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Regular responses to selection

2. RECOMBINATION AND ACCELERATED RESPONSE

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1. INTRODUCTION

Thoday & Boam (1961) have described the results of a selection experiment in which four related lines of *Drosophila melanogaster* produced remarkably similar responses to selection for high sternopleural chaeta number (Fig. 1). Two of these, dp 1 and dp 2, each homozygous for the mutant gene *dp* produced almost identical responses. dp 1 originated from F_2 *dp*/*dp* segregants from a cross of a *dp*/*dp* stock × inbred Oregon wild type. Initially 19 chaetae, mean chaeta number rose to 24 chaetae in generation 20 at a steady but decreasing rate, then rose rapidly to 28 chaetae in generation 24, and then more slowly to a plateau at about 30 chaetae. dp 2 was taken from dp 1 at generation 9 and subjected to 15 generations of back selection to which it did not respond. Then, put once again under forward selection, it responded exactly as dp 1 except that the final chaeta number was somewhat lower. dp 6 was derived from dp 2 at the end of its back selection phase and maintained for many generations of mass culture before it was placed under selection for high chaeta number. It responded very much like dp 1 and dp 2, but the response was a little delayed and more sudden. The fourth line, vg 4, which had ancestry in common with the dp lines (see Thoday & Boam, 1961) also showed a similar response, though after reaching 30 chaetae it continued to respond until it reached 37 chaetae. Figure 1 of Thoday & Boam (1961) is reproduced here for easy reference.

The present paper is concerned with attempts to explain the rapid rise of chaeta number from about 24 to 28 chaetae in these four lines.

It is axiomatic that a regular response to selection of this kind must involve effects of the relevant gene complexes that are expressed in heterozygotes. The investigations reported here are therefore largely concerned with the effects on chaeta number of single genomes, chromosomes and parts of chromosomes derived from these lines, in flies heterozygous for these genomes (etc.).

2. METHODS

The assay experiments began in 1957 and involved many tests with many markers, their original aim being to find suitable markers for critical tests. The principles developed in the course of these investigations and those of Thoday & Boam (1959),

* Work done while holding an M.R.C. studentship.

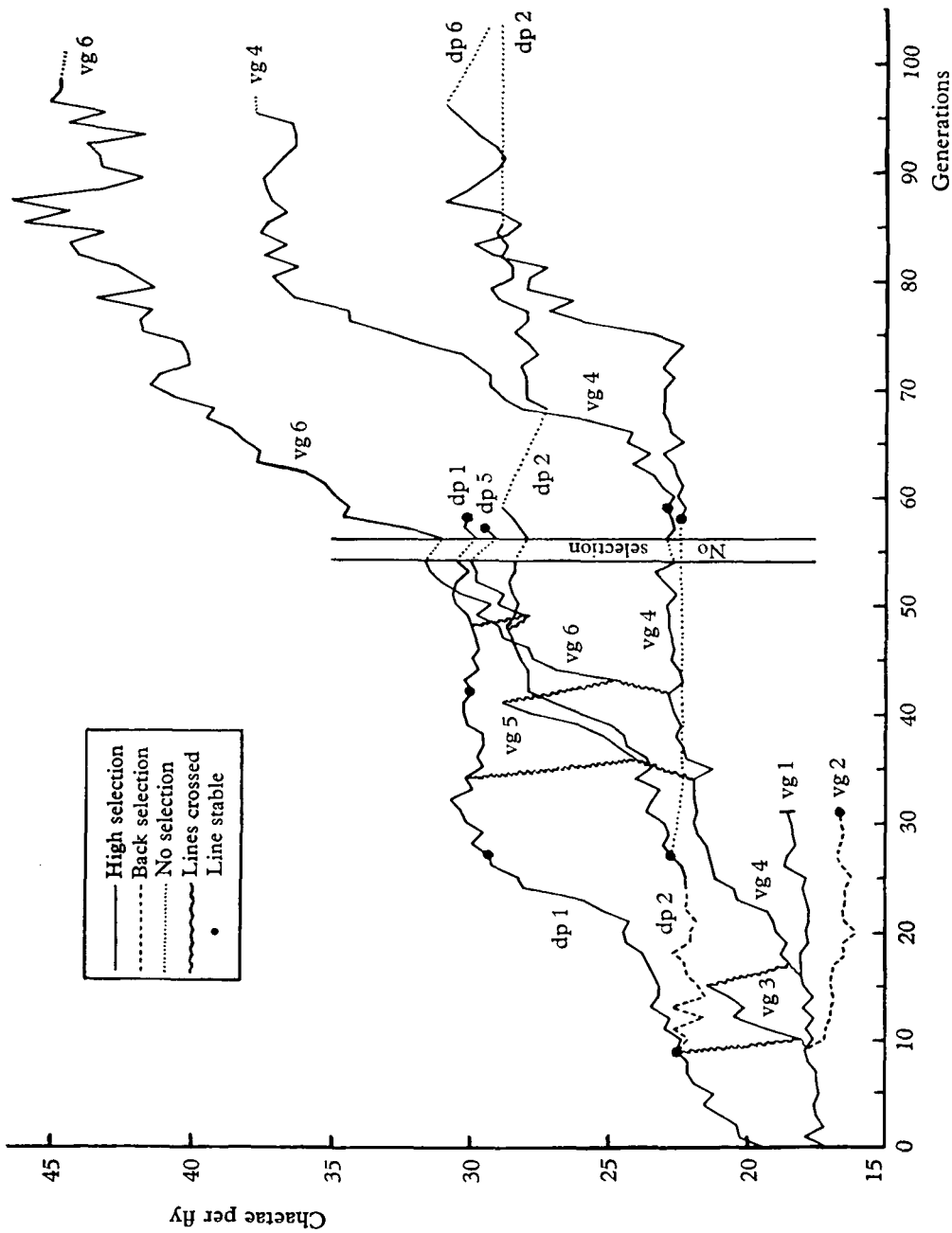

Fig. 1. The selection lines. From Thoday & Boam (1961).

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Gibson & Thoday (1962) and Wolstenholme & Thoday (1963) have been discussed by Thoday (1961).

