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# A Pair of Closely Linked Genes Controlling High Scutellar Chaeta Number in *Drosophila*

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**Summary.** 1. A line selected for high scutellar chaeta number reached a mean of about 16 chaetae in females and 13.5 in males at the 69th generation of selection following an accelerated response to selection which commenced at generation 65 and added five chaetae. 2. The accelerated response can probably be explained in terms of two recessive high chaeta number genes 1.05 cM apart, and which are located between *po* and *vg* on chromosome II. The gene closest to *vg* was found to be scabrous, *sca*, which causes rough eyes when homozygous and has a pleiotropic effect on scutellar chaeta number. The gene was found in one of the strains used in setting up the selection lines. 3. The results are discussed in relation to other theories of control of the scutellar chaeta system.

## Introduction

Although the genetics literature abounds with selection experiments, there have been rather few attempts at analyzing responses of selection to the level of genetic loci. Thoday (1961) and his colleagues arrived at a more specific location of polygenic differences built up in selection experiments for sternopleural chaeta number (see also Spickett and Thoday, 1966) by sophisticated breeding techniques (see Lee and Parsons, 1968). One pair of loci 20cM apart situated on the second chromosome amply justified their attempts. If + represents more and − fewer chaetae, −−/−− homozygotes were found to be lethal as were all individuals carrying the ++ chromosome. This is a remarkable position effect extending over 20 cM since +−/−+ is viable and ++/−− is lethal (Gibson and Thoday, 1962). A pair of high chaeta number loci situated 2 cM apart have been found on chromosome 3, which in this case did not show interactions but were both fully dominant (Wolstenholme and Thoday, 1963). Such precise information adds to our knowledge of quantitative genetics, complementing the approach of biometrical analyses. Other examples, with some general comments, appear in Lee and Parsons (1968).

In this paper a line selected for high scutellar chaeta number will be described, with the genetic analysis of part of the response to selection. The scutellar chaeta number system differs from that of other quantitative systems in *Drosophila* in that it shows little phenotypic variation from the normal four chaetae. Even so, there have been a number of reports of genotypic variability for the trait (Payne, 1918; Sismanidis, 1942; Hosgood and Parsons, 1967). The limited phenotypic variation is thought to be a result of developmental canalization, where a variety of underlying genotypes produce the same phenotype. Existing variability can, however, be exploited in directional selection experiments by basing selection on strains set up from single inseminated females in the wild, which have a high frequency of flies with more than the normal four scutellar chaetae (Hosgood, MacBean and Parsons, 1968). The variability between such strains has been shown to be mainly under the control of additive genes, which presumably arose from genetic differences between the founder females (Parsons and Hosgood, 1967).

Hosgood, MacBean and Parsons (1968) commenced four replicated selection lines derived from sixteen single inseminated female strains collected in the wild:

1. the strain having the highest mean scutellar chaeta number,
2. a hybrid population derived from four strains having the highest means,
3. a hybrid population of all 16 strains, and
4. the strain having the lowest mean.

Directional selection was carried out in each generation by selecting from the 100 flies of each sex scored, the 10 of each sex having the highest numbers of scutellar chaetae to provide the next generation. Extremely rapid responses were found in 1 and 2 above such that one replicate of 2 showed a continuous accelerated response to generation 12. This is the line 2 *A* in Figures 1 (♀) and 2 (♂). These extremely rapid responses were interpreted as being due to the segregation of high scutellar number genes in the base populations, which on selection rapidly increased in frequency producing the observed response. Line 4 *A C* in the figures represents a control line for 4 above.

## The Selection Line to be Analysed

At generation 12, line 2 *A* ceased to respond to selection, although there was an extra chaeta added at generation 22 (Hosgood, MacBean and Parsons, 1968). At generation 17 line 2 *A V* was established

by a cross between 10 females of line 2 *A* with a mean scutellar chaeta number of 10.50, and 10 males from one of the replicates of the controls from line 3 having a mean of 4.00 . Line 2 *A* died out at generation 53 due to a bacterial infection. At generation 65 line 2 *A V* began an accelerated response to generation 69, during which time the chaeta number went from 11 to 16 in females. The males paralleled the females, although the actual chaeta numbers were somewhat lower. After this, the line plateaued at about 16 chaetae in females and 13.5 in males. After the accelerated response the variability between generations was much lower than before the response. Two possible interpretations come to mind, namely either the line has become homozygous for chaeta genes and such variation as is observed is environmental, or that there is further genetic variability, which natural selection acts against, so that the plateau represents a balance between directional selection for higher chaeta number and natural selection for lower chaeta number. Some support for the latter hypothesis comes from a line set up at generation 75 where 10 randomly chosen individuals of each sex were allowed to mate and this was continued over a number of generations (line 2 *A V R* — Figures 1 and 2). In both sexes the chaeta number decreased, by about 1.5 chaetae in the males and 2.0 in the females, relative to 2 *A V*, and once reached the levels were maintained. In other words this may be the level at which there is either homozygosity or a balanced situation. Back selection was not carried out. Detailed genetic localization studies were carried out during the plateau from generations 70 to 94, during which time it seems reasonable to assume that the genotype remained constant for scutellar chaeta number.

