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Developmental Origin of Even Spacing Between the Microchaetes of *Drosophila melanogaster*

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Abstract

In *D. melanogaster*, evenness of spacing is a prominent feature of the patterns of microchaetes. As a general explanation of this characteristic, Wigglesworth has suggested a scheme whereby some epidermal cells become singled out (determined) at random to differentiate bristles, and the same potential in neighbouring cells is suppressed by the determined ones. Though this model may be satisfactory for other epidermal regions, the spacing between bristles within some of the mid-dorsal thoracic acrostichal rows is too even to be accommodated.

Introduction

One of the most obvious pattern features of *Drosophila melanogaster* microchaetes is a tendency for many of these bristles to be regularly spaced. A simple plausible explanation of this feature was suggested by Wigglesworth (1940, 1953): once an epidermal cell is finally committed toward bristle differentiation it suppresses the realization of any similar potentials in neighbouring cells. If bristles form at random within this restrictive framework, then adjacent bristles are spaced apart and the overall distribution is characterized by a degree of spacing uniformity.

The original form of this hypothesis (the two-dimensional random model) assumes a particular region of epidermis to be a sheet of equipotent cells, and the singling out of only some cells to differentiate bristles is a locally occurring process. In *D. melanogaster*, this latter assumption may require modification. Ursprung (1967) has suggested that the final determination of bristle cells is made not locally at the adult sites but rather within the imaginal discs, and that these determined cells migrate to their adult positions. The cell lineage studies of Murphy and Tokunaga (1970) do not lend support to this extreme view, though some singling out (partial determination) may occur in the imaginal discs (Garcia-Bellido and Merriam 1971*a*, 1971*b*) in which case the final determination of bristles may be made in a subpopulation of epidermal cells. But irrespective of when bristle determination occurs, Wigglesworth's scheme could play a crucial role locally, either in the final determination or in guiding cells to their proper locations. In the former case it is bristle cells that are determined, in the latter the bristle sites.

Wigglesworth's hypothesis has previously been investigated in relation to the two-dimensional pattern of microchaetes on the abdominal tergites of *D. melanogaster* (Claxton 1964). By incorporating the scheme in a simulation program, it was possible to generate patterns of points on paper with the same quantitative distribution characteristics as those of the microchaetes. Here a similar investigation was undertaken with some of the microchaetes in the dorsocentral region of the *D. melanogaster*

mesonotum. These bristles are arranged in longitudinal (acrostichal) rows, and in each row bristles tend to be evenly spaced. The results suggest that within-row spacing is not compatible with Wigglesworth's scheme when it is incorporated in a one-dimensional random model.

The Model

The outcome of this model was easily simulated. On a line (arbitrarily 1000 units in length), a point (bristle) was fixed by choosing a random number between 0 and 1000. This number, along with r adjacent and consecutive numbers on either side of it ($r = 5$ was convenient) were then ineligible for subsequent choice from the random number set. This process was repeated and further points added to the line until all values between 0 and 1000 were ineligible for subsequent choice. The mean and standard deviation of distances separating successive points were then calculated. A very similar analysis, but one based theoretically, was undertaken by Bánkövi (1963), and a simple modification of his results making them applicable to the current model showed the ratio of standard deviation to mean (i.e. coefficient of variation) to compare closely with theoretical expectation.

Similar parameters were obtained for bristles within the first five acrostichal rows (counting from the mid-dorsal line laterally). For each of 30 ♂ and 30 ♀ flies (an Oregon-R strain of wild type *D. melanogaster*), the dorsal thorax was dissected from the remainder of the body, prepared histologically, and mounted upright on a glass slide. The measurement of distances between adjacent bristles was aided by the use of a projection microscope. Errors due to curvature of the mesonotum were ignored (but see later) though their significance was minimized by confining measurements to a comparatively flat region of the mesonotum lying between two imaginary parallel lines: one connecting the posterior dorsocentral macrochaetes, the other located anteriorly a distance twice that between the anterior and posterior dorsocentrals. The within-row standard deviations of distances between bristles were obtained utilizing analysis of variance techniques, and these along with mean values (arbitrary units) are listed in Table 1. Corresponding standard deviations from the simulated pattern were calculated by adjusting the single model value in proportion to the real and model means; in effect the listed model standard deviations are those for model means identical with real means. F values, drawing statistical comparisons between squares of model and real standard deviations, are also included in Table 1.

Results and Discussion

It is unrealistic to suppose that all cells committed to bristle differentiation have identically sized fields of suppression around them or that the fields are perfectly circular in shape. These features could, however, be simulated by including in the model variable r values (e.g. normally distributed) rather than constant ones, with the result that the standard deviation of distances between successive points increases relative to the mean. Thus the model standard deviations given in Table 1 are estimates of the absolute minima that a random model can produce. At the same time, the standard deviations of distances between bristles almost certainly include components additional to those that would be imparted by the simple model. Ignoring curvature of the mesonotum, regular longitudinal gradients in bristle spacing (e.g. smaller spacing anteriorly than posteriorly), and possible interactive effects between bristles

in different rows as well as between the dorsocentral macrochaetes and some of the neighbouring microchaetes, all have the potential of contributing to the measured variability in spacing. Therefore the following conclusions that result from standard deviations for the model being larger than those for distances between bristles, are, if anything, conservatively based.

