EFFECTS OF DISRUPTIVE SELECTION

VII. A THIRD 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. The high chaeta number flies were heterozygous for third chromosomes increasing chaeta number, and third chromosomes with an intermediate effect on chaeta number. The low chaeta number flies were heterozygous for low and intermediate second chromosomes. The third chromosomes of low chaeta number flies and the second chromosomes of high chaeta number flies had intermediate effects.

Gibson and Thoday (1962*a*) have analysed the second chromosomes of this population and have shown that the low second chromosomes contained two low chaeta number genes and that they were produced by recombination from the two repulsion chromosomes.

The present paper describes the results of the corresponding analysis of the high third chromosomes.

2. MATERIALS AND METHODS

Assays of chaeta number have mostly been made in 4 × 1 in. vial cultures, using normal oatmeal agar medium. Cultures were raised at 25° C.

We have studied third chromosomes extracted from the D⁺ population at generation 42 when the mean chaeta numbers of the high and low halves of the population differed by 2.1 chaetæ. The extraction procedure and the identification of the differing classes of third chromosome are described below.

The extraction and early assays were made with the *y bw st* stock that was used by Thoday and Boam (1959). Various other marker and balancer stocks have been used: these will be described with the results. Figure 1 shows the third chromosome markers that have been used in the investigations. Most of the marker stocks were made up or obtained as the need became apparent. There was no time to inbreed them to reduce heterogeneity.

3. EXTRACTION OF CHROMOSOMES

Thoday and Boam (1959) used four single pair cultures each generation to maintain the D⁺ population. Two, H₁ and H₂, represented male lines selected for high chaeta number. The other two, L₃ and L₄, represented male lines selected for low chaeta number. Of the high male lines, only H₁ proved to contain high third chromosomes. The results to be described below concern chromosomes extracted

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from eight high chaeta number flies, four selected from H_1 and three from H_2 . Four (two males and two females) were taken from each male line but one of the H_2 females failed to produce progeny.

Each of these eight original flies was mated to the $y\ bw\ st$ stock. Ten males from the progeny of each, but twenty from the progeny of the successful H_2 female were mated singly to $y\ bw\ st$ females to provide 80 genomes extracted from D^+ .

The chaetæ were counted on five flies of each sex of each of the four eye colour classes of the testcross progeny. The usual analyses were made of the data from the homozygous $bw\ st$ progeny to test for effects of the X chromosomes and for heterogeneity of the $y\ bw\ st$ stock, with the same negative results as were obtained by Thoday and Boam (1959) and by Gibson and Thoday (1962a).

FIG. 1.—The markers used in the investigation. (Locations from Bridges and Brehme, 1946.)

Differences of chaeta number arising from chromosomes II and III are detected in the segregations from bw and st . There was a wide variety of third chromosome effects, and most of the higher chromosomes were from H_1 genomes, but the distinction between different classes of third chromosomes was not as clear cut as it was in the assay of Thoday and Boam (1959), neither were the deviations as large.

On the basis of this data, three "classes" of third chromosome were selected, "low", "dubious" and "high" and reassayed by a further testcross to $y\ bw\ st$ to determine their reality. Most of the selected "low" class remained low ($< +1$ chaetæ). Most of the "high" class remained "high". The "dubious" chromosomes mostly fell into the range of the "high". Analyses of variance showed no significant difference distinguishing the high from the dubious class, but a highly significant difference ($P < 0.001$) distinguished these from the low class. These results were consistent with the view that there are but two classes of third chromosome, but they differed from those of Thoday and Boam (1959) in three respects. Thoday and Boam found a dubious class, their high class gave deviations of $+3$ chaetæ, and their low class gave no negative deviations. The last two of these may be accounted for by change in the $y\ bw\ st$ stock and in environmental conditions. The first could not.