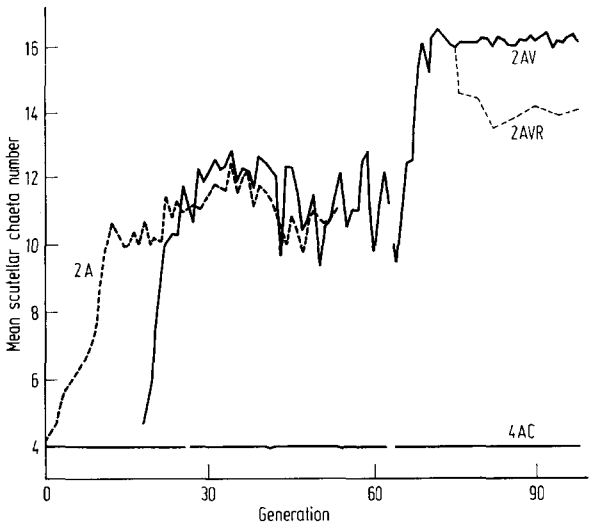


Fig. 1. Selection response in females in lines 2 *A*, 2 *A V* and 2 *A V R*. See text for derivation of these lines and the control line, 4 *A C*

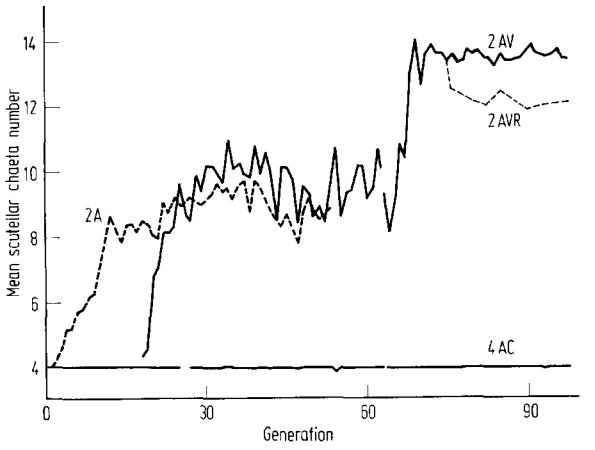


Fig. 2. Selection response in males in lines 2 *A*, 2 *A V* and 2 *A V R*. See text for derivation of these lines and the control line, 4 *A C*

## Genetic Analysis

At the same time as the accelerated response began, a rough eye phenotype appeared in 2 *A V*, and by generation 68 all flies were rough eyed. By reciprocal crosses with 4 *A C*, it was shown that the gene(s) controlling rough eye was recessive. Backcrossing the $F_1$ females to 2 *A V* females confirmed this giving 116 normal: 109 rough ($\chi_1^2$ for a 1:1 ratio = 0.22), (Table 1) indicating the likelihood of a single recessive gene.

Table 1. *Mean scutellar chaeta numbers in the $F_1$'s between 2 A V and 4 A C and backcrosses of this $F_1$ to 2 A C*

| Parent | | $F_1$* | | Backcross | | | |
|---|---|---|---|---|---|---|---|
| Female | Male | Females | Males | Females | | Males | |
| 2 *A V* | 4 *A C* | 5.05 | 4.80 | Normal | Rough | Normal | Rough |
| | | | | 6.59 | 11.73 | 5.67 | 9.53 |
| 4 *A C* | 2 *A V* | 4.70 | 4.13 | (71)+ | (64) | (45) | (45) |

* All the $F_1$ means are based on 40 flies.
+ The number of flies scored is given in the brackets.

The difference in scutellar chaeta number between rough and normal eye types in the backcross was about 5 chaetae, which is a major portion of the accelerated response. In the $F_1$ chaeta numbers were found somewhat in excess of 4, showing that the increase in chaeta number is mainly but not entirely controlled by recessive genes. The backcross progeny show rather high chaeta numbers for flies with normal eyes, as would be expected since their genomes would have a higher proportion of the selected line than the $F_1$'s.

The first detailed genetic analysis was carried out at generations 27 and 28 using the procedure of Kearsey and Kojima (1967), in line 2 *A*. Using 4 *A C* as a control and neglecting the *Y* and fourth chromosomes, eight true-breeding combinations of the three major chromosomes from 2 *A* and 4 *A C* can be synthesized using techniques involving marked multiple inversion stocks. The inversion stock used

was *Basc*; *Cy ds*$^{33k}$/*Pm*; *H*/*Sb*. The pure breeding stocks are then crossed in various ways to form 27 female and 18 male homozygous and heterozygous combinations. In Table 2a mean chaeta numbers are given for these combinations and in Table 2b the analyses of variance. The major additive effect is in chromosome 2, with significant effects in the other chromosomes, 3 being more important than the *X*. There are significant but lesser effects due to dominance in chromosomes 2 and 3. The magnitude of the second chromosome additive effect makes it difficult not to postulate the involvement of one or more major genes in the increased chaeta level of 2 *A*.

The next analysis followed the methods of Mather (1941, 1942 — see Lee and Parsons, 1968 for review). Localization to a specific chromosome is achieved by a comparison of the three major chromosomes of 2 *A V* as heterozygotes and homozygotes to analogous chromosomes of the inversion tester stock above. The tester stock was crossed to 2 *A V* and then the resultant $F_1$ crossed back to 2 *A V* which gives eight phenotypes in which the line 2 *A V* is represented as a homozygote or heterozygote for each of the three major chromosomes in turn. This method is only fully efficient in detecting recessive genes in the selected chromosome, but since from Table 1, much of the genetic activity in 2 *A V* is controlled by recessive genes, this is not a severe limitation. Flies were taken from generation 72 of selection, and the analysis of variance is given in Table 3 again showing significant effects for all chromosomes which are largest for chromosome 2. Certain interaction components are also significant. The relatively high contribution of the *X* in the male may be related to the hemizygous nature of the chromosome comparison. Because in both of the above analyses chromosome 2 had produced the most significant effects, it was decided to investigate this chromosome in detail to achieve a more definitive level of localization of genetic activity.