It would be profitless here to describe the whole series of experiments. We will confine our attention mainly to two types, one intended to assess which of the chromosomes were concerned in the responses, the other directed to analysis of third chromosomes. The markers that were used in the exploratory stages of the investigation are, however, included with those referred to in this paper in Table 1.

Table 1. *Chromosome III markers used in the investigations*

Locus	Location*
ve	0·2
se	26·0
h	26·5
eyg	35·5
rt^2	37·0
Gl	41·4
th	43·2
st	44·0
cp	45·3
p	48·0
Sb	58·2
e	70·7

* From Bridges & Brehme (1946).

The two main classes of experiment are genome assays in which at least chromosomes II and III are marked and *ve h eyg cp* breeding programmes designed to analyse Chromosome III effects. The first involve simple test cross assays to determine which chromosome has the greater chaeta number effect when heterozygous, such as Thoday & Boam (1959) used with homozygous *bw st* or *y bw st* stocks.

The second type of breeding programme is complex and derives from the elegant methods of Breese & Mather (1957). The genome assays indicated that the main effects on chaeta number of dp 1 or dp 2 genomes were associated with a region of chromosome III, and assays with the other markers suggested the relevant genes lay between *se* and *rt*. A *ve h eyg cp* homozygous stock was therefore bred especially for this investigation. It is viable, fertile and behaves very consistently. We are indebted to Mr T. B. Boam who did the breeding work involved in making this chromosome. It was only made once so that the *ve h eyg cp* stock is homozygous in this region apart from any mutation that may occur.

We would stress that an elaborate breeding programme such as this is not essential. In fact Wolstenholme & Thoday (1963) have successfully located chaeta number genes with much simpler programmes. The present technique is, however, more satisfactory inasmuch as it eliminates the markers from chromosomes to be assayed, and was in any case forced upon us by the need to use *h* as one of the markers, because we found *ve se eyg cp* homozygotes infertile, and *se* is seldom classifiable in *eyg* homozygotes.

The *ve h eyg cp* chromosome was used to synthesize chromosomes combining parts of third chromosomes from inbred Oregon wild-type (one of the foundation stocks for all the lines) and parts of third chromosomes from the line to be investigated. The breeding programme was as follows, the particular example given being that necessary to synthesize a number of chromosomes combining the left half of the left arm from Oregon with the right half of the left arm from dp 1. Modifications to make other *synthetic chromosomes* are obvious. The *ve h eyg cp* chromosome is designated vc. Segments of dp 1 chromosome are designated H, those of Oregon chromosome L.

1	vc/vc ♀ × dp 1	vc/vc ♀ × Oregon
2	$\frac{\text{dp 1}}{\text{vc}}$ ♀ × $\frac{\text{vc}}{\text{vc}}$	$\frac{\text{O}}{\text{vc}}$ ♀ × $\frac{\text{vc}}{\text{vc}}$
3	$\frac{\text{vc}}{\text{vc}}$ ♀ × $\frac{\textit{ve h}\ \text{HH}}{\text{vc}}$	$\frac{\text{vc}}{\text{vc}}$ ♀ × $\frac{\text{LL}\ \textit{eyg cp}}{\text{vc}}$

A number of each of these '*derived*' chromosomes were preserved heterozygous in males by perpetual testcross to vc/vc.

4	$\frac{\textit{ve h}\ \text{HH}}{\text{vc}} \times \frac{\text{LL}\ \textit{eyg cp}}{\text{vc}}$
5	$\frac{\textit{ve h}\ \text{H H}}{\text{L L}\ \textit{eyg cp}} \times \frac{\text{vc}}{\text{vc}}$
6	$\frac{\text{vc}}{\text{vc}} \times \frac{\text{LLHH}}{\text{vc}}$

LLHH is the *synthetic* chromosome, and was preserved by further test crosses to vc/vc females.

A number of 'synthetic' chromosomes was made from each 'derived' chromosome.

These synthetic chromosomes were then assayed by test crossing the synthetic/vc males to vc/vc females. Counts were made of ten wild-type progeny of each sex. When a class of synthetic chromosomes proved variable, '*retest*' assays were made by test crossing synthetic/vc males in further generations to determine the number of subclasses with respect to chaeta number they fell into, according to the principles described by Thoday (1961). At each such assay HHHH and LLLL chromosomes were also used as controls.

In the process of breeding the synthetic chromosomes some part of the *ve h eyg cp* chromosome may of course be included. If this latter chromosome differed from the Oregon chromosome in chaeta number genes of importance, this could affect the results. However, tests of the results obtained from several 'derived' Oregon/*ve h eyg cp* chromosomes of each relevant type provided no evidence of variety among these derived chromosomes (see appendix). Hence it is presumed that the Oregon and *ve h eyg cp* third chromosomes have substantially similar chaeta number genes.

Further, the fact that dp 6 (see p. 12) gives results differing from those obtained with dp 1, dp 2, vg 4 and vg 6 indicates that the latter results are not a consequence of differences distinguishing Oregon and *ve h eyg cp*.

3. MATERIAL

In the assays we have used the selected lines dp 1, dp 2, dp 6, vg 4 and vg 6. In addition we have used the *vg*/*vg* and the *dp*/*dp* stocks that were used as foundations for the lines together with the Oregon inbred line that was their other ancestor. Two other lines, to be known as the dp and vg 'base lines' were used in some assays. The first is a 'mass no-selection' line (see Thoday & Boam, 1961) derived from dp 2 just after it was put under forward selection at the end of its back selection period. This is the line from which dp 6 was later taken. The other is a comparable mass no-selection line taken from vg 4 at generation 59. Both these base lines have means of 22 chaetae and represent the stable period that occurred before the accelerated response in the history of the selection lines (see Fig. 1).

Most of the assays to be described below were made in Cambridge two years or more after selection in the lines had ceased. When the senior author moved from Sheffield the lines were maintained without selection by Mr Boam until facilities for *Drosophila* work became available in Cambridge. During this period the chaeta-number of vg 4 and vg 6 fell to 35 and 40 chaetae respectively. dp1, dp 2 and dp 6 were stable.

