Regular responses to selection

3. INTERACTION BETWEEN LOCATED POLYGENES

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1. INTRODUCTION

Thoday, Gibson & Spickett (1964) have described the results of genetic analyses of the third chromosomes of a number of related high sternopleural chaeta-number lines of *Drosophila melanogaster* that were established by Thoday & Boam (1961). These analyses used the principles for locating polygenes described by Thoday (1961), and showed rather clearly that lines dp 1, dp 2, vg 4 and vg 6 all had third chromosomes that increased chaeta number, and that much of this increase was attributable to two small regions, fairly readily separable by recombination, and lying between the markers *h* (26·5 centiMorgans) and *eyg* (35·5 centiMorgans). Evidence was presented suggesting that the regular accelerated responses taking the lines dp 1, dp 2 and vg 4 from about 24 to about 28 chaetae per fly, were made possible by recombinational events that brought together the 'high' alleles at these two loci.

The lines dp 1 and dp 2 ceased to respond to selection at about 30 and 29 chaetae per fly respectively. The lines vg 4 and vg 6 both responded to selection beyond this level. vg 4 produced the high chromosome III itself and reached a mean of 37. vg 6, which acquired the high chromosome III from dp 1 by hybridization of dp 1 with vg 4 before that latter line had produced it, rose to a maximum of 45 chaetae per fly. Thoday and Boam argued that most of the vg 4 response from 30 chaetae per fly to 37, and a corresponding part of the response of vg 6 above 30 must have common causes in genetic material that was present in vg 4 before, but could not be exploited by selection until after, the high chromosome III had been introduced into or produced in that line. In other words this genetic material must interact with one or both of the chromosome III loci.

This paper describes genetic analyses of genomes extracted from these two lines vg 4 and vg 6. It will be seen that the larger part of the differences between vg 6 and Oregon and between vg 4 and Oregon, Oregon being an inbred wild-type ancestor of all these lines, is explicable in terms of a few locatable polygenes, between some of which there are pronounced interactions.

We propose to use the term 'located polygene' for genetic factors located according to the principles of Thoday (1961). It is intended as a non-committal

term to designate genes affecting a polygenic character which, though they cannot be handled by simple Mendelian methods, *have been* located with sufficient accuracy in linkage maps for their individual effects to be open to study. They may prove to be tightly linked complexes, or effective factors (Mather, 1949) and may have built up as such during the course of a selection experiment. On the other hand, some of them may prove to be single functional units in addition to behaving as single units of inheritance in the experiments during which they were located. We have little idea how often unlocated polygenes* will prove to be locatable. In all probability locatable polygenes are a non-random sample comprising the most extremely effective genes or linked complexes of a continuous spectrum which ranges down to genes of vanishingly small effect.

2. MATERIALS

The selection experiment of Thoday & Boam (1961) had to be stopped when the senior author moved to Cambridge in 1959. The lines were thereafter maintained without selection by six-pair transfer in Sheffield by Mr Boam until facilities became available in Cambridge at the end of 1960. By this time a small part of the selection responses had been lost, but the lines appeared to have become stable under natural (relaxed) selection. The means of the lines when these assays began were vg 4, 35·6 and vg 6, 39·2 chaetae per fly, in bottle culture at 25°C. Heritability tests of these lines have given negative results indicating that they are substantially homozygous at loci affecting chaeta number.

As with the assays of chromosome III described by Thoday, Gibson & Spickett (1964) the genomes from these lines have been compared with our standard Oregon inbred line, which was one of the ancestors of all the lines of Thoday & Boam (1961), and with marker stocks similar in mean to Oregon. When these assays began the mean of Oregon was just over 20.

Other stocks used will be referred to when the results are described.

3. RESULTS

(i) *Genome assays*

Four types of assay were made to test which chromosomes were contributing to the high chaeta-number of the selected lines: y bw st F_1 male testcrosses; y bw st F_2's; whole chromosome assays using balancers; and small scale assays using a chromosome IV marker. No chromosome IV effect was detected and it is therefore ignored below.

(a) y bw st F_1 male testcrosses

These testcrosses are carried out in exactly the same way and using the same inbred *y bw st* stock as was used by Thoday & Boam (1959). F_1 + or y, $bw/+$, $st/+$

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* In view of the contemporary fashion for over hasty coining of new terms for genetic concepts, we are tempted to suggest, but hope no one will use, the terms 'neon' and 'krypton' for located and unlocated polygenes!

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males are obtained from reciprocal crosses and testcrossed to *y bw st* females. Chaeta numbers of the different marker classes of progeny are recorded. The difference of chaeta-number distinguishing red-eyed from brown-eyed progeny, together with that distinguishing scarlet-eyed from white-eyed assesses the effect of chromosome II: that distinguishing red from scarlet and brown from white assesses chromosome III. Interactions between chromosomes can be also assessed. Such assays are, however, limited in that they cannot detect recessive effects. The summarized results are listed in Table 1.

Table 1. *Mean chaeta numbers of progeny of* y bw st F_1 *male test crosses of vg 4 and vg 6 flies*

		Second and third chromosomes from vg 4 or 6			
		None	One II	One III	One II, one III
			Eye colour		
		White	Scarlet	Brown	Red
vg 4	♀ $y/+$	20·6	20·8	23·2	23·4
	♀ y/y	19·7	19·6	22·7	23·4
	♂ y	19·4	20·1	22·6	22·8
	♂ $y*$	19·3	19·2	22·1	22·5
vg 6	♀ $y/+$	21·7	22·1	26·2	27·1
	♀ y/y	19·6	20·0	22·7	23·2
	♂ y	19·3	18·9	22·8	23·1
	♂ $y*$	19·0	18·9	22·2	23·2

* From reciprocal crosses.

It is clear from these results that the two lines have high third chromosomes with similar effects on *y bw* backgrounds. There are suggestions that both lines show some effect of chromosome II, but that this is largely dependent on the presence of the high chromosome III. There are indications also of effects of chromosome I, and there is a very striking interaction between chromosome I of vg 6 and chromosome III of vg 6.

(b) y bw st F_2 assays

y bw st F_2 assays, if the F_2 individuals are progeny tested to distinguish heterozygotes from homozygotes for the wild-type alleles of the markers, can inform us about the homozygous effects of the different chromosomes from the lines under test. They are easy to do but are of course crude in the sense that information must be lost through recombination in the F_1 females separating chaeta-number genes from the markers. This is especially important for chromosomes I and II since *y* is at the tip of X, and *bw* is very near the end of linkage group II. The results will not therefore be described in detail here, for they give less information than the results, described below, of the assays using balancers. It is however of

interest that the results were qualitatively consistent with those obtained using balancers, and that the technique, though crude, was surprisingly informative and could therefore be used with other species in which balancers are not available but marker genes are. The results confirmed the existence of high chaeta-number genes on chromosomes II and III of both lines, and of a high chaeta-number effect of chromosome I from vg 6, and of positive interactions between II and III, and between vg 6 I and III. The difference of mean chaeta number distinguishing wild-type homozygotes from *y bw st* homozygotes was 5·6 in the vg 6 F_2 and 4·3 in the vg 4 F_2.

(c) *Genome assays using balancers*

(i) *Chromosomes II and III*. In order to provide more accurate assessment of the contributions of different chromosomes from vg 4 and vg 6 and of interchromosome interactions to the chaeta-number difference distinguishing these lines from Oregon, stocks were bred using the balancer chromosomes CyL^4 and *Mé* in the usual ways. CyL^4 and *Mé* were first put on an Oregon background.