The procedure follows that of Thoday (1961) and his colleagues who arrived at a more specific location of polygenic differences built up in selection experiments for sternopleural chaeta number. From a back-cross of a female, heterozygous for a multiply-marked chromosome and the selection line, to a male homozygous for the multiply-marked chromosome, recom-

Table 2a. *Mean scutellar chaeta numbers for all 27 female and 18 male homozygous and heterozygous chromosome combinations, between chromosomes from line 4 AC (A chromosomes) and selection line 2 A (B chromosomes). Chromosomes taken from generations 27/28*

| Chromosomal constitution (females) | | | Females | Males |
|---|---|---|---|---|
| *X* | 2 | 3 | | |
| *A* | *A* | *A* | 4.00 | 4.00 |
| *H* | *A* | *A* | 4.02 | |
| *H* | *A* | *H* | 4.04 | |
| *H* | *H* | *A* | 4.12 | |
| *A* | *A* | *H* | 4.02 | 4.00 |
| *A* | *A* | *B* | 4.20 | 4.10 |
| *A* | *H* | *B* | 4.90 | 4.22 |
| *A* | *H* | *A* | 4.04 | 4.01 |
| *A* | *B* | *A* | 5.60 | 5.18 |
| *H* | *B* | *A* | 6.45 | |
| *A* | *H* | *H* | 4.04 | 4.01 |
| *A* | *B* | *H* | 6.02 | 5.18 |
| *A* | *B* | *B* | 7.78 | 6.84 |
| *H* | *H* | *H* | 4.18 | |
| *B* | *A* | *A* | 4.00 | 4.00 |
| *B* | *A* | *H* | 4.00 | 4.02 |
| *B* | *H* | *H* | 4.74 | 4.26 |
| *H* | *A* | *B* | 4.38 | |
| *B* | *A* | *B* | 4.46 | 4.46 |
| *B* | *H* | *B* | 6.28 | 5.61 |
| *B* | *H* | *A* | 4.24 | 4.00 |
| *B* | *B* | *A* | 6.36 | 5.56 |
| *B* | *B* | *H* | 6.88 | 6.30 |
| *H* | *H* | *B* | 5.66 | |
| *H* | *B* | *H* | 4.90 | |
| *H* | *B* | *B* | 8.62 | |
| *B* | *B* | *B* | 9.98 | 7.89 |

Table 2b. *Analyses of variance for the various effects of the three major chromosomes in line 2A based on the data in table 2a*

| Source of variation | Chromosome | d.f. | Females | | Males | |
|---|---|---|---|---|---|---|
| | | | M. S. | *F* | M. S. | *F* |
| additive (a) | *X* | 1 | 637.3 | 21.67** | 489.9 | 24.24** |
| | 2 | 1 | 6,840.6 | 232.59*** | 6,142.7 | 303.94*** |
| | 3 | 1 | 2,551.4 | 86.75*** | 2,030.6 | 100.48*** |
| dominance (d) | *X* | 1 | 62.5 | 2.13 | — | — |
| | 2 | 1 | 673.5 | 22.90** | 987.0 | 48.84** |
| | 3 | 1 | 829.9 | 28.22*** | 256.5 | 12.69* |
| *a* × *a* | *X* × 2 | 1 | 116.6 | 3.96 | 127.4 | 6.30 |
| | *X* × 3 | 1 | 272.7 | 9.27* | 357.5 | 17.69* |
| | 2 × 3 | 1 | 151.9 | 5.17 | 271.5 | 13.43* |
| *d* × *d* | *X* × 2 | 1 | 0.8 | 0.03 | — | — |
| | *X* × 3 | 1 | 61.4 | 2.09 | — | — |
| | 2 × 3 | 1 | 8.6 | 0.29 | 16.1 | 0.79 |
| *a* × *d* | *X* × 2 | 1 | 77.2 | 2.62 | 0.0 | 0.00 |
| | 2 × *X* | 1 | 58.3 | 1.98 | — | — |
| | *X* × 3 | 1 | 0.9 | 0.03 | 0.8 | 0.04 |
| | 3 × *X* | 1 | 51.8 | 1.76 | — | — |
| | 2 × 3 | 1 | 125.1 | 4.25 | 45.4 | 2.25 |
| | 3 × 2 | 1 | 2.6 | 0.09 | 78.1 | 3.87 |
| Error | | 8 | 29.4 | | 20.2 | |

Note. Error M.S. for males has 4 degrees of freedom.
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

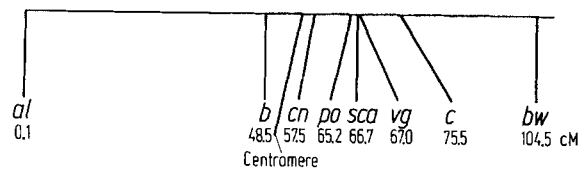


Fig. 3. Chromosome 2 markers used in the investigation (map locations — Lindsley and Grell, 1967)

binants occur which are recognized by the segregation of the marker genes. Such recombinant chromosomes can be tested to determine how many classes can be distinguished with reference to the quantitative trait under discussion.