A further testcross was therefore carried out using twelve third chromosomes, whose classification had proved similar in the first two tests. Three were of the "high" class, three were of the "dubious" class, and three were of the "low" class. Another three were chosen because they had given deviations less than zero. Two cultures were obtained testing each chromosome. The results are given in fig. 2. They are clear cut and show two classes of third chromosome only.

None of the high chromosomes came from H_2 , and the H_1 flies segregated both classes of chromosome, which is in agreement with the

FIG. 2.—Final classification of third chromosomes into high and low classes. Genomes selected at the earliest test as having high (points), dubious (solid triangles), low (circles) and extra-low (open triangles) third chromosomes. D values in this and later figures are the differences of mean chaeta number between flies heterozygous for the chromosomes under test and flies containing only the relevant "standard" marked chromosomes.

results of Thoday and Boam. The lack of a real class among the dubious chromosomes corresponding to the recombinant class of Thoday and Boam is not surprising. Thoday and Boam assayed 20 genomes from H_1 females and found two such chromosomes. The present extraction involves the same number of genomes from H_1 females and chance alone could readily account for the difference.

Following these investigations a few well tested "high" and "low" third chromosomes were preserved for analysis. They were maintained heterozygous with *st* in males which were crossed each generation to γ *bw st* females.

4. INVESTIGATIONS OF "HIGH" THIRD CHROMOSOMES

(i) Viability Tests

Gibson and Thoday (1962*a*) found that the low chaeta number second chromosomes from D⁺ are homozygous lethal. A test was made

that should have detected this or any extreme viability disturbances caused by the "high" third chromosomes. High/*se cpe* females and males were mated together, and the progeny were classified for the markers using further progeny testing where necessary. The frequencies of the marker classes of progeny give a close fit to the expectations based on the map distances given in Bridges and Brehme (1944), and supply no evidence that any part of the high chromosomes gives exceptionally low viability.

Gibson and Thoday (1962*a*) made good use of the lethal effect of their low second chromosomes. The lack of evidence for lethality of the high third chromosomes made it impossible to use the short cuts these authors used in their investigations. Further investigation proceeded along the lines discussed by Thoday (1961), involving test crosses to marker stocks with the aim of locating the high chaeta number factor or factors that distinguish these "high" chromosomes from those in marked stocks of more normal chaeta number.

(ii) Preliminary testcross results

The first testcross was done against the marker chromosome *stp* to determine in which arm of chromosome III the high chaeta number factor or factors occur. Four of the high third chromosomes were combined in females with *stp* and the females were test crossed to *stp* males. Equal numbers of progeny of the four marker classes were picked up and assayed for chaeta number with the results listed in table 1.

The differences of chaeta number distinguishing the ++ and *stp* classes of progeny are around 2 chaetæ, agreeing with the deviations obtained in the *y bw st* assays. Among the recombinant classes, the difference from *stp* is largely recovered in the *st+* flies indicating that the high chaeta number factor or factors lie to the right of the locus *st*. There is some indication of difference between the different high chromosomes but no *+ / st* × chromosomes interaction.

Similar testcrosses were made using the standard *se cpe*. These gave rise to difficulty for the *se cpe* chromosomes proved to give unusually low chaeta numbers. "High" third chromosomes give about 3.6 chaetæ more than *se cpe* chromosomes and "low" third chromosomes give 1.7 chaetæ more.

The low effect of the *se cpe* chromosomes proved to arise from the *cp* region. When this was accounted for the results confirmed that the factor or factors distinguishing high from low D⁺ chromosomes is to the right of *cp* (*cp* is near *st*, see fig. 1), and demonstrated at least part of it to be to the left of *e* because the *cp+* and the *+e* classes of progeny both had chaeta numbers intermediate between the parental ++ and *cp e* classes. The *+e* class had the higher chaeta number indicating the high factor(s) to be nearer *cp* than *e*.

