam's population was derived have been tested. 46 proved to be $\underline{+-}$ and 2 $\underline{-+}$. The wild stock is therefore heterozygous for repulsion linkage chromosomes from which the polymorphism could be built up by recombination and selection.

7. It is pointed out that this adds to the evidence that non-randomness of linkage phase must be considered seriously in theoretical quantitative genetics.

12. REFERENCES

BEARDMORE, J. A. 1960. Developmental stability in constant and fluctuating temperatures. *Heredity*, *14*, 411-422.

BREESE, E. L., AND MATHER, K. 1957. The organisation of polygenic activity within a chromosome in *Drosophila*. I. Hair characters. *Heredity*, *11*, 373-395.

CAVALLI, L. L., AND MACCACARO, G. A. 1952. Polygenic inheritance of drug resistance in the bacterium *Escherichia coli*. *Heredity*, *6*, 311-332.

DOBZHANSKY, TH. 1946. Genetics of natural populations. XIII. Recombination and variability in populations of *Drosophila pseudoobscura*. *Genetics*, *31*, 269-290.

GIBSON, J. B., AND THODAY, J. M. 1959. Recombinational lethals in a polymorphic population. *Nature*, *184*, 1593-1594.

HARRISON, B. J., AND MATHER, K. 1950. Polygenic variability in chromosomes of *Drosophila melanogaster* obtained from the wild. *Heredity*, *4*, 295-312.

MATHER, K. 1943. Polygenic inheritance and natural selection. *Biol. Revs.*, *18*, 32-64.

MILLICENT, E., AND THODAY, J. M. 1961. Effects of disruptive selection. IV. Gene flow and divergence. *Heredity*, *15*, 119-217.

MISRO, B. 1949. Crossing over as a source of new variation. Proc. VIII Int. Cong. Genet. *Hereditas* (Suppl.), 629-630.

THODAY, J. M. 1960. Effects of disruptive selection. III. Coupling and repulsion. *Heredity*, *14*, 35-49.

THODAY, J. M., AND BOAM, T. B. 1959. Effects of disruptive selection. II. Polymorphism and divergence without isolation. *Heredity*, *13*, 205-218.

THODAY, J. M., AND BOAM, T. B. 1961. Regular responses to selection. I. Description of responses. *Genetical Research*, *2*, 161-176.