4. RESULTS

(i) *Genome assays*

The first assay of this type was made in 1957 using generation 62 dp 1 flies and a *bw st* stock. Reciprocal F_1s were obtained and the males test crossed to the *bw st* stock, there being four replicate cultures of each type of test cross. The results are given in Table 2. The test cross progeny of the two reciprocal F_1s are distinguished because in one the females are heterozygous for an X chromosome from dp 1, whereas in the other the X chromosomes all derive from the *bw st* stock. They are also distinguished in that the Y chromosomes have different origins. There are no significant cross, sex, sex × cross, cross × genotype or sex × genotype components in the analysis of variance in Table 2 indicating that dp 1 and the *bw st* stock are not distinguished by their sex chromosomes.

The *bw*/*bw* and *bw*/+ flies have significantly different chaeta numbers, but the difference lies in the direction that the second chromosomes of dp 1 give lower chaeta numbers than do those of the *bw st* stock, hence the second chromosome cannot be responsible for the high chaeta number of dp 1. The third chromosomes of dp 1 on the other hand give distinctly higher chaeta numbers than do those of the *bw st* stock. It seems clear that the major cause of the accelerated response of dp 1 must lie in chromosome III. The difference distinguishing +/*st* and *st*/*st* flies was 3·175 chaetae.

Table 2. *Mean chaeta numbers obtained in assay of dp 1 against* bw st

Cross	Marker types of test cross progeny			
	+	*bw*	*st*	*bw st*
bw st ♀ × (*bw st* ♀ × *dp* 1 ♂)	23·825	24·100	20·700	21·250
bw st ♀ × (dp 1 ♀ × *bw st* ♂)	23·500	24·3500	20·250	20·875

Source	Analysis of variance		
	n	Mean square	*P*
+ /*st*	1	838·51	V. small
+ /*bw*	1	32·51	< 0·001
Interaction	1	0·20	
Crosses	1	2·11	
Genotypes × crosses	3	3·95	
Sex	1	1·01	
Sex × crosses	1	3·20	
Sex × genotypes	3	3·76	
Cultures	6	9·80	< 0·05
Cultures × genotypes	18	4·27	
Other interactions	27	3·15	
Between flies within sex, culture and genotype	253	2·85	

Similiar tests at this time gave similar results for dp 2 and vg 6 which had then only just passed a mean of 30 chaetae. Tests were also made of vg 4 then at the 'base line' value of 22 chaetae, the dp 'base line', Oregon, stock-vg and stock-dp third chromosomes. The dp stock proved heterogeneous for chromosome III, the others showed no evidence of heterogeneity. The differences of chaeta number distinguishing + /*st* from *st*/*st* flies are listed in Table 3.

Table 3. *Chromosome III effects*

Differences in chaetae per fly distinguishing + /*st* from *st*/*st* flies (data from 1957)

Line	Difference
dp 1	3·2
dp 2	2·5
vg 6	3·1
vg base line	1·4
dp base line	1·3
Oregon	(0·5)
dp stock*	(−0·1) and 1·1
vg stock	(−0·2)

* Two different values: the first for 4 chromosomes from a dp stock female; the second for 4 chromosomes from a dp stock male. Each group was homogeneous. The difference between groups is significant ($P < 0·01$).

Differences bracketed are not significantly different from zero.

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It should be noted that these were only small preliminary assays. They were however informative in a number of respects. First, the high lines all have high third chromosomes. Secondly, the base lines both have intermediate third chromosomes, whereas both the vg stock and the Oregon line have third chromosomes indistinguishable in effect from the *st* third chromosomes. Thirdly, the dp stock contained at that time at least two classes of third chromosome, one of which had effects similar to those of the 'base line' chromosomes, the other being similar to *st* and Oregon.

Other results of these assays were as follows:

1. Assays of dp 1 and dp 2 together showed that there was no significant difference between their third chromosome's effects on chaeta number.

2. The vg 6 assay did not produce evidence of an effect of second chromosomes, suggesting that vg 6 second chromosomes then had a slightly higher chaeta number effect than those of dp 1.

3. There was at that time an X chromosome effect of about 0·6 chaetae in vg 6. These last two points will not be considered further in this paper which is concerned solely with the rise of the lines to about 30 chaetae.

These assays showed that at that time dp 1 and dp 2 had a third chromosome effect in common, and if their accelerated responses had a common cause the acceleration must have been caused by change of chromosome III. There followed some extensive investigation of the third chromosomes in the lines dp 1, dp 2 and vg 6 (it will be noted that at that time vg 4 and dp 6 had not yet produced their accelerated responses), in which various markers were used. All assays agreed in leading to the conclusion that the third chromosomes of these lines all had high chaeta number factors in the *se-rt* region, and the investigation was delayed for the breeding of the *ve h eyg cp* chromosome and further delayed by the senior author's move to Cambridge.

(ii) ve h eyg cp *assays*

(*a*) dp 1

We will discuss in detail the results obtained in the *ve h eyg cp* assays which involved third chromosomes from dp 1.

dp 1 was the first line investigated with the *ve h eyg cp* breeding programme. Five of each class of derived chromosomes (see p. 4) from both dp 1 and Oregon were made. Two of each kind of synthetic chromosome were made from each of the 5×5 appropriate combinations of derived chromosomes.

The results are summarized in Fig. 2, which gives the histograms of culture means obtained with the different classes of synthetic chromosomes, together with those of LLLL and HHHH chromosomes which were put through the relevant parts of the synthesis process and backcrossed to the *ve h eyg cp* stock to the same extent as the synthetic chromosomes.

The HLLL chromosomes give a histogram similar to that of LLLL, and the LHHH histogram is similar to that for HHHH. We conclude that the main distinction between HHHH and LLLL chromosomes cannot involve genes much to

the left of the locus *h*. The LLLH and HHHL histograms are also similar to those for LLLL and HHHH respectively, so that the relevant genes cannot be much to the right of the locus *eyg*. The HHLL and LLHH histograms are clearly of a new type.