From these breeding programmes, stocks of the following genomic compositions were obtained, where O represents an Oregon chromosome, and V one derived from the line under test.

$$\frac{O}{\overline{O}} \frac{V}{\overline{V}} \frac{V}{\overline{V}}, \quad \frac{O}{\overline{O}} \frac{O}{\overline{O}} \frac{V}{\overline{V}}, \quad \frac{O}{\overline{O}} \frac{V}{\overline{V}} \frac{O}{\overline{O}}.$$

These stocks, Oregon and intercrosses will provide all combinations of chromosomes II and III.

Six male flies from vg 4 and six from vg 6 were used separately. One stock of each kind was made from each original male fly. These six separately-bred sets of stock are referred to below as 'sub-lines'.

Before these stocks could reasonably be used for chaeta-number assays of whole chromosomes from the lines, it was necessary to test the efficacy of the balancers used in breeding them. Heritability tests were therefore made on the O/O V/V O/O and O/O O/O V/V stocks, using assortative mating. No significant heritabilities were found within stocks.

Assays of chaeta number do however indicate significant differences between the replicates or sub-lines of these stocks. There are significant differences between sub-lines and significant genotype sub-line interactions (Tables 2 and 3). However it is clear that these differences between sub-lines are trivial by comparison with those arising from 'genotypes', that is from differences between Oregon and V chromosomes. It would in fact be surprising if the replicate stocks were not different since there is no reason to expect the selected lines to be completely isogenic even though, when tested, they showed no significant heritability. The far greater mean squares associated with genotypes (Tables 2 and 3) clearly indicate substantial similarity between the genomes derived from any one line in replicate breeding programmes. Most of the high chaeta-number genes in the selected lines must be homozygous.

Table 2. *Mean chaeta numbers of flies with various vg 4 autosomes expressed as differences from Oregon*

Number of vg 4 chromosomes II	Number of vg 4 chromosomes III		
	0	1	2
0	20·4	+4·4	+6·1
1	+1·3	+5·8	+8·8
2	+1·7	+6·3	+10·5

Analysis of variance of culture totals

Source	Degrees of freedom	Mean squares	Probability
Genotypes	8	58,749	Small
Sub-lines	5	82	< 0·001
Sexes	1	61	< 0·05 > 0·01
Genotypes × sub-lines	40	127	< 0·001
Genotypes × sexes	8	587	< 0·001
Sub-lines × sexes	5	234	< 0·001
Genotypes × sub-lines × sexes (error)	40	13	

Table 3. *Mean chaeta numbers of flies with various vg 6 autosomes expressed as differences from Oregon*

Number of vg 6 chromosomes II	Number of vg 6 chromosomes III		
	0	1	2
0	19·9	+5·0	+6·6
1	+2·0	+5·9	+9·7
2	+1·8	+6·7	+11·2

Analysis of variance of culture totals

Source	Degrees of freedom	Mean squares	Probability
Genotypes	8	65,741	Small
Sub-lines	5	663	< 0·001
Sexes	1	31	
Genotypes × sub-lines	40	200	< 0·001
Genotypes × sexes	8	814	< 0·001
Sub-lines × sexes	5	92	> 0·2
Genotypes × sub-lines × sexes (error)	40	77	

Tables 2 and 3 list the differences of chaeta number distinguishing flies with various combinations of V autosomes from Oregon (O/O, O/O, O/O) flies. The two tables are very similar. Both vg 4 and vg 6 have high chaeta-number third chromosomes, both have high chaeta-number second chromosomes, and these chromosomes show interaction.

Table 4 gives the results of partitioning the eight degrees of freedom for geno-types from Tables 2 and 3. The assays were designed in the form of a 3^2 factorial

Table 4. *Factorial analysis of genotype sums of squares from Tables* 2 *and* 3

Source	Degrees of freedom	Mean square vg 4	P	Mean square vg 6	P
Chromosome II	1	50,827	Small	51,681	Small
Chromosome II dominance	1	2,340	< 0·001	3,842	< 0·001
Chromosome III	1	400,214	Small	451,092	Small
Chromosome III dominance	1	5,221	< 0·001	4,528	< 0·001
Chromosome II × III	1	8,965	< 0·001	9,436	< 0·001
Chromosome II × dominance III	1	2,417	< 0·001	3,631	< 0·001
Chromosome III × dominance II	1	1	—	142	—
Dominance Dominance Interaction	1	3	—	1,583	< 0·05
Error (Genotypes × sub-lines)	40	127		200	

experiment, the nine genotypic classes representing every combination of three dosages of the two chromosomes. The sums of squares attributable to genotypes may therefore be partitioned fully to give estimates of the components of variance contributed by additive and dominance effects of each chromosome, and by additive and dominance interchromosomal interactions.

The analyses show that in both the lines there are effects of chromosome II and of chromosome III and that both of these chromosomes show dominance. There is an interaction between the main effects of chromosome II and chromosome III. Chromosome II also acts as a dominance modifier of chromosome III in both the lines. There is a suggestion of dominance interaction between chromosomes II and III in the line vg 6 and this is the only term suggesting any difference between the lines.

These results confirm that there are in both vg 4 and vg 6 genetic factors showing pronounced positive interaction for chaeta-number as predicted by Thoday & Boam (1961).

(ii) *Chromosome I.* The *y bw st* testcross and F_2 assays indicated a strong effect of chromosome I from vg 6 together with an interaction between chromosomes I and III. Using an attached X $(\widehat{y}\,\widehat{y})$ stock and the usual balancers for chromosomes II and III, X chromosomes from vg 4 and vg 6 were bred into stocks with an Oregon background. Again six sub-lines of each type were made. Table 5 gives the results of assays of females of these stocks, of Oregon and of hybrids between them and Oregon. No significant effect of the vg 4 X emerged. There is a strong effect of the vg 6 X, with no significant evidence of dominance. The vg 6 X added 1·3 chaetae per fly when heterozygous and 3·2 when homozygous. Corresponding assays of males agreed, the observed effect of the vg 6 X when hemizygous being 2·8 chaetae per fly.

Table 5. *Mean chaeta numbers of Oregon females and females with vg 4*
or vg 6 X chromosomes on an Oregon background

	Number of X chromosomes from the vg line		
	0	1	2
vg 4	20·0	19·8	20·0
vg 6	20·0	21·2	23·1

Analyses of variance of culture totals

vg 4	Degrees of freedom	Mean square	P
Genotypes	2	58	> 0·05
Sub-lines	5	42	> 0·05
Genotypes × sub-lines (error)	10	23	
vg 6			
Additive component	1	12,224	< 0·001
Dominance component	1	234	> 0·05
Total genotypes	2	6,190	< 0·001
Sub-lines	5	55	> 0·05
Genotypes × sub-lines (error)	10	51	

Table 6. *Interactions between chromosomes I and II from vg 6*

Mean chaeta numbers

Number of chromosomes II from vg 6	X chromosome		Difference
	Oregon	vg 6	
0	19·8	22·7	+2·9
1	20·9	24·2	+3·3
2	21·4	24·6	+3·2

Analysis of variance of culture totals

Source	Degrees of freedom	Mean square	P
Chromosome I	1	35,281	Small
Chromosome II	2	4,162	Small
Interaction	2	49	> 0·05
Total genotypes	5	8,740	Small
Sub-lines	5	9	—
Genotypes × sub-lines (error)	25	40	

These stocks were further used together with the chromosome II and III stocks to breed up the combinations of vg 6 and Oregon chromosomes V, V/O, O/O; V, V/V, O/O; V, O/O, V/O; and V, O/O, V/V.

