PATTERNS OF ABDOMINAL TERGITE BRISTLES IN WILD-TYPE AND SCUTE *DROSOPHILA MELANOGASTER*

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THE large sensory bristles which constitute part of the complex of specialized epidermal structures in *Drosophila*, occupy quite definitely fixed positions; the fact the systematists have been able to individually name many of these bristles (e.g. FERRIS 1950) attests to the regularity with which they develop at specific sites. For this reason alone the *Drosophila* epidermis and the bristles it supports are of interest to biologists concerned with the regulatory mechanisms responsible for localized differentiation, and for the appearance of definitely shaped organs and tissues arranged in orderly patterns.

The numbers and the spatial arrangement of these large bristles seem to depend on at least two developmental systems which are to some extent independent of each other. The analysis of genetic mosaics made by STERN (1954a) for example, led him to conclude that bristle initiation is preceded in the sequence of development by a prepattern which determines specifically the sites where bristles may appear. Whether or not the potential for bristle differentiation at these specific sites is ultimately realised then depends on some state of the integument which decides the ability or competence of its constituent cells to respond to the prepattern.

In contrast, the smaller bristles on the *Drosophila* integument often exhibit only a regularity in the size of the spacing intervals between them. Despite this distinctive pattern characteristic, MAYNARD SMITH and SONDHI (1961), mainly for reasons of economy, have suggested that it too may depend on specific prepatterns. In this respect, however, WIGGLESWORTH (1940, 1953) has proposed a simple, yet attractive, developmental model giving an explanation for even spacing between integumentary structures, and the question arises whether WIGGLESWORTH's competitive model might not be more appropriate than specific prepatterns in relation to the arrangement of the smaller bristles in *Drosophila*. Following from STERN's (1954a) conclusions that the location of dorsocentral bristles depends partly on competitive influences between them, CLAXTON (1964) discussed a way in which the competitive model might be superimposed upon and interwoven with the concept of prepatterns. For this reason there need be little or no loss of economy if the patterns of small and large bristles are determined in different ways. Both can be accommodated within the basic prepattern-competence framework, and it is necessary only that the prepattern be nonspecific

if the mechanism incorporated in the competitive model is to have a primary pattern determining function.

The development of bristle patterns is influenced by a number of mutant genes. The effects of many of those which have been studied, for example, *achaete* (STERN 1954a,b), *scute* (STERN and SWANSON 1957—cited by GOTTLIEB 1964; YOUNG and LEWONTIN 1966), *hairy* (STERN 1954b), *engrailed* (TOKUNAGA 1961), and *theta* (STERN 1956b—cited by GOTTLIEB 1964), have been interpreted in terms of a basic shift in the ability or competence of epidermal cells to respond to the prepattern. Although relatively little attention has been given to the smaller bristles of *Drosophila*, YOUNG and LEWONTIN (1966) based their conclusions on a study of the abdominal sternite bristles. Thus, as one would predict, in cases where mutant genes alter the pattern characteristics of small as well as of large bristles, both effects can be accommodated by a change in one or other of the components (in this case competence) of the prepattern-competence complex.

The present work is concerned with an examination of some pattern and size characteristics of the abdominal tergite bristles in *scute* and wild-type flies. Of special interest was the possibility that regional gradients of the characteristics on individual tergites might reflect localized differences of the type induced when *scute* is substituted for its wild-type allele.

MATERIALS AND METHODS

A mating plan was designed originally so that legitimate comparisons could be made between *scute* (*sc*), *Hairy-wing* (*Hw*), and wild-type flies, separate stocks of which had previously been maintained with random breeding. The present paper is not concerned with the *Hairy-wing* mutants, and although the symbolism of the mating scheme diagrammed in Figure 1 is accordingly simplified, it appears more complex than would have been necessary if designed only for a two-way comparison. The two initial matings were between *scute* males and yellow, *Hairy-wing* females, and between yellow, *Hairy-wing* males and *scute* females; a stock of white-eye mutants was later introduced into the plan so that after two more generations, heterozygous *scute* females could be distinguished from homozygous wild-type females. Recombination between the white-eye and *scute* loci has been estimated to be about 1.5% (BRIDGES and BREHME 1944), and with the small numbers of flies which were included in the final sample, it was improbable that crossing over between these loci would have invalidated the use of white eye as a suitable marker gene. Although no extensive search was made, no *sc w* males were discovered in the populations from which samples were drawn.

As near to 48 hours after hatching as was possible, flies were collected at random from the final four populations which were produced. Each fly was individually anaesthetised, and weighed immediately. Following this procedure the abdomen of each fly was dissected from the head and thorax, and after rupturing the ventral surface of the abdomen it was placed in a drop of Depex mounting fluid on a glass slide. The abdominal contents, the sternites (when still attached), and all but the 2nd, 3rd, 4th and 5th tergites were then removed with dissecting needles, and a cover slip was placed over the remaining four tergites. By applying gentle pressure to the mount the tergites were carefully flattened, and they appeared finally as four distinct and approximately rectangular areas of cuticle each with its intact bristles lying on the cuticular surface and pointing posteriorly.