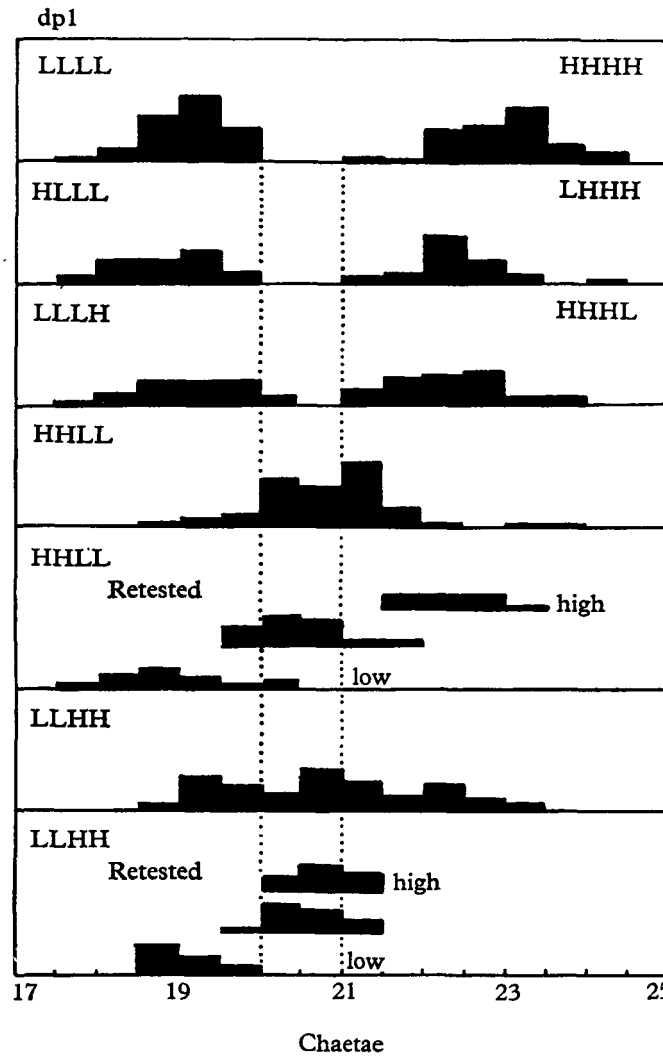

Fig. 2. Distribution curves of mean chaeta numbers for third chromosomes synthesized from Oregon and dp 1, together with results of retests of the extreme and intermediate HHLL and LLHH chromosomes.

They give means intermediate between those for HHHH and LLLL and they overlap the HHHH and LLLL distributions.

These results either mean that there is one high chaeta number locus between *h* and *eyg*, or that there are more loci. If there is only one, 'retests' (further progeny testing to *ve h eyg cp* ♀♀) should show the HHLL and the LLHH chromosomes to fall into only two classes corresponding to HHHH and LLLL. If there were two,

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very close to *h* and *eyg*, the retests should show only one class intermediate between HHHH and LLLL. If there were two, both between *h* and *eyg*, there should be three classes of HHLL and of LLHH chromosome, that containing both high chaeta number genes, that containing one only, and that containing neither. The first class should give a distribution like HHHH, the second an intermediate distribution, and

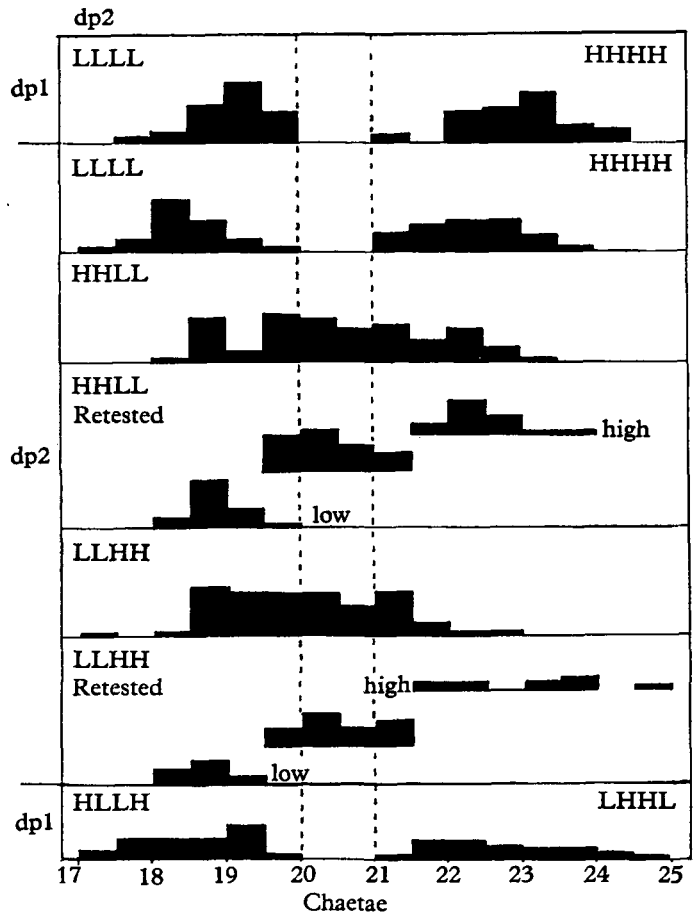

Fig. 3. As Fig. 2, for dp synthetic chromosomes. The control results for dp 1, and the HLLH and LHHL dp 1 results are included for comparison. Synthetics involving *ve-h* and *eyg-cp* recombination were not made from dp 2 chromosomes.

the third one like LLLL. Two 'high', two 'medium' and two 'low' chromosomes of each kind were therefore chosen for further progeny testing to *ve h eyg cp* females. The HHLL retest results show unequivocally that there are at least three real classes, two similar to the 'parental' classes and one intermediate. The LLHH retests give intermediate and low distributions, but the high LLHH chromosomes chosen for retest were clearly misclassified. It would have been surprising had classification been perfect.

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As a further test that the loci we are interested in all lie between the markers *h* and *eyg* 24 HLLH and 24 LHHL chromosomes were synthesized by putting LHHH and HLLL chromosomes from dp 1 through the *ve h eyg cp* programme once more. The resulting distribution curves are figured at the bottom of Fig. 3. It can be seen that the HLLH chromosomes are like LLLL and the LHHL chromosomes are like HHHH. There is no good evidence that any relevant loci effective in heterozygotes lie outside the *h-eyg* region of the dp 1 chromosome III.