TABLE 1

Mean chaeta numbers of the various classes of progeny resulting from the *st p* testcrosses. (All flies heterozygous *st p*)

Chromosomes tested	Type of progeny			
	<i>st p/st p</i>	<i>++/st p</i>	<i>+ p/st p</i>	<i>st +/st p</i>
A ₄	20.94	22.70	20.74	22.36
A ₉	20.32	21.96	20.32	21.80
B ₄	20.60	22.57	20.70	22.47
D ₉	20.14	22.46	21.14	22.36
Joint	20.49	22.41	20.73	22.22
Deviation from standard	...	+1.92	+0.24	+1.73

Analysis of variance

Source	<i>n</i>	M.S.	P
Left arms (<i>+/st</i>)	1	8.2350	> 0.50
Right arms (<i>+/p</i>)	1	521.9010	< 0.001
Left arms × right arms (<i>+/st</i> × <i>+/p</i>)	1	0.1686	...
Genotypes	3	176.7682	< 0.001
Chromosome stocks	3	12.6653	< 0.01 > 0.001
Sex	1	42.5350	< 0.001
<i>+/st</i> × chromosomes	3	2.6134	> 0.05
<i>+/p</i> × chromosomes	3	0.7359	...
<i>+/st</i> × <i>+/p</i> × chromosomes	3	4.6154	> 0.05
Genotypes × chromosomes	9	2.6549	> 0.05
Genotypes × sex	3	2.6975	> 0.05
Chromosomes × sex	3	0.5601	...
Genotypes × chromosomes × sex	9	4.0421	> 0.05
Error (between individuals)	688	2.4595	
Total	719		

(iii) *th st cp Sb* testcrosses

Similar testcrosses were then made using a *th st cp Sb/th st cp* marker stock, the high/*th st cp Sb* females being mated to *th st cp* homozygous males. Recombinants separating *th* from *st* or *st* from *cp* were not taken for assay. The results are summarised in table 2.

The difference of chaeta number between the parental classes of progeny is 2.62, rather higher than that obtained in the *y bw st* and *st cp* assays, but of the same order. The *th st cp Sb* chromosomes are therefore similar in effect to low second chromosomes, and the high factors in the tested chromosome cannot be far outside the markers *th* and *Sb*.

Both marker recombinant classes have means intermediate between the parental classes, indicating that at least part of the high chaeta effect is brought about by a factor or factors located between the loci *cp* and *Sb*.

More precise information is to be obtained by progeny testing the recombinant chromosomes in further testcrosses. Since, however, the *th st cp* recombinant chromosomes are combined in flies with a *th st cp* standard chromosome they are indistinguishable in further breeding experiments. Some 12 months later therefore, the experiment was repeated, but the *th st cp Sb*/High females were testcrossed to *th cp*

TABLE 2

Mean chaeta numbers of the various classes of progeny resulting from the first th st cp Sb testcross. (All flies heterozygous th st cp)

Type of progeny			
<i>th st cp Sb</i> (Standard)	++++	<i>th st cp</i> +	+++ <i>Sb</i>
18.00	20.62	18.95	19.19
Deviation	+2.62	+0.95	+1.19

males. The results are in conformity with those of the first *th st cp Sb* testcross, with the exception that there are larger deviations (table 3).

Since the *th st cp Sb* classes in both testcrosses have the same mean chaeta number, this larger deviation arises from the high chromosome. The same high chromosome had been used in both tests having been

TABLE 3

Mean chaeta numbers of the various classes of progeny resulting from the second th st cp Sb testcross. (All flies heterozygous th cp)

Type of progeny			
<i>th st cp Sb</i> (Standard)	++++	<i>th st cp</i> +	+++ <i>Sb</i>
17.86	21.16	18.86	19.59
Deviation	+3.30	+1.0	+1.73

preserved in males in the interim. It must therefore have mutated at some locus increasing chaeta number by about 0.5 chaetæ during the time that separated the two experiments. This increased chaeta number is recovered in the +++ *Sb* recombinants. The mutant must be located near the *th st cp* markers, for the *th st cp* + recombinants have the same chaeta number as in the first testcross done before the mutant arose.

