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Studies on the Scutellar Bristles of *Drosophila melanogaster* III.* Long-term Selection for High Bristle Number in Three further Lines Derived from the Oregon-RC Strain, Correlated Responses in Abdominal Bristles, and Changes in Regulation of the Scute Locus

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Abstract

Results of selection over 135 generations for high scutellar bristle number in three further lines derived from Oregon-RC complement those on the four lines reported previously (Sheldon and Milton 1972). All lines differed widely in pattern of scutellar response, correlated response in abdominal and posterior central scutellar bristles, sex differences, and behaviour on relaxing selection, though the selection limits reached in the present three lines were lower than in the previous lines.

The early scutellar response supports the conclusion in the previous paper that early selection was mainly for poor regulation of the scute locus at the canalization level of control, since the probit span of the four-bristle class decreased as the mean scutellar number increased in the selection lines, and increased in the relaxed lines as the mean scutellar score decreased.

Introduction of the sc^1 allele into the selection lines confirmed the earlier findings that the selected background increased scutellar bristles in sc^1 to a much lesser extent than in sc^+ flies, and that considerable interaction of sc^1 and sc^+ with selected background occurred. The degree of dominance of sc^+ over sc^1 was increased in two lines out of five and decreased in one line. The latter indication of breakdown in the dominance level of control was not supported by results of the effect of an extra dose of sc^+ in males, where all lines were similar to the unselected level.

The correlated response in abdominal bristles was positive, similar in size in the three lines, and smaller than in the previous lines. The changes in abdominal score on relaxation of selection for scutellar bristles do not correlate well with the variable reductions in scutellar score across 10 relaxed lines. Positive, zero or negative genetic correlations between the two characters seem to be possible depending on the line and stage of response. In relation to abdominal scores, changes in dominance of sc^+ over sc^1 occurred but were not correlated with the dominance changes for scutellar bristles. The effect of an extra dose of sc^+ on abdominal bristles was similar in all six lines observed, adding 2·6–4·1 bristles, much smaller than the scutellar effect, where $sc^+/sc^+ \cdot Y$ males exceeded sc^+/sc^+ females.

Introduction

Results of long-term selection for high scutellar bristle number in four related lines derived from the Oregon-RC wild-type strain were reported in a previous paper (Sheldon and Milton 1972). Sheldon (1968) following Rendel *et al.* (1965) and Rendel (1967, 1968, 1969) had held the hypothesis that canalization at four bristles in wild type is accomplished by genetic regulation of the scute locus and that selection for high bristle number merely changed the minor gene background without affecting the canalization genotype. In contrast to this, Sheldon and Milton (1972) interpreted

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their results and, by inference, those of the above authors, at least in the early generations of selection, as being due to selection for poor regulators of the scute locus, i.e. the canalization genotype was altered. Latter (1970) and Scowcroft and Latter (1971) had also concluded that selection for high scutellar bristle number had altered the canalization genotype. The three selection lines to be reported in this paper were run at the same time as the ones in the 1972 paper and provide further evidence in support of the interpretation in that paper. As in the previous study, correlated responses in abdominal bristles are also reported, as well as indications of changes in dominance at the scute locus.

Materials and Methods

The Oregon-RC wild-type stock was the base population for the three selection lines. All fly populations were cultured in 150-ml bottles containing standard wheatmeal–treacle–agar medium, seeded with live yeast, at a temperature of 25 ± 1°C. The details of the different lines follow.

Line Y1

Line Y1 was started from the 1962 sample of Oregon-RC, the scutellar scores of which were given by Sheldon (1968). In each generation flies with the highest scutellar bristle score were selected. Up till generation 48 the procedure usually followed was to use five cultures per generation, with five female and three to five male parents per culture, mated at random. The numbers of flies scored per generation varied between 100 and 900 of each sex, averaging about 280 in both females and males. The proportion selected as parents varied, therefore, between 4·8 and 20·8% in females and 2·2 and 26·3% in males, apart from the parents of generation 1 which were less than 1% of the base population (0·8% in females, 0·2% in males). From generation 48 to generation 94 the system was as follows: five cultures, 120 of each sex scored from a pool of equal numbers from each culture, 40 of each sex selected and mated at random eight pairs per culture. From generation 95 to generation 138 only 60 of each sex were scored and 30 of each sex selected. After generation 138, selection was partially relaxed, only one generation in three being scored and selected as in the preceding period.

The number of abdominal bristles on the fifth sternite in females and males was scored every generation except 2 and 8 up till generation 48, then at generations 52, 58, 62, 67, 72, 92, 110 and 155. In the earlier generations a variable number up to 46 was scored per scutellar bristle class. After generation 20 a maximum of 25 per scutellar class was scored, in most cases five per class per bottle, except for the peripheral classes when such numbers could not be obtained. In the generations in which abdominals were scored all progeny were scored for scutellars, resulting in a doubling or trebling of the selection intensity in those generations.

Relaxed lines (Y1 Random 1 and Y1 Random 2) were taken from the selection line at generations 42 and 81. They were maintained by unselected random matings in five cultures per generation using eight pairs of parents per culture. They were scored periodically for scutellar and abdominal bristles.

Line Y2

Line Y2 began as an abortive attempt to establish a low-bristle selection line from the same foundation population sample as line Y1, using as first-generation parents 11 males and four females with three bristles and 24 females with four bristles. However, in four generations only two females and two males with three bristles occurred out of some 10000 flies scored and they had no progeny when mated, so the attempt to establish a low line was abandoned. Generation 3, however, had contained a higher proportion of five-bristle flies than the base population. These were used to begin a new high line (Y2), the first effective generation of which will continue to be called generation 4 so as to be in step with line Y1. This generation had three cultures, each having 10 females and three to four males with five bristles as parents. From then till the end of the experiment line Y2 was managed the same as line Y1, including scoring abdominal bristles and running two relaxed lines, Y2 Random 1 and Y2 Random 2. In line Y2 very short, fine, posterior central (p.c.) bristles began to appear after a number of generations and were recorded but not included in the scutellar bristle

score for the purposes of selection of parents or evaluating the main response to selection. The reasons for this approach were given by Sheldon (1968) and Sheldon and Milton (1972). The same procedure for p.c. bristles was followed in line A5 below, but they did not occur in line Y1.

Line A5

The foundation population of line A5 was a sample of Oregon-RC larvae given a heat shock at 37°C for 11 h beginning when they were 78 h old in uncrowded cultures, since previous work with scute flies had shown this to be a temperature-sensitive period (Sheldon and Rendel, unpublished data). This treatment resulted in about 65% mortality but also a very large increase in the proportion of flies with more than four bristles. About 1140 larvae were treated (60 per culture) and yielded adult flies with the following distribution of scutellar bristle number:

No. scutellar bristles	3	4	5	6	7
Females	0	154	52	16	1
Males	0	161	17	1	0

The first selected generation came from the flies with more than four bristles, cultured in eight bottles each with eight female and two male parents. However, generation 1 contained only 15 females with five bristles out of 938 and one male with five out of 907 scored, i.e. no higher than unselected untreated Oregon-RC. Generation 2 was produced in seven cultures, one having the 15 females with five bristles and 10 males with four bristles as parents and the other six cultures each having five females and five males with four bristles. Generations 3–9 had three, two, one, one, four, five and four cultures respectively, depending on the number of females available with more than four bristles. Between four and nine females were used per culture, mated to males with more than four bristles supplemented by ones with four bristles. The need to use any males with four bristles ceased at generation 8. Generations 10–40 had five cultures usually with five or six pairs of selected parents, but, as the matings of the selected parents were assortative in this line, occasional cultures had as few as one or as many as 10 female parents. Flies with the highest bristle number were selected in sufficient numbers to make up this number of cultures. The intensity of selection in this period varied between 2·3 and 23·2% in females and from 2·3 to 25·8% in males. Generations 41–94 and 95–135 and subsequent partial relaxation were managed in the same way as the equivalent periods in lines Y1 and Y2.