Table 3. *Analyses of mean scutellar numbers based on the Basc; Cy $ds_{33}{}^{k}$/Pm; H/Sb testcross (see text), based on 25 flies for each of four replicates*

a) Analysis of variance

| Source of variation | d. f. | *F* | |
|---|---|---|---|
| | | Females | Males |
| X | 1 | 168.0*** | 655.8*** |
| 2 | 1 | 1982.5*** | 953.6*** |
| 3 | 1 | 268.9*** | 68.3*** |
| X×2 | 1 | 21.6*** | 89.9*** |
| X×3 | 1 | 19.7*** | 1.4 |
| 2×3 | 1 | 45.6*** | <1 |
| X×2×3 | 1 | 21.6*** | 4.0 |
| Replicates | 3 | <1 | 1.5 |
| Error | 21 | | |

***$P < 0.001$

b) Effects of chromosomes on bristle score

| Chromosome | Female | Male |
|---|---|---|
| 1 | +1.26 | +2.84 |
| 2 | +4.34 | +3.43 |
| 3 | +1.60 | +0.92 |

The various chromosome 2 markers used in this investigation are given in Figure 3. The first localization study, using the techniques above, involved the marker stock *al cn bw* which spans the whole chromosome. The effects of the regions may be estimated by a factorial method analogous to that in Table 3 described for localizing genetic activity to chromosomes. The factorial analyses of variance indicates significant genetic activity in the *al* and *cn* regions, which is positive in the *cn* region, but negative in the *al* region (Table 4). Thus the region of interest for the selection of high chaeta number is *cn*, especially as the *al* region is negative with reference to the standard *al cn bw* marker stock. The cross produces eight phenotypes, being variously homozygous and heterozygous for *al*, *cn* and *bw* and in order to find out more about rough eyes these eight phenotypes as females, were crossed in turn to 2 *A V* males. Both rough and normal eyed flies come from all crosses where the $cn^{+}$ allele is present in the offspring, but only 25 out of 827 of flies where the *cn* allele is present show rough eyes (Table 5). This indicates the likelihood of the gene being in the region of *cn*, especially as no such division is apparent for the *al* and *bw* loci. The 25 rough eyed *cn* flies occurred for the maternal phenotype *al cn* + indicating that recombination is possible between the *cn* locus and the rough eye locus, and that the rough eye locus probably lies to the right of *cn*.

Table 4. *Analyses of mean scutellar chaeta numbers based on the al cn bw testcross. 25 flies were scored per replicate*

| Source of variation | d.f. | *F* | |
|---|---|---|---|
| | | Females | Males |
| a) Analysis of variance | | | |
| *al* chromosomes | 1 | 64.8*** | 157.9*** |
| *cn* chromosomes | 1 | 119.9*** | 157.9*** |
| *bw* chromosomes | 1 | 3.7 | 3.9 |
| *al*×*cn* | 1 | 0.10 | 28.0** |
| *al*×*bw* | 1 | 17.5** | 15.8** |
| *cn*×*bw* | 1 | 41.5*** | 85.8*** |
| *al*×*cn*×*bw* | 1 | 17.5** | 10.9* |
| Replicates | 1 | 0 | 1.8 |
| Error | 7 | | |

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

b) Effects of the chromosome regions on chaeta score

| Chromosome | | |
|---|---|---|
| *al* | −0.25 | −0.18 |
| *cn* | +0.34 | +0.19 |
| *bw* | +0.06 | +0.03 |

Table 5 also shows that an increase of about three chaetae occurs for rough eyed progeny compared with normal eyes. This is somewhat below the five chaeta increase of the accelerated response, and would seem to indicate that the accelerated response is due to the gene for rough eyes, and/or to a gene(s) closely linked with it.

The *cn* region was further investigated by using the marker stock *b cn c bw*. The markers *b cn* and *cn c*

Table 5. *Progeny check on region localization data. Females of phenotypic classes crossed with 2 A V males (see text). Mean scutellar chaeta numbers are given, and the numbers in brackets, after means, indicate the total number and number of rough eye flies observed*

| Maternal phenotype | | | Females | | Males | |
|---|---|---|---|---|---|---|
| | | | (normal eye) | (Rough eye) | (Normal eye) | (Rough eye) |
| *a* | *cn* | *bw* | 5.72 ( 85) | — | 4.48 (101) | — |
| *al* | *cn* | + | 5.70 (128) | 8.77 (13) | 4.48 (151) | 6.92 (12) |
| *al* | + | *bw* | 6.50 ( 88) | 9.25 (41) | 5.20 ( 76) | 8.40 (34) |
| *al* | + | + | 5.75 ( 81) | 8.40 (39) | 4.60 ( 79) | 7.10 (42) |
| + | *cn* | *bw* | 5.65 ( 91) | — | 4.53 ( 87) | — |
| + | *cn* | + | 6.15 ( 97) | — | 4.55 ( 87) | — |
| + | + | *bw* | 6.40 ( 70) | 8.90 (32) | 5.10 ( 76) | 7.60 (34) |
| + | + | + | 5.40 (159) | 8.75 (75) | 4.75 (134) | 7.71 (71) |

Table 6a. *Mean scutellar chaeta numbers of various classes produced in the b cn c bw test cross (1) b cn region* (All flies are heterozygous *b cn*).