When the mounting fluid had hardened, each preparation was placed in a projection microscope, and the magnified images of the 3rd and 4th tergites were drawn on paper. The location of each bristle socket was also marked, and bristle length (the distance between its distal tip and

FIGURE 1.—The mating plan designed originally to draw comparisons between wild type, scute, and Hairy-wing flies. The latter mutants are not discussed in the present paper, and the symbolism of the plan is simplified accordingly.

the centre of its circular socket) was measured and recorded. In view of the fact that the cover slip and glass slide were firmly and closely attached to one another it was not possible for the bristles to be curved appreciably in the plane at right angles to the slide, and any small curvature here was ignored in the measurements of bristle length. In the plane of the glass slide the bristles were usually approximately straight, but in exceptional cases a bend or distinct curve in a bristle was taken into account when its length was measured.

Relatively rarely a bristle was dislodged from its socket and even more rarely it was lost, presumably in the preparation of the mounts. The empty socket was drawn as usual in relation to others, and, therefore, the records of bristle numbers were unaffected, but missing bristles did not enter into any results which concerned length, e.g. the average length of bristles on each tergite.

The anterior and lateral edges of the tergite were defined by the boundary where pigmentation and, therefore, sclerotization ended. The corresponding boundary on the posterior edge of the tergite was, however, more difficult to locate precisely, and not infrequently its continuity was interrupted, presumably due to stresses imposed during the mounting process. In addition, there was often some transverse folding of the cuticle near the posterior tergite edge. For the present purposes, therefore, the posterior tergite boundary was taken as the distinct transverse

FIGURE 2.—For the purposes of estimating tergite size and determining the regional variation in bristle characteristics, a rectangle, ABCD, was drawn on the tergite boundaries (heavy line) and divided by a series of lines parallel to its long and short axes into 35 sub-regions of equal size. The symbolism T_1 , T_2 , T_3 , T_4 , T_5 and L_1 , L_2 , L_3 , L_4 , defines the transverse and longitudinal tergite subdivisions respectively.

line separating heavily and lightly pigmented cuticle; this line was usually on or immediately behind the posterior transverse row of large tergite bristles. In both scute and wild-type flies, only rarely were there any tergite bristles located outside these defined boundaries.

An approximate estimate of tergite size was obtained by constructing a rectangle on the previously drawn tergite boundaries, and calculating the area contained within it. The rectangle was also divided into 35 sub-regions of equal size by drawing four transverse and six longitudinal evenly spaced lines parallel to its long and short axes respectively (Figure 2), and the resulting subdivisions defined by the lettering system given in Figure 2, were subsequently used as the basis for determining the regional variation in bristle number and bristle length on the tergite.

The six longitudinal and three most posterior transverse lines were also used in determining the proportionate size of the tergite surface covered by bristles (the bristle band). To achieve this the edges of the bristle band were first established. Taking the longitudinal lines in turn, and with repeated selection of two bristle sockets from the two adjacent tergite subdivisions, the most anterior point of intersection between the lines joining socket pairs and the particular longitudinal line was marked on the latter. Having in this way obtained a point on each of the six longitudinal lines, the distances of these from the posterior boundary of the tergite were measured and expressed as proportions of the total axial length of the tergite. The same procedure was adopted on the two ventral edges of the tergite, but here, the most ventral intersects were marked on the three most posterior transverse lines used for dividing the tergite into sub-regions, and the distances between the corresponding intersects on right and left sides of the body were expressed as proportions of the transverse width of the tergite. These fractional distances are presented later in Table 2. The posterior edge of the bristle band on each tergite was, in all cases, taken to coincide with the defined posterior tergite boundary.

Along the posterior edge of the band of bristles on each tergite there was a row of bristles which appeared distinctly larger than others located anteriorly in the band, and it was intended initially to examine the members of this row separately. In this respect, however, difficulties arose, and contrary to the earlier belief, measurements showed that the tergite bristles could not always be separated into two distinct populations on the basis of their length. Although the very largest bristles were found to be confined to the posterior edge of the bristle band, and although frequency distributions of bristle length showed there to be two bristle populations on each tergite, the range of length in either population usually overlapped the other so that there was a continuous gradation from the shortest to the longest bristles. The frequency distributions for the parameter bristle length, are illustrated in Figure 3, and these show clearly that there was no division between the two bristle populations with different mean lengths. Each of the

FIGURE 3.—Frequencies of bristle length, illustrating in each case, that the tergite bristle population is a composite of two overlapping distributions.

distributions was constructed by dividing the range of length values on each tergite into equal divisions on either side of the mean length for that tergite, and recording the numbers of bristles falling into each division. For wild type and scute separately, the numbers of bristles in corresponding divisions were summed over the third and fourth tergites of 20 male and 20 female flies, and these results are plotted in Figure 3.