These results are astonishingly clear and show that the dp 1 third chromosome is distinguished from the Oregon third chromosome by two regions with effects increasing chaeta number, both between *h* and *eyg*. Each may be complex of course, but they are two effective factors in Mather's (1949) sense. Designating the dp 1 chromosome as + + in Mather's (1943) system and the Oregon as − −, the medium HHLL chromosomes must be + − and the medium LLHH chromosomes must be − +. The distribution curves for these chromosomes are very alike, so that we must conclude the two effective factors are similar in quantitative effect. The distributions are also approximately intermediate between those for HHHH and LLLL, so no pronounced interactions are indicated.

(*b*) dp 2

In the breeding of synthetic chromosomes from dp 2, attention was concentrated on recombinants involving the *h-eyg* region. Only one of each class of Oregon derived chromosome was used, 20 of each class of dp 2 derived chromosome being used. From each dp 2 derived chromosome, four synthetic chromosomes were made.

The results are illustrated in Fig. 3. They are essentially the same as those obtained with dp 1 except that no misclassification was revealed in the progeny tests. We conclude that dp 2 contains essentially the same effective factors as dp 1.

(*c*) vg 4 *and* 6

The comparable assays of third chromosomes from the lines vg 4 and vg 6 were made somewhat differently. First, only one synthetic chromosome was made from any one chromosome 'derived' from the Vg/*ve h eyg cp* heterozygotes, 30 derived chromosomes of each kind being used. Secondly, the progeny tests were carried out on all (instead of extreme and intermediate samples) of the synthesized HHLL and LLHH chromosomes. Thirdly, as these lines have effective chaeta number genes on other chromosomes, chromosome III was first put on an Oregon background. The results are given in Figs. 4 and 5. There is no doubt that these two lines contain third chromosome genes similar to those in dp 1 and dp 2.

vg 6 was made by hybridizing vg 4 and dp 1 well before vg 4 produced its accelerated response (Fig. 1). It was therefore expected by Thoday & Boam (1961) that vg 6 would have derived from dp 1 the genes responsible for the delayed response of dp 1. vg 4 on the other hand, though it had ancestry in common with dp 1 (see Fig. 1) clearly produced the chromosome concerned with the delayed response of itself.

Comparison of Figs. 4 and 5 shows how very similar the third chromosome produced in vg 4 and the third chromosome transferred from dp 1 and vg 6, must be.

vg 4 and vg 6 (see Fig. 1) rose to much higher chaeta numbers than the dp lines. We know these further rises to be due to genes on chromosomes II and I. The evidence for this will be published and discussed in a later paper.

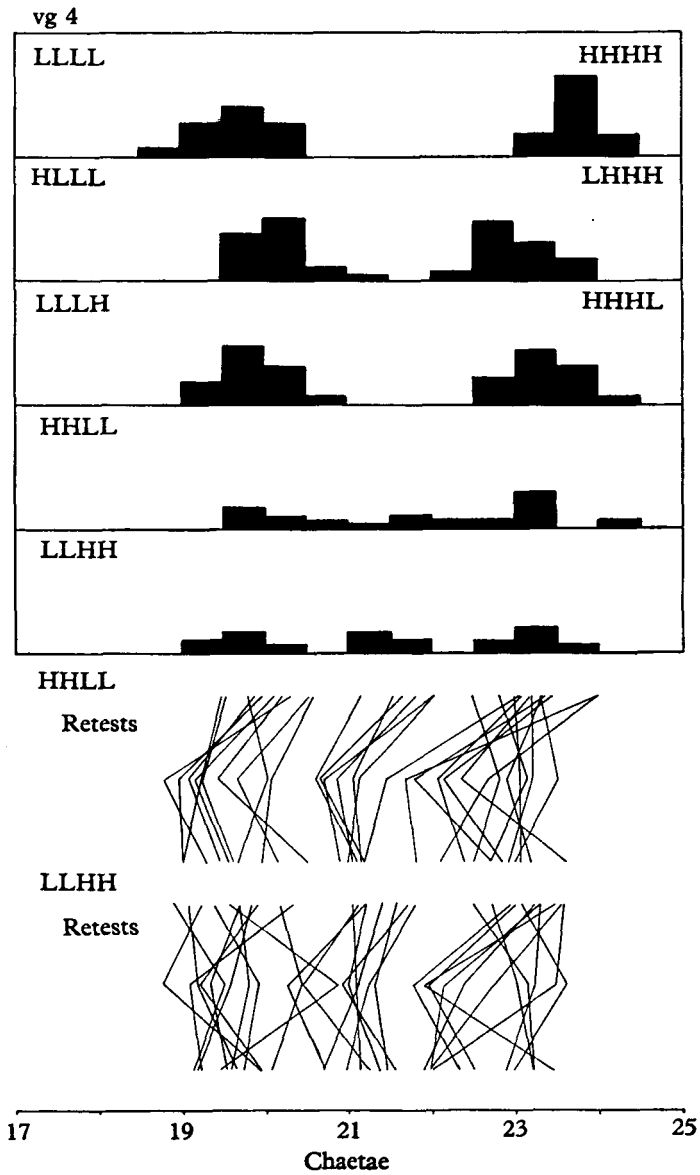

Fig. 4. As Fig. 1 for vg 4 synthetic chromosomes. Progeny testing of HHLL and LLHH synthetics was made on the whole sample; the results for each individual chromosome are presented in the form of a line connecting the mean at first assay with the means in two consecutive generations of progeny testing.

