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# Analyses of some Genes from Abdominal Bristle Number Selection Lines in *Drosophila melanogaster*

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**Summary.** Effects on abdominal bristle number were determined for three mutations, *scabrous-like, dark hairy margin* and *scabrous*, and for some second chromosome recessive lethal genes prevalent in various irradiated and unirradiated selection lines derived from an outbred population. The relationship between the effect of a lethal gene on a quantitative character and the equilibrium frequency of the lethal in a line selected for that character was examined.

Recently it has been suggested that a large proportion of the response in selection lines may be due to effects of a relatively small number of genes (Spickett and Thoday 1966, Robertson 1966). In *Drosophila*, special techniques, such as chromosomal analysis using marked inversion stocks, have long been used in the study of the genetics of quantitative characters, *e.g.* Mather (1942), Sismanidis (1942), Robertson and Reeve (1953), Fraser and Scowcroft (1965) and Scowcroft (1966). In the last decade methods have been developed which, although laborious, enable individual loci affecting metric characters to be studied and mapped. Thoday (1961) outlined a scheme for locating polygenes and measuring their effects. This method has been used for detailed analyses of sternopleural bristle number selection lines by Thoday, Gibson and Spickett (1964) and Spickett and Thoday (1966). Inter-varietal chromosome substitution lines can be used for similar analysis of quantitative characters in wheat, *e.g.* Law (1966, 1967).

On a less sophisticated level, the basis of the response in selection lines can be at least partly revealed by an examination of the effects of visible mutants which reach high frequencies. In a group of irradiated and unirradiated lines derived from the Canberra population, an outbred strain of *Drosophila melanogaster*, Hollingdale and Barker (1971) found three mutants which became prevalent in different lines which were being selected for increased abdominal bristle number. Lethal analysis of the same lines revealed several recessive lethals present at high frequencies. These three visible mutants and some of the second chromosome lethals were studied in some detail, to determine why they had become prevalent.

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## Methods and Results

### *1. scabrous-like (scal)*

This is a recessive semi-lethal, semi-sterile mutant on chromosome II, mapped at $11.7 \pm 0.3$ centi-Morgans (Barker and Hollingdale 1970). Females are almost completely sterile. In homozygotes the eyes are roughened, and slightly bulging; bristle number is increased (see later); the wings are abnormal, tending to be broad, slightly spread and curved, with disturbed venation — longitudinal vein *L* II is irregular and the posterior crossvein is abnormal, sometimes with a section missing, sometimes with an extra piece of vein associated with it. Wing effects tend to be more pronounced in females. Emergence was delayed in the line in which this mutant was found (line *SO.2*, unirradiated, Hollingdale and Barker 1971).

When a mutant with a phenotype similar to *scabrous* (Lindsley and Grell 1968) was first discovered in line *SO.2*, an attempt was made to establish a subline lacking the mutant. This was unsuccessful as the mutant gene was retained in the subline even though flies with the mutant phenotype were excluded before selecting individuals with high abdominal bristle number. The subline was terminated after three generations of selection. The *scabrous-like* segregants in the final generation had abdominal bristle numbers of $45.2 \pm 0.7$ and $37.6 \pm 0.5$ for the fifth sternite in females and the fourth sternite in males respectively; the corresponding means for wild-type segregants were $34.7 \pm 0.2$ and $28.1 \pm 0.2$. The mutant also increases scutellar bristle number; the *scabrous-like* segregants had an average of 6.5 bristles in females and 5.7 in males compared with 4 bristles in wild-type flies.

At generation 61, wild-type flies from line *SO.2* were scored for abdominal bristle number and individually progeny tested to determine their genotypes at the *scabrous-like* locus, by mating scored flies with

a *scal/Cy* stock. In a culture with no *scabrous-like* progeny and less than twenty non-*Cy* progeny the *SO.2* parent was classed as semi-sterile; its genotype at the mutant locus could not be determined. (Twenty wild-type progeny was an arbitrary limit, chosen because occasionally cultures were found with about this number of wild-type progeny and one *scabrouslike* offspring.) This introduced a bias, important only for *SO.2* females, since the heterozygote class included cultures with less than twenty non-*Cy* progeny, whereas the homozygote wild-type class did not. However, as shown in Table 1, mean bristle number of semi-sterile females was intermediate between the means for heterozygotes and wild homozygotes and the gene frequency in females, 33.1%, was similar to that in males, 33.7%. The over-estimate of the heterozygote effect of *scabrous-like* in females was therefore unlikely to be large.

Table 1. *Effect of scabrous-like, scal, on bristle number in line SO.2 at generation 61; x = mean abdominal bristle number (fifth sternite in females, fourth in males), n = scored and progeny tested*

| Genotype | Females | | Males | |
|---|---|---|---|---|
| | *x* | *n* | *x* | *n* |
| +/*scal*° | 42.7±0.3 | 82 | 34.3±0.3 | 87 |
| +/+ | 40.8±0.4 | 42 | 32.9±0.4 | 42 |
| Semi-sterile | 41.5±0.4 | 32 | 32.0 | 1 |
| Sterile | 44.8±0.8 | 4 | 34.8±1.2 | 10 |
| Frequency of *scal* | 33.1% | | 33.7% | |
| Heterozygote effect | 1.9±0.5 | | 1.4±0.5 | |
| Phenotypic standard deviation | 2.9 | | 2.6 | |

° Genotype at the *scabrous-like* locus in fertile, wild-type individuals was determined by examining their progeny for *scabrous-like* segregants.