The occurrence of this mutant is interesting but unfortunate from

the point of view of the present investigation. However, since it does not lie far enough to the right of *cp* to affect the chaeta number of the *th st cp*+ recombinant class, it cannot seriously interfere with the progeny tests designed to determine whether the *cp-Sb* region of the high chromosome contains one or more than one high chaeta-number factor.

Thoday (1961) has discussed the use of marker tests with progeny testing of recombinant classes to determine the number of loci concerned in a chaeta number difference. Briefly, if one or more chaeta loci lie between the markers used the variance between the recombinant classes should be less than that between the parental classes, but the variance within recombinant classes should be greater than that within parental classes. If there is only one locus between the markers, progeny testing should show each of the recombinant marker classes to fall into two chaeta number classes. If there are two loci there should be three chaeta number classes, there being one class of each marker recombinant class that has one but not the other high chaeta number gene. Progeny testing is more powerful and informative than variance comparison.

Progeny tests were made by mating *th st cp*+/*th cp* and +++ *Sb*/*th cp* males to *st p* standard females. Five flies of each sex and genotype from each culture were assayed for chaeta number. Fig. 3 shows the differences of chaeta number distinguishing the *th st cp*+/*st p* or +++ *Sb*/*st p* from the homozygous *st p* flies in two consecutive generations of progeny testing for three classes of marker recombinant chromosome, those having D-values greater than +1.5 chaetae, those having values between +1.5 and +0.6 and those having values lower than +0.6 at the original assay. There is, as is to be expected, some misclassification, but there is no doubt that both marker recombinant classes contain at least three chaeta-number classes, and hence that there are at least two high chaeta-number genes between *cp* and *Sb* in the "high" chromosomes.

A further series of testcrosses was made to determine whether more than two loci may be involved. Ninety-five *th st cp*+/*th cp* recombinants were taken at random from the progeny of the high/*th st cp Sb* × *th cp*/*th cp* testcross. From these eight males of each of five classes were selected, according to their chaeta number deviations from their *th st cp Sb*/*th cp* sibs, and progeny tested to *st p* females, 30 flies of each sex and genotype being assayed for chaeta number from each resulting culture. Their chaeta number deviations as selected, and in the progeny test are given in fig. 4. Though selected as five groups, they seem readily to classify in the fourth progeny test, which was more extensive and involved replicates, into the same three groups as are seen in fig. 3.

These and other retests of similar nature provide clear evidence that three classes of *th st cp*+ recombinant chromosome are obtainable and no good evidence that more classes are to be obtained. Combining

FIG. 3.—The results of two consecutive progeny tests of *cp-Sb* marker recombinant chromosomes. The three classes of chromosome were chosen as having given high, intermediate and low D values at first assay.

A *th st cp+* chromosomes
B *+++ Sb* chromosomes

all the data the mean chaeta number deviations (from *st cp*) are for the three classes $+0.3122$, $+1.4156$ and $+2.7320$ chaetæ per fly. The $+1.4156$ class does not differ in chaeta number from the mean of the other two classes ($t_{88} = 1.1994$ $P > 0.2$).

We conclude therefore that the high D^+ third chromosomes are distinguished from the standard markers we have used and hence from the similar chaeta number "low" D^+ third chromosomes by two high chaeta number factors lying between *cp* and *Sb*, and that each heterozygous high factor adds about 1.2 chaetæ per fly to mean chaeta number.

Comparable progeny tests of the $+++Sb$ recombinant class have been made, but they are less extensive and complicated by the presence

FIG. 4.—Extensive progeny test results of *th st cp*+ recombinants selected as five chaeta number classes to test whether more than three classes occur.

of the mutant factor referred to above. Ten $+++Sb$ /recombinant chromosomes selected to cover the range of such chromosomes had been preserved, and these were progeny tested over four generations, after arbitrary division into four classes. The results are presented in fig. 5. There is a strong suggestion that the four classes are real for there seem to be two classes of chromosome lying between the three low and the one high chromosome. Nevertheless that there are at least three classes is clear, and the lack of evidence for more than three among the *th st cp*+ recombinants clearly supports the view that the fourth class of $+++Sb$, if real, arises from segregation of the irrelevant mutant factor.