The number of abdominal bristles on the fifth sternite was scored at generations 8, 10, 11, every alternate generation till 35, then 38, 46, 51, 57, 61, 71, 91, 110 and 155. Six relaxed lines, A5 Random 1–6, were taken from the selection line at generations 41, 80, 92, 93, 94 and 95, and managed in the same way as in the relaxed lines of Y1 and Y2.

sc^1 *and* $sc^+ \cdot Y$ *Backcrosses*

From generation 43, the scute (sc^1) mutant was backcrossed for 10 generations into the selection lines after which the segregating scute-locus genotypes were scored for both scutellar and abdominal bristles. From about generation 135, an extra dose of sc^+ attached to a *Y* chromosome was backcrossed for 18 generations into the selection lines. The backcross lines were scored for scutellar bristles each generation, and for scutellar and abdominal bristles in the last backcross generation.

Following the procedure in previous papers (e.g. see Rendel 1967; Sheldon and Milton 1972), the scutellar bristle data were transformed to the probit scale to aid analysis of changes in canalization and dominance.

Results

Scutellar Bristles

Fig. 1 shows the response in scutellar bristles and the extent of regression of response in relaxed lines in lines Y1, Y2 and A5. The three lines follow response patterns which are different from each other and from the lines M, M2, and M3 reported earlier (Sheldon and Milton 1972). Only two of the six relaxed A5 lines (Random 1 and Random 2) are shown in Fig. 1*c* because the others overlap

them. The scores of the other four are given in Table 1. The relaxed lines vary in pattern of regression but are similar in the long periods of stability, though at somewhat different levels. None regressed back to base-population level. The p.c. bristles

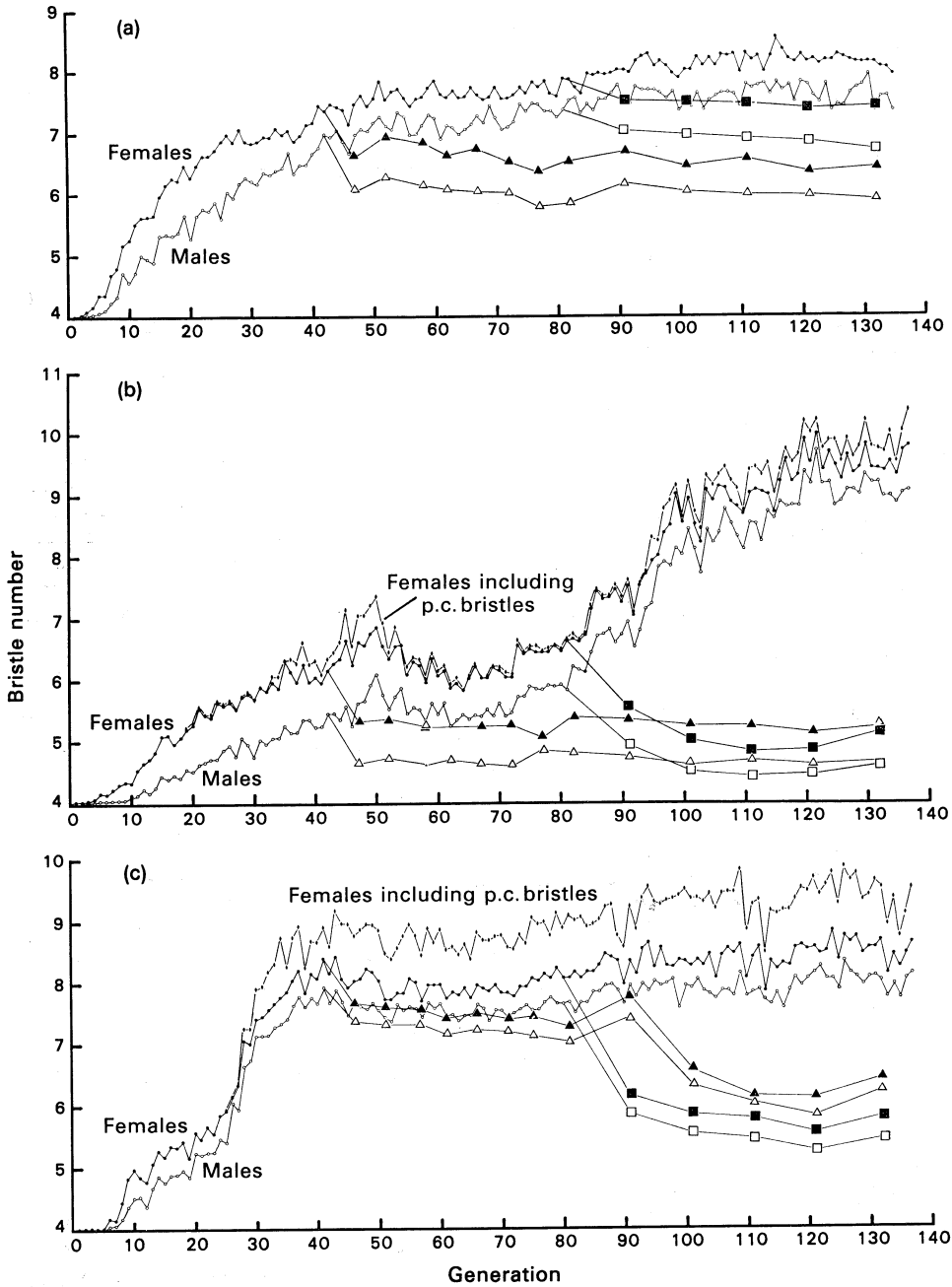

Fig. 1. Scutellar bristle selection response in lines Y1 (*a*), Y2 (*b*) and A5 (*c*). Relaxed line Y1 Random 1 and Y2 Random 1 derived at generation 42, Y1 Random 2 and Y2 Random 2 at generation 81, A5 Random 1 at generation 41, and A5 Random 2 at generation 80.

are not shown for the relaxed lines in Fig. 1 but the frequency of p.c. bristles declined similarly in all lines as the scutellar bristle number decreased.