*scabrous-like* thus became prevalent in selection line *SO.2* because of a sizable heterozygote effect on bristle number (0.7 standard deviations for females, 0.5 for males) and was maintained at a high frequency for more than thirty generations despite the sterility of mutant homozygote females. The equilibrium between natural and artificial selection prevented the frequency of *scabrous-like* rising much above the maximum expected for a lethal gene, and although mutant females were not deliberately discarded, they were never sufficiently numerous to create difficulty in maintaining the line.

### 2. *dark hairy margin* (*dhm*)

This is a recessive mutant on chromosome III, mapped at 43.2 centiMorgans (Barker and Hollingdale 1970). In homozygotes, the whole wing appears darker than wild-type, wing veins are thicker and the margin more hairy; bristle number is increased (see later); wings tend to spread slightly and in some cultures wing abnormalities reduce viability. The mutant became fixed in line *SO.3* (unirradiated, Hollingdale and Barker 1971).

At generation 38 all selected flies in line *SO.3* were *dark hairy margin* phenotype, so a subline, *SO.3+*, was initiated using a random sample of wild-type flies from line *SO.3* mated to a random sample of *dark hairy margin* flies. In subsequent generations mutant homozygotes were discarded from the subline before selecting for abdominal bristle number under the same selection regime used in *SO.3*. Mean bristle numbers in line *SO.3* and its subline *SO.3+* are shown in Figure 1; bristle number means in sublines after five generations of relaxation of selection are also included. Although *SO.3+* was considerably below *SO.3* for some time, prevention of fixation of *dark hairy margin* did not limit the response of *SO.3+*. Over the last generations, the mean of *SO.3+* was increasing faster than the mean of *SO.3*.

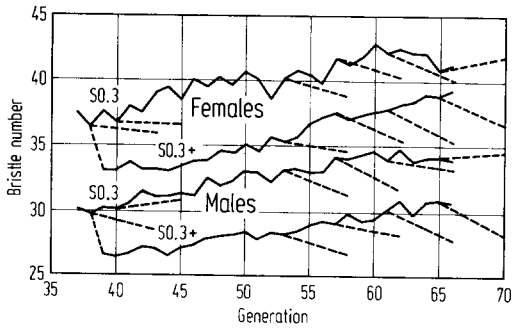


Fig. 1. Response of line SO.3 and its subline SO.3+ to selection for increased abdominal bristle number. Broken lines indicate periods of relaxation of selection

The effect of this gene on abdominal bristle number was examined by progeny testing flies scored for bristle number. A relaxed selection subline taken from line *SO.3* at generation 29 and grown under normal culture conditions for one generation was used for the first progeny test. A second progeny test was carried out using flies from generation 40 of subline *SO.3+*. Results of both progeny tests are given in Table 2. The *dark hairy margin* homozygotes were higher in abdominal bristle number than wild-type flies but of the four estimates of heterozygote effect only that for females from line *SO.3+* differed significantly from zero. This estimate, 1.1 ± 0.5 bristles, is about half the phenotypic standard deviation for wild-type females of line *SO.3+* at the time of the progeny test. This small heterozygote effect must have been sufficient to account for retention of the mutant in subline *SO.3+*, and its initial increase in frequency in selection line *SO.3*. The mutant also increased scutellar bristle number;

Table 2. *Effect of dark hairy margin, dhm, on abdominal bristle number; genotype was determined by test mating scored individuals*

| Line | Generation | Genotype | Mean bristle number | | Number scored | |
|---|---|---|---|---|---|---|
| | | | Females | Males | Females | Males |
| *SO*.3 | 30 | *dhm/dhm* | 34.6±0.5 | 29.5±0.4 | 23 | 26 |
| | | +/*dhm* | 31.4±0.3 | 26.0±0.2 | 69 | 80 |
| | | +/+ | 32.2±0.4 | 25.6±0.5 | 31 | 23 |
| *SO*.3+ | 40 | *dhm/dhm* | 36.4±0.6 | 30.5±0.4 | 23 | 37 |
| | | +/*dhm* | 33.2±0.3 | 26.4±0.3 | 73 | 56 |
| | | +/+ | 32.1±0.4 | 26.1±0.4 | 26 | 30 |

at generation 27 scutellar bristle number means for twenty-five pairs of mutant homozygotes were 5.9 bristles in females and 4.5 bristles in males, as compared with 4 bristles for wild-type flies. Whether the bristle number and wing morphology effects were associated because of true pleiotropy (single locus) or linkage (two loci, wing mutant in coupling with a 'high bristle' gene) remained undetermined.

### 3. *scabrous* (*sca*)

This mutant, located at 66.7 centiMorgans on chromosome II, has a large effect on bristle number, and disturbs the eye facets. It became fixed in line *SR*.2 (irradiated, Hollingdale and Barker 1971) and had previously been found in other abdominal bristle number selection lines, including some from the same Canberra base population (Table 3). Alleles in different lines varied in their effects on the bristle systems and in the degree of distortion of the eye surface. Some alleles also had effects on fertility, although the allele in *SR*.2 was fully fertile and the line retained good productivity even at extremely high mean bristle number. Although the effect on bristle number is large, the gene usually has not become obvious in selection lines until selection has raised bristle number above the level of the base population. In