| | Progeny type | | |
|---|---|---|---|
| *b cn* (standard) | ++ | *b*+ | +*cn* |
| 4.05 | 5.00 | 4.68 | 4.23 |
| Deviations from *b cn* | +0.95 | +0.63 | +0.18 |

Analysis of Variance

| Source | d.f. | *F* |
|---|---|---|
| +/*cn* | 1 | 25.43*** |
| +/*b* | 1 | 3.20 |
| +/*cn* × +/*b* | 1 | 0.24 |
| Genotype | 3 | 9.62*** |
| Sex | 1 | 5.93* |
| Genotype × Sex | 3 | 0.53 |
| Error | 80 | |
| Total | 87 | |

Table 6b. *Mean scutellar chaeta numbers of various classes produced in the b cn c bw test cross (2) cn c region.* (All flies are heterozygous *cn c*)

| | Progeny type | | |
|---|---|---|---|
| *cn c* (standard) | ++ | *cn* + | + *c* |
| 4.08 | 4.97 | 4.35 | 4.41 |
| Deviation from *cn c* | +0.89 | +0.27 | +0.33 |

| Source | d. f. | *F* |
|---|---|---|
| +/*cn* | 1 | 17.81*** |
| +/*c* | 1 | 13.64*** |
| +/*cn* × +/*c* | 1 | 1.74 |
| Genotype | 3 | 11.06*** |
| Sex | 1 | 11.76*** |
| Genotype × Sex | 3 | 0.49 |
| Error | 128 | |
| Total | 135 | |

* $P < 0.05$, *** $P < 0.001$

were taken in pairs so that the regions in the centromeric regions could be considered. The *bw* region was not considered as previous analyses showed little effect in this region. The data for *b cn* in Table 6a show that only $cn^+$ produces a significant effect relative to the marker region, as $b^+$ and *b* regions do not differ significantly. From the techniques of Thoday (1961), mean chaeta numbers of ++ and *b*+ are similar, as are + *cn* and *b cn*, which seems to indicate that the chaeta locus might be nearer to the *cn* locus than the *b* locus (Table 6a). For *cn c*, however, both $cn^+$ and $c^+$ produced significant effects relative to the marker regions (Table 6b). Both $cn^+$ and $c^+$ have higher chaeta numbers than the corresponding mutant regions. This is in accord with genetic activity probably to right of the *cn* locus as already suggested, and between *cn* and *c*.

Similarly to the *al cn bw* testcross, four randomly chosen males from each of the eight possible phenotypes made up from the *b*, *cn* and *c* loci were backcrossed to 2 *A V* females (Table 7), and once again it can be concluded that the rough eye gene is in the vicinity of *cn*, and the presence of rough eyes in progeny from *b cn*+ phenotypes supports the location of the region of activity between *cn* and *c*. In fact it can be seen that this table is analogous in form to Table 5, except that *al* is replaced by *b* and *bw* by *c*, thus limiting the region of activity compared with the previous experiment.

Between *cn* and *c*, the only reported mutant with a rough eye phenotype is the recessive gene scabrous (*sca*) (Lindsley and Grell, 1967). On crossing *sca sca* flies with 2 *A V* reciprocally, the $F_1$ produced only rough eyes. It was noted at this stage that the stock *sca sca* flies had chaeta numbers greater than four. Thus means of 5.59 and 4.49 were obtained for 100 females and males respectively. This suggests a pleiotropic effect of *sca* on scutellar chaeta numbers. Rather than investigate the detailed effects of this locus at this stage, it was decided to continue with further genetic localization studies.

The next marker stock taken was *vg c*. It was found (Table 8) that classes ++ and +*c* were similar, as were *vg*+ and *vg c*. There was significant activity associated with $vg^+$, suggesting that the region of activity is close to the *vg* locus and perhaps to its left. The *po vg* stock was then taken, and is particularly useful as the markers closely span the *sca* locus (Table 9). It was found that the means of the recombinant classes *po*+ and +*vg* were intermediate between the two parental extremes, and furthermore the $po^+$ and $vg^+$ alleles differed significantly from the corresponding alleles of the marker chromosomes. Both of these results are in accord with genetic activity between the two loci.

Progeny testing enables an estimate of the mini-

Table 7. *Progeny check on sub region of cn region. Males of phenotypic classes crossed with 2 A V females (see text). Mean scutellar chaeta number based on n = 25 except when fewer flies were available. The numbers in brackets, after means, indicate the total number and the number of rough eye flies observed*

| Paternal phenotype | | | Females | | Males | |
|---|---|---|---|---|---|---|
| | | | (Normal eye) | (Rough eye) | (Normal eye) | (Rough eye) |
| + | + | + | 7.04 (106) | 11.88 (54) | 5.96 ( 96) | 10.28 (51) |
| *b* | + | + | 6.00 (124) | 12.08 (59) | 4.08 (113) | 8.96 (56) |
| + | + | *c* | 6.84 (100) | 11.08 (44) | 5.36 ( 93) | 9.72 (40) |
| *b* | + | *c* | 5.60 (144) | 9.52 (68) | 5.08 (120) | 9.08 (63) |
| + | *cn* | + | 6.15 (113) | — | 5.03 ( 91) | — |
| *b* | *cn* | + | 7.03 (118) | 10.84 (13) | 5.63 (107) | 9.70 (10) |
| + | *cn* | *c* | 5.30 (105) | — | 5.28 ( 93) | — |
| *b* | *cn* | *c* | 5.65 (114) | — | 5.00 (103) | — |