Table 1. Mean scutellar bristle number in the four relaxed lines A5 Random 3–6

Generation of relaxation	Mean scutellar bristle number in line A5: Random 3		Random 4		Random 5		Random 6	
	Female	Male	Female	Male	Female	Male	Female	Male
Origin	8·1	7·8	8·5	8·0	8·6	7·9	8·2	8·0
10	7·0	6·7	7·9	7·6	7·9	7·6	7·8	7·6
20	6·4	6·1	6·8	6·5	7·8	7·4	7·6	7·3
30	6·4	6·1	6·5	6·2	7·9	7·4	7·5	7·1
41	6·1	5·9	6·3	6·0	7·6	7·1	7·3	6·9
51–54	6·2	6·0	6·6	6·1	6·6	6·2	6·5	6·2

Probit transformation of the data represented in Fig. 1 provides distributions of probit distances spanned by each scutellar bristle class, but only those for the four-bristle class are given in Tables 2 and 3. The probit width of the canalized

Table 2. Distances in probits (σ) spanned by the four-scutellar bristle class in lines Y1, Y2 and A5

Total number of flies scored in each generation is given in parentheses. Blank spaces indicate that no estimates were available

Generation	Width in probits (σ) of four-scutellar bristle class in: Line Y1	Line Y2	Line A5
Male			
0	5·80	5·80	5·80
1	5·43(955)		
2	5·01(728)	5·67(1535)	
3			5·50(474)
4	4·56(401)	4·80(460)	
5		4·54(376)	
7			4·27(970)
8			3·97(659)
9		4·31(423)	
11			3·17(646)
12		3·62(419)	
17			2·65(786)
21			2·12(626)
23		2·53(325)	
27			1·50(571)
35		1·63(124)	
43		2·02(550)	
50		1·21(228)	
62		2·16(1043)	
Female			
0	5·40	5·40	5·40
2		5·03(1435)	
4		4·20(410)	
5	3·34(475)		
15	0·96(120)		
19			2·29(946)

four-bristle class in the base population is about $5 \cdot 4\sigma$ in females and $5 \cdot 8\sigma$ in males. The results given here are mostly for males. The female results for the relaxed lines are similar to the male results (Table 3) except that their higher mean score and lower incidence of flies with only three bristles allow fewer estimates of the probit width of the four-bristle class.

Table 3. Distances in probits (σ) spanned by the four-scutellar bristle class in males of lines Y2 Random 1 and Random 2, and A5 Random 1 and Random 2

Total number of flies scored in each generation is given in parentheses. Blank spaces indicate that no estimates were available

Generation	Width in probits (σ) of four-scutellar bristle class in:			
	Y2 Random 1	Y2 Random 2	A5 Random 1	A5 Random 2
47	2·71(372)			
51			0·39(582)	
52	2·64(861)			
58	3·07(881)			
72	2·98(648)			
77	2·72(776)			
82	2·54(1230)			
91	2·50(780)			1·39(815)
101		3·24(1592)		1·51(845)
111	2·89(718)			1·63(841)
121	3·00(608)	3·37(975)	1·43(986)	1·92(1026)
132	2·81(518)			1·87(697)
143		3·38(1033)		2·23(879)

Lines Y2 and A5 and their relaxed lines clearly follow the same trend in the four-bristle class as observed in the papers by Sheldon and Milton (1972), Sheldon(1968) and Rendel *et al.* (1965). As the mean bristle number increases, flies with three bristles persist and the probit width of the four-bristle class decreases. This trend can be seen in greater detail over a longer period in these two lines than in the lines previously reported, as their mean bristle number increases more slowly. However, line-Y1 males have no estimates of probit width of the four-bristle class after generation 4, though the rate of change in mean bristle number and the numbers of flies scored in line Y1 are similar to those in line M3 of Sheldon and Milton (1972), where eight (decreasing) estimates were available in males up to generation 28 and one estimate of $2 \cdot 26\sigma$ in females at generation 8. The occurrence of single three-bristle females in generations 5 and 15 of line Y1 (Table 2) indicates that the same process is occurring here but, with the numbers scored, there should have been more estimates of the four-bristle class available. There was a similar shortage of such estimates in females in lines Y2 and A5. Data for the Y1 Random lines do not provide further information on this point because their mean bristle scores did not regress sufficiently to obtain estimates of the probit width of the four-bristle class in either males or females. However, the males in Y2 and A5 random lines (including A5 Random 3, 4 and 6) have many reduced estimates of the four-bristle class width as the mean regresses to five bristles or less.

Changes in the probit spans of the other bristle classes reflect changes in variance and any tendency to developmental stability (canalization) in classes other than four bristles. However, these data are not presented here, as they were in the previous

Table 4. Means and frequency distributions of scutellar bristle number for all genotypes in the scute backcross populations

Genotype	No. of individuals in scutellar bristle class:											Arithmetic	Probit mean
	0	1	2	3	4	5	6	7	8	9	10	mean	
Line Y1													
+/+					96	104	125	102	84	5		5·98	0·29σ above 5,6 threshold
+/*sc*					336	157	155	48	13			4·94	0·51σ below 5,6 threshold
sc/*sc*	4	21	174	67	13							2·23	0·56σ below 2,3 threshold
+/*Y*					335	263	266	273	166	11		5·78	0·11σ above 5,6 threshold
sc/*Y*	54	123	271	68	12							1·74	1·03σ below 2,3 threshold
Line Y2													
+/+					66	163	275	146	50	3		5·94	0·45σ above 5,6 threshold
+/*sc*				2	717	191	57	10				4·34	1·51σ below 5,6 threshold
sc/*sc*		3	139	92	16							2·48	0·17σ below 2,3 threshold
+/*Y*				3	541	748	481	104	28	3		5·12	0·57σ above 4,5 threshold
sc/*Y*	1	30	367	100	11							2·18	0·78σ below 2,3 threshold
Line A5													
+/+					2	27	184	130	122	31	4	6·90	0·19σ above 6,7 threshold
+/*sc*				1	182	171	375	69	8			5·44	1·31σ below 6,7 threshold
sc/*sc*		11	106	100	78							2·83	0·26σ above 2,3 threshold
+/*Y*				6	88	239	456	406	309	37	3	6·46	0·03σ below 6,7 threshold
sc/*Y*	26	86	239	108	58							2·17	0·46σ below 2,3 threshold

paper, because the relatively small changes involved do not warrant it. In summary, line Y1 shows little change in variability but some concentration of flies in the eight-bristle class. Line Y2 shows some increase in the variance in the period of renewed response after generation 90. It has a slight concentration in the six-bristle class, which does not persist in the relaxed lines, but none in the eight-bristle class. Line