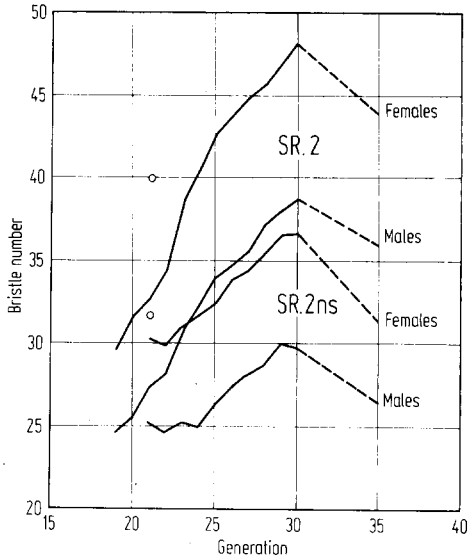


Fig. 2. Response of line SR.2 and its subline SR.2ns to selection for increased abdominal bristle number. Broken lines indicate relaxation of selection. Open circles indicate bristle number of female (upper circle) and male *scabrous* segregants at generation 21

*SR*.2 rough-eyed *scabrous* homozygotes were not detected until generation 15.

McBride and Robertson (1963) suggested that a low frequency of *scabrous* in the base population and a small effect on bristle number in heterozygotes could explain why no *scabrous* individuals were observed in their lines until after about ten generations of selection. They observed a large difference in bristle number between *scabrous* homozygotes and heterozygotes in a high genetic background ($F_1$ between a *scabrous* phenotype selection line and the base population, backcrossed twice to the selection line), but they did not determine the heterozygous effect of their allele. McBride and Robertson also compared *scabrous* and wild-type $F_2$ segregants in a

Table 3. *Occurrence of scabrous in abdominal bristle number selection lines*

| Reference | Selection line | | Generation *scabrous* discovered | Base population | Comments |
|---|---|---|---|---|---|
| | Selection intensity | Pairs of parents | | | |
| McBride and Robertson (1963) | 10% | 10 | c. 10 | outbred Kaduna | 2 related lines. |
| Clayton and Robertson (1964) | 20% | 10 | >5 | outbred Kaduna | Irradiated line. *sca/sca* females infertile. |
| Jones (1967) | 20% | 10 | 10 | outbred Canberra | Irradiated line and subline. |
| Kitagawa (1967) | 20% | 6 | c. 13 | $F_2$ of two inbred lines | Irradiated line. |
| Jones, Frankham and Barker (1968) | 40% | 10 | 27 | outbred Canberra | |
| Rathie and Barker (1968) | 20% | 20 | 10 | outbred Canberra | |
| This paper | 50% | 100, later 80 | 15 | outbred Canberra | Irradiated line. |

low genetic background (two backcrosses to their base population) and found that their *scabrous* homozygotes still had a large advantage in bristle number. This data did not fully exclude the possibility that the various effects of the gene were dependent on background. A backcrossing programme to measure the bristle number effect of the gene in a low background would involve recognition of homozygous segregants by their eye phenotype. If the degree of expression of the eye phenotype were also dependent on background, such a procedure would ensure that modifiers enhancing the expression of *scabrous* were retained because of selection in each segregating generation. And modifiers producing an extreme eye phenotype could also increase bristle number in *scabrous* homozygotes. Another pleiotropic effect, female infertility, was expressed only in a particular background (or the Kaduna population harboured two distinct *scabrous* alleles, or the infertile allele in Clayton and Robertson's (1964) line was induced or altered by the radiation treatment).

Effects of the *scabrous* allele found in line *SR.2* were examined in several ways. A non-*scabrous* subline, *SR.2ns*, was established from *SR.2* at generation 21. It was not irradiated. Selection was carried out as in *SR.2*, but only wild-type flies were scored for bristle number. Selection response in the subline is compared in Figure 2 with the response of *SR.2* until generation 30, when selection was discontinued in the subline. Also at generation 21, forty pairs of *scabrous* flies were scored for abdominal bristle number (the circles in Figure 2) and for scutellar bristle number. The scutellar bristle number mean was $5.2 \pm 0.2$ bristles in females and $4.4 \pm 0.1$ in males. At this stage the frequency of *scabrous* homozygotes in *SR.2* was still low, but the gene became fixed within a few generations. In subline *SR.2ns*, where *scabrous* was in effect an artificial recessive lethal gene, mutant homozygotes were produced at an appreciable frequency each generation. In some generations a record was kept of the number of homozygous flies discarded during scoring (Table 4). On the assumption that a recessive lethal gene is maintained in a selection line solely through its effect on the selected character, its equilibrium frequency in the line is determined by the magnitude of its (heterozygous) effect on the selected character and the selection intensity (see Appendix).

Table 4. *Frequency estimates for scabrous homozygotes in subline SR.2ns before selection*

| Generation | Females | Males | Combined |
|---|---|---|---|
| 23 | 6/46 | 5/45 | 0.12 |
| 27 | 9/109 | 16/116 | 0.11 |
| 28 | 18/118 | 10/110 | 0.12 |
| 29 | 9/109 | | |
| 30 | 10/100 | 5/96 | 0.08 |

Table 5. *Mean abdominal bristle number of flies from subline SR.2ns at generation 30, classified according to their genotype at the scabrous locus*

| Genotype | *sca/sca* | *+/sca* | *+/+* |
|---|---|---|---|
| Females | $47.4 \pm 0.6$ (17)[+] | $37.8 \pm 0.6$ (40) | $36.4 \pm 0.3$ (82) |
| Males | $40.2 \pm 1.0$ (10) | $30.8 \pm 0.4$ (51) | $28.8 \pm 0.3$ (66) |

[+] Bracketed figure is the number scored and progeny tested.