It was in fact only this evidence that led us to search back and find the indication that the high chromosome we had used for the most extensive tests had mutated.

FIG. 5.—Progeny tests of $+++Sb$ recombinants to test whether they comprise four chaeta-number classes.

(iv) *Resynthesis of the high factor*

The above results indicate that the high third chromosomes of the D^+ population have high chaeta number effects because they possess two factors a^H and b^H located between cp and Sb , the chromosomes with which we have compared them being $a^L b^L$ at these loci. It follows that the third, medium, class of recombinant $th\ st\ cp+$ chromosome should be $a^L b^H$ at these loci, and the corresponding class of $+++Sb$ chromosomes should be $a^H b^L$. (There are of course two

"medium" classes of +++Sb chromosome, one having 0.5 more chaetæ than the other because it possesses the irrelevant mutant: both should be $a^H b^L$).

If then we make females heterozygous for these two types of chromosome they should produce among their progeny two classes of *th st cp Sb* recombinant chromosome, low and medium, and two classes of ++++ recombinant, medium and high.

Two such tests have been done. In the first we used one of the lower medium +++Sb (Sb 27) chromosomes with a medium *th st cp* + chromosome (th 55). In the second we used one of the higher +++Sb (Sb 30) chromosomes with a different *th st cp* + medium chromosome. The heterozygous females were made using a *Me'* balancer and testcrossed to *th cp*. Heterozygous males were also made and testcrossed

TABLE 4

Mean chaeta numbers of the various classes of progeny produced in the th 55/Sb 27 and th 86/Sb 30 testcrosses. (All flies heterozygous th cp)

Testcross	Sex of heterozygous parent	Type of progeny			
		<i>th st cp</i> +	+++ Sb	<i>th st cp Sb</i>	++++
th 55 × Sb 27	♀	20.13	20.14	19.58	20.23
	♂	19.54	19.57	...	...
th 86 × Sb 30	♀	19.83	20.93	19.37	21.46
	♂	20.11	20.96	...	...

as controls. Fifty of each sex of each genotype were assayed from each cross. The means of the progeny are given in table 4. When due allowance is made for the high chaeta number mutant in Sb 30, the two results are in reasonable agreement.

The parental classes of both female and male testcrosses give similar results. The recombinant classes differ in chaeta number from the parental classes in the expected directions.

Variance comparisons should reveal the effect of recombination in producing extreme chaeta number classes among the marker recombinants (Thoday, 1961), but this test proves insensitive in this case and no significant differences of variance emerge. Progeny tests of selected males, provide a much more sensitive test. Their results are illustrated in fig. 6. These progeny tests were done by selecting the ten extreme low and ten extreme high males of each marker class of progeny of the th 55/Sb 27 testcross. They demonstrate no heritable difference between the extremes of the marker parental classes and a clear heritable difference between the extremes of the marker recombinant classes, the *th st cp Sb* chromosomes containing

FIG. 6.—Progeny tests of the chromosomes produced in the resynthesis tests.
(For description see text, p. 423.)

five resynthesised “low” chromosomes, and the ++++ class containing eight resynthesised “high” chromosomes. Table 5 gives analyses of variance demonstrating the significance of these results.