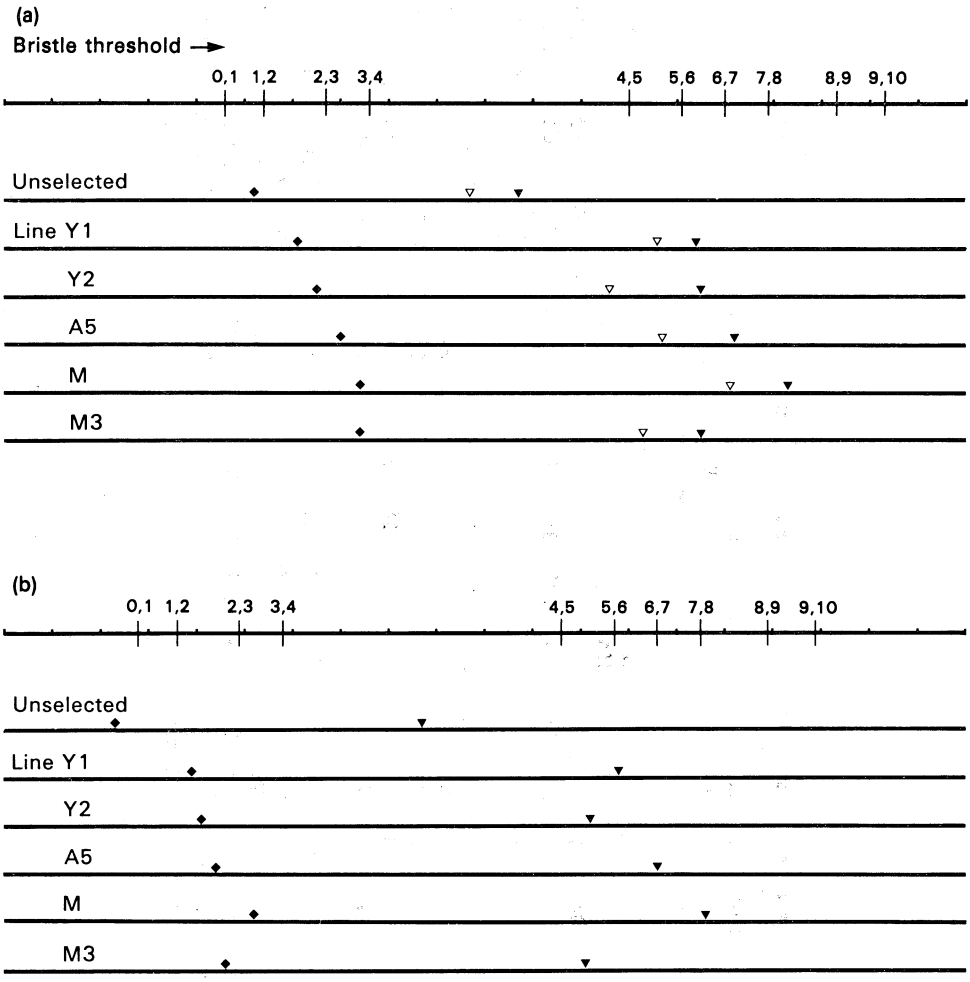

Fig. 2. Common probit scale of scutellar Make values, as used by Sheldon and Milton (1972), showing values for scutellar bristle-class thresholds and the positions of the genotypes *sc*/*sc*, *sc*/*Y*, +/*sc*, +/*Y* and +/+ in unselected flies and in the scute backcross populations of lines Y1, Y2, A5, M, M3. 1 division = 1 probit (1σ). (*a*): ♦ Female *sc*/*sc*. ▽ Female +/*sc*. ▼ Female +/+. (*b*): ♦ Male *sc*/*Y*. ▼ Male +/*Y*.

A5 shows some increase in variance from the beginning of the period of accelerated response after generation 25 until about generation 60. It decreases in the period around generation 66 and increases again after that. There is an indication of some concentration in the six-bristle class, reflecting a potential barrier to selection advance before, but not after, generation 25, and a further small concentration at eight bristles, the plateau level, after generation 30. The A5 relaxed lines, A5 Random 1,

2, 3, 4, 5 and 6, in general, retain this small excess of flies in the six-bristle class and also in the eight-bristle class when it is measurable.

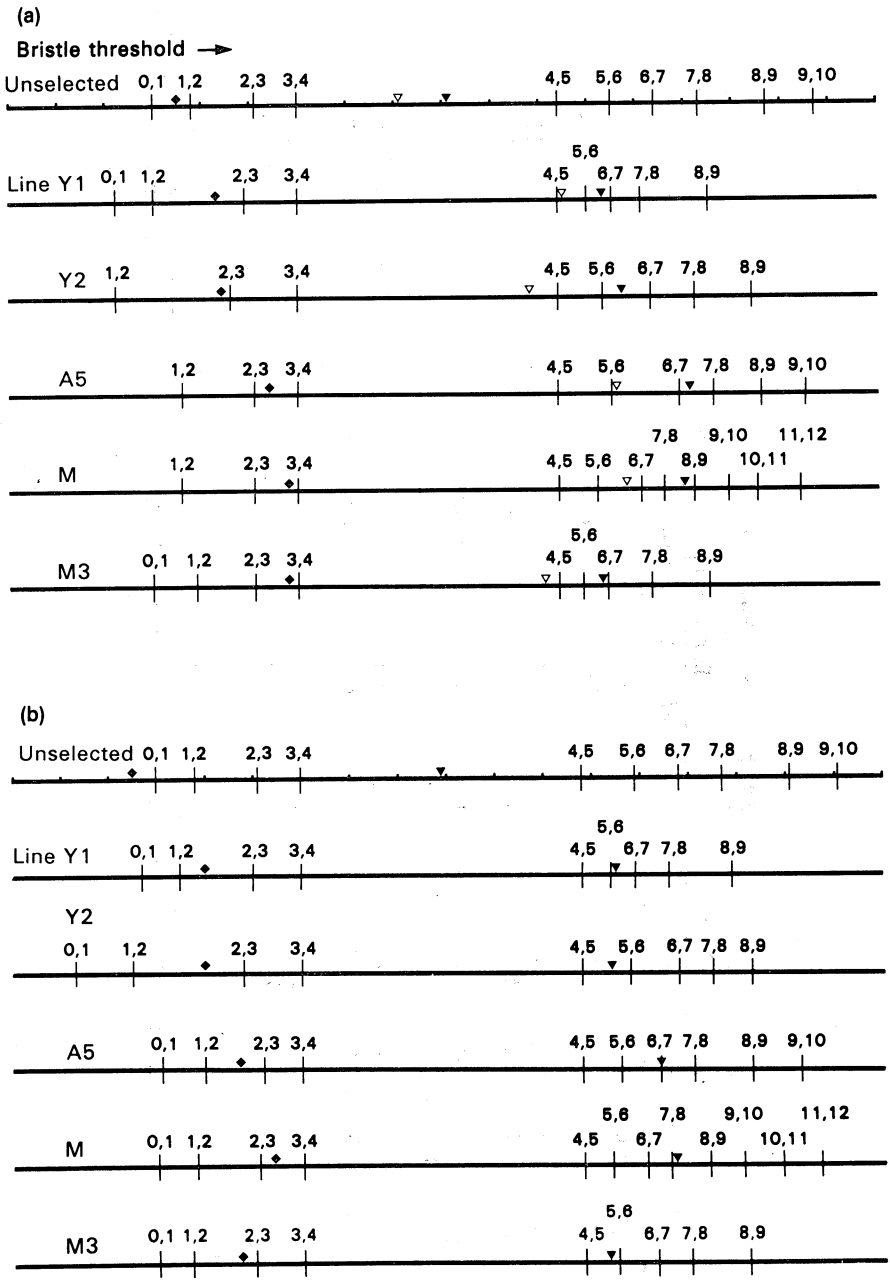

Fig. 3. Positions of the genotypes *sc/sc*, +/*sc*, +/+, *sc/Y* and +/*Y* in unselected flies and in the scute backcross populations of lines Y1, Y2, A5, M, M3, plotted on probit scales having common 3,4 and 4,5 bristle thresholds (the unselected level) but individual line values for the other bristle thresholds. 1 division = 1 probit (1σ). (*a*): ♦ Female *sc/sc*. ▽ Female +/*sc*. ▼ Female +/+. (*b*): ♦ Male *sc/Y*. ▼ Male +/*Y*.