A moderate heterozygous effect of about 0.5 to 1.0 phenotypic standard deviations (of the *SR.2ns* wild-type population) would be adequate to maintain the mutant homozygotes at frequencies in the range of those in Table 4.

Flies of generation 30 in subline *SR.2ns* were used to measure the effect of *scabrous* on bristle number, determining genotype at the *scabrous* locus by test-mating wild-type scored flies with *scabrous* flies from line *SR.2* (Table 5). The proportionate effects of this *scabrous* allele in homozygotes and heterozygotes were respectively 1.3 and 1.0 in females and 1.4 and 1.1 in males. Jones (1967) found that his *scabrous* gene increased bristle number by larger factors of 1.65 in homozygotes and 1.2 in heterozygotes for both females and males in the background of his selection line. The eye phenotype of his allele was also more extreme than that of the *SR.2* allele. If the size of the bristle effect is the same irrespective of background genotype, as suggested by McBride and Robertson (1963), it is difficult to see why the first homozygotes were not usually detected earlier than indicated in Table 3. A heterozygote effect (from Table 5) of $1.4 \pm 0.6$ or $2.0 \pm 0.5$ bristles in one-segment abdominal bristle score is about equal to a base population standard deviation, and the minimum initial frequency of a gene derived from the base population is $1/4n$ ($n$ = number of pairs of parents in the selection line). In smaller selection lines such a gene could not persist for long at a low frequency. It seems likely that selection increases the frequency of modifiers enhancing the effects of *scabrous*, *i.e. scabrous* is a 'minor' gene in an unselected background. Alternatively, *scabrous* could be a synthetic mutant, perhaps a small duplication, that has arisen several times. Either hypothesis could explain the variation between different alleles found in lines derived from common base populations. An attempt was made to screen the Canberra cage population for the presence of *scabrous*. On two occasions, approximately one year and two years after initiation of the selection lines, virgin flies raised from egg samples taken from the Canberra population were individually mated to stocks containing the *scabrous* allele of *SR.2*. No *scabrous* progeny were detected in 2086 tests. The frequency of *scabrous* in the base population was therefore apparently less than 1 in 4,000. (In the first sample, in which 1179 individuals were tested, the tester stock was also homozygous for the

mutant *dark hairy margin*; no *dark hairy margin* progeny were detected.)

The effect of the *scabrous* allele was also examined in a background largely that of the Canberra base population. Stocks derived from the generation 32 lethal test of *SR*.2 males (Hollingdale and Barker 1971) and carrying third chromosome lethals but free of second chromosome lethals, yet still segregating for the *Curly* chromosome, were examined for degree of expression of the *scabrous* eye phenotype. Of these stocks, one was classed as *H*, high expression, and the others as *L*, low expression. Second chromosomes of some of these stocks were substituted into the Canberra background using the usual inversion chromosome techniques. The crossing programme ensured that the first (*X*) and third chromosomes of each substitution stock were a mixture of a large number of base population chromosomes. The one high expression stock (*sca H*) and a mixture of eight low expression stocks (*sca L*) were used to form two chromosome substitution stocks, Can; *sca H*; Can and Can; *sca L*; Can. These stocks were crossed reciprocally with the Canberra population and one hundred pairs of each stock and of each heterozygous genotype were scored for abdominal bristle number (Table 6). There were significant differences between the two *scabrous* stocks presumably due to differences in the second chromosome genotype. The *sca H* stock was more extreme in eye expression, scutellar bristle number (mean 6.8 bristles in females, compared with 4.8 bristles in the *sca L* stock) and heterozygous effect on abdominal bristle number in females, but less extreme in its homozygous effect in females and males. Its abdominal bristle number variances were also higher than those of the *sca L* stock, although this difference was significant only for homozygous males. The *sca H* stock was unusual — it was chosen on the basis of its extreme eye phenotype. But the differences between *sca H* and *sca L* show that linked modifiers can alter the effects of this major gene.

In the *sca L* stock, substitution of base population first and third chromosomes for those of *SR*.2 reduced mean bristle number by about 10 bristles, since the line mean at generation 32 was 48.5 in females and 39.3 in males. (Mean bristle number of the eight generation 32 males which provided the *sca L* second chromosomes was also 39.3.) In both females and males, a single *SR*.2 *scabrous* second chromosome from the *sca L* stock increased abdominal bristle number in the Canberra background by a factor of 1.1, and two of these chromosomes increased it by a factor of 1.7 above the base population level (bristle number mean 22.6 in females, 18.1 in males). The proportion of these effects attributable to the *scabrous* locus itself is not known as other second chromosome genes which acted as modifiers enhancing the bristle number effect of *scabrous* could have been present. As the first and third chromosomes together had a ten bristle effect, the effect of two second chromosomes, apart from the *scabrous* locus, could have been as much as five bristles. The substitution experiment thus measured the effect of *scabrous* in a low, but not completely unselected, genetic background. The problem of the slow initial increase in frequency of this major gene in abdominal bristle number selection lines remained unresolved.