No attempt was made to obtain direct estimates of bristle density on individual tergites, either for the purpose of studying density gradients within a single tergite or for establishing density differences in different genotypes. Particularly in the former case where density would have to be based on very small numbers of bristles, some of the estimates might have been seriously biased depending on how the edges of the bristle band on each tergite were located. Possibly density may be better estimated by measuring the mean distance from any bristle to its nearest neighbour (c.f. CLARK and EVANS 1954), but once again it is likely that edge effects may bias the results. In addition, this method may not be valid for between-genotype comparisons because of JOHNSTON'S (1966) evidence that the product of root density and mean distance to nearest neighbour is lower in scute than in wild-type flies. Some implications concerning regional gradients of bristle density can, however, be made on the basis of the relative bristle numbers within the equally sized sub-regions of each tergite. In addition, between genotype comparisons of density were approximated from the average figures for bristle number, tergite area, and the proportionate size of the tergite surface developing bristles.

TABLE 1

Average values (and standard errors) of tergite bristle number, tergite length (axial), tergite width (transverse), tergite area, and fly weight, together with the differences between wild type and scute expressed as percentages of the values in wild type.

Each figure a mean for 20 flies

	Wild type				Scute				Percentage reduction in scute			
	Male Seg. 3	Male Seg. 4	Female Seg. 3	Female Seg. 4	Male Seg. 3	Male Seg. 4	Female Seg. 3	Female Seg. 4	Male Seg. 3	Male Seg. 4	Female Seg. 3	Female Seg. 4
Bristle number	116.1 ± 2.0	114.1 ± 1.5	130.2 ± 2.2	131.4 ± 2.1	83.5 ± 2.5	86.8 ± 2.2	104.5 ± 2.5	104.8 ± 2.8	28.1	23.9	19.8	20.3
Tergite length (μ)	2372 ± 24	2433 ± 31	2464 ± 27	2538 ± 29	2216 ± 24	2218 ± 25	2395 ± 20	2420 ± 24	6.6	8.8	2.8	4.7
Tergite width ($\mu \times 10^{-1}$)	1145 ± 9	1133 ± 9	1474 ± 11	1486 ± 10	1110 ± 10	1099 ± 10	1402 ± 11	1415 ± 12	3.1	3.0	4.9	4.8
Tergite area ($\text{mm}^2 \times 10^4$)	2715 ± 46	2757 ± 53	3633 ± 60	3773 ± 64	2459 ± 46	2438 ± 44	3358 ± 54	3424 ± 54	9.4	11.6	7.6	9.3
Fly weight (mg)	0.901 ± .018		1.388 ± .044		0.820 ± .018		1.193 ± .038		8.9		14.1	

RESULTS

Bristle number: There were fewer tergite bristles on the third and fourth abdominal segments of scute than of wild-type flies (Table 1). The reduction in bristle number, expressed as a proportion of the bristle number in wild-type flies, varied from about 20% in the females to about 28% on the third segment of males. However, a comprehensive variance analysis of the bristle number data did not reveal any statistically significant interaction term so that the differences in these percentage reductions were evidently attributable to sampling variation. The only statistically significant ($P < 0.01$) classifications in the analysis were those associated with wild-type/mutant and male/female differences, the nature of which are apparent in Table 1.

The area of each tergite was not measured exactly but a comparison of the dimensions of the rectangular regions drawn on the tergite boundaries (see Figure 2) suggested that tergite size was smaller in scute than in wild-type flies. The widths and lengths of these rectangles given in Table 1 show the consistently smaller average dimensions which were obtained in the mutants, and they indicate that the reduction in tergite size brought about by the scute gene was of the order of 10%. This result was in line with the body weight records (Table 1) which suggested that scute has a depressant effect on growth. There was no evidence that the scute gene influenced the approximate coincidence of the posterior transverse row of large bristles and the line separating darkly and lightly pigmented cuticle on each tergite. Therefore, the latter, which for the present purposes defined the posterior boundary of the tergite, served also in all cases as the location of the posterior edge of the band of tergite bristles. In the manner described previously, the positions of the anterior and ventral edges of this band were established by the intersects these made with the artificial lines dividing the tergite into a fixed number of sub-regions. At places corresponding with the six longitudinal lines and the three most posterior transverse lines of subdivision, the lengths and widths of the bristle band were accordingly measured, and these results, expressed as proportions of the length and width of the tergite respectively, are given in Table 2. These values were subsequently used to make a diagrammatic comparison of the relative locations of the bristle band edges in scute and wild type (Figure 4), and to calculate the proportionate size of the tergite surface where bristles developed. The results show that the distribution of tergite bristles was relatively less extensive in scute than in wild-type flies and the differences between them were associated generally with the anterior edge of the bristle band, particularly in the central dorsal and ventral tergite regions where the edge was quite markedly indented posteriorly in the mutants.