Table 5. Summary of genotype comparisons shown in Figs 4 and 5

Line		Distance in probits (σ) from unselected base					Distance in probits (σ) within lines			Dominance ratio
		sc/*sc*	*sc*/*Y*	+/*sc*	+/*Y*	+/+	+/*Y*−*sc*/*Y*	+/*sc*−*sc*/*sc*[A]	+/+−+/*sc*[B]	(+/+−+/*sc*)/(+/*sc*−*sc*/*sc*)
Y1	C[C]	0·9	1·6	3·9	4·1	3·7	8·9	7·5	0·8	0·106
	I[D]	0·8	1·5	3·4	3·6	3·2	8·5	7·2	0·8	0·111
Y2	C	1·3	1·8	2·9	3·5	3·8	8·1	6·1	1·9	0·311
	I	0·9	1·5	2·7	3·4	3·6	8·3	6·4	1·9	0·297
A5	C	1·8	2·1	4·0	4·9	4·5	9·2	6·7	1·5	0·224
	I	1·9	2·2	4·5	4·5	5·0	8·7	7·2	1·5	0·208
M	C	2·2	2·9	5·4	5·9	5·6	9·4	7·7	1·2	0·156
	I	2·3	2·9	4·7	4·8	4·9	8·3	7·8	1·2	0·172
M3	C	2·2	2·3	3·6	3·4	3·8	7·5	5·9	1·2	0·203
	I	2·3	2·2	3·0	3·4	3·2	7·6	5·5	1·2	0·218
Unselected base	C						6·4	4·6	1·0	0·217

[A]First dose of +. [B]Second dose of +. [C]On common probit scale as in Fig. 4. [D]On individual line probit scales as in Fig. 5.

Table 4 gives the scutellar scores of the scute backcross populations. As with lines M and M3 in Sheldon and Milton (1972), even 10 generations of backcrossing were not sufficient to bring the mean bristle scores of the backcross populations right up to the level of the selection lines. The $+/+$ bristle means in Y1 and A5 backcrosses were about 1·5 and 1·2 bristles below the corresponding selection line, but the Y2 backcross was only about 0·7 bristles below.

If we take the unselected population segregating for scute which was used in Sheldon and Milton (1972) as the base line for the scute segregants, and unselected Oregon-RC as the base line for the $+/+$ and $+/Y$ segregants, and use the probit scales of bristle Make values used by Sheldon and Milton (1972), we can draw the set of comparisons between the lines which is given in Fig. 2. Lines M and M3 from the previous paper are included for comparison. In addition, since the actual probit widths of the bristle classes both below the 3,4 threshold and above the 4,5 threshold vary considerably between lines, the set of comparisons can also be made using the actual probit values occurring in each line and using the four-bristle class in unselected material as the common reference point (Fig. 3).

Table 5 summarizes the movement of the different selected genotypes from the unselected base on the two different probit scales; also the effects of the first ($+/sc-sc/sc$) and second ($+/+-+/sc$) substitutions of $+$ for *sc*. Regardless of the probit scale used it is clear: (i) that only about half or less of the response in $+$ flies in a backcross line is reflected in its *sc* flies; and (ii) that the ranking of selected backgrounds is different in $+$ and *sc* flies; both of which confirm the indications already provided by lines M and M3 in Sheldon and Milton (1972). The ratios of the effects of second and first substitutions of $+$ for *sc* show a very large increase in degree of dominance of $+$ over *sc* in Y1, a relatively large increase in M and decrease in Y2, and little change in M3 and A5. The values given for M and M3 in the previous paper were incorrect because $+/sc$ had been positioned on the probit scale in relation to a different bristle-class threshold from that used to position $+/+$.

The effects of an extra dose of sc^+ are shown by the data in Table 6, where $sc^+ \cdot Y$ males are always above the $+/+$ females in the same backcross line. The level of scutellar bristles in the backcross lines did not reach the level in the corresponding selection line despite the 18 backcross generations, though this effect was not as marked as in the previous paper (Sheldon and Milton 1972). The effect of an extra dose of sc^+ is taken as the difference between females and $sc^+ \cdot Y$ males in the backcross line added to the expected difference between females and males in the selection line at the level of phenotype of backcross females. The score of males is increased approximately 1·5–2·0 bristles by the extra dose of sc^+, varying slightly with the background, and being slightly less than in the previous paper where the selected lines had higher bristle levels.

Abdominal Bristles

The correlated responses in abdominal bristle number are shown in Fig. 4*a* for the Y1 lines, in Fig. 4*b* for the Y2 lines, and in Fig. 4*c* for the A5 lines. In general the abdominal responses tend to parallel the scutellar responses, except that (i) the late response of about two scutellar bristles in line Y2 (generations 95–120) is accompanied by very little extra increase in abdominal bristles, and (ii) relaxation causes

Table 6. Comparison of scutellar bristle means of the selection lines and their $sc^+ \cdot Y$ backcrosses

Means of selection lines were each based on about 120 flies, except those marked with an asterisk for which 25 flies were used. Means of the backcross lines were based on only 25 flies except for the last generation

Backcross generation	Mean No. of scutellar bristles in: Line Y1		Y1 backcross		Line Y2		Y2 backcross		Line A5		A5 backcross	
	+/+	+/Y	+/+	+/+ · Y	+/+	+/Y	+/+	+/+ · Y	+/+	+/Y	+/+	+/+ · Y
1	7·90	7·68	6·40	7·96			7·92	9·24			6·72	7·64
2			6·20	8·12			7·40	8·64			6·36	8·12
3	8·18	7·38	7·28	7·84	9·56	8·86	8·00	9·84	8·42	7·71	7·00	8·72
4			6·70	8·28			8·36	9·44			7·28	8·20
5			6·96	8·31			8·32	9·40	8·40	7·77	7·56	8·76
6	7·73	7·36	6·88	8·24	9·54	8·83	8·40	9·56	8·47	7·83	7·56	8·84
7			6·24	7·84			8·48	9·16			7·96	8·92
8			7·28	8·44			7·92	9·40			7·40	8·80
9	7·73	7·35	6·60	7·84	9·53	9·08	8·65	9·36	8·15	7·81	7·72	9·00
10			6·48	8·00			8·20	9·72			7·84	8·32
11			6·28	7·72			8·52	9·44			7·80	8·80
12	7·76	7·13	7·12	8·08	9·35	9·07	8·60	9·52	7·84	7·28	7·40	8·24
13			6·88	7·92			7·96	9·40			7·04	8·20
14	7·70*		6·84	7·88	9·10*		8·64	9·48	7·95*		7·64	8·80
15	7·74	7·23	6·72	8·28	9·39	8·90	8·48	9·60	8·13	7·83	7·44	8·64
16	7·90*		6·48	8·12	9·63*		9·20	9·72	8·17*		7·48	8·36
17	7·85*		6·92	7·76	9·12*		8·72	9·52	7·88*		7·80	8·52
18	7·67	7·01	6·60	8·00	9·18	8·59	8·56	9·44	7·59	7·24	7·44	8·68
19			6·93	7·88			8·83	9·54			7·03	8·31

rather more reduction in the abdominal bristles than in the scutellar bristles in the A5 random lines. Differences between the lines are quite small compared with the lines reported in Sheldon and Milton (1972). The tendency for a positive correlation, within generations, of abdominal score with scutellar bristle class is also much less marked than in that paper, so the mean scores of abdominal bristles within