Table 1. Means (arbitrary units) and standard deviations (s.d.) of distances between adjacent bristles within five acrostichal rows on each side of 30 ♂ and 30 ♀ wild type *D. melanogaster*, together with corresponding standard deviations of distances between adjacent points located on a line with a simulation model (see text)

Each mean distance is followed (in parentheses) by the number of individual distance measurements. *F* values compare squares of corresponding model and real standard deviations, with appropriate degrees of freedom (d.f.)

		Row 1	2	3	4	5
30 ♂	Mean	6·40 (302)	6·88 (297)	6·59 (255)	5·93 (132)	5·60 (136)
	s.d.	1·081	1·736	1·378	1·172	0·820
Model	s.d.	1·397	1·508	1·439	1·295	1·223
F value	*F*	1·670*	<1·0	1·090	1·221	2·224*
	d.f.[A]	131, 242	131, 237	131, 195	131, 75	131, 76
30 ♀	Mean	6·09 (332)	6·14 (330)	6·08 (283)	5·82 (149)	5·26 (150)
	s.d.	1·096	1·519	1·314	1·263	0·899
Model	s.d.	1·330	1·341	1·328	1·271	1·148
F value	*F*	1·473*	<1·0	1·021	1·013	1·631*
	d.f.	131, 272	131, 270	131, 223	131, 90	131, 90

* $P < 0{\cdot}01$.

[A] The second value for each degrees-of-freedom pair is usually 60 (30 flies × 2 sides) less than the corresponding number of distance measurements, though this rule varies for row 4 where fewer than two bristles (and hence no measurements) occurred on three of the 60 ♂ sides and on one of the 60 ♀ sides.

The four statistically significant *F* values in Table 1 show that bristle spacing in rows 1 and 5 is more even than could result from a random determinative sequence. For rows 3 and 4 the spacing between bristles is also less variable than is characteristic of the model though the differences fail to reach statistical significance. The latter situation is reversed for row 2.

Although the degree of regularity in the spacing between row-2 bristles, for example, is less than that in rows 1 and 5, it seems unlikely that the fundamental pattern-determining mechanisms are different in the two cases. More probably the same processes operate in all rows though their determinative effects are secondarily modified, either only in some rows or to varying extents in different rows.

If the random model is abandoned, with what can it be replaced? An alternative is suggested by the segmental gradients which were discovered initially in *Rhodnius prolixus* by Locke (1959), but which are probably a fundamental feature of all insects (Lawrence 1973). As well, in *D. melanogaster*, there are posteroanterior gradients of decreasing bristle length and increasing bristle density, not only on the abdominal tergites (Claxton 1967) but also on the abdominal sternites and mesonotum. If pattern determination follows gradients too, so that the random element in the previous models is replaced by a regular longitudinally oriented sequence, then much

greater regularity in spacing could be achieved, particularly in rows. Further, a sequence model in two dimensions appears (from preliminary unpublished studies) to adequately simulate the distribution of abdominal tergite bristles, provided the variability in normally distributed radii of fields of suppression (specified as a coefficient of variation = $c.v._r$) is increased from 13·7% (the most appropriate value for the two-dimensional random model) to about 16%.

In this context, $c.v._r$ is presumably a reflection of inherent random variability in those biochemical and physical properties of the epidermis on which the product of Wigglesworth's scheme depends. One would expect this variability to be characteristic for an individual at least, and possibly also the species. Thus it is interesting that the value of $c.v._r = 16\%$ agrees closely with the coefficient of variation of distances between successive bristles ($c.v._d$) in acrostichal rows 1 and 5. If bristle spacing in these rows is determined wholly by processes like those envisaged in the one-dimensional sequence model, then $c.v._r$ should equal $c.v._d$, and from Table 1 $c.v._d$ ranges from about 14% (row 5, ♂) to 18% (row 1, ♀) with a mean of 16·7%.

Another advantage of the sequence models is that they avoid the dilemma of occasional simultaneous determination in two adjacent or nearby cells with the result that two bristles develop side by side. Though this type of development occurs with the scutellar macrochaetes in selected stocks (e.g. Sheldon 1968), it is not typical, even at low frequency, for the abdominal or thoracic microchaetes of normal flies.

As an explanation for the even spacing between bristles within the acrostichal rows, the one-dimensional sequence model is not completely satisfactory. In this analysis the rows have been examined individually and in isolation, yet because the distance between rows is (on average) smaller than the spacing between bristles within rows, the opportunity for interactive effects between adjacent rows cannot be ignored. Though any one of several different modifications of the current one-dimensional sequence model may overcome this difficulty, these are as yet too speculative to form the basis of serious discussion. It thus seems that even spacing between pattern elements may, in some cases at least, be the result of a more complex process than was originally conceived by Wigglesworth.

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