For the reasons given earlier, individual values of bristle density were not measured on each tergite. The estimates of density obtained when average bristle number was divided by the average size of that area of the tergite where bristles developed (Table 2), are probably biased, but assuming the bias to be of a similar magnitude in mutant and wild-type flies, these estimates suggest that bristle density was lower in scute than in wild type. From more direct evidence, JOHN-

FIGURE 4.—The relative locations of the anterior and ventral edges (wild type, full lines; scute, dotted lines) of the regions on each tergite where bristles develop. For comparative purposes the tergites (rectangles) have all been drawn the same arbitrary size.

STON (1966) reached the same conclusion, and in view of the fact that the scute gene reduced fly size (as measured both by tergite area and body weight, c.f. Table 1), it seems likely that bristle density differences between scute and wild type may have been even greater at and immediately after the time of bristle initiation than they were in the adult flies.

Bristle number may be expressed by the product of three factors—(1) bristle density, (2) the fractional size of the tergite surface which develops bristles, and (3) the area of the tergite. The present investigation suggests that a reduction in all three of these factors contributes to the bristle number differences between wild type and scute.

The results of scoring bristle numbers within the sub-regions of each tergite

TABLE 2

Values and standard errors for A, the axial length of the bristle band expressed as a proportion of tergite length at the longitudinal lines of division between L_1 and L_2 , L_2 and L_3 , L_3 and L_4 (c.f. Figure 2) and B, the transverse width of the bristle band expressed as a proportion of tergite width at the transverse lines of division between T_1 and T_2 , T_2 and T_3 , T_3 and T_4

	Wild type				Scute			
	Seg. 3	Male	Seg. 4	Female	Seg. 3	Male	Seg. 4	Female
A. L_1 and L_2	0.654 ± .006	0.654 ± .005	0.614 ± .007	0.614 ± .007	0.627 ± .008	0.645 ± .008	0.611 ± .006	0.598 ± .007
L_2 and L_3	0.708 ± .006	0.698 ± .005	0.686 ± .007	0.699 ± .006	0.669 ± .010	0.684 ± .009	0.677 ± .009	0.693 ± .011
L_3 and L_4	0.718 ± .006	0.712 ± .009	0.707 ± .009	0.730 ± .007	0.559 ± .016	0.593 ± .013	0.633 ± .010	0.654 ± .010
B. T_1 and T_2	0.970 ± .002	0.964 ± .002	0.977 ± .001	0.977 ± .002	0.962 ± .003	0.960 ± .002	0.977 ± .002	0.981 ± .001
T_2 and T_3	0.973 ± .002	0.976 ± .001	0.973 ± .001	0.983 ± .001	0.966 ± .003	0.923 ± .002	0.973 ± .001	0.984 ± .001
T_3 and T_4	0.952 ± .005	0.943 ± .011	0.937 ± .013	0.894 ± .026	0.853 ± .025	0.874 ± .022	0.911 ± .015	0.816 ± .054
Fractional area of tergite developing bristles	0.659	0.653	0.640	0.648	0.609	0.630	0.613	0.615
Bristle density (no./mm ²)	649	634	560	537	558	565	508	498

These values each of which represents the mean of 20 flies, were used to calculate the accompanying figures for the fractional area of the tergite which develops bristles. The latter, and also the average figures for bristle number and tergite area given in Table 1, were used to obtain the overall estimates of bristle density.

FIGURE 5.—Average numbers of bristles on the tergite sub-regions where bristles developed. The heaviest lines correspond with T_1 (c.f. Figure 2), and decrease in thickness through T_2 and T_3 to the dotted lines representing T_4 .

are shown graphically in Figure 5. There was no *a priori* reason to believe that the distribution of tergite bristles would be bilaterally asymmetrical so that the corresponding records of bristle numbers on the right and left sides of the tergites were averaged.

Disregarding for the moment the results pertaining to the fourth transverse and fourth longitudinal subdivisions, Figure 5 illustrates clearly that there was a general gradient of bristle number which increased anteriorly in wild-type flies. Because the tergites were partitioned into a fixed number of sub-regions, their size being variable with, and proportional to, tergite size, but constant for individual tergites, this regional gradient of bristle number directly reflects a gradient of bristle density.

Bristle number was not highest in the most anterior tergite sub-regions where bristles developed (the sub-regions included within the fourth transverse subdivision), but Figure 4 shows that in wild-type flies the anterior edge of the band of bristles on each tergite was itself located (on average) within the fourth transverse subdivision. Therefore, it is not valid to expect that the relationship between bristle number and relative bristle density should be applicable in this subdivision of the tergite as it is in the more posterior transverse subdivisions, and it is possible that the anterior increase in bristle density may be more extensive than the variation in bristle number suggests.

A second exception to the general anterior increase in bristle number concerned the results for the tergite sub-regions within the fourth longitudinal subdivision. Here there was no indication of any posterior-anterior gradient in bristle number which was consistent in both sexes and in both the third and fourth abdominal segments of wild-type flies.