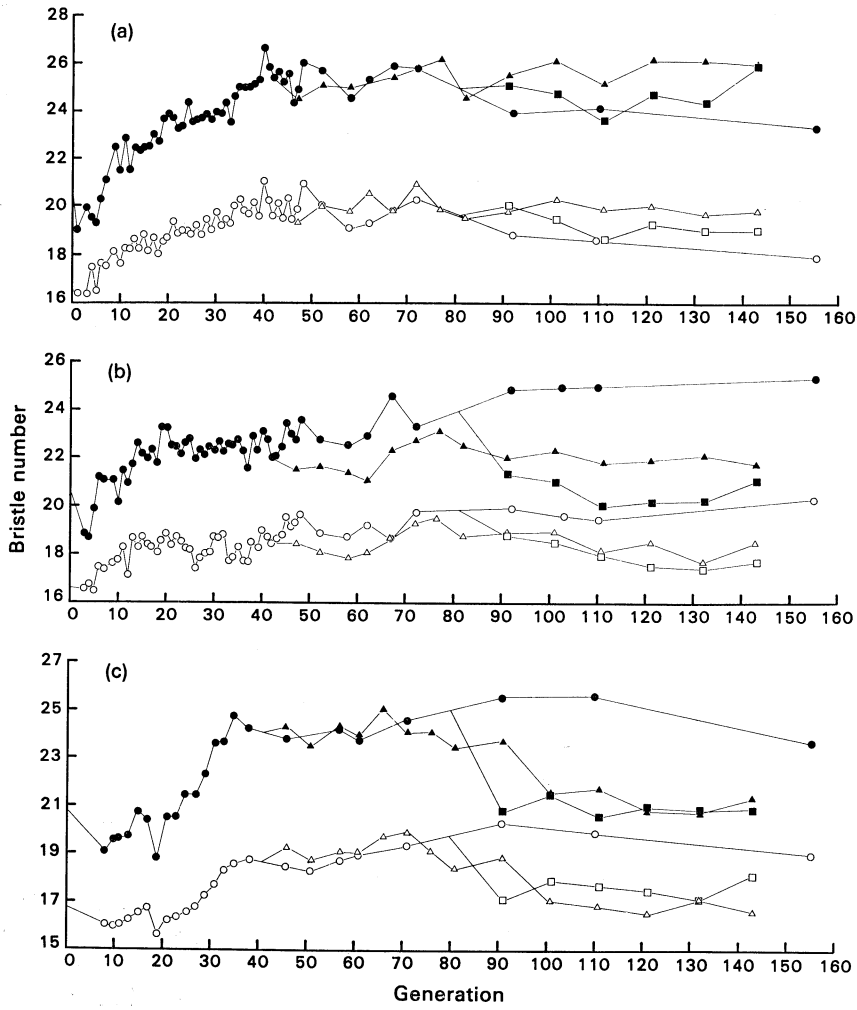

Fig. 4. Abdominal bristle correlated response in lines Y1 (*a*), Y2 (*b*) and A5 (*c*). ● Females. ○ Males. Relaxed line Y1 Random 1 and Y2 Random 1 derived at generation 42, Y1 Random 2 and Y2 Random 2 at generation 81, A5 Random 1 at generation 41, and A5 Random 2 at generation 80.

scutellar bristle classes are not presented here. The abdominal score in a particular scutellar bristle class also tends to increase in these lines as the mean scutellar level increased with selection, similar to the previous paper. The difference between male and female abdominal means is similar in the three lines in spite of differences between male and female scutellar means.

The abdominal scores of all genotypes in the scute backcross populations are given in Table 7. The unselected abdominal means are 6·5, 8·6, 16·2 and 20·4 in *sc*/*Y*, *sc*/*sc*, +/*Y* and +/+ respectively (Young and Sheldon 1965). In terms of actual number of bristles, the abdominal responses in scute (*sc*) flies are similar to those in the wild-type (+) segregants, in contrast to the situation for scutellar bristle responses, both here and in Sheldon and Milton (1972). The abdominal scores of heterozygous (+/*sc*) females are also higher than those of the +/*Y* males, as in the previous paper. The ratio of the effect of the second to the first substitution of *sc* by sc^+ can be calculated from Table 7 as 0·149, 0·157 and 0·167 in lines Y1, Y2 and A5 respectively, indicating a decrease compared with the unselected level, taken as about 0·25 in the previous paper though it is possibly as low as 0·217.

Table 7. Mean abdominal bristle scores within scutellar bristle classes in the scute backcross populations

Blank spaces indicate that no estimates were available

Genotype	Mean No. abdominal bristles within scutellar bristle class: 0	1	2	3	4	5	6	7	8	9	10	Weighted mean
Line Y1												
+/+					22·5	23·6	24·2	24·3	25·6	26·6		24·0
+/*sc*					21·7	23·1	22·9	22·9	22·1			22·5
sc/*sc*	12·0	11·8	12·7	12·2	11·2							12·4
+/*Y*					18·8	19·3	19·9	20·4	20·5	20·2		19·7
sc/*Y*	9·2	9·6	10·0	10·3	10·6							9·9
Line Y2												
+/+					22·3	22·9	23·2	24·2	25·2	23·0		23·4
+/*sc*				14·0	21·6	21·9	22·6	22·8				21·7
sc/*sc*		11·7	10·6	11·2	12·0							10·9
+/*Y*				18·7	19·1	19·4	19·5	20·2	20·4	19·3		19·4
sc/*Y*	9·0	9·0	9·1	9·3	9·1							9·1
Line A5												
+/+					23·5	21·0	21·9	24·1	24·4	24·2	24·8	23·2
+/*sc*				20·0	19·8	20·8	22·6	23·5	23·3			21·6
sc/*sc*		9·0	11·2	12·7	12·4							12·0
+/*Y*				15·8	16·5	17·0	18·0	19·6	19·6	19·4	18·3	18·5
sc/*Y*	9·1	9·2	9·6	9·8	10·1							9·6

The abdominal scores of +/+ and +/+ · *Y* in the $sc^+ \cdot Y$ backcross populations are given in Table 8. Data for lines M, M3, M2 and M2·2 are also included. Expected values of normal males (+/*Y*) have been estimated from the usual male–female difference in abdominal bristles in that particular line. The effect of an extra dose of sc^+ in males is similar in all lines, adding between 2·6 and 4·1 bristles, which is not large enough to exceed the +/+ female score, in contrast to the effect of an extra dose of sc^+ in males on scutellar bristles.