These stocks, together with others described above, permit investigation of the interactions between X and II and X and III in males. Females were not generated for this would have involved more complex breeding programmes. The X in the male stocks was maintained against $\widehat{y}\,y$. The Y chromosomes were from Oregon. Again six sub-lines were used.

Table 7. *Interactions between chromosome I and III from vg 6*

Mean chaeta numbers

Number of chromosomes III from vg 6	X chromosome		Difference
	Oregon	vg 6	
0	20·0	23·3	+3·3
1	24·7	30·8	+6·1
2	26·3	33·8	+7·5

Analysis of variance of culture totals

Source	Degrees of freedom	Mean square	P
Chromosome I	1	113,232	⎫
Additive III	1	165,848	⎪ Small
Dominance III	1	17,442	⎬
I × additive III	1	4,150	⎭
I × dominance III	1	399	< 0·05
Total genotypes	5	61,110	Small
Sub-lines	5	50	
Interaction (error)	25	70	

Table 6 gives the results for X and II, Table 7 those for X and III. Analysis of variance showed no significant interaction between the vg 6 X and II. There is, however, pronounced interaction between the vg 6 X and III, the effect of X being much greater in the presence of III. In these assays the hemizygous vg 6 X added 3·3 chaetae when both chromosomes III were from Oregon, 6·1 chaetae when chromosome III was heterozygous and 7·5 chaetae when both were from vg 6.

The results of these assays are included in Table 8. The genome assays account for all but five of the chaetae distinguishing vg 4 from Oregon, and all but one of those distinguish vg 6 from Oregon. The small effect of the vg 4 X detected in the *y bw st* assays, if real, may account for some of the additional chaetae.

Table 8. *Estimates of the effects of whole chromosomes from the lines vg 4 and vg 6 in chaetae per fly*

Source	vg 4	vg 6	
Oregon mean	20·4	19·9	⎫
Chromosome II homozygous	+1·7	+1·8	⎪
Chromosome III homozygous	+6·1	+6·6	⎬ Tables 2 and 3
Increment from II, III interactions	+2·7	+2·8	⎪
Total effect of II + III	+10·5	+11·2	⎭
X chromosomes homozygous	—	+3·3	⎫ Table 7
Increment from X, III interactions	?	+4·2	⎬
Total = expected mean of lines	30·9	38·6	
Observed mean of lines	35·6	39·2	

(ii) *Chromosome assays*

(a) *Chromosome III*

As mentioned in the introduction, assays of chromosome III have been described by Thoday, Gibson & Spickett (1964). They show that the third chromosomes of vg 4 and vg 6 each contain two high chaeta-number locatable polygenes lying between the marker loci *h* and *eyg*. No evidence was obtained suggesting that regions outside the 10 map units between *h* and *eyg* had, at least when heterozygous, effects on chaeta-number differing from those of the comparable regions of Oregon third chromosome.

In the process of Thoday, Gibson and Spickett's investigations stocks were obtained containing the left high chaeta-number gene without the right, and the right without the left. These stocks have been used in investigations of the interactions with chromosomes I and II and will be referred to below. The left gene will be referred to as 3a: the right as 3b.

Table 9 gives the effects of the two locatable polygenes derived from vg 4 as they influence flies with Oregon first and second chromosomes. There is no evidence of interaction, and the two loci when homozygous are similar in effect. The effect of 3a homozygous is +3·1 chaetae, that of 3b homozygous is +3·3 chaetae, and of the two together +6·4. It will be observed that these effects account for all of the effect of chromosome III detected in the genome assays (Table 8).

Table 9. *Effects of 3a and 3b from vg* 4

Mean chaeta numbers

Number of 3b genes	Number of 3a genes		
	0	1	2
0	20·0 (= Oregon)	22·6	23·3
1	22·2	24·7	25·6
2	23·2	25·4	26·4 (= vg 4 III)

Analysis of variance of culture totals

Source	Degrees of freedom	Mean square	P
3a additive	1	32,100	Small
3a dominance	1	2,499	< 0.05
3b additive	1	39,204	Small
3b dominance	1	2,882	< 0.01
3a × 3b interactions	4	80	—
Total genotypes	8	9,530	Small
Sub-lines	5	158	—
Genotypes × sub-lines (error)	40	343	

(*b*) *Chromosome II*

In order to locate the chromosome region(s) responsible for the chromosome II effects, the marker chromosome *dp cn bw* was used. The evident interaction between chromosomes II and III meant that it was necessary to make separate assays of chromosome II, one in the presence of and one in the absence of the vg line third chromosomes. The *dp cn bw* chromosome was first put on an Oregon background.

The assays were made according to the principles described by Thoday (1961) and used in various ways by Gibson & Thoday (1962), Wolstenholme & Thoday (1963) and Thoday, Gibson & Spickett (1964).

Assays of *dp cn bw* against Oregon provided no notable evidence of differences affecting chaeta number distinguishing these stocks. The assays of chromosome II on an Oregon background could therefore be made by simple testcross of O/O, V/V, O/O stocks to the O/O *dp cn bw/dp cn bw* O/O stock, followed by progeny testing of each recombinant male by further testcrossing to *dp cn bw* for as many generations as seemed necessary to determine whether any marker class of chromosome falls into relatively clear chaeta-number classes. Similar testcrosses of O/O, V/V, V/V stocks to a O/O *dp cn bw/dp cn bw* V/V stock, obtained by further breeding, assay chromosome II in the presence of the V chromosome III.

Figs. 1 and 2 show the results of the assays in the presence of Oregon third chromosomes, Figs. 3 and 4 those obtained in the presence of vg line third chromosomes. The results for vg 4 and vg 6 are similar. Each histogram presents the means, each based on 20 flies of each sex, for 20 chromosomes of the appropriate marker class.

Considering first the results obtained in the Oregon third chromosome background, there are three classes of histogram. Those of *dp cn bw* and *dp cn H* (where *H* represents a region of the V chromosome II) form one group. Their mean is low, the distribution appears unimodal and the variance is low. The histograms of *H H H* and *H H bw* form another group in which the mean is high, the distribution appears unimodal and the variance is low. The histograms of *H cn bw* and *dp H H* form another group inasmuch as the means are intermediate between those of the other two groups, the variances are high and there is an appearance of bimodality in the distributions. The reciprocal recombinant classes differ in that the histogram of *dp H H* is biased towards higher chaeta numbers whereas that of *H cn bw* is biased towards lower chaeta numbers.