An anterior-posterior gradient of bristle number was evident in scute as well as in wild type, but it was not so extensive in the former. Figure 5 shows that, except on the fourth tergite of scute males, bristle number in the first and second longitudinal subdivisions increased anteriorly from the first to the third transverse subdivisions, and in females the gradient included the third longitudinal subdivision. The failure for the gradient to extend to the fourth transverse subdivision was probably due to the fact that in scute the anterior boundary of the bristle band on each tergite was indented posteriorly on the dorsal tergite surface, and the indentation in males entered (on average) into the third transverse subdivision (Figure 4). In scute as well as in wild-type flies then, there was a general gradient of bristle density which increased anteriorly, and which possibly extended nearer to the anterior edge of the bristle band on each tergite than the sub-regional results for bristle number indicated.

There was no evidence of any general transverse gradient in bristle number, but within each of the transverse subdivisions there were some consistent trends. In the first transverse subdivision for example, bristle number, and therefore bristle density, was highest in sub-region T_1L_4 and, except in wild-type females, lowest in sub-region T_1L_2 . Except for the third segment of scute females, bristle number was highest at the ventral edges of the tergite in both the second and third transverse subdivisions, and there was a consistent increase in bristle number moving from L_3 to L_4 within the second transverse subdivision. When the results for males and females and for the third and fourth tergites were averaged, the pattern of bristle number values within the first, second, and third transverse subdivisions was remarkably similar in scute and wild type flies although the

FIGURE 6.—A comparison between wild type and scute of the subregional values for bristle number averaged over the third and fourth tergites of males and females.

values themselves were of course smaller in the mutants. This comparison is drawn with histograms in Figure 6, which illustrates again the posterior-anterior gradients in bristle number extending from the first to the third transverse subdivisions.

Bristle length: Measurements of bristle length are given in Table 3, and these show that the average length was smaller in scute than in wild-type flies, smaller in males than in females, and smaller on the third than on the fourth tergite.

TABLE 3

Average values (and standard errors) of bristle length (microns) and the differences between wild type and scute expressed as percentages of the values in wild type.

Each figure a mean for 20 flies

	Male		Female	
	Seg. 3	Seg. 4	Seg. 3	Seg. 4
Wild type	641.1 $\pm$ 7.4	664.3 $\pm$ 8.5	668.8 $\pm$ 6.2	701.3 $\pm$ 6.6
Scute	610.5 $\pm$ 6.2	627.0 $\pm$ 5.5	620.6 $\pm$ 6.3	657.1 $\pm$ 7.7
Percentage reduction in scute	4.8	5.6	7.2	6.3

Variance analysis showed that these differences were all statistically significant ($P < .01$); in the same analysis, neither of the two-factor interaction classifications involving the difference between wild type and scute was significant, although the single three-factor interaction involving this difference was significant at the 1% level of probability.

FIGURE 7.—Average lengths of bristles on the tergite sub-regions where bristles developed. The heaviest lines correspond with T_1 (c.f. Figure 2), and decrease in thickness through T_2 and T_3 to the dotted lines representing T_4 .

Variation in bristle length within each tergite was investigated in the same way as was the regional variation in bristle number. With the methods described previously the tergite was divided into 35 sub-regions in each of which an average bristle length was obtained. The results are illustrated in Figure 7.

In the first and most posterior transverse subdivision, bristle length values were relatively large; this subdivision contained the row of bristles which are, even before measurement, apparently larger than others on the tergite. Proceeding anteriorly from the second to the fourth transverse subdivisions, there was a general decrease in bristle length. Bristle length in the first transverse subdivision was greatest near the ventral edges of the tergite and it decreased towards the fourth longitudinal subdivision located on the dorsal surface of the tergite. In each of the remaining transverse subdivisions where bristles developed, length was shorter in the first than in the second longitudinal subdivision, but thereafter in the third and fourth subdivisions, the changes in bristle length were variable. A notable contrast here between wild type and scute was the more

FIGURE 8.—A comparison between wild type and scute of the sub-regional values for bristle length averaged over the third and fourth tergites of males and females.

general tendency for a decline in bristle length from the second to the fourth longitudinal subdivisions in scute flies, suggesting that there may be regional differences in the length changes caused by the mutant gene. In spite of this, however, the histograms depicted in Figure 8 illustrate that scute and wild type share numerous similarities in the general pattern of bristle length variation over the tergite.

The regional length differences between wild type and scute are illustrated graphically in Figure 9 which reveals several regularities on both the third and fourth tergites of males and females. In all cases, for example, the length differences between wild type and scute increased in magnitude from the second to the fourth transverse subdivisions within the first longitudinal subdivision. With few exceptions, the length differences in any transverse subdivisions were greater in the fourth than in the first longitudinal subdivision. The existence of these regularities also suggests that the reduction in bristle length caused by the scute gene is variable on different regions of the tergite. In this respect, however, there was no evidence that the bristle length differences were closely associated with the regional pattern of bristle length in wild type; the correlations of these two parameters which were evaluated for the third and fourth tergites and for males and females separately, and which were exclusive of the results from the first transverse subdivision, although positive, were not statistically significant.