Discussion

These results for three high scutellar bristle selection lines, each derived independently from Oregon-RC, are to be viewed in conjunction with the results for the four selection lines already reported, which had been derived ultimately from one mating pair of Oregon-RC (Sheldon and Milton 1972). (We should point out a printing error on the second page of that paper. Line M3 was derived from matings among

Table 8. Mean abdominal bristle scores within scutellar bristle classes in the $sc^{+} \cdot Y$ backcross populations

Genotype	Mean No. abdominal bristles within scutellar bristle class: 4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	Weighted mean
Line Y1																	
+/+ ♀♀	22·1	23·2	23·0	23·8	23·9	24·3	24·7										23·6
+/+ · *Y* ♂♂	—	21·5	20·5	21·6	21·5	22·9	23·4										21·9
Expected level +/*Y* ♂♂																	18·2
Line Y2																	
+/+ ♀♀	—	—	23·8	23·1	23·9	24·8	26·4	26·0	28·0								24·8
+/+ · *Y* ♂♂	—	—	—	23·0	23·3	23·0	23·7	23·3	24·3								23·4
Expected level +/*Y* ♂♂																	19·7
Line A5																	
+/+ ♀♀	20·9	22·4	22·6	23·2	24·8	25·5	26·2	29·0									23·6
+/+ · *Y* ♂♂	—	—	19·5	20·4	21·4	24·4	23·8	26·4									22·2
Expected level +/*Y* ♂♂																	19·0
Line M																	
+/+ ♀♀	—	26·6	26·4	28·4	31·4	34·6	34·4	34·4	34·7	34·9	32·0	35·0	—	—	30·0		31·4
+/+ · *Y* ♂♂	—	—	24·6	24·3	23·7	29·0	30·0	31·6	30·6	31·6	32·8	31·0	35·0	—	—		28·1
Expected level +/*Y* ♂♂																	24·0
Line M3																	
+/+ ♀♀	23·3	23·0	24·3	27·3	29·4	29·5	29·8	29·4	31·2								28·0
+/+ · *Y* ♂♂	—	19·0	21·4	21·8	23·9	27·6	27·8	29·3	28·2	29·0	30·0						26·4
Expected level +/*Y* ♂♂																	23·5
Line M2																	
+/+ ♀♀	—	—	21·0	23·3	24·6	25·9	27·6	26·8	27·5	29·1	29·0	30·0	29·0	31·0	—	—	26·5
+/+ · *Y* ♂♂	—	—	19·0	21·1	21·4	22·2	23·1	24·6	25·2	24·9	25·6	25·4	25·7	29·0	27·5	31·0	24·1
Expected level +/*Y* ♂♂																	21·5
Line M2·2																	
+/+ ♀♀	23·3	24·0	23·8	25·2	26·9	26·8	27·0	29·9	31·1	29·7	31·0	28·5	—	—	—	—	28·0
+/+ · *Y* ♂♂	—	—	22·0	21·0	22·8	24·4	23·4	24·2	25·3	25·2	27·5	27·9	28·6	27·9	26·0	—	24·7
Expected level +/*Y* ♂♂																	—

the progeny of the same single-pair mating from which line M started. The beginning of M3 was six single-pair cultures of female with four bristles by male with four bristles, not male with five as stated in the paper.) The approach in these studies has not been a biometrical genetic one, with designed comparisons of different selection intensities, mating systems, population sizes, etc. We have been more concerned to select extreme phenotypes by any suitable procedure to enable us to study the genetic basis of the variation, of the canalization at four scutellar bristles in wild-type flies, and of the correlated responses in other bristle systems. Many features of the response to selection or relaxation in the different lines are interesting in themselves and will be discussed, but the question of first importance is the nature of the selection response in relation to the genetic basis of canalization at four bristles.

Regulation of the Scute Locus

The lines of evidence supporting the hypothesis that canalization at four bristles is brought about by genetic regulation of the scute locus (Rendel *et al.* 1965) have recently been summarized by Rendel (1979). The results in this paper significantly reinforce the conclusion of our previous paper (Sheldon and Milton 1972) that the early selection response in our lines is due mainly to selection for poor regulation of the scute locus. The detailed argument leading to this conclusion need not be repeated here. The evidence is the consistent trend, across all lines, for the probit span of the four-bristle class to decrease (to as low as 1σ) as the mean bristle number increases above four bristles, and to increase again in the relaxed lines as the mean regresses towards four bristles.

The second main area of agreement with results of the previous paper is the absence of a major response component due to breakdown of the first or dominance level of regulation at the scute locus, which appears to be independent of the second or canalization level of control. While there are changes in the dominance ratio in three out of the five lines in Table 5, the biggest one is of increased dominance of + to *sc* in line Y1. One of the others, line M, is in the same direction. The third one, line Y2, does show reduced dominance, which indicates that the dominance level of regulation of the scute locus has begun to break down. However, this is not supported by the results on the effect of an extra dose of sc^+ (Table 6). Here line Y2 shows an effect no higher than the other two lines in this paper, the four lines in the previous paper and the low, unselected and high lines of Rendel *et al.* (1965). This comparison between the scute (sc^1) backcross results and the $sc^+ \cdot Y$ backcross results has reduced value because of the long time difference between them. Nevertheless, apart from the apparent reduction in dominance in Y2, there is nothing in the results of either paper indicating a breakdown of the dominance level of control.

As it is difficult to see why selection for high number of scutellar bristles in $+/+$ and $+/Y$ flies can lead to increased dominance of + over *sc* (line Y1, Table 5) some further insight into the question of dominance changes may be gained by looking at the interactions in Table 5 in more detail. In the previous paper two points were made about the effect of the wild-type selected backgrounds on scute flies. Firstly the selected backgrounds had less effect on scute (sc^1) flies than on wild-type (sc^+) flies. Secondly there was an interaction of sc^1 and sc^+ with selected background. Specifically, sc^1 flies in line M3 background scored higher, relative to line M sc^1 flies, than expected on the basis of the scores of sc^+ in both backgrounds. Table 5 shows that these

two points apply generally across the five lines, in agreement with other authors (Fraser and Green 1964; Fraser *et al.* 1965; Fraser 1966; Miller and Fraser 1968). In our lines response in sc^1 flies is usually less than half that in their sc^+ sibs, line M3 being the exception with the scutes at about two-thirds the level of the wild-type response. At the other extreme in line Y1 *sc*/*sc* females show only about one-quarter of the wild-type response, while +/*sc* flies also breach a fairly general rule in showing a slightly higher, instead of lower, response than +/+. The effect of the first substitution of + for *sc* is therefore increased on two counts while the effect of the second substitution of + for *sc* is reduced, the net result being a much higher degree of dominance of + over *sc*. On the other hand line Y2 has a relatively low level of response in *sc*/*sc* females, similar to line Y1, but its +/*sc* females have moved much less than in the other lines. Therefore the effect of the first substitution is reduced and the effect of the second substitution increased, yielding a larger dominance ratio, i.e. a lower degree of dominance of + over *sc*. While this analysis of the situation exposes the complexity of the data it comes no closer to providing an explanation of the possible mechanisms involved. The extremes represented by Y1 and Y2 may simply be random correlated responses in a wide spectrum of possible modifier-gene responses. Line M3 is just as extreme in a different respect, namely the highest relative response in scute flies, but the net result is to have the same dominance ratio as the base population. Therefore, the indication in line Y2 of a beginning of breakdown in the dominance level of control at the scute locus, opening up new potential for a higher level of phenotype in +/+ flies, gains no support from the rest of the data.

Variability between Lines in Scutellar Response

While the limits of response in the three lines reported here were not as extreme as those in the previous paper, the general picture is still one of considerable variability between lines in pattern of response. Taking all lines in both papers into account we find they are all different. Line Y1 in this paper appears to be quite similar to line M3 until the latter's accelerated response about generation 120. They are, however, different in at least two other respects. Line Y1, unlike all the other lines, does not have p.c. bristles as a correlated response, and its relaxed lines do not regress as much as those of line M3.