Table 6. *Means and variances for abdominal bristle number of scabrous second chromosomes in the Canberra background; H = high, L = low expression of the eye phenotype*

| Genotype | Mean | | Variance | |
|---|---|---|---|---|
| | *sca/sca* | *+/sca* | *sca/sca* | *+/sca* |
| *sca H* females | 37.3±0.3 | 27.1±0.2 | 11.0 | 6.0 |
| *sca H* males | 29.2±0.3 | 21.0±0.2 | 8.7 | 3.3 |
| *sca L* females | 38.5±0.3 | 25.7±0.2 | 8.4 | 5.0 |
| *sca L* males | 30.6±0.2 | 20.6±0.2 | 6.0 | 2.7 |

### 4. *SO.3 lethals* $II_A$ *and* $II_B$

At generation 32, these lethals had frequencies of 29.0% and 17.7% respectively and none of the tested second chromosomes carried both lethals. This situation could represent an incipient balanced-lethal system, although both lethals were present at less than the expected equilibrium frequency for a single lethal carried by all selected parents. Non-occurrence of the coupling phase could be purely a chance effect, but if the lethals arose on different chromosomes the repulsion phase could persist for a considerable number of generations. Temporary linkage disequilibrium has often been postulated as an important cause of irregularities in selection response (Mather and Harrison 1949).

The effect of lethal $II_A$ and lethal $II_B$ on abdominal bristle number was studied by progeny testing flies scored at generation 47. At the same time fitness of the genotypes was investigated by measuring the productivity of the test cultures. Genotype was determined by mating virgin flies from line *SO*.3 with one of the lethal stocks and determining the ratio of *Cy*: + flies among the progeny. Ratios were classified as 2:1 (parent heterozygous for the lethal) or 1:1 (parent free of the lethal), although some cultures with intermediate ratios were unclassifiable. Each male from line *SO*.3 was progeny tested for both lethals. A male was kept for two days in a culture vial with two females of one stock, and then transferred to a second culture vial containing two females of the other stock for a further two days. Half the males were mated to stock $II_A$ first and half to stock $II_B$ first, in order to eliminate any possible bias in productivity due to age.

Table 7 gives the results of classifiable progeny tests and estimates of the frequencies of lethals $II_A$ and $II_B$ derived from the progeny test data. The double heterozygotes among males classified at both loci could include both coupling and repulsion phase heterozygotes. There was no evidence of develop-

Table 7. *Results of progeny test for second chromosome lethals $II_A$ and $II_B$ in line SO.3 at generation 47*

| Classification | | Mean bristle number | Mean progeny number | Number of individuals classified |
|---|---|---|---|---|
| $II_A$ locus | | | | |
| Females | $+/II_A$ | 40.9±0.4 | 101±7 | 55 |
| | +/+ | 40.0±0.3 | 123±7 | 89 |
| Males | $+/II_A$ | 32.6±0.3 | 252±7 | 64 |
| | +/+ | 32.0±0.3 | 274±8 | 90 |
| $II_B$ locus: | | | | |
| Females | $+/II_B$ | 40.1±0.4 | 119±7 | 54 |
| | +/+ | 40.1±0.3 | 121±7 | 69 |
| Males | $+/II_B$ | 32.5±0.3 | 234±8 | 63 |
| | +/+ | 32.0±0.2 | 267±7 | 87 |
| Both loci: | | | | |
| Males | Double heterozygotes | 32.9±0.5 | 252±8 | 23 |
| | $++/II_A+$ | 32.4±0.4 | 269±7 | 35 |
| | $++/+II_B$ | 32.2±0.5 | 259±7 | 32 |
| | ++/++ | 31.9±0.3 | 269±8 | 47 |

ment of a balanced lethal system but both lethals had been maintained in the line at appreciable frequencies for at least fifteen generations. The frequency of lethal $II_A$ decreased from 29% to 20% and that of lethal $II_B$ increased from 18% to 21% during this period, when the selection intensity was mostly 33.3%. (For several generations prior to generation 32 and after generation 45, the selection intensity was 20%; Hollingdale and Barker 1971.) Lethal $II_B$ may have arisen or become important in the selection line later than lethal $II_A$; this could explain the absence of the coupling phase at generation 32 and the continued increase in frequency of lethal $II_B$ from generation 32 to generation 47. Assuming that the observed gene frequencies of about 20% were equilibrium frequencies achieved when the selection intensity was 33.3%, and that these lethals were maintained in the line solely through their effects on the selected character, each lethal gene would be expected to have a heterozygous effect of 0.37 phenotypic standard deviations. As the estimate of the standard deviation at generation 47 in line *SO.3* was 2.7 in females and 2.3 in males, the difference in mean bristle number between the lethal heterozygote and the wild-type homozygote was expected to be approximately 1.0 bristles in females and 0.85 bristles in males.

Estimates of lethal gene frequency (%):

| | Females | Males | Combined |
|---|---|---|---|
| Lethal $II_A$ | 19.1 | 20.8 | 20.0 |
| Lethal $II_B$ | 22.0 | 21.0 | 21.4 |

The data for males classified at both loci showed no disturbance of genotype frequencies from the expected frequencies, so the separate progeny tests for each