Figure 9 shows that the magnitude of the bristle length differences between wild type and scute was generally smaller in the first than in other transverse subdivisions, and in one of the sub-regions (T_1L_2) on the fourth tergite of males

FIGURE 9.—Average bristle length differences between wild type and scute on the tergite sub-regions where bristles developed. The heaviest lines correspond with T_1 (c.f. Figure 2), and decrease in thickness through T_2 and T_3 to the dotted lines representing T_4 .

and on the third tergite of females, average bristle length was greatest in the mutants. Particularly in view of the fact that the first transverse subdivision contained the largest bristles, this result was somewhat surprising, but further investigation provided a logical basis for these peculiarities.

Although the bristles on each tergite were not entirely separable into two populations on the basis of their length, an attempt was nevertheless made to approximate this partition. Separately for the third and fourth tergites of males and females and of wild type and scute, the eight corresponding average values of bristle length were increased by 25%, and the resulting figures served as division points for assigning any particular bristle to one or other of the two bristle populations. For each of the tergite sub-regions in the first transverse subdivision, the number of larger bristles was expressed as a fraction of the total number in that sub-region, and subsequently eight sets of averages of these proportions were obtained. Each set, consisting initially of seven proportion values corresponding with the seven longitudinal tergite subdivisions was then reduced to four values by combining the results for right and left sides of the tergite. The comparisons which could then be made showed that the proportion values were often higher in the mutants, and this was invariably the case for the subregion T_1L_2 where the length differences between wild type and scute were relatively small or otherwise negative. Moreover, the wild type-scute differences in the proportion values were found to be negatively correlated with the corresponding wild type-scute differences in bristle length, and the correlation coefficient was significantly different from zero ($r = -0.88$, $df = 14$, $P < 0.001$). Thus the tergite sub-regions where average bristle length was greater or only slightly less in scute than in wild type were those sub-regions in which the fractional numbers of larger bristles showed the most significant increases from wild type to scute. Without reference to accompanying changes in the proportional numbers of large and small bristles, therefore, the average length differences between wild type and scute in some of the sub-regions of the first transverse subdivision have limited biological significance. In particular, it is probable that here, as well as elsewhere on the tergite, the scute gene causes a consistent reduction in bristle length.

DISCUSSION

The present examination together with JOHNSTON's (1966) objective comparisons show that the spacing intervals between the abdominal tergite bristles are markedly regular in size in both wild-type and scute flies. Also both studies have demonstrated that the scute gene reduces tergite bristle density and in view of the fact that the scute flies were smaller than their comparably aged wild-type sibs it is likely that the magnitude of the density difference is even greater at the time of bristle initiation than it is in adult flies. Thus, any hypothesis concerning the determination of tergite bristle patterns has to accommodate a density change while maintaining regular spacing. WIGGLESWORTH's competitive model would readily explain these features in either one of two ways.

The function of the competitive model depends on the ability of differentiating

bristle cells to suppress in their neighbours any realization of the potential these also have for differentiation. Thus bristle density is determined largely by the average distance to which the suppression radiating from each of the developing bristles is effective, and increases or decreases in this average distance would produce inverse alterations in density. Changes of this type would not be expected to influence the relative sizes of the spacing intervals between bristles.

The proper functioning of the competitive model seems to require that bristle initiation proceeds over a period of time, in which case bristle density would also depend on the duration of initiation. It is quite possible that significant density changes could be produced in this way without any major disturbance of the regular spatial arrangement of bristles (cf. CLAXTON 1964).

Thus, if the state of epidermal competence influences either the effectiveness of differentiating bristles to suppress differentiation in nearby cells, or the period over which bristle initiation takes place, or both, then WIGGLESWORTH's competitive model would satisfactorily account for the pattern characteristics of tergite bristles as well as the pattern changes induced by the scute gene. The question now arises, does the concept of *specific* prepatterns also provide a simple account of these features?

The relationship between prepattern and competence can be represented diagrammatically by a horizontal plane intersecting conical "peaks" which form part of an irregular surface, the prepattern. Within this framework, the height of a peak is proportional to the differentiating inducement offered by the prepattern at this particular site, and the level of the intersecting plane, competence, determines the strength or height that the prepattern must reach if it is to promote a response. Thus when competence is reduced, for example, the change can be simply represented by raising the plane which intersects the prepattern, with the result that differentiation now requires stronger inducement from the prepattern. Provided some peaks in the prepattern are lower than others it is possible they may be "submerged" if competence is reduced, and one might expect in these circumstances that bristle density would be correspondingly lowered. This expectation is fulfilled in scute. But in so far as the regular spacing of the tergite bristles is largely, if not entirely, maintained in the face of a density change, the lowest peaks in the prepattern could not be distributed randomly in the prepattern landscape. Instead, they would have to bear a definite spatial relationship with each other and with higher peaks, and it would be necessary to envisage that the prepattern is organized in this respect as well as in relation to the two-dimensional arrangement of the peaks.