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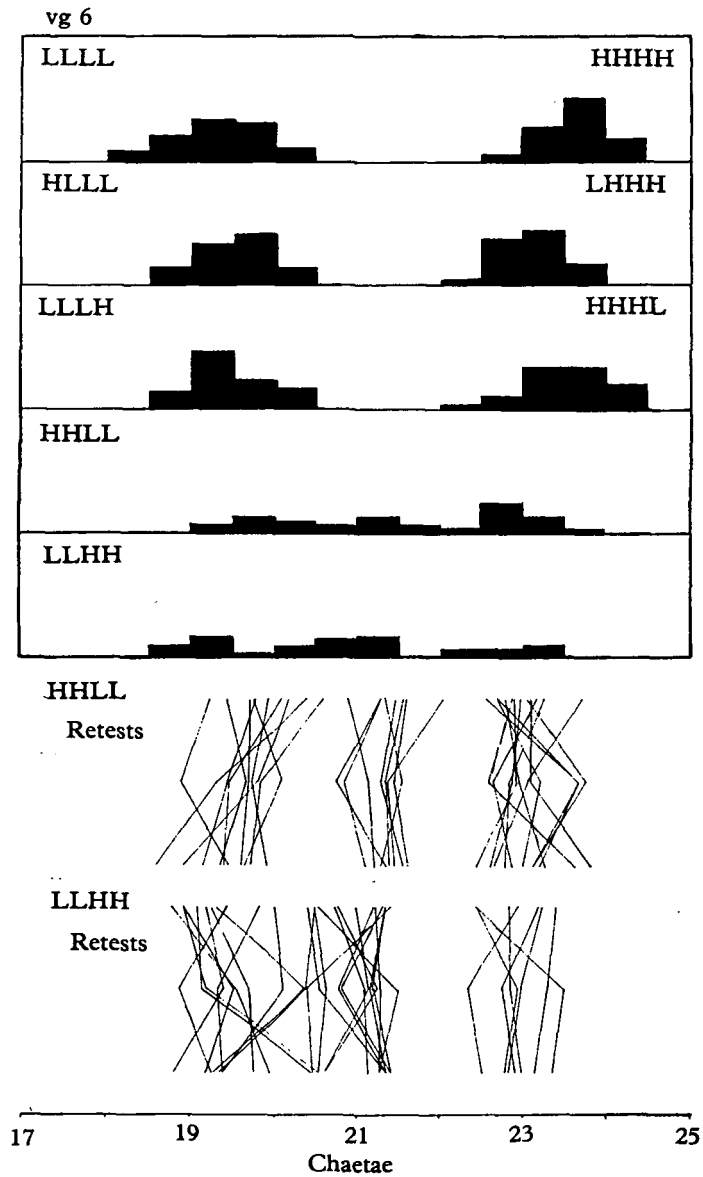

Fig. 5. As Fig. 4 for vg 6 synthetic chromosomes.

(*d*) dp 6

y bw st assays of dp 6 show that it is different from the other dp lines. Correspondingly it also gives quite different *ve h eyg cp* results. They indicate some difference associated with chromosome III, but both *h-eyg* recombinant classes appear to be homogeneous which would imply two loci close to *h* and *eyg* or one on either side of the *h-eyg* region. The dp 6 chromosome III evidently differs from that of the other lines and it will not be considered further in this paper.

(iii) *Location of the factors*

The vg 4 and vg 6 data, since only one synthetic was made from each derived chromosome and progeny tests of all the synthetic chromosomes were made, allow us to attempt to estimate the position of the loci. Table 4 gives the necessary data derived from the progeny tests given in Figs. 4 and 5. The few chromosomes that show some misclassification have been placed in the most plausible class.

Let p_1 be the proportion of the *h-eyg* linkage map separating *h* from the left chaeta locus, p_2 that separating the two chaeta loci, and p_3 that separating the right chaeta locus from *eyg*: $p_1+p_2+p_3=1$. Then, allowing for the two recombinations involved, the proportions of the synthetic chromosomes falling into each chaeta-number class will be as given in Table 4.

Table 4. *Frequencies of* $++$, $+-$ *or* $-+$, *and* $--$ *chromosomes obtained in the h-eyg recombinant classes in the* ve h eyg cp *tests*

	Class of chromosome							
	HHLL				LLHH			
Line	$++$	$+-$	$--$	Total	$--$	$-+$	$++$	Total
vg 4	13	6	10	29*	11	8	11	30
vg 6	13	8	9	30	12	11	7	30
Total	26	14	19	59	23	19	18	60
e	p_3^2	$p_2(p_2+2p_3)$	$p_1(2-p_1)$	1	$p_3(2-p_3)$	$p_2(p_2+2p_1)$	p_1^2	1

* One vg 4 HH *eyg cp* derived chromosome was lost.

Then

the frequency of $++$ HHLL added to that of the $--$ LLHH will be $2p_3$
the frequency of $++$ LLHH added to that of the $--$ HHLL will be $2p_1$
the frequency of $+-$ HHLL added to that of the $-+$ LLHH will be $2p_2$

Dr A. R. G. Owen informs us that this simple method of estimation has almost as high efficiency as a more sophisticated maximum likelihood estimate he has designed. It gives 29·3 and 31·8 centimorgans as the positions of the two chaeta-number loci.

These are the best estimates of the location of the factors with which we are concerned. We do not give estimates of their errors, for they are not in fact fully consistent with the data. If we use them to calculate expectations for the data of Table 4 we find that they predict less $++$ and more $--$ chromosomes among both HHLL and LLHH synthetics than we observed. This inconsistency suggests something peculiar in the recombinational phenomena in this region and will have to be left unexplained until further investigation is possible. Meanwhile we would stress that we do not attach much importance to precise location save that the loci both lie between *h* and *eyg* and are separable. The conclusions to which we attach importance

are that there are two loci between *h* and *eyg* and hence fairly closely linked, and that there is no material of comparable importance affecting chaeta number and distinguishing the Oregon and selected third chromosomes outside the *h-eyg* region.

(iv) *Homozygous effects*

The extent to which the two loci we have revealed provide a sufficient account of the selection responses we are seeking to explain depends considerably on the chaeta number of flies homozygous for + + chromosomes. On this point we have conflicting evidence.

The first results were obtained in one of the early pilot assays of dp 1 third chromosomes made in 1957. dp 1 females were mated to a homozygous *st p* stock and F_1 females were test crossed to *st p*, these markers being close to and on either side of the centromere. From the test cross progeny two pairs of + *p*/*st p* flies were set up and the male progeny of each were assayed for chaeta number. The st^+ males were progeny-tested to distinguish homozygotes from heterozygotes at the marker locus. The results are given in Table 5. The cultures were infertile and the data are few, but the results strongly suggested that the left arm of chromosome III was responsible for the whole of the selection response.