Each culture and hence each 'derived' chromosome of the *dp H H* and *H cn bw* classes was further progeny tested by mating males to *dp cn bw*. Details of the results are shown in the figures. These further progeny tests confirm the bimodality of the distributions of culture means from the marker classes *dp H H* and *H cn bw*. Each therefore comprises two chaeta-number classes. The high chaeta-number class is similar, as regards chaeta number, to the parental *H H H* class. The low chaeta-number class is similar to the parental *dp cn bw* class. Since we obtain both parental chaeta-number classes in each of the *dp-cn* reciprocal marker recom-

binant classes it follows that all the chaeta-number difference distinguishing the parental classes is associated with genetic material lying between these two markers. Since there is only evidence for two chaeta-number classes in each of these marker recombinant classes it may be concluded that both vg 4 and vg 6
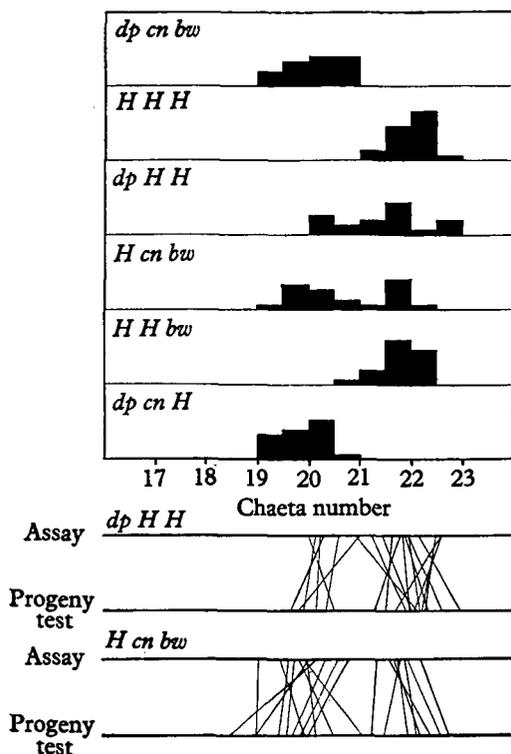
Fig. 1. Assay of chromosome II of vg 4 against *dp cn bw* with chromosome III from Oregon. The histograms show the distribution of mean chaeta numbers given by the indicated marker classes of chromosomes. The lower part of the figure shows how repeatable the means of *dp-cn* recombinant chromosomes were on retesting by further backcross to the *dp cn bw* stock.

second chromosomes are distinguished from Oregon by a single high chaeta-number locatable polygene.

The frequencies of the different chaeta-number classes among the *dp + +* and *+ cn bw* marker recombinant chromosomes derived from vg 4 and vg 6 are set out in Table 10.

The results for the comparable assay of the second chromosome of vg 4 when the third chromosomes are from vg 4 are given in Fig. 3, and those of the similar assay of vg 6 in Fig. 4. The results for the two lines are similar, and they are also qualitatively similar to those of the assays of chromosome II on the Oregon background. The culture means are of course higher, due to the presence of the high chaeta-number producing third chromosomes, and the difference between the

chaeta classes is greater. The figures show much more clearly than those for the assay on Oregon background that, in both vg 4 and vg 6, there is a single locatable polygene promoting high chaeta-number and lying between *dp* and *cn*. The frequencies of the two chaeta-number classes among the *dp + +* and *+ cn bw* marker
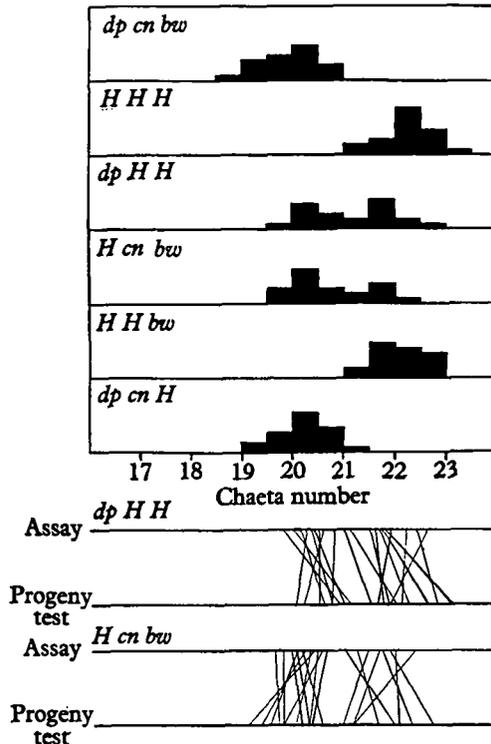
Fig. 2. Assay of chromosome II of vg 6 against *dp cn bw* with chromosome III from Oregon. The histograms show the distribution of mean chaeta number given by the indicated marker classes of chromosomes. The lower part of the figure shows how repeatable the means of *dp-cn* recombinant chromosomes were on retesting by further backcross to the *dp cn bw* stock.

classes are given in Table 10. X^2 analysis does not reveal any linkage heterogeneity between vg 4 and vg 6, or between the assays made on Oregon and high third chromosome background. There can be little doubt that the same locatable polygene is revealed in each of these four assays.

The proportion of the map distance between *dp* and *cn* that separates *dp* from the chaeta locus is $(52 + 49)/160 = 0.63$. *dp* is at 13.0 and *cn* is at 57.5 (Bridges & Brehme, 1944) so that we estimate the chaeta locus as at $13.0 + 0.63 \times (57.5 - 13.0) = 41.1$. The standard error, similarly estimated, is ± 1.7 map units.

The 'locus' on chromosome II has behaved as a unit of inheritance in these assays. There remains however a possibility that this 'locus' is complex. This is to say that the line chromosome II and the *dp cn bw* or Oregon chromosome II might differ at two or more closely linked loci, their heterozygote being say $+ +/- -$.
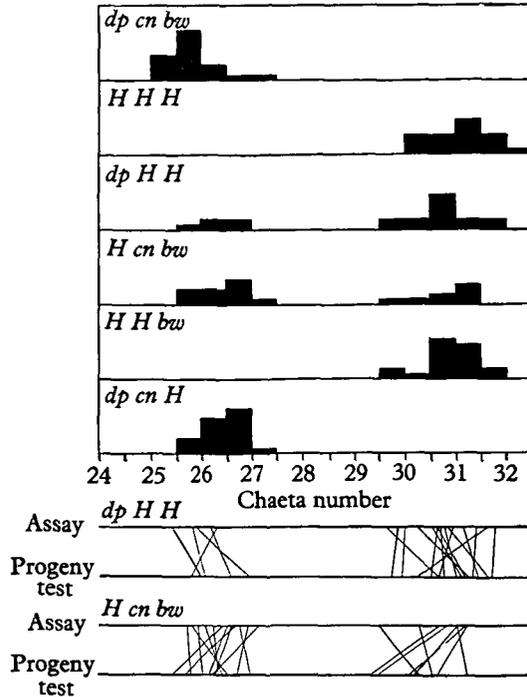
Fig. 3. Assay of chromosome II of vg 4 against *dp cn bw* with chromosome III from vg 4. The histograms show the distribution of mean chaeta number given by the indicated marker classes of chromosomes. The lower part of the figure shows how repeatable the means of *dp-cn* recombinant chromosomes were on retesting by further backcross to the *dp cn bw* stock.

Table 10. *Frequencies of 'high' and 'low' chaeta-number chromosomes among the* dp-cn *recombinants from the crosses used to assay chromosome II*

| Marker class | H cn bw | | dp H H | |
Chaeta class	High	Low	High	Low
vg 4 II (Oregon III)	8	12	11	9
vg 6 II (Oregon III)	8	12	14	6
vg 4 II (vg 4 III)	8	12	15	5
vg 6 II (vg 6 III)	7	13	12	8
Total	31	49	52	28

$\chi^2_{(3)}$ for linkage heterogeneity $= 0.94$. $P > 0.8$.