Table 8. *Mean scutellar chaeta numbers of various classes produced in the vg c test cross* (All flies are heterozygous *vg c*)

| | Progeny type | | |
|---|---|---|---|
| *vg c* (standard) 4.00 | ++ 4.52 | *vg*+ 4.08 | +*c* 4.42 |
| Deviation from *vg c* | +0.52 | +0.08 | +0.42 |

Analysis of Variance

| Source | d. f. | *F* |
|---|---|---|
| +/*vg* | 1 | 26.86*** |
| +/*c* | 1 | 1.18 |
| +/*vg* × +/*c* | 1 | 0.02 |
| Genotype | 3 | 9.35*** |
| Sex | 1 | 3.27 |
| Genotype × Sex | 3 | 1.09 |
| Error | 192 | |
| Total | 199 | |

*** $P < 0.001$

Table 9. *Mean scutellar chaeta numbers of various classes produced in the po vg test cross* (All flies are heterozygous *po vg*)

| | Progeny types | | |
|---|---|---|---|
| *po vg* (standard) 4.00 | ++ 4.43 | *po*+ 4.30 | +*vg* 4.20 |
| Deviations from *po vg* | +0.43 | +0.30 | +0.20 |

| Source | d. f. | *F* |
|---|---|---|
| +/*po* | 1 | 4.61* |
| +/*vg* | 1 | 12.02*** |
| +/*po* × +/*vg* | 1 | 0.25 |
| Genotypes | 3 | 5.62*** |
| Sex | 1 | 4.61* |
| Genotypes × Sex | 3 | 0.97 |
| Error | 152 | |
| Total | 159 | |

* $P < 0.05$, *** $P < 0.001$

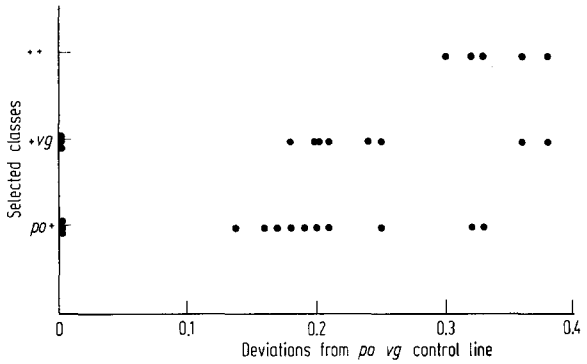


Fig. 4. Progeny tests of randomly chosen parental and recombinant *po vg* chromosomes

mum number of loci involved to be made by a consideration of the number of chaeta classes among the tested recombinant chromosomes. Essentially $n + 1$ classes indicate $n$ loci (Thoday, 1961). Recombinant chromosomes were randomly chosen from each recombinant class and tested against *po vg* females. The results are given in Figure 4 and show three distinct classes, indicating two loci between *po* and *vg*. It is unlikely that more than two loci are involved because of the short distance between *po* and *vg*. We can therefore write the selection line as $+HH+$ and the control lines as *po LL vg*. Thus the initial cross to produce the recombinants is $\frac{+\,HH\,+}{po\ LL\ vg} \times \frac{po\ LL\ vg}{po\ LL\ vg}$. If we call the region *po-L* segment 1, *L-L* segment 2, and *L-vg* segment 3, three possibilities occur for each recombinant genotype (Table 10). Assuming *sca* to be one of the loci, then if it is next to *vg* its effect will be observed in the intermediate class of *po*+ recombinants i.e. *po L H*+, and not in the intermediate class of +*vg* recombinants i.e. +*HLvg*. The two loci, *sca* and the second locus (designated *chaeta* locus) have similar effects (Fig. 4), and furthermore, the distribution of the extreme parental and recombinant classes indicates that the loci are largely additive.

Table 10. *Phenotypes showing recombination from the cross* $\frac{+\,HH\,+}{po\ LL\ vg}$ ♀ × $\frac{po\ LL\ vg}{po\ LL\ vg}$ ♂

| | +*vg* | Chaeta number | *po* + | Chaeta number |
|---|---|---|---|---|
| Cross over in segment 1 | + *LL vg* | Low | *po HH* + | High |
| Cross over in segment 2 | + *HL vg* | Medium | *po LH* + | Medium |
| Cross over in segment 3 | + *HH vg* | High | *po LL* + | Low |

Segment 1, *po-L*; Segment 2, *L-L*; Segment 3, *L-vg*.

In order to look further at the situation, the recombinant males in Table 10, were crossed to 2 *A V* females. For the +*vg* males we expect:

$\frac{+\,LL\ vg}{po\ LL\ vg}$ ♂ × $\frac{+\,HH\,+}{+\,HH\,+}$ ♀ → $\frac{+\,LL\ vg}{+\,HH\,+}$, $\frac{po\ LL\ vg}{+\,HH\,+}$ normal eyes and only one chaeta number class (low) (1)

$\frac{+\,HL\ vg}{po\ LL\ vg}$ ♂ × $\frac{+\,HH\,+}{+\,HH\,+}$ ♀ → $\frac{+\,HL\ vg}{+\,HH\,+}$, $\frac{po\ LL\ vg}{+\,HH\,+}$ normal eyes with two chaeta number classes (intermediate) (2)

$\frac{+\,HH\ vg}{po\ LL\ vg}$ ♂ × $\frac{+\,HH\,+}{+\,HH\,+}$ ♀ → $\frac{+\,HH\ vg}{+\,HH\,+}$ rough eyes