Table 5. *Homozygous effect of the left arm of a dp 1 third chromosome*

	Number of flies and mean chaeta numbers			
	Test 1		Test 2	
Genotype	*N*	Mean	*N*	Mean
st^+/st^+	8	30·8	6	29·5
st^+/st	13	24·8	19	23·7
st/st	6	19·2	9	20·3

A further pilot assay was made a little later. In this dp 1 females were mated to *dp*/*dp Mé*/III males derived from a dp 1 × *Mé*/*se* rt^2 *th* cross. The Moiré progeny gave a mean of 23·5 chaetae and the non-Moiré a mean of 29·9 chaetae, again suggesting that the homozygous effect of chromosome III from dp 1 raised chaeta number to about 30, the mean of dp 1 itself.

These pilot assay results were deemed sufficient for the time being and further assays of the homozygous effect of chromosome III were left until more was known of the relevant genes. In 1961 further assays were made using dp 1 HHHH and Oregon LLLL chromosomes that had been through the *ve h eyg cp* breeding programme. They gave results in sharp contrast to those given above, for the dp 1 HHHH homozygotes had a mean chaeta number of 26, these third chromosomes being tested on *ve h eyg cp* stock background. Values of 26·50 and 26·48 were obtained from flies homozygous for third chromosomes from vg 4 and vg 6 on Oregon backgrounds.

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These assays and the pilot assays reported above differed in two ways. First the genetic backgrounds differed, for the pilot assays were made with mixed dp 1 and *st p* or *Mé/se rt*^{2} *th* stock backgrounds, whereas in the later assays negligible parts of the background can have come from the selected lines. It seems unlikely that this could account for the discrepancy, as the background of the pilot assays cannot have involved a large proportion of dp 1 genes. Second, in the pilot assays the cytoplasm is wholly derived from dp 1, whereas in the later assays none of the cytoplasm came from the selection lines. It therefore seemed possible that there might be some cytoplasmic factor either with an additive effect on chaeta number or interacting with the chromosome III. This hypothesis seemed the more plausible since a cytoplasmic factor raising chaeta number by 0·3 chaetae had already been shown to distinguish one of the female lines of dp 1 in its earlier history (Thoday, 1958).

The possibility that there might be a cytoplasmic effect in the dp 1 line was tested in 1962. Reciprocal crosses were set up between dp 1 and *Mé/Ly*. From both kinds of cross F_1 *Mé* females were mated to dp 1 males, and fifteen flies of each sex of the four genotypes were assayed from each kind of cross. The data enable a comparison to be made between the effect of the dp line cytoplasm and the Mé stock cytoplasm. The results (Table 6) show that there is no significant difference depending on cytoplasm.

Table 6. *Mean chaeta numbers of progeny obtained by crossing F_1 Mé/dp 1 females of each reciprocal cross to dp 1 males*

	Marker non-*Mé*		Genotype *Mé*	
Cytoplasms	*dp/dp*	*+/dp*	*dp/dp*	*+/dp*
From dp 1	31·1	30·9	22·3*	22·9
From *Mé* stock	30·0	31·1	24·0*	23·4

* These two values were each based on only 6 flies as the *dp/dp Mé* flies were semi-lethal at the pupal stage.

However, the homozygotes had a mean chaeta number of 30·5, which is close to that of dp 1 itself, not to that of + + homozygotes obtained from the *ve h eyg cp* synthetic chromosomes. We have not yet been able to explain this discrepancy. It would seem most likely that, additional to the genes we have located in chromosome III, there must be others that only have detectable effect when homozygous. They do not come through the *ve h eyg cp* programme so that we would suppose them to be in the right arm of the chromosome. However, this does not fit the results of the *st p* test, unless we presume that they are far distal in the right arm and therefore are usually included in *+p* recombinants as a result of a second cross-over in the right arm itself.

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5. DISCUSSION

In their discussion of the responses to selection that occurred in these lines Thoday & Boam (1961) argued that the delayed or accelerated responses to selection that occurred in dp 1, dp 2, dp 6 and vg 4 and took these lines from means of about 24 to 28 chaetae in a few generations must have had their origin in a recombinational event which produced from + −/− + heterozygotes the coupling + + chromosome.

Though it seems clear that they were wrong in assuming that the same recombination was involved in dp 6 as in the other lines, the assays reported here suggest that they were essentially right about dp 1, dp 2 and vg 4. The third chromosomes from each of these lines are distinguished from those of the Oregon inbred line third chromosomes by two high chaeta number factors at about 30·2 and 32·6 cMs. These two factors together, when heterozygous with the Oregon − − chromosome, raise chaeta number by a little less than 4 chaetae as compared with homozygous Oregon chromosomes on a similar background. Each separate + factor increases chaeta number by about half this amount and they seem when heterozygous to have approximately equal effects, and to show no pronounced interaction.

The dp and the vg base lines, in 1957 both contained third chromosomes that have effects on chaeta number comparable with those of the + − and − + chromosomes derived from the selection lines in the *ve h eyg cp* breeding programmes. The Oregon inbred stock used in the establishment of the lines and in the assays is by definition − − at the two loci, and the vg stock likewise behaves as − −. On the other hand, the dp stock that was used with Oregon to establish dp 1 from which all the dp lines derive was when assayed evidently heterozygous for at least two classes of chromosome III, one giving chaeta numbers similar to − −, the other giving chaeta numbers characteristic of + − or − +.

These facts are essentially what was to be expected if the general explanation of the delayed responses put forward by Thoday & Boam (1961) were sound, and they enable us to suggest the following account of the essential genetic changes that seem to have occurred in these lines before they reached the chaeta numbers at which the accelerated responses occurred.

At its initiation dp 1 seems likely to have derived − − third chromosomes from its Oregon parent. It may also have derived − − chromosomes from its dp stock parent. If, however, we may presume that the evidence that the dp stock was heterogeneous indicates that it contained + −, − + or both types of third chromosome, then dp 1 may also have derived either + − or − + chromosomes or both from this stock. The simplest assumption is that the higher chaeta number third chromosomes we know to have been in the dp stock were of both types and, hence, that at its initiation dp 1 contained at least one of each of these repulsion type chromosomes among the 16 third chromosomes in the foundation flies. Selection for high chaeta number would then lead to an increase of the frequency of the + − and − + chromosomes at the expense of the − − chromosomes, but the probability of + −/− + heterozygous females occurring would be low as most selected flies would be − − heterozygotes. By generation 9, when the line reached the level of 22

chaetae, the frequency of − − chromosomes must have been much reduced. Perhaps only + − and − + remained, which might explain the relative stability of the line at this level against both natural selection and the back selection exercised in the first 15 generations of the history of dp 2, for recombination would be necessary for the line to respond further either forward *or backward* at these loci. Subsequent selection in dp 1 and the later forward selection in dp 2 would in due course pick out and raise the frequency of + + recombinants until, after the level of 24 chaetae was reached, + + chromosomes would be sufficiently frequent for + + homozygotes to occur and be selected. Then we would expect rapid establishment of homozygosity for + + and consequent stability of the lines at the 28 to 30 chaetae level.