Recombination within this complex would produce chromosomes with intermediate effects on chaeta number, unless there be very strong interactions between the sub-loci. The assays of chromosome II on the high III background are sufficiently clear-cut (Figs. 3 and 4) for us to be reasonably sure that no such intermediate chromosomes were produced. These assays together involved 80 *dp-cn* marker crossover chromosomes. If the locus were complex then r_2/r_1 of the marker crossovers should be crossovers within the complex locus, where r_2 is the 'length' of the

complex locus and r_1 the map distance (44·5 units) between *dp* and *cn*. Since no intermediate chromosomes were observed among 80 chromosomes we conclude that $X^2_{(1)}$ would be 3·841 if r_2 were 2·038 map units* and, hence, that the probability that r_2 is larger than 2·038 map units is 0·05. The located polygene clearly occupies a very restricted region of linkage map II.
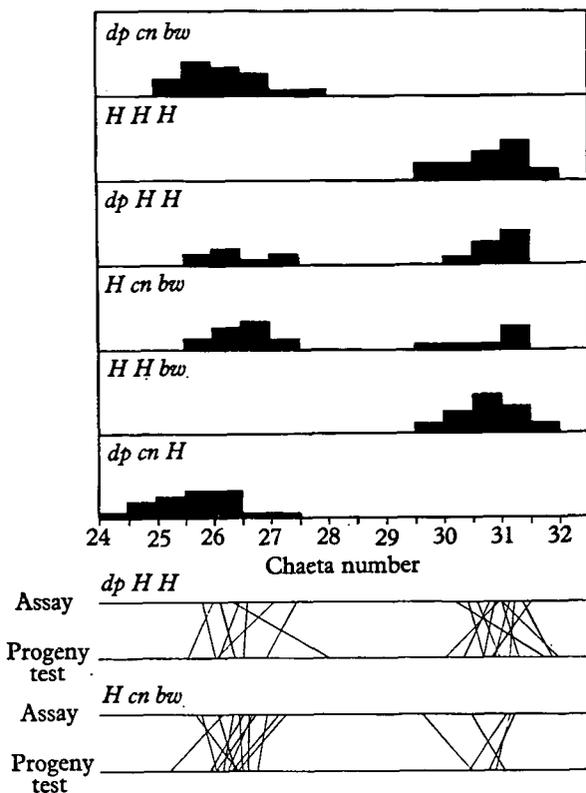
Fig. 4. Assay of chromosome II of vg 6 against *dp cn bw* with chromosome III from vg 6. The histograms show the distribution of mean chaeta number given by the indicated marker classes of chromosomes. The lower part of the figure shows how repeatable the means of *dp-cn* recombinant chromosomes were on retesting by further backcross to the *dp cn bw* stock.

The observed effect in these assays of the located polygene in chromosome II when heterozygous with *dp cn bw* is about 1·75 chaetae when the third chromosomes are Oregon and 4·75 when the third chromosomes are from the vg lines. The effects of whole second chromosomes heterozygous (Tables 2 and 3) were 2·0 and 1·3 with Oregon III and 3·1 and 2·7 with chromosome III from the vg lines. The estimated effect of the located polygene agrees well with that of the whole chromosome II when the third chromosomes are Oregon. The estimated effect of the

* The general formula, when no intermediates have been observed, is $r_2 = r_1\chi^2/(N+\chi^2)$, where N is the number of chromosomes tested and $\chi^2_{(1)}$ has the value giving the chosen probability.

located polygene is larger than that for the whole chromosome when the third chromosomes are from the vg line. It seems likely that the effect of the chromosome II located polygene, varies rather much with minor variations of environment (culture conditions, etc.) or genetic background. It is perhaps for this reason also that the *y bw st* testcross assays (p. 98) gave so little evidence of a chromosome II effect in the absence of the vg lines' third chromosomes.

(c) Chromosome I

Chromosome I was assayed in males on an Oregon background using the marker *y cv v f*, an attached X stock being used to progeny test the marker recombinant males. No attempt was made to use chromosome III from the vg line for these assays. The breeding programme was as follows:

Let chromosome I from the stocks under test be termed *H H H H*.

$$\frac{y\ cv\ v f}{y\ cv\ v f}\ \female \times H\ H\ H\ H.$$

$$\frac{y\ cv\ v f}{H\ H\ H\ H}\ \female \times y\ cv\ v f.$$

Amongst the progeny will be:

$$
\begin{array}{ll}
y\ cv\ v f & H\ H\ H\ H \\
H\ cv\ v f & y\ H\ H\ H \\
H\ H\ v f & y\ cv\ H\ H \\
H\ H\ H f & y\ cv\ v\ H.
\end{array}
$$

These derived chromosomes are preserved and multiplied for assay by crossing to attached X females.

Twenty derived chromosomes of each type were made and the chaetae of twenty male flies from a culture of each derived chromosome were counted. This was done for vg 6 and vg 4, and also for Oregon inbred, in order to test whether the markers or closely linked loci in the marker chromosome had any effect on chaeta-number differing from that of Oregon.

Figure 5 gives the results of assaying Oregon against *y cv v f*. It will be seen that the histogram for any class of derived chromosome containing the gene *f* has a lower mean than the corresponding reciprocal class with *f*+. The mean difference associated with *f* (or with closely linked genetic material) is 0·5 chaetae per fly. In the assay of the vg X chromosomes therefore we must correct for this difference between the marker chromosome and Oregon. This has been done by subtracting 0·5 chaetae from the mean observed for the *f*+ classes of genotype before drawing up the histograms for the vg 6 X assay given in Fig. 6.

Fig. 6 shows clearly that all the marker recombinant classes combining regions of the marker X chromosome with regions of the vg 6 X chromosome have mean chaeta numbers intermediate between those of the parental chromosomes. This indicates that there must be at least two chaeta-number loci distinguishing the two parental classes. Progeny testing of the *y cv v* + recombinant class suggests
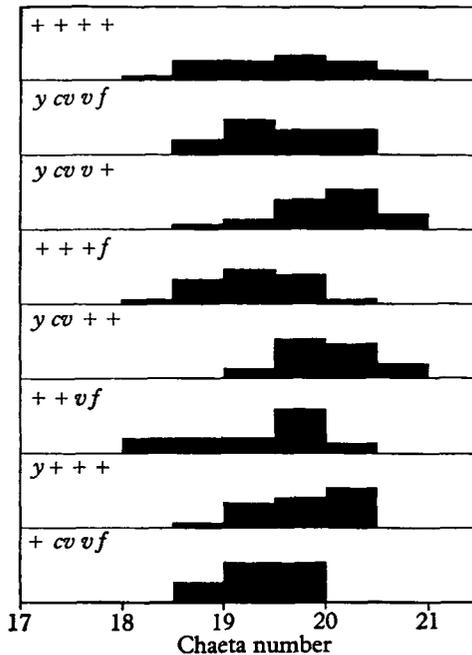
Fig. 5. Assay of Oregon X against *y cv v f*. Each histogram shows the distribution of mean chaeta numbers given by the indicated marker class of chromosomes.

that it falls into two chaeta-number classes one like *y cv v f* the other intermediate. Progeny tests of the reciprocal marker class + + + *f* agree in suggesting that there are two classes, one intermediate, the other high like + + + +. Again progeny tests of *y* + + + suggest an intermediate and high class and of + *cv v f* suggest intermediate and one or two low chaeta-number chromosomes.

These results are not quite as clear as those obtained in the chromosome II assays on an Oregon background. In view of the interaction between chromosomes I and III of vg 6 it would have been desirable to reassay chromosome I on the vg 6 third chromosome background, for it seems likely that this would have made the results much clearer, as it did with chromosome II. However, such assays were not made. The results for chromosome I, therefore, rest on a less sure basis than those for chromosome II.

However, the results as they are clearly show that the minimum requirement to explain this difference between the vg 6 X and the Oregon X is one locus between *y* and *cv* and another between *v* and *f*. Taking the most plausible classification of the marker recombinant chromosomes from the progeny test results gives 2·4 ± 0·51 cMs and 51·5 ± 0·92 cMs for the map position of the two loci. There is no evidence that these loci interact. The effect of each locus is + 1·5 chaetae per fly in hemizygous males on an Oregon background.