TABLE 5

Analyses of variance of the results obtained on retesting chromosomes selected from each of the four marker classes of progeny produced in the th 55/Sb 27 testcross

Genotype	Source	Sd^2	n	M.S.	F	P
<i>th st cp +</i>	High/low	0.0782	1	0.0782	...	...
	Error	3.8612	18	0.2145		
	Total	3.9394	19			
<i>+++ Sb</i>	High/low	0.0180	1	0.0180	...	...
	Error	1.4970	18	0.0832		
	Total	1.5150	19			
<i>th st cp Sb</i>	High/low	2.4852	1	2.4852	10.9916	<0.01, >0.001
	Error	4.0692	18	0.2261		
	Total	6.5544	19			
<i>++++</i>	High/low	6.1650	1	6.1605	17.0557	<0.001
	Error	6.5015	18	0.3612		
	Total	12.662	19			

The existence of two separable chaeta loci between *cp* and *Sb* is confirmed.

It should be noted that the mean chaeta numbers of the flies containing the five resynthesised intermediate *th st cp Sb* chromosomes,

and of the eight resynthesised ++++ chromosomes differ by 2.5 chaeta, showing that nothing of the high chaeta number effect of the high D⁺ chromosome has been lost in the process of breakdown and resynthesis.

5. MORE PRECISE LOCATIONS

Rough estimations of the positions of the two chaeta number loci indicate them to be at about 48.0 and 53.0 cMs on the linkage map, but for precise location testcrosses have been made using markers nearer these positions than are *cp* and *Sb*. Several of the marker stocks obtained and used proved too heterogeneous and hence difficult to use though none of the results conflict with those to be described below, and all of them helped us to determine which markers should be used.

Two of the test crosses proved to involve reasonably adequate marker stocks. These involved a *cu kar* stock and a *p cu* stock (see fig. 1).

(i) The *cu kar* testcross

Thirty flies of each sex of each marker genotype were obtained from the progeny of high/*cu kar* ♀ × *cu kar* testcrosses. The means and

TABLE 6

Mean chaeta numbers of the various classes of progeny produced in the cu kar testcross. (All flies heterozygous cu kar)

<i>cu kar</i> (standard)	Type of progeny		
	++	<i>cu</i> +	+ <i>kar</i>
18.43	19.85	18.83	19.16
Deviations	+1.42	+0.40	+0.73

Analysis of variance

Source	<i>n</i>	M.S.	P
+/ <i>cu</i>	1	45.9375	<0.001
+/ <i>kar</i>	1	17.6042	<0.005
+/ <i>cu</i> × +/ <i>kar</i>	1	1.2042	>0.05
Genotypes	3	21.582	<0.001
Sex	1	63.4952	<0.001
Genotypes × sex	3	1.4627	>0.05
Error (individuals)	232	1.7313	
Total	239		

deviations of the progeny are given in table 6. The deviations are less than in the testcrosses described earlier indicating that the *cu kar* third chromosomes give rather high chaeta numbers. Nevertheless

the means suggest that part of the high chaeta effect of the high chromosomes is recovered in each marker recombinant class, the majority being in the $+kar$ class.

FIG. 7.—Progeny tests of *cu-kar* recombinant chromosomes chosen at random.

These results suggest that of the two loci "a" lies to the left of *cu* and "b" lies between *cu* and *kar*. Progeny tests of 20 $+kar$ and 15 $+cu$ chromosomes (chosen at random) give the results illustrated in fig. 7,

FIG. 8.—Large scale progeny tests of selected *cu-kar* recombinant chromosomes with $++$ controls.

which are consistent with this view, implying as it does that the $+kar$ chromosomes should comprise the two genotypes $a^H b^H$ and $a^H b^L$ and that the *cu* $+$ chromosomes should comprise the two genotypes $a^L b^L$ and $a^L b^H$. Further extensive progeny tests (fig. 8) of selected samples of these chromosomes confirm that each marker recombinant

class involves two chaeta number classes, and that the high+*kar* recombinants are comparable with high chromosomes tested at the same time on a comparable genetic background. The locus *b* therefore lies between the loci *cu* (50.0 cM) and the locus *kar* (52.0 cM). Since it is clearly readily separable from both markers we place it at $51 \pm \text{cM}$.