The developmental mechanisms which determine the shape of the prepattern landscape are unknown. However, with respect to the prepatterns thought to govern the arrangements of macrochaetes and ocelli on the head, and of chaetae on the legs of *Drosophila*, MAYNARD SMITH and SONDHI (1961) and HOLLINGSWORTH (1964) have proposed variations of a determinative system investigated theoretically by TURING (1952). But whatever the mechanisms may be, the organization required of a prepattern specific for individual tergite bristles seems to place additional demands on them. For this reason the relative simplicity of

WIGGLESWORTH's competitive model appears to be a more favourable scheme by which to explain the characteristic pattern of tergite bristles as well as the pattern changes which result when the mutant gene *scute* is substituted for its wild-type allele.

The smaller number of tergite bristles in *scute* flies was due in part to the proportionately smaller size of the region on each tergite where bristles developed, and it was found in particular that the anterior edges of the bristle bands were located more posteriorly in the mutants. This alteration is somewhat analogous to the way in which the normal sex combs in male *Drosophila* are modified in triploid (2X 3A) intersexes (STERN 1956a; HANNAH-ALAVA and STERN 1957), and it can be accommodated by a prepattern-competence model, similar to the one proposed by these workers. The model, which is illustrated in Figure 10, depicts the prepattern as a raised plane falling abruptly at the posterior tergite edge but sloping down more gently towards the anterior tergite edge. Thus, if competence is represented by a horizontal plane intersecting the prepattern, and if the height of this plane is inversely related to the level of competence, then the common region of intersection of the two planes (the region determined for bristle development) will narrow in width from the anterior edge as competence is reduced.

Two observations are of interest with respect to the prepattern shape in a transverse direction across the tergite. First, the anterior edge of the bands of tergite bristles were moved posteriorly in *scute* flies to the greatest extent on the dorsal surface of the tergites so it is possible that the transverse contour of the prepattern is correspondingly lower in these than in more lateral and ventral regions. Second, the longitudinal edges of the bristle bands on the ventral tergite surfaces were not displaced to any great extent when *scute* was substituted for its wild-type allele so that a transverse cross section of the prepattern probably falls sharply at its ventral edges.

From their comparisons of the sex combs in males and intersexes of *Drosophila*

FIGURE 10.—The proposed shape of the tergite bristle prepattern in longitudinal cross-section.

melanogaster, STERN (1956a) and HANNAH-ALAVA and STERN (1957) concluded that the response of intersex cells to a constant prepatter was, with regard to the numbers of sex comb teeth and the lengths of the rows of these teeth, intermediate between the response in males and the absence of a response in females. In relation to the lengths and widths of the individual teeth in each comb, however, there were no systematic differences between males and intersexes, and it appeared that differentiation at this level was an all-or-none process. On the other hand, HOLLINGSWORTH (1964) found that individual sex-comb teeth as well as the size of the sex combs themselves were affected in intersexes of *D. subobscura*, and in particular, the teeth were "progressively less male-like towards the proximal end" of a comb. In addition, HOLLINGSWORTH reported variation between the different intersexual grades so that the most male-like intersexes had "the longest sex combs with the most male-like teeth". In conformity with these results, but in contrast to those concerning the sex combs of *D. melanogaster* intersexes, the scute gene was found to cause a reduction in tergite bristle length so that a change in the response of epidermal cells to the prepatter has in this case influenced a size characteristic of the individual pattern elements as well as the extent of the pattern itself. The scute gene has in other words affected both the initiation of differentiation and at least one aspect of the course which differentiation takes. There was some evidence that the extent to which the scute gene reduced bristle length was variable on different regions of the tergite. However, this variability did not appear to be associated with the regional differences in length which were found in wild-type flies so that either competence is not reduced uniformly by the scute gene or, alternatively, and possibly more likely, competence as defined for the present purposes, may be only one of a number of interacting factors which affect bristle length.

The investigation of regional variation in bristle length showed that, with respect to this character, there were some major gradients on the tergite which were generally similar in wild type and scute. The most significant of these gradients was one wherein bristle length decreased continuously in a longitudinal direction from the first to the fourth transverse subdivisions. Therefore, in so far as the competence difference between wild type and scute was associated with a difference in bristle length, one may logically enquire if the gradients in length on the tergites of both wild-type and scute flies are also associated with corresponding gradients in competence.

The model for the relationship between prepatter and competence in a longitudinal cross section of a tergite (Figure 10) made no provision for this possibility, although it is nevertheless clear that a prepatter of constant height intersected by a line sloping down towards the posterior tergite boundary would be an alternative, yet adequate, model to account for the effects of scute. With the latter alternative, which is basically similar to one discussed in relation to the development of extra sex combs in *Drosophila melanogaster* (TOKUNAGA and STERN 1965), the longitudinal gradients of competence and bristle length could also be correlated. But the apparent greater versatility of the second model is not necessarily a recommendation for its adoption. One may argue, for example, that

bristle length might depend not so much on a particular level of competence as on the height of the prepatter relative to this level. With this assumption the two models are equally versatile. The important conclusion is that the regional variation in bristle length on individual tergites may be at least partly due to regional differences similar in nature to the differences between wild type and scute.