$\frac{+\,HH\,+}{po\ LL\ vg}$ normal eyes (high) (3)

Table 11. *Mean scutellar chaeta number in $F_1$ for crosses between po vg male recombinants and 2 AV females. Recombinant deviations based on figure 4. Means based on 20 flies scored unless otherwise specified in brackets*

| Deviation from 4 chaetae | Recombinant Class | Females (Normal eye) | Females (Rough eye) | Males (Normal eye) | Males (Rough eye) | Combined deviation class (Normal eye) | Combined deviation class (Rough eye) |
|---|---|---|---|---|---|---|---|
| High | + + | 4.72 (25) | 9.00 (25) | 4.72 (25) | 8.76 (25) | 4.72 | 8.88 |
| | + *vg* | 4.90 | 8.90 | 4.80 | 8.75 | | |
| | | 4.85 | 8.50 | 4.60 | 8.60 | 4.79 | 8.74 |
| | *po* + | 4.75 | 8.70 | 4.80 | 8.55 | | |
| | | 4.90 | 9.25 | 4.70 | 8.70 | | |
| Intermediate | + *vg* | 5.40 (30) | — | 5.17 (30) | — | | |
| | | 5.23 (30) | — | 5.06 (30) | — | 5.24 | -- |
| | | 5.13 (30) | — | 5.03 (30) | — | | |
| | | 5.53 (30) | — | 5.37 (30) | — | | |
| | | 2 lines unproductive | | | | | |
| | *po* + | 4.94 (13) | 7.50 ( 8) | 4.77 (13) | 7.25 ( 8) | | |
| | | 4.89 (18) | 7.75 | 4.87 (15) | 7.13 (16) | | |
| | | 4.80 | 7.55 | 4.85 | 7.25 | | |
| | | 4.90 | 6.85 | 4.80 | 7.20 | 4.82 | 7.34 |
| | | 4.90 | 7.75 | 4.80 | 7.35 | | |
| | | 4.80 | 7.15 | 4.85 | 7.65 | | |
| | | 4.57 (14) | 7.33 (19) | 4.72 (19) | 7.06 (17) | | |
| | | 1 line unproductive | | | | | |
| Low | *po vg* | 4.48 (25) | — | 4.48 (25) | — | | |
| | + *vg* | 4.95 | — | 4.90 | — | 4.48 | -- |
| | | 4.80 | — | 4.70 | — | | |
| | | 1 line unproductive | | | | | |
| | *po* + | 4.70 | — | 4.65 | — | 4.81 | -- |
| | | 4.80 | — | 4.95 | — | | |
| | | 1 line unproductive | | | | | |

Table 11 gives the results and the eye phenotypes confirm this. In cross (3) equal numbers of rough and normal eyes would be expected ($\chi^2_1$ for 1:1 = 0.05 based on 169 flies). For cross (2) on combining the data for Table 11 with other data, of similarly tested recombinant chromosomes, a bimodal distribution was found for chaeta numbers, as would be expected since the *chaeta* locus is homozygous in one genotype and heterozygous in the other. For *po*+ males we expect:

$$\frac{po\ LL\ +}{po\ LL\ vg}\ \text{(low)}\ \delta \times \frac{+\ HH\ +}{+\ HH\ +}\ \text{♀} \rightarrow \begin{cases} \dfrac{po\ LL\ +}{+\ HH\ +} \\ \dfrac{po\ LL\ vg}{+\ HH\ +} \end{cases} \begin{matrix} \text{normal eyes} \\ \text{and only one} \\ \text{chaeta class} \end{matrix} \quad (1)$$

$$\frac{po\ LH\ +}{po\ LL\ vg}\ \text{(intermediate)}\ \delta \times \frac{+\ HH\ +}{+\ HH\ +}\ \text{♀} \rightarrow \begin{matrix} \dfrac{po\ LH\ +}{+\ HH\ +} \\ \dfrac{po\ LL\ vg}{+\ HH\ +} \end{matrix} \begin{matrix} \text{rough eyes} \\ \text{normal eyes} \end{matrix} \quad (2)$$

$$\frac{po\ HH\ +}{po\ LL\ vg}\ \text{(high)}\ \delta \times \frac{+\ HH\ +}{+\ HH\ +}\ \text{♀} \rightarrow \begin{matrix} \dfrac{po\ HH\ +}{+\ HH\ +} \\ \dfrac{po\ LL\ vg}{+\ HH\ +} \end{matrix} \begin{matrix} \text{rough eyes} \\ \text{normal eyes} \end{matrix} \quad (3)$$

The eye phenotypes in Table 11 confirm this. In crosses (2) and (3) rough and normal eyes would be expected to be in a 1:1 ratio ($\chi^2_1 = 0.69$ and $= 2.22$ respectively based on 523 and 180 flies).

Looking now at the actual scutellar chaeta numbers, the high class shows pooled mean chaeta number differences between rough and normal of 4.07 chaetae. In the intermediate class (*po*+) the difference was 2.52 which is less as would be expected, since in the former case *chaeta* is homozygous and in the latter, heterozygous. The +*vg* intermediate class was bimodal, and the mean difference between the two means from the two modes within the distribution was estimated as 1.64 chaetae, which provides an estimate of the effect of the *chaeta* locus (*H/H vs H/L*). The two means from the two modes of this distribution were 6.04 and 4.40 chaetae respectively. The other normal eyed flies in Table 11 all have chaeta numbers at about the lower figure, as would be expected since they should be heterozygous for the *chaeta* locus.