*Heterozygous effect[+] of a recessive lethal gene at equilibrium in a selection line; q = equilibrium frequency of the lethal gene, m, p = proportion of population selected; for $(q')^2$, see text*

| | q | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| p | 0.01 | 0.05 | 0.10 | 0.15 | 0.20 | 0.25 | 0.30 | 0.32 |
| 0.010 | 0.00 | 0.02 | 0.05 | 0.10 | 0.16 | 0.26 | 0.49 | 0.72 |
| 0.050 | 0.00 | 0.03 | 0.05 | 0.12 | 0.20 | 0.33 | 0.61 | 0.86 |
| 0.100 | 0.01 | 0.03 | 0.08 | 0.14 | 0.23 | 0.38 | 0.69 | 0.97 |
| 0.125 | 0.01 | 0.03 | 0.08 | 0.15 | 0.25 | 0.40 | 0.73 | 1.01 |
| 0.166 | 0.01 | 0.04 | 0.09 | 0.17 | 0.28 | 0.44 | 0.79 | 1.06 |
| 0.200 | 0.01 | 0.04 | 0.09 | 0.18 | 0.30 | 0.48 | 0.82 | 1.11 |
| 0.250 | 0.01 | 0.04 | 0.11 | 0.20 | 0.32 | 0.52 | 0.89 | 1.16 |
| 0.333 | 0.01 | 0.05 | 0.13 | 0.23 | 0.37 | 0.59 | 0.98 | 1.25 |
| 0.400 | 0.01 | 0.06 | 0.14 | 0.26 | 0.42 | 0.66 | 1.07 | 1.33 |
| 0.500 | 0.01 | 0.07 | 0.18 | 0.33 | 0.51 | 0.79 | 1.20 | 1.45 |
| 0.600 | 0.02 | 0.09 | 0.22 | 0.40 | 0.64 | 0.96 | 1.38 | 1.60 |
| $(q')^2$ | | | 0.01 | 0.03 | 0.06 | 0.11 | 0.18 | 0.22 |

[+] Mean phenotype of $+m$ heterozygotes minus mean phenotype of ++ homozygotes, expressed in $\sigma_P$ units.

lethal locus were presumably free of any bias due to effects at the other lethal locus. The significance of the differences in mean bristle number between genotypes was examined by calculating one-tailed *t*-tests, using selection line estimates of the variance (99 degrees of freedom). Mean bristle number of heterozygotes was significantly larger than that of wild-type homozygotes for females classified at the lethal $II_A$ locus ($P < 0.05$). In males, mean bristle number of double heterozygotes was significantly larger than the mean for males free of both lethals ($P < 0.05$). Means of male heterozygotes classified at only one locus were not significantly larger than the means of the corresponding homozygotes ($0.1 > P > 0.05$, in both cases) but the observed differences between the heterozygotes and their homozygotes were also not significantly smaller than 0.85 bristles. A larger scale progeny test would be required to detect a heterozygous effect as small as 0.85 bristles.

The progeny test results indicated that lethals $II_A$ and $II_B$ were maintained in line *SO.3*, but not solely through their heterozygous effects on abdominal bristle number. In females, the heterozygous effect of lethal $II_A$ was about large enough, but lethal $II_B$ had no effect on bristle number; its effect was significantly less than 1.0 bristles. The heterozygotes must have had another selective advantage over wild-type homozygotes. Progeny production of females heterozygous for lethal $II_B$ was the same as that of the homozygotes, although for other heterozygotes (Table 7) progeny production was lower than in corresponding homozygotes. However, it must be remembered that 25% of all zygotes produced by heterozygotes in progeny test cultures were lethal. Relative progeny production of lethal locus genotypes is dependent on the frequency of the lethal gene in the population. When a lethal gene is very rare in the population ($q \doteq 0.0$), none of the progeny of a lethal heterozygote would be homozygous at the lethal

locus, so that the progeny production of the heterozygote may be greater by a factor of 1.3 than the value obtained in a test-cross to the lethal stock ($q = 0.5$). At intermediate gene frequencies progeny production of heterozygotes relative to homozygotes would be increased proportionately. As the frequency of lethal $II_B$ among selected parents in line *SO.3* was about 0.25, heterozygous females had an advantage in terms of progeny production. Progeny production of the heterozygotes at both lethal loci relative to their homozygotes may have been an important component of fitness when the lethal gene frequencies were low. Other fitness components, such as competitive effects between genotypes, were not measured.

The selective effects operative at the two lethal loci have been interpreted above assuming that the selection line was in equilibrium at generation 47. However, mean bristle number was still increasing slowly. Genotype $X$ environment interactions and epistatic interactions between the lethal loci or with other components of the genotype (such as the mutant *dark hairy margin*, segregating at generation 32, fixed before generation 47) were also assumed to be negligible.

*5. SO.1 lethal $II_A \equiv$ SR.3 lethal $II_A$*

This lethal was present in two selection lines at generation 32 at intermediate frequencies, 12% in *SO.1* and 24% in *SR.3*. Lines *SO.1* (unirradiated) and *SR.3* (irradiated, Hollingdale and Barker 1971) were selected for an extra generation at the conclusion of the selection experiment and flies of this last generation (generation 67) were used to examine the effect of the lethal on bristle number by the same method as that used for *SO.3* lethals $II_A$ and $II_B$. One hundred pairs of each line were scored for abdominal bristle number and individually test-mated to a stock carrying a *SO.1* lethal $II_A$ chromosome balanced over the *Curly* inversion chromosome. The numbers of *Curly* and normal winged progeny were counted and the ratio classified as 1:1 (scored parent free of the lethal) or 2:1 (scored parent heterozygous for the lethal). The ratio was classified as 2:1 in only one of approximately three hundred and seventy completed tests. Neither selection line had retained the lethal over the thirty-five generations which had elapsed since the lethal test. Whatever selective advantage the lethal had, whether due to increased bristle number in the heterozygote or to an increase in some fitness component, the advantage was only temporary. Despite reaching appreciable frequency in two separate lines, its selection must be attributed to favourable linkage or epistatic interactions.