As well as reducing bristle length, the scute gene was responsible for a decrease in bristle number. However, the investigation of the regional variation in bristle number was suggestive of a general density gradient increasing in a longitudinal cross section of the tergite from the posterior to the anterior edges of the bristle band. Clearly then in this case, there is no argument in favour of the idea that the height of the prepatter in relation to the level of competence accounts both for the effect of the scute gene and for the longitudinal gradients in bristle density. If there were, one would have expected bristle density to be higher in scute than in wild type. However, the present investigation was not directed at the developmental stage when the bristles were initiated. It is conceivable that the scute gene, by its influence on competence, may reduce the period of time over which the bristles are initiated. Could it be, therefore, that the reduction in bristle density is brought about in this way in spite of a reduction in the extent to which the developing bristles inhibit the initiation of others? With this possibility in mind, work is now in progress to compare the developmental sequences of bristle initiation in wild-type and in scute flies.

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SUMMARY

The numbers, lengths, and distributions of bristles on the third and fourth abdominal tergites were compared in wild-type and scute *Drosophila* with the object of determining whether the changes brought about by the mutant gene could be accommodated within the framework of prepatter and competence systems. There was a general reduction in bristle number and bristle length in scute flies, and the proportional area of the tergite where bristles developed was also smaller in the mutants. The nature of the latter change suggested a hypothetical model for the shape of the prepatter in a longitudinal cross section of the tergite, but the model's use in accounting simultaneously for bristle density and bristle length differences between wild type and scute, and for regional gradients of length and density on individual tergites, was found to be limited.

LITERATURE CITED

- BRIDGES, C. B. and K. S. BREHME, 1944 The mutants of *Drosophila melanogaster*. Carnegie Inst. Washington Publ. 552.
- CLARK, P. J., and F. C. EVANS, 1954 Distance to nearest neighbour as a measure of spatial relationships in populations. *Ecology* 35: 445-453.

- CLAXTON, J. H., 1964 The determination of patterns with special reference to that of the central primary skin follicles in sheep. *J. Theoret. Biol.* **7**: 302-317.
- FERRIS, G. F., 1950 External morphology of the adult. pp. 368-419. *Biology of Drosophila*. Edited by M. DEMEREC. Wiley, New York.
- GOTTLIEB, F. J., 1964 Genetic control of pattern determination in *Drosophila*. The action of Hairy-wing. *Genetics* **49**: 739-760.
- HANNAH-ALAVA, A., and C. STERN, 1957 The sexcombs in males and intersexes of *Drosophila melanogaster*. *J. Exptl. Zool.* **134**: 533-556.
- HOLLINGSWORTH, M. J., 1964 Sexcombs of intersexes and the arrangement of chaetae on the legs of *Drosophila*. *J. Morphol.* **115**: 35-52.
- JOHNSTON, N. V., 1966 The pattern of abdominal microchaetae in *Drosophila*. *Australian J. Biol. Sci.* **19**: 155-166.
- MAYNARD SMITH, J., and K. C. SONDHI, 1961 The arrangement of bristles in *Drosophila*. *J. Embryol. Exptl. Morphol.* **9**: 661-672.
- STERN, C., 1954a Genes and developmental patterns. *Proc. 9th Intern. Congr. Genet. (Caryologia. suppl. Vol.)*: 355-369. — 1954b Two or three bristles. *Am. Scientist* **42**: 213-247. — 1956a Genetic mechanisms in the localized initiation of differentiation. *Cold Spring Harbor Symp. Quant. Biol.* **21**: 375-381. — 1956b The genetic control of developmental competence and morphogenetic tissue interactions in genetic mosaics. *Arch. Entwicklungsmech. Organ.* **149**: 1-25.
- STERN, C., and D. L. SWANSON, 1957 The control of the ocellar bristle by the scute locus in *Drosophila melanogaster*. *J. Fac. Sci. Hokkaido Univ.* **13**: 303-307.
- TOKUNAGA, C., 1961 The differentiation of a secondary sex comb under the influence of the gene engrailed in *Drosophila melanogaster*. *Genetics* **46**: 157-176.
- TOKUNAGA, C., and C. STERN, 1965 The developmental autonomy of extra sex combs in *Drosophila melanogaster*. *Develop. Biol.* **11**: 50-81.
- TURING, A. M., 1952 The chemical basis of morphogenesis. *Phil. Trans. Roy. Soc. Lond. B.* **237**: 37-72.
- WIGGLESWORTH, V. B., 1940 Local and general factors in the development of "pattern" in *Rhodnius prolixus* (Hemiptera). *J. Exptl. Biol.* **17**: 180-200. — 1953 Determination of cell function in an insect. *J. Embryol. Exptl. Morphol.* **1**: 269-277.
- YOUNG, S. S. Y., and R. C. LEWONTIN, 1966 Differences in bristle making abilities in scute and wild type *Drosophila melanogaster*. *Genet. Res.* **7**: 295-301.