(ii) *The p cu testcross*

Precisely comparable testcrosses were done with the loci *p* (48.0 cM) and *cu* to give greater precision to the location of "a". Two of the

TABLE 7
Mean chaeta numbers of the progeny produced in the p cu testcrosses.
(All flies heterozygous *p cu*)

Chromosomes tested	Type of progeny			
	<i>p cu</i> (Standard)	++	<i>p</i> +	+ <i>cu</i>
B ₄	19.20	21.52	20.80	20.38
D ₉	19.26	21.22	20.90	19.66
Joint mean	19.23	21.37	20.85	20.02
Deviation	...	+2.14	+1.62	+0.79

Analysis of variance

Source	<i>n</i>	M.S.	P
+/ <i>p</i>	1	42.9025	<0.001
+/ <i>cu</i>	1	220.5225	<0.001
+/ <i>p</i> × +/ <i>cu</i>	1	1.8225	...
Genotypes	3	88.4158	<0.001
Chromosomes	1	4.0325	>0.05
Sex	1	16.4075	.05 >0.01
+/ <i>p</i> × chromosomes	1	8.7025	.05
+/ <i>cu</i> × chromosomes	1	1.3225	...
+/ <i>p</i> × +/ <i>cu</i> × chromosomes	1	0.9025	...
Genotypes × chromosomes	3	3.6425	>0.05
Genotypes × sex	3	2.1292	...
Chromosomes × sex	1	0.0025	...
Genotypes × chromosomes × sex	3	1.2625	...
Error (individuals)	384	2.8531	
Total	399		

high D⁺ chromosomes (one the same as that used with *cu kar*) were used. The results are given in table 7. Again each marker recombinant class contains part of the high chaeta effect but this time the *p*+ class is the higher.

Progeny tests of ten chromosomes (chosen at random) of each recombinant class and ten ++ parental chromosomes gave the results

illustrated in fig. 9. The $p+$ recombinants overlap the $++$ data and the low values are given by the $+cu$ recombinants.

These results are consistent with the view that the $p+$ recombinants comprise the two classes $a^H b^H$ and $a^L b^H$ and the $+cu$ recombinants the two classes $a^L b^L$ and $a^H b^L$. Further progeny testing of

FIG. 9.—Progeny tests of p - cu recombinant chromosomes, chosen at random, together with $++$ controls. Two high chromosomes (B_4 and D_9) were used to generate recombinants.

FIG. 10.—Large scale progeny tests of selected p - cu recombinant chromosomes with $++$ controls.

selected extreme recombinant chromosomes (fig. 10) leaves no doubt of the reality of the two classes of $+cu$ chromosome, though the distinction of the two classes of $p+$ is less clear. The lower $p+$ data agree well with the higher $+cu$ but of the higher $p+$ only one is of clearly higher chaeta number. Bearing in mind the error variance (fig. 10) of the $++$ parental control results this is not surprising.

In view of this, two $p+$ chromosomes of each putative class were subjected to a further progeny testing for two further generations on an extensive scale. The higher gave mean chaeta deviations about 2.3 chaetæ which were not significantly different from those given by $++$ and the lower gave mean chaeta deviation about 1.4 that were significantly different from both the high $p+$ ($p < 0.001$) and the $++$ parental deviations ($p < 0.01$).

It is concluded that the locus "a" lies between p (48 cM) and cu (50 cM) at $49 \pm$ cM.

6. DOMINANCE TESTS

From the chromosomes described on p. 420, a high $th\ st\ cp+$ and a low $th\ st\ cp+$ chromosome, both extensively tested, were chosen and made homozygous using the Me' balancer. The two resulting stocks and their F_1 were assayed for chaeta number on a large scale with the results summarised in table 8. It is clear that the two high chaeta

TABLE 8

Mean chaeta numbers of each type of progeny produced in the dominance test

Type of progeny		
HH/HH	HH/LL	LL/LL
22.1167	22.2222	20.4667

number factors together are completely dominant to the two low factors.