As in the previous paper, the number of p.c. bristles in lines Y2 and A5 never exceeds two and their size remains small and fine, as in M3. It is only in later generations under intermittent selection, when the mean scutellar number increases in all lines to at least 12, that the size of the p.c. bristles increases, as already reported in lines M, M2 and M2·2. Line Y1 also begins to have p.c. bristles from about generation 220, when it begins to respond rapidly from a plateau level of about 8·5 scutellar bristles in females (unpublished data). Details of these later responses in all lines will be given in a later report.

The three lines reported in this paper have plateau or stasis levels which are partly similar to, partly different from, those of the previous four lines. In females they are 5·7 (Y2), 6·8 (Y2), 7·6 (Y1), 7·9 (A5), 8·1 (Y1, A5), 8·5 (A5), 9·0 (Y2), 9·6 (Y2). Taking all lines into consideration, the range of stasis levels shown in the previous paper is widened, though there is some clustering around eight bristles. However, our lines show only a slight reduction in variability and increased con-

centration of flies in the eight-bristle class compared with quite a large effect in some of Latter's lines (Latter 1966, 1970). Other authors' results have tended to agree with ours in showing no strong association between stasis level and increased probit widths of particular bristle classes (e.g. Fraser *et al.* 1965).

There is considerable variation in final bristle level of the relaxed lines. However, almost all have an initial, relatively short period of regression followed by a long period of stability, frequently at levels well above that of the base population. This was also found by Latter (1963, 1966, 1970) who interpreted regression on relaxation as being appreciable and rapid and due to homeostatic behaviour associated specifically with the canalized character. However, in many cases in our results the regression was neither appreciable nor rapid. In fact out of 15 relaxed lines in both papers line M Random 1 was the only one which regressed rapidly right back to a level close to the base population level. Of more significance in our results perhaps is the more rapid regression on relaxation, usually following a more rapid response, in the lines derived from the very narrow base (M, M2, M3) compared with those from a broader base (Y1, Y2, A5). If response in populations derived from one or only a few extreme individuals involves new or rare, less-balanced gene combinations (cf. Fraser 1965), it is reasonable to expect that regression or homeostatic behaviour will be more pronounced in them than in populations of broader base, the latter having a better opportunity for a balance to be achieved between artificial and natural selection. In relation to the stasis levels the stable levels realized by the relaxed lines extend the spectrum even further, e.g. 5·0–5·7 (Y2 Random 1 and 2, M3 Random 1 and 2, A5 Random 2), 6·0–6·6 (Y1 Random 1, A5 Random 1,3,4,5,6), 7·2–7·8 (Y1 Random 2, A5 Random 1,5,6), and 9·0 (M Random 2). Taken together with results from other authors, the so-called stasis levels range almost continuously from 5 to 14 bristles, which tends to reduce the importance earlier authors have attached to particular bristle levels in the response pattern (e.g. Fraser *et al.* 1965; Fraser and Scowcroft 1965; Latter 1966; Scowcroft 1966).

Probit Analysis

It is recognized that the validity of analysing the variable, total scutellar bristle number, rather than its component sites, by the probit method has been questioned (Robertson 1965; Scowcroft *et al.* 1968; Latter 1970; Scowcroft and Latter 1971). Rendel (1965) and Finlay (1965) presented evidence in favour of the view that there is in fact regulation of total bristle number, and Rendel (1979) has recently discussed the question again. We do not propose to discuss the pros and cons at this stage because we do not consider that interpretation of our results in the present context, based on analysis of total bristle number, is materially affected by the problem. In the previous paper (Sheldon and Milton 1972) we also drew attention to the inadequacy of the method (Rendel 1963) for analysing, across scute and wild-type flies, the correlation between abdominal and scutellar bristles in terms of competition for common bristle-making resources. In this paper differences in probit scale between lines (Fig. 5) introduced further difficulties of interpretation, e.g. the increases in probit span of the two-bristle class in *sc* flies, especially in line Y2. We do not yet understand what this means and propose to defer consideration of these questions until we have presented elsewhere the results for low selection lines and for crosses between high and low lines.

Correlated Responses in Abdominal Bristles

The pattern of abdominal responses is simpler than in the previous paper. There is a positive correlated response, of similar size in the three lines but smaller at the same scutellar bristle mean than in the previous three lines M, M2 and M3. It is not surprising, therefore, that the tendencies for positive correlation within generations between abdominal score and scutellar-bristle class, and for abdominal score in a particular scutellar-bristle class to increase as the scutellar mean increases over generations, are less marked than in the previous paper (Sheldon and Milton 1972).

The changes in abdominal bristle score in the relaxed lines provide one of the most variable features of the comparison between lines. In two relaxed lines, M Random 1 and 2, the regression in abdominal mean follows that of scutellar bristles very closely. In three others, Y2 Random 2 and A5 Random 1 and 2, the abdominal bristle mean regresses to base population level, though the scutellar bristle means regress only about half way. In A5 this is consistent with the early correlated response pattern in selection line A5 itself, no increase in abdominal bristles having occurred until the scutellar bristle mean was above five. In the other relaxed lines M3 Random 1 and 2, Y1 Random 1 and 2, and Y2 Random 1, the regression in abdominal bristles was less than in scutellar bristles. In fact the abdominal bristle scores of the Y1 random lines finished at higher levels than line Y1 itself, the latter having shown a reduction in abdominal bristles after generation 72. In these five relaxed lines presumably the extra abdominal bristle response retained was due to genes increasing abdominal bristles but not scutellar bristles, which were accumulated during selection for scutellar bristles because they were linked to the genes for high scutellar bristles. Thus the correlated response picture across all selection and relaxed lines reflects at different stages positive, zero or negative genetic correlations between the two characters (cf. Rendel 1963).

There was no correlation between the changes in dominance of sc^+ over *sc* measured on abdominal bristles and those measured on scutellar bristles. The comparison of dominance ratios is as follows:

	Y1	Y2	A5	M	M3	Unselected
Scutellar	0·11	0·31	0·22	0·16	0·21	0·22
Abdominals	0·15	0·16	0·17	0·22	0·11	0·22

The reason for the decreases in abdominal dominance ratio seems to be a proportionately smaller effect of the second dose of +. For abdominal bristles there was little interaction between *sc* and + and the selected backgrounds, in contrast to the situation for scutellar bristles. A tentative view in the previous paper was that genes responsible for the dominance level of control at the scute locus provide less control in one tissue and tighter control in another tissue. The overall results reinforce the view of tissue specificity in dominance modification, which was demonstrated by Ohh (1968).

The effect of an extra dose of sc^+ on abdominals in the $sc^+ \cdot Y$ backcross experiments (Table 8) correlates reasonably well with the dominance data, the lowest dominance ratio line, M3, having the lowest effect of an extra sc^+ (2·9 bristles) in these lines. However, the much smaller effects of an extra dose of sc^+ on abdominal bristles than on scutellar bristles further emphasizes the character or tissue specificity of the dominance and dosage compensation control mechanisms. Finally, as with the scutellar data, these data on abdominal bristles give no indication of a breakdown

in dominance control, which, if it occurred, would presumably enable the phenotype to be pushed more easily to higher levels.

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