The results of assaying the vg 4 X chromosome indicated that this chromosome raises mean chaeta-number by 0·75 on an Oregon background and that this effect is due to genetic material between the markers *v* and *f* which locates at 51·0 ± 1·0.

These results are not reproduced here because the whole chromosome assays of vg 4 failed to detect an effect of X. It is, however, of interest that the location is the same as that of one of the located polygenes in the vg 6 X and the effect is half
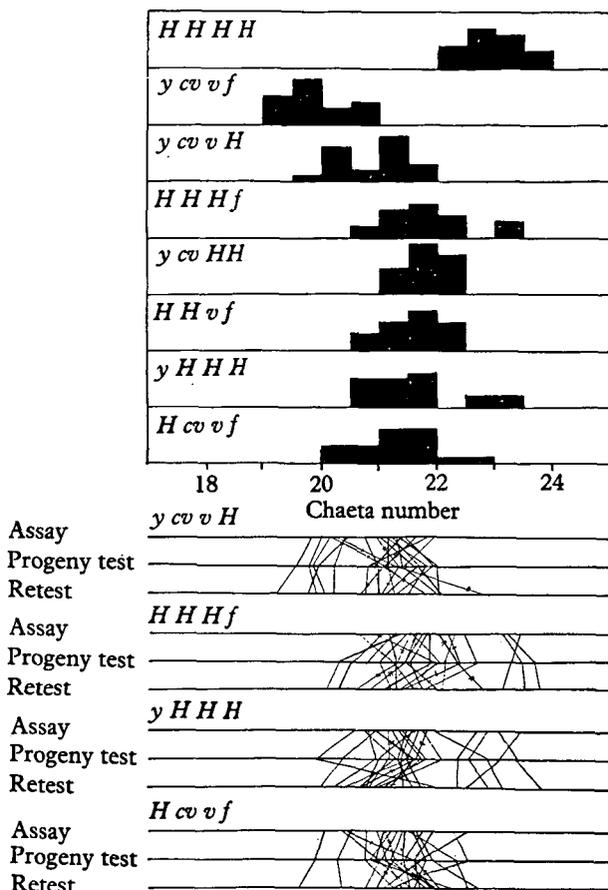
Fig. 6. Assay of vg 6 X against *y cv v f* on an Oregon background. The histograms show the distribution of mean chaeta number given by the indicated marker classes of chromosomes. The lower part of the figure shows how repeatable the means of *dp-cn* recombinant chromosomes were on retesting by further backcross to the *y cv v f* stock.

as great. This suggests that this vg 6 locus may be complex, one component of it being present in vg 4.

(iii) *Factor interactions*

The genome assays showed that there were interactions between chromosome II and III of vg 4, and both between I and III and between II and III of vg 6.

Assays have been made using stocks derived from the location experiments to determine which of the loci are involved in these interactions.

(a) II, III interactions

Stocks containing the gene 3a from vg 4 but not the gene 3b, together with the second chromosome of vg 4, were assayed in a 3^2 factorial experiment with Oregon stocks. The X chromosome was from Oregon. Likewise stocks containing the second chromosome and 3b but not 3a from vg 4 were used in a similar 3^2 factorial experiment. The results are given in Tables 11 and 12. Six sub-lines were used as usual. There is good agreement between the two assays where they should agree. There is significant interaction between II and both 3a and 3b but that involving 3b is much smaller than that involving 3a.

Table 11. *Interaction of chromosome II and locus 3a from vg 4*

Mean chaeta numbers

Number of chromosomes II	Number of 3a chromosomes		
	0	1	2
0	20·3	22·8	23·5
1	21·9	26·2	27·2
2	22·1	26·4	27·9

Homozygous effect of: II alone + 1·8, 3a alone + 3·2.

II + 3a + 7·6. 'Interaction effect' + 2·6.

Analysis of variance of culture totals

Source	Degrees of freedom	Mean square	P
Chromosome II additive	1	152,230	
Chromosome II dominance	1	30,840	
3a additive	1	331,584	Small
3a dominance	1	35,382	
Additive × additive	1	15,606	
Other interactions	3	1,128	> 0·05
Total genotypes	8	70,923	Small
Sub-lines	5	372	—
Genotypes × sub-lines (error)	40	885	

It will be observed that these assays give estimates of the effects of the loci in both chromosomes that agree well with those obtained in the whole chromosome assays (Table 8).

(b) I, III interactions

Comparable assays were made by crossing Oregon and vg 6 X stock females to the 3a and 3b stocks. The results are given in Table 13. There is no interaction between chromosome I and 3b but 3a and chromosome I do interact, the combination of the vg 6 chromosome I and 3b heterozygous with Oregon adding almost three chaetae more than the sum of the independent effects of the two chromosomes.

I

Table 12. *Interaction of chromosome II and 3b from vg 4*

Mean chaeta numbers

Number of chromosomes II	Number of 3b chromosomes		
	0	1	2
0	19·9	22·5	23·3
1	21·7	24·4	24·9
2	21·8	24·6	25·4

Homozygous effect of: II alone + 1·9, 3b alone + 3·4.

II + 3b + 5·5. 'Interaction effect' + 0·2.

Analysis of variance of culture totals

Source	Degrees of freedom	Mean square	P
II additive	1	122,616	⎫
II dominance	1	11,022	⎬ Small
3b additive	1	168,920	⎪
3b dominance	1	19,094	⎭
Additive × additive	1	7,108	< 0·01
Other interactions	3	268	—
Total genotypes	8	32,385	Small
Sub-lines	5	546	—
Genotypes × sub-lines (error)	40	782	

Table 13. *Interaction between vg 6 X, 3a and 3b in males*

Chromosome III	Oregon X	vg 6 X	Difference
Oregon/Oregon	19·9	23·1	+3·2
3a/Oregon	22·4	28·5	+6·1
Oregon/Oregon	20·4	23·4	+3·0
3b/Oregon	22·4	25·3	+2·9

Entries are mean chaeta numbers

(iv) *The combined effects of the located factors*

Assays done at different times and in different ways have generally given rather uniform estimates of the effects of the various locatable polygenes on chaeta number.

The two located polygenes in chromosome III have equal effects, they do not interact and their combined effect when homozygous on an Oregon background is + 6·4 chaetae (p. 104) as compared to + 6·1 for the intact chromosome III (p. 103).[*] The factor in chromosome II when heterozygous on an Oregon background increases chaeta number by + 1·75 chaetae (p. 109), as compared to + 1·3 and 2·0 (Tables 2 and 3) for the intact chromosome II. When the factor on chromosome II

[*] It should be pointed out here that Thoday, Gibson & Spickett (1964) reported two differing classes of experimental result for chromosome III. Most of their assays agreed very well with those described in this paper. Three of their assays, however, suggested a much greater effect of the third chromosome from dp 1 when homozygous.

was assayed on a high chromosome III homozygous background its heterozygous effect was +4·75 chaetae (p. 109), which is greater than the corresponding figure obtained for the whole chromosome assay (Tables 2 and 3). The assays of whole chromosome II and its interactions with the 3a and 3b stocks (Tables 11 and 12) agree closely with the whole chromosome assays, giving a combined II III interaction increment of +2·75 chaetae. Rather than overestimate the effects of the located polygene in chromosome II we will use these latter estimates.

The two located polygenes on chromosome I of vg 6 each add about 1·5 chaetae per fly when on an Oregon background. The whole vg 6 X chromosome interacts with 3a so that when 3a is heterozygous, vg 6 X hemizygous adds 6·2 chaetae per fly.