Further tests were then made using $th\ st\ cp\ a^H\ b^L$ and $th\ st\ cp\ a^L\ b^H$ chromosomes derived by further breeding to determine the dominance relations of the separate loci. The results are summarised in table 9. Both high loci are fully dominant and the two have quantitatively indistinguishable effects on chaeta number. There is no evidence that they interact. We are indebted to Dr J. B. Gibson who bred these chromosomes and made this test.

7. DISCUSSION

The results described in this paper show that the high chaeta number third chromosomes responsible for the polymorphism established in Thoday and Boam's (1959) selection line are distinguished from "normal" chromosomes by two fully dominant factors at 49 and 51 centimorgans each raising chaeta number by 1.2 per fly. The alternative third chromosomes in the line give flies with chaeta numbers similar to those of the standards used for locating these genes and are presumably therefore $a^L\ b^L$ in genotype.

These investigations provide a sufficient explanation of the maintenance of the polymorphism in Thoday and Boam's population, but, unlike those of Gibson and Thoday (1962a) do not explain the origin of the polymorphism. Further, the few third chromosomes we have tested from the "Dronfield" wild stock from which the polymorphic population was derived, appear to be $a^L b^L$ at these loci. Though it seems likely that Dronfield originally contained $a^H b^L$ and $a^L b^H$ chromosomes and that the $a^H b^H$ chromosomes were produced

TABLE 9
Mean chaeta numbers of genotypes differing at the a and b loci

	HH	HL	LH	LL
HH	21.52	21.45	21.21	21.26
HL		19.66	21.55	19.36
LH			20.00	19.56
LL				18.57

Analysis of variance of culture totals

Source	n	M.S.	P
$a^H + b^H/a^H$ or b^H/L only *	2	135912.2	Small
Residual genotypes	7	1230.9	≈ 0.2
Total genotypes	9	31160.1	Small
Cultures error	18	727.8	
Total	27		

* Comparison is HH/HH+HH/HL+HH/LH+HH/LL+HL/LH versus HL/HL+LH/LH+HL/LL+LH/LL versus LL/LL to give two degrees of freedom.

by recombination as implied by Thoday and Boam (1959), we cannot provide any evidence for this, and the origin of one or both the high genes by mutation during the selection experiment is also possible.

Though these investigations fail to provide a complete explanation of the origin of the polymorphism under study, they do provide a further striking demonstration of the possibility of locating at least some of the genes of quantitative genetics. Only four loci have been shown to be involved in the double polymorphism that was established by disruptive selection: the two in chromosome II of Gibson and Thoday (1962a) and the two demonstrated here.

The two loci on chromosome II were shown to have remarkable properties (Gibson and Thoday, 1962b), and their lethal interaction

aided location considerably. The two loci described in the present paper gave more difficulty for though of smaller effect they had to be located on the basis of chaeta number alone. Despite the need to use heterogeneous marker stocks, and the lack of a sufficiently homogeneous stock with markers close to and on both sides of the important region of the linkage group, it proved possible to show that this region is between positions 48 and 52 on linkage group III and to demonstrate two separable loci with equal effects on chaeta number. There is no evidence that they interact, and both appear fully dominant. Disruptive selection must have maintained these two in coupling linkage phase, against recombination pressure, the two "dubious" chromosomes of Thoday and Boam (1959) being examples of the repulsion products of recombination.

Such precise information adds to our understanding of quantitative genetics and will, we hope, encourage others to attempt to locate such genes and thus provide information complementing that obtainable from biometrical analyses.

8. SUMMARY

1. The high chaeta number third chromosomes responsible for the polymorphism established by Thoday and Boam by disruptive selection have been shown to contain two high chaeta number genes at 49 and at 51 centimorgans.

2. Each is fully dominant, they do not interact, and they each add about 1.2 chaetæ per fly.

3. Disruptive selection must have preserved the two loci heterozygous in the coupling phase of linkage.

9. REFERENCES

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