## Discussion

Results of selection for increased abdominal bristle number in lines derived from the Canberra population and selected under various regimes of population size and selection intensity (Frankham, Jones and Barker 1968, Jones *et al.* 1968) and the comparison of the effects of radiation on response in Canberra selection lines of small size (Jones 1967) and larger size (Hollingdale and Barker 1971) have been interpreted as evidence for the existence in the base population of rare genes with large effects on bristle number. Within any one line a major part of the response could be attributed to genes at relatively few loci. It has been suggested that the effects of some of these major genes have been enhanced during the course of selection, by interaction with modifiers that have been increased in frequency by the selection process. Over the long term, replicate lines differentiate because of successions of chance events involving sampling and recombination.

Although bristle number is not correlated with fitness in the base population (Latter 1963), there is generally a decline in productivity in long-term selection lines. During a period of rapid response, deleterious genes linked to the 'large' gene may also increase in frequency, and perhaps become fixed in the line. In the case of a lethal gene linked to a gene with an appreciable effect on the selected character, linkage in the coupling phase will keep the alleles at both loci segregating at intermediate frequencies until recombination allows fixation of the bristle gene and elimination of the lethal by natural selection. This seems a likely explanation of the observations on *SR.3* lethal $II_A$; perhaps a recombination, which disrupted the linkage of this lethal to a 'large' gene, occurred soon after generation 30, as the coefficient of variation dropped to a lower level by generation 40. Some 'large' genes themselves could be deleterious, *e.g.* females homozygous for *scabrous-like* in *SO.2* were sterile, because of either true pleiotropy or operational pleiotropy, *i.e.* a very close linkage which was not broken during the course of the experiment.

Chromosomal substitution analyses of lines selected for various bristle systems in *Drosophila* have shown that interactions between different chromosomes can be an important component of selection response. Mather and Harrison (1949) found interaction between the first and third chromosomes, related to level of response in a series of abdominal bristle number selection lines. Scowcroft (1966) found favourable interactions between chromosomes in selection lines which had reached a high level for scutellar bristle number, but in lines showing less response the interactions often opposed the effects of single chromosomes. Frankham (1969) analysed two widely divergent abdominal bristle number selection lines and their base population, using marked inversion chromosomes to create the stocks required, but scoring bristle effects in substitution lines free of the marked chromosomes. He found some significant interactions between chromosomes but these were small as compared with the large main effects of individual chromosomes. Interactions within a chromosome may

also be important, but detection of these is dependent on location of the loci with appreciable effects on the selected character. Mather (1942) found that third chromosomes in his high and low abdominal bristle number lines differed in one region, and that the rest of this chromosome was essentially the same as the initial population third chromosome. Spickett and Thoday (1966) compared two selection lines with an ancestral inbred line and located genes with appreciable effects on sternopleural bristle number on each of the major chromosomes; strong interaction between one of the third chromosome genes and a second chromosome gene was sufficient to account for the interaction found between the whole chromosomes in a preliminary chromosome substitution experiment. They also found that some of the "locatable polygenes" had qualitatively distinguishable developmental effects. Visible mutations with large bristle number effects found in some of our unirradiated and irradiated selection lines were comparable to Spickett and Thoday's sternopleural bristle number genes, except that pleiotropic effects were more extreme. None of the selection lines was examined in sufficient detail here to determine the relative importance of interactions in their responses to selection, but the presence of visible mutants and high-frequency lethals indicated that the genetic basis of the response in each line was complex, and differed between replicate lines.

Persistence of lethal genes at intermediate to high frequencies in the selection lines has been discussed assuming that the effects at each locus were independent of the rest of the genotype. However, it would be more realistic to assume that interactions were important, as indicated by the observed complex allelic relationships; some chromosomes apparently carried several different lethals. Also, occasional inconsistencies were found in the allelism pattern (Hollingdale 1969), caused by semi-lethality of some combinations of alleles at a lethal locus, or due to synthetic lethals, or to clustering of lethals in limited regions on the chromosome. Falk and Lifschytz (1968) discussed the non-random localization of lethals on *Drosophila* chromosomes and suggested that inconsistencies found by Salceda (1967) in the allelism relationships between lethal second chromosomes resulted from clustering of small and overlapping lethal deletions in short chromosome regions. Salceda's lethals included some with apparent heterotic effects in heterozygotes as they were present at intermediate frequencies in Sankaranarayanan's (1964, 1965, 1966) experimental populations, three with a history of irradiation and one unirradiated. Linkage relationships between lethals reaching intermediate or high frequencies in selection lines therefore could be important.

Nei (1964a, b) found that linkage equilibrium for two lethal loci would be unusual but stable non-trivial equilibria were possible if the single heterozygote at each locus, or the double heterozygote, or both, had a selective advantage. For an initial population containing two lethals at low frequency (one of each single lethal heterozygote in each hundred individuals) with a recombination value of 0.01, Nei (1964b) showed that, for symmetric fitness models, change in gene frequency occurred rapidly, but followed a period of apparent stability if the product of the selective values of double heterozygotes and individuals free of both lethals exceeded the product of the selective values of individuals heterozygous at one locus or the other. These results were obtained with deterministic models, and the selective values used were artificially large, although perhaps not unrealistic where the selective advantage of a lethal heterozygote in a selection line is derived from a large heterozygous effect of the lethal gene on the selected character. Although *SR*.4 lethals $III_A$ and $III_B$ (Hollingdale and Barker 1971) were not analysed to determine their heterozygous effects on bristle number, they must have had large selective values to have attained frequencies of 40% in the absence of the coupling phase. Nei's calculations showed that linkage between these lethals need not have been very close. If each single heterozygote had a selective value twice that of the lethal-free homozygote, and if there were epistatic interaction such that the double heterozygote was twice as fit as the single heterozygotes, in less than twenty generations an initial population with these rare lethals occurring in different individuals, but only five map units apart, would give rise to an equilibrium population with each lethal gene at a frequency of 38% and with only 3% of coupling phase chromosomes.