Since the vg stock third chromosomes behave as if they were − −, the line vg 1 (see Fig. 1) at its start seems likely to have been − − in all its third chromosomes. These seem to have been the key loci, and this would explain the relative lack of response of this line to selection. However, if our explanation of the dp 1 response is sound, vg 4 will have had a very good chance of starting with + − and − + chromosomes derived from dp 1 since it was originated from a cross of vg 1 and dp 1 at a time when dp 1 had 22 chaetae. Thereafter selection would eliminate − − from vg 4 and leave it heterozygous for + − and − + and capable of the accelerated response.

Thoday & Boam (1961) in discussing the regularity of these responses put forward a hypothesis in terms of two chromosomes + − and − + whose relative frequency might be changed by selection if they had somewhat different effects on chaeta number. They suggested that this might give rise to regularity, for the change of frequency would be correlated with change of mean chaeta number and would necessarily change the frequency of + −/− + heterozygotes and hence the probability of occurrence of recombinants. Their calculations, however, suggested that it was unlikely that this could produce such regular responses as those observed. A similar hypothesis invoking + −, − + and − − in the base population is more likely to be sound, for reduction of the frequency of − − chromosomes would clearly have a more pronounced effect on the probability that + −/− + females would occur.

It seems to us that this is the essence of the story of these accelerated responses to selection and it is our view that the chromosome analyses published here add greatly to the evidence that recombination converting repulsion into coupling linkages is of great importance in selection experiments.

Our evidence is the more clear because the picture it reveals is so relatively simple, involving as it does only two 'loci' of approximately equal effect that do not interact at least when heterozygous. That so simple a result should emerge must act as a warning to us against too readily assuming that responses to selection for a polygenic character will necessarily involve large numbers of loci. We are, however, aware that we have, so far, only sought for the simple aspects of the picture. It should also be borne in mind that these investigations, during which the principles we use for location of polygenes that are described in Thoday (1961) were initially developed, were started just because the selection results were such that a simple explanation seemed likely.

Though we think we have uncovered the essential explanation of the accelerated responses of dp 1, dp 2 and vg 4 (we are not concerned in this paper with the further response of vg 4 beyond 30 chaetae (Fig. 1)), we do not presume we have provided by any means a complete explanation of the responses by demonstrating these two loci. There is in fact evidence that we have not and that other factors must be involved. Viability effects and viability modifiers are clearly concerned for all the lines gave difficulty after the accelerated responses had occurred and in some of the assays there has been difficulty in obtaining adequate numbers of some of the more extreme genotypes. We think it also probable that competition effects were involved such that for example $++/+-$ flies might be more viable in the absence of than in competition with, say, $+-/-+$ flies. There is also the conflicting evidence concerning the effects of $++$ when homozygous. Further, the assays of dp 6 do not seem at present consistent with the view that dp 6 contains $+-$, $-+$ or $++$ chromosomes.

We hope later to obtain more critical evidence concerning these probable complexities. For the present, however, it seems clear that the origin of the $++$ chromosome by recombination was the essential cause of the accelerated responses in dp 1, dp 2 and vg 4.

We feel that the results so far obtained fully justify our view that serious attempts to locate the genes that occur in selected lines are essential if the effects of selection (and the nature of polygenes, see Spickett, 1963) are to be more completely understood.

SUMMARY

1. It has been shown that the lines dp 1, dp 2, vg 4 and vg 6 of Thoday & Boam (1961) each have two high sternopleural chaeta number genes or 'effective factors' between *h* and *eyg* in chromosome III. Their line dp 6 does not contain these two genes.

2. Lines derived from ancestors of dp 2 and vg 4 before the latter produced their accelerated responses have third chromosomes affecting chaeta number as if they had only one or other of these genes.

3. Of the three stocks from which all the lines derived, one, Inbred Oregon, lacks these genes. The second, *vg*/*vg*, has third chromosomes similar in effect to Oregon. The third, *dp*/*dp*, was heterogeneous, having a class of third chromosomes similar in effect to those of Oregon and a class similar to those having one high gene.

4. It is suggested that the history of the accelerated response in dp 1, dp 2 and vg 4 was as follows. Initially most of these third chromosomes were $--$ at the two loci, but a minority (derived from the dp/dp stock) were $+-$ and $-+$ (where $+$ indicates the allele increasing chaeta number. Selection would reduce the frequency of $--$, and hence increase the proportion of $+-/-+$ heterozygotes and the probability of recombination to produce $++$. Origin and multiplication of $++$ would account for the accelerated response.

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APPENDIX: TESTS OF VARIETY AMONG THE DERIVED CHROMOSOMES

The region of interest is that between *h* and *eyg*. 5 + + *eyg cp* Oregon derived chromosomes were used in all combinations with 5 *ve h* + + dp 1 derived chromosomes. Two synthetics were assayed from each of the 25 combinations. Analysis of variance of the mean results for the 25 classes of synthetics shows that the dp 1 derived chromosomes vary significantly whereas the Oregon derived chromosomes do not (Table A). Likewise 5 *ve h* + + Oregon derived chromosomes were used with 5 + + *eyg cp* dp 1 derived chromosomes with similar though less clear-cut results (Table B).

Table A

Source	*n*	Mean square	*P*
dp 1 derived	4	6807·1	< 0·01
Oregon derived	4	438·0	—
Interaction (error)	16	1062·5	—

Table B

dp 1 derived	4	2030·76	< 0·05
Oregon derived	4	1424·46	> 0·05
Interaction (error)	16	541·96	—