There is some evidence of a locatable polygene in the X of vg 4 with an effect of +0·75 chaetae on an Oregon background. It is not known whether it interacts with chromosome III.

Ignoring possible second-order interactions, the five located polygenes in vg 6 account for chaeta-number increments by comparison with their alleles in Oregon which sum to 17·5 chaetae. Thus they account for 87·5% of the difference distinguishing vg 6 from Oregon. Most of the deficit appears to be lost in the genome assays as well as the location assays. We therefore have a surprisingly complete account of the differences between our selection lines and Oregon in terms of the few locatable polygenes we have been able to detect.

There is another approach to assessment of the extent to which our location techniques have led to a complete explanation of the difference between the selection lines and Oregon in terms of located polygenes. This is to calculate the means expected of F_1 and F_2, and the variance contributed in F_2 by segregation of the known genes assuming that only the known genes are segregating in F_2 and that all genotypes are equally viable. Comparisons may then be made with observed data.

Suitable data have been obtained from the cross vg 4 × Oregon in a quite independent experiment done by Mr Arnold (unpublished). Ignoring recombination between the loci 3a and 3b and the possible effect of the vg 4 X chromosome, assuming that all F_1 variance is environmental and that environmental variance in F_2 is the same as that of F_1, and taking 20·4 as the mean of Oregon, we obtain:

	Mean	Variance
F_1	26·4	σE^2
F_2	25·6	$\sigma E^2 + 8·1$

Mr Arnold's data give

	Mean	Variance	Variance difference
F_1	26·9	5·4	—
F_2	25·6	15·5	10·1

The three known loci distinguishing Oregon and vg 4 account for the means very well and for 80% of the difference between F_2 and F_1 variances. This seems very satisfactory.

(v) *Qualitative effects of the located polygenes*

In the experiments designed to locate the factors each of them has behaved as a single locus. Such location experiments, however, can never establish that each locus is single in the ordinary sense of a functional unit of genetic material. Location of polygenes as described here can never do more than establish that the loci are behaving as units within the limits defined by the experimental methods used. These units may in fact be complexes, or effective factors in Mather's (1949) terminology. Indeed there is slight evidence suggesting that one of those on the vg 6 X may be (p. 111). On the other hand there is evidence that three of the located polygenes may be quite distinct functional units since Spickett (1963, 1965) has shown that they affect chaeta number in qualitatively distinct ways.

By studying chaeta *pattern* instead of chaeta number, Spickett was able to show that the factor 3a increases the weight of flies and the area of the sternopleurite, and increases chaeta number generally, not only in the whole sternopleurite, but also in other regions of the flies. The increase of weight is brought about by increase of cell number. By contrast 3b and the chromosome II gene had effects restricted to particular regions of the sternopleurite. 3b increased the number of microchaetae in the ventral areas of the sternopleurite. The located polygene on chromosome II had a very local effect in the dorsal region where three macrochaetes normally lie. In fact its effect was confined to the region of the middle macrochaete. In the absence of the gene 3a this factor usually adds, on each side of the fly, a microchaete, which is almost always in this region. In the presence of 3a it usually leads to the replacement of the macrochaete by several microchaetae, apparently by delaying initiation of chaeta development at the macrochaetal site.

The finding that the factor 3a increases fly weight by comparison with its Oregon allele led to the discovery of a further factor in chromosome III of the line vg 4, for this line does not differ significantly in weight from Oregon. Location of fly-weight polygenes showed that the effect of 3a on weight of vg 4 is compensated by a weight-reducing factor to the left of the locus h which has no detectable effect on chaeta number and reduces weight by reducing cell size.

Thus the third chromsome of vg 4 may be designated $-^w +^{wb} +^b$ and that of Oregon $+^w -^{wb} -^b$ where w refers to an effect on weight and b to one on bristle number, and $+$ and $-$ indicating the direction of effect. We cannot of course say whether the factor 3a is affecting weight (or cell number) and chaeta number through being a complex locus or through pleiotropy. But the complex of three loci seems to us to provide a beautiful example of the type of overlapping linked polygenic system affecting more than one character which is the essence of Mather's (1943) theory of polygenic systems. Even if the 3a locus be supposed to be pleiotropic this is so, for we would then have a system where the presumably undesirable pleiotropic effect—on cell number, hence fly size—is compensated for by the cell-size factor so that the effect on chaeta number, desirable in terms of the artificial selection experiment, could be exploited. In this connection it should be stressed

that Mather does not postulate that polygenes cannot have pleiotropic effects, though his stressing that pleiotropy is 'almost useless as a concept for application in biometrical genetics' and his demonstrations that correlated responses to selection are often due to linkage have often been taken as implying his disbelief in pleiotropic action of any polygenes despite his statements to the contrary (e.g. Mather, 1943, p. 55).

4. DISCUSSION

The results presented in this paper and its predecessor explain by far the greater part of the difference distinguishing vg 6 with about 40 sternopleural chaetae per fly from Oregon inbred with 20 chaetae in terms of five genetic factors, some of which show pronounced interaction. Three of these factors explain a large part of the difference between vg 4 with 35 chaetae per fly and Oregon, and two of them explain a large part of the difference between the lines dp 1 and dp 2, each with about 29 chaetae per fly, and Oregon. Of these five factors three, that on chromosome II, and the two on chromosome III are rather precisely located in the linkage groups, and these have been shown to affect chaeta number in qualitatively distinguishable ways. The two on chromosome I that are exclusive to the line vg 6 are less well studied and might well be more readily resolvable by further analysis into more factors. Nevertheless the simple hypothesis of five factors, each occupying not more than about 2 map units, adequately explains most of the difference between the selected lines and their Oregon wild-type ancestor.

This picture conforms closely with that to be derived from the results of similar analyses of lines produced by disruptive selection reported by Gibson & Thoday (1962) and Wolstenholme & Thoday (1963) in which differences produced by selection were shown to be, at least largely, explicable in terms of a few located polygenes of relatively large effect. The effect of these genes is not sufficient for them to be detected by classical Mendelian methods but they are large enough, relative to environmental variance and other sources of variance, to permit them to be handled with some precision using breeding programmes designed according to the principles described by Thoday (1961). This having been done, their distinct developmental effects are open to study (Spickett, 1963, 1965).

It has been pointed out before (Thoday, Gibson & Spickett, 1964) that good arguments can be put forward for supposing that the selection lines described and analysed in this series of papers may be exceptional, for the analysis was only undertaken in the first place because the regularity of responses in the related lines seemed to suggest it would be possible. Likewise disruptive selection seems likely to exploit relatively effective loci and the factors located in the analysis of the disruptive selection lines are unlikely to be a random sample of 'polygenes'.

Further, it must be stressed that the picture revealed in this paper is not to be regarded as a picture relevant to the number of loci controlling sternopleural chaeta-number. It is only a picture of the number of loci distinguishing our selection lines from one of their ancestors. Indeed, the results of analyses of second

chromosomes from disruptive selection lines by Gibson & Thoday (1962), together with some unpublished results Dr Gibson has obtained in assays of second chromosomes from the lines described by Millicent & Thoday (1961), provide a clear warning in this connection. All these disruptive selection lines were derived by selection from the wild stock 'Dronfield', itself originating from one wild female, fertilized before capture. Gibson & Thoday (1962) analysed a low chaeta-number second chromosome and found two relevant loci. Gibson has analysed two low and one high chaeta-number second chromosome from three lines of Millicent & Thoday. Each of these also differed from the standard *dp cn bw* marker used in assay at two loci. But at least four and almost certainly six loci are necessary to account for the combined results. It would seem that any one of these selection experiments has exploited only a limited number of the chaeta-number genes segregating in the 'wild' stock from which they came. The character is much more polygenically controlled in this stock, but the difference between selected chromosomes and those against which they were tested depended in the main on relatively few genes.