Analyses of individual genes such as those presented here emphasize the complexity of relationships between genotypes and the response patterns they produce under selection; the more detailed work of Thoday and his colleagues has also demonstrated this in the sternopleural bristle system. By seeking out individual genes, even if it is only possible to identify those with reasonably large effects, more is being learnt about this genetic variation governing the expression of quantitative characters and about relationships between fitness and the metric characters under selection.

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## Appendix

### Equilibrium Gene Frequency and Heterozygote Effect of a Recessive Lethal Gene

Let $q'$ be the frequency of an autosomal recessive mutant gene, $m$, in a random mating population.

Mutant homozygotes, $m\,m$, are lethal prior to birth (or eclosion), or are removed artificially. Let $q$ be the frequency of gene $m$ among survivors at birth. The population can be represented thus:

| Genotype | $++$ | $+\,m$ | $m\,m$ |
|---|---|---|---|
| Frequency among zygotes | $(1-q')^2$ | $2q'(1-q')$ | $(q')^2$ |
| Frequency among survivors | $1-2q$ | $2q$ | 0 |
| Fitness of survivors | 1 | $1+s$ | |

$$q' = q/(1-q)\,, \quad q = q'/(1+q')\,.$$

Applying the usual procedure (*e.g.* Falconer 1960, Li 1967), genotype frequencies in the selected population are

$$++ \quad \frac{1-q'}{1+q'+2sq'} \quad \text{or} \quad \frac{1-2q}{1+2sq}$$

$$+\,m \quad \frac{2q'(1+s)}{1+q'+2sq'} \quad \text{or} \quad \frac{2q(1+s)}{1+2sq} \quad \ldots\ (1)$$

and at equilibrium the change in gene frequency

$$\Delta q' = \Delta q = 0$$

$$\therefore\ q' = s/(1+2s) \qquad q = s/(1+3s)$$

$$s = q'/(1-2q') \qquad s = q/(1-3q) \ldots \quad (2)$$

As $s$ increases, $q'$ approaches 0.5 and $q$ approaches 0.3, the frequencies expected when all selected individuals are heterozygous at the mutant locus.

Now the selection coefficient, $s$, may be determined by the heterozygous effect of the mutant gene on a quantitative character under selection. The normal distribution of the character in the whole population (variance $= \sigma_P^2$) is composed of two overlapping normal distributions corresponding to the two genotypes, $++$ and $+\,m$. These component distributions are assumed to have equal variances, $\sigma^2$, and means which differ by $x\,\sigma$ units. $\sigma^2$ is the environmental variance plus the residual genetic variance. The phenotypic variance in the whole population, $\sigma_P^2$, is thus the sum of $\sigma^2$ and the genetic variance due to segregation at the mutant locus, $\sigma_m^2$.

$$\sigma_P^2 = \sigma^2 + \sigma_m^2$$

To find $\sigma_m^2$ in terms of $\sigma^2$, consider the following table:

| Genotype | Value | Frequency |
|---|---|---|
| $++$ | 0 | $1-2q$ |
| $+\,m$ | $x\,\sigma$ | $2q$ |

$$\therefore \quad \sigma_m^2 = 2q\,x^2\sigma^2 - (2q)^2\,x^2\sigma^2 = 2q\,(1-2q)\,x^2\sigma^2$$

and

$$\sigma_P^2 = \sigma^2\,[1 + 2q\,(1-2q)\,x^2]$$

or

$$\sigma = \sigma_P \sqrt{1 + 2q\,(1-2q)\,x^2} \ldots \quad (3)$$

From (1) and (2) above, the frequencies of the two egnotypes in the selected sample, in terms of the equilibrium gene frequency, are

| | | |
|---|---|---|
| | $++$ | $(1-3q)/(1-q)$ |
| and | $+\,m$ | $2q/(1-q)$ . |

Let the selected sample be a proportion $p$ of the whole population, excluding $m\,m$ homozygotes. The selected homozygotes then have frequency $p\,(1-3q)/(1-q)$ in the whole population, and a frequency of $p\,(1-3q)/(1-2q)\,(1-q)$ among the component population of homozygotes (since homozygotes are fraction $(1-2q)$ of the whole population). Similarly, selected heterozygotes have a frequency of $p/(1-q)$ among the component population of heterozygotes. Evaluating these expressions for given values of $p$ and $q$, and referring to a normal distribution table (*e.g.* Table A4 of Steel and Torrie 1960), the truncation point can be located in terms of its distance, in $\sigma$ units, from the mean of each component distribution. The difference between the mean values of homozygotes and heterozygotes, in $\sigma$ units, is then easily obtained and converted to $\sigma_P$ units using (3) above. The following table gives the magnitude of the heterozygous effect needed to maintain a recessive lethal gene at equilibrium frequency $q$ when a proportion $p$ of the population is selected each generation.

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