The known gene locations are *po* (65.2), *sca* (66.7) and *vg* (67.0) giving a *po-vg* map distance of 1.8 cM. The three classes in the recombinant classes in Figure 4 represent crossovers in the 3 regions, and totalling the numbers in this Figure gives five crossovers in the *po-chaeta* region, fourteen in the *chaeta-sca* region and five in the *sca-vg* region. Adding in further data of similarly tested recombinant chromosomes gives nine, twenty-four and eight respectively. Dividing these into 1.8 cM gives a map:

| *po* | *chaeta* | *sca* | *vg* |
|---|---|---|---|
| 0.40 | 1.05 | 0.35 | |

which corresponds quite well with that published (Lindsley and Grell, 1967) for the three genes previously located, *po*, *sca* and *vg*.

## Discussion

Two closely linked loci, *chaeta* and *sca*, which are just over 1 cM apart probably provide the basis of the accelerated response to selection between generations 65 and 69. The two loci when homozygous for high chaeta genes add about five chaetae, between them, and of these *sca sca* homozygotes add proportionately more than *chaeta* homozygotes. Presumably before the accelerated response, there were some repulsion genotypes $\frac{+\ sca}{chaeta\ +}$ in the population, and the accelerated response followed a recombinational event to produce *chaeta* — *sca* chromosomes. This leads us to enquire as to the origin of the two loci. In the genetic background of 2 *A V* are 16 strains set up from single inseminated females. These have been investigated and in strain 1 (Hosgood, MacBean and Parsons, 1968) a single *sca sca* fly was found. Subsequent pairwise crosses of phenotypically normal flies of this strain with homozygous *sca sca* flies produced scabrous and normal eye progeny in a 1:1 ratio for 2 males from a sample of 34 productive males of strain I and for 1 female in the 35 productive females of strain I tested. All remaining crosses produced phenotypically normal flies. In other words 3 of a sample of 69 flies tested were heterozygous *sca*/*sca*$^+$ indicating an allelic frequency of approximately 2% for *sca* in the strain genome.

While it is possible that this frequency varies considerably from that in the original population, it is clear the *sca* gene did not arise by mutation during the course of selection. So far as *chaeta* is concerned, obtaining evidence of this type was not sought partly because of the complex breeding program involved. In any case, whatever the origin of *chaeta*, it is likely that recombination in the repulsion genotype as above occurred. A more remote possibility is that the *chaeta* gene became homozygous during the initial accelerated response at generation 18, and that the accelerated response between generations 65 and 69 involved *sca* and another locus elsewhere in the genome. Unfortunately tests on genetic architecture were not carried out prior to generation 65, except for the Kearsey-Kojima analysis at generations 27 and 28 which showed chromosome 2 to be of importance in the response to selection at that stage.

Therefore, in conclusion, two loci have been located which are involved in the response to selection and they are tightly linked. By the breeding techniques used they can be mapped accurately. This provides no difficulty with *sca*, but *chaeta* is a gene known only to have a quantitative effect on chaeta number. Such precise information about chaeta determining loci adds to our knowledge of quantitative genetics, and shows the problems of applying biometrical models to observed data. The scutellar chaeta system is one of peculiar difficulty from the biometrical point of view because of the strong canalization of many strains at four chaetae which means that many genotypes show the same phenotype (see Rendel, 1967).

From the literature it is clear that the genetic architecture of scutellar chaeta number may be no less complex than sternopleural chaeta number for which Beardmore (1970) cites the work of Davies (Ph. D. thesis, 1969) as indicating 16 loci. Beardmore (1970) suggests that when taken in conjunction with other work, this may indicate 20 or more loci involved in the sternopleural chaeta system. It has furthermore been shown by Spickett (1963) that different genes affect chaeta patterns on the sternopleural plate.

For scutellar chaeta number, Whittle (1969) carried out a complete chromosomal analysis of two homozygous stocks having increased scutellar chaeta numbers in terms of comparisons with an inbred stock having four chaetae. There were differences in all chromosomes of both stocks, which were located to three factors in one stock and five in the other. Further, a factor or factors causing a change in pattern without a change in chaeta number was found. Fraser's (1970) model postulates a system with two major loci namely scute *sc*, and extravert *xvt* on chromosome 3. These loci are modified in expression by $\alpha$ and $\beta$ modifiers respectively. Mutation at the *sc* locus leads to a reduction of chaetae below the normal four, while mutation at *xvt* results in an increased chaeta number. Substitution of *sc* for *sc*$^+$ suppresses *xvt* and the $\beta$ modifiers (Miller and Fraser, 1968), while substituion of *xvt* for *xvt*$^+$ suppresses the expression of the $\alpha$ modifiers in the presence of *sc*$^+$. Fraser (1967) concluded that the normal four chaetae result from a stabilization of development because of a complex interaction mechanism between these loci and their modifiers. A further locus tufted (*tft*) increases the number of scutellar chaetae markedly (Fraser, 1970), and Fraser suggested that this locus may be a regulator of a locus determining the upper limits of the number of scutellar chaetae, such that a mutation at *tft* removes the repression and allows the chaeta number to continue at an unregulated pace. Rendel (1967, 1968) has discussed the scutellar bristle system in terms of more molecular considerations, but although this may provide answers in the long term, we feel that our results are difficult to put in such terms.

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