Neither is the picture we have revealed in this paper necessarily at all complete in terms of the number of loci relevant in the selection experiment itself. Not only had some of the effects of selection been lost before the assays were done, but two theoretical considerations suggest that locating polygenes can only reveal part of the story.

First, as has been pointed out by Thoday (1963), assaying any selected chromosome against a single standard may not detect all the loci directly affecting chaeta number which were responsible for the results of selection. For example, we have shown the difference between the vg 4 chromosome II and that of the *dp cn bw* stock (and by implication Oregon) to be inherited as a single factor. But two (or more) loci could have been involved in the origin of the high chaeta-number chromosome II. Our results do not exclude the possibility that this chromosome may have been produced by a recombination that combined two + genes one of which is actually present in the *dp cn bw* chromosome and Oregon, the other being derived from, say, the vg-stock ancestors of the lines. The High/*dp cn bw* heterozygote used for assay would then be ++/+− and we would only locate the heterozygous locus. In locating polygenes in the way we have done, then, we may only have located as few as half of the loci directly affecting chaeta number that were relevant in the selection experiment.

Second, we must remember that, if any of the chaeta-number loci affect fitness (or are extremely tightly linked to fitness loci), there may be interactions affecting fitness with other loci themselves affecting fitness but not chaeta number. These latter fitness loci would influence the variance of chaeta number in a population, but could not be located by study of chaeta number with our techniques. There may therefore be genes that are chaeta-number genes from the point of view of population genetics, but are not chaeta-number genes from the point of view of developmental genetics. In other words, these genes would affect the frequency distribution of chaeta-number classes in the population without playing any

chaeta-number controlling roles in the development of the individual. Bearing in mind that Breese & Mather (1957, 1960) have shown complex interlinkage of viability and chaeta-number loci, we must suppose that fitness interactions of this kind are likely to occur quite often. The number of loci influencing response to selection for chaeta number may then be much larger than the number directly
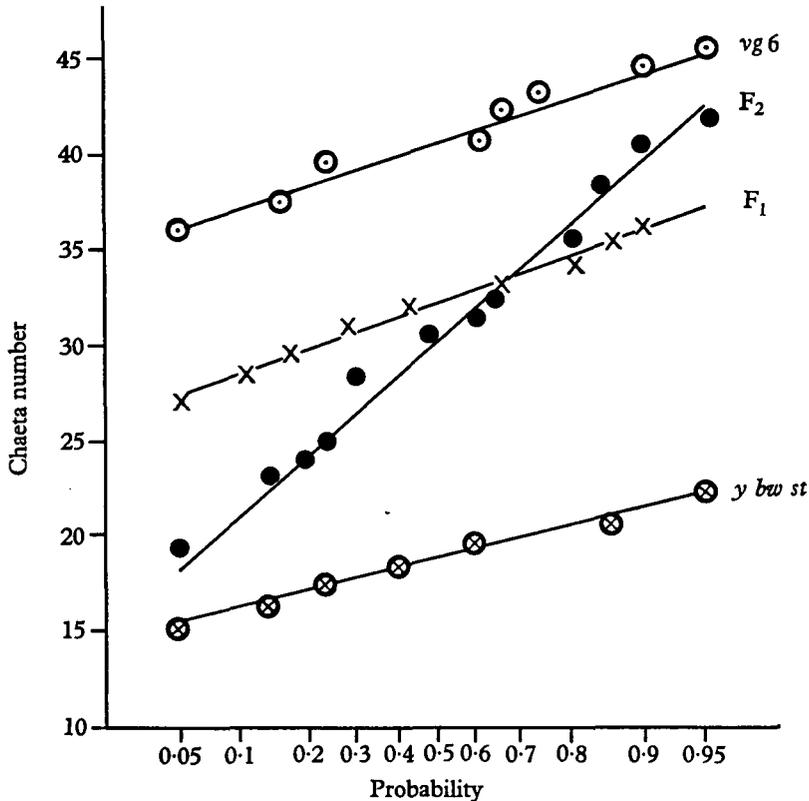
Fig. 7. Cumulative frequency distributions of chaeta number in vg 6, *y bw st*, their F_1 and F_2, plotted on probability paper. Normal distributions should be linear when represented in this way. Higher variances give steeper slopes.

affecting chaeta number. Some biometrical techniques may detect the contribution of the interacting viability genes to chaeta-number variance. Locating polygenes by study of chaeta number alone could not.

These considerations provide a clear warning to anyone inclined to over-generalize from the results presented in this paper and its predecessors in this series. For this reason no attempt will be made here to discuss the bearing of these results on general theory of continuous variation. We do however feel it necessary to point out that we have been concerned with continuous variation in this paper. In Fig. 7 the data for the vg 6 × *y bw st* F_2 assays are plotted on probability paper.

It will be seen that the distributions for parents F_1 and F_2 are all reasonably near normal and that the F_2 has much the greater variance. Bearing in mind that there is no crossing over in male *Drosophila*, and hence that only four units of segregation exist in the male parent of any F_2, few would doubt on this evidence alone that the difference between the chaeta number of vg 6 and that of *y bw st* is polygenically determined. Yet most of the difference is determined by a few loci, each of relatively large effect, some having strong interactions. Students of continuous variation must therefore ask in each particular case how much of the genetic variance they are interested in can be explained in terms of a few locatable polygenes and their interactions. It is a mistake to assume that large numbers of genes will always be involved.

SUMMARY

1. This paper describes further investigations of the high sternopleural chaeta-number lines of *Drosophila melanogaster* established by directional selection by Thoday & Boam (*Genet. Res.* 2, 161). The lines are vg 4 with a mean of 35·6 and vg 6 with a mean of 39·2 chaetae per fly.

2. Two locatable polygenes, 3a and 3b, distinguish the line third chromosomes from those of Oregon inbred (mean about 20·5, an ancestor of all the lines). These two genes are both located between the markers *h* and *eyg* and do not interact.

3. There is one locatable polygene at $41\cdot1 \pm 1\cdot7$ centiMorgans distinguishing the line second chromosomes from those of Oregon. There is no evidence that this gene is a linked complex, and, if it be a linked complex, it is unlikely to occupy more than 2 map units of the second linkage group. It interacts strongly and positively with the gene 3a.

4. These three genes account for 80% of the genetic variance of the vg 4 × Oregon F_2.

5. Two separate regions at $2\cdot4 \pm 0\cdot5$ and $50\cdot5 \pm 0\cdot9$ centiMorgans distinguish the vg 6 X chromosome from that of Oregon. They do not appear to interact. Together they interact strongly and positively with gene 3a.

6. These five genes account for 87·5% of the chaeta-number difference between vg 6 and Oregon.

7. The locatable polygenes on chromosomes II and III each have qualitatively distinguishable developmental effects.

8. It is pointed out that, though the genetics of these lines may be unusually simple, the results indicate that attempts to locate specific genes and study their individual effects should be made more often by students of continuous variation. Since the location of the polygene in chromosome II was done using marker genes 45 map units apart, such studies may be practicable even in species whose linkage groups are much less well marked than those of *Drosophila melanogaster*.

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