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Some Quantitative Features of *Drosophila* Sternite Bristle Patterns

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Abstract

The arrangement of bristles on the third sternite of *D. melanogaster* is characterized by a tendency towards even spacing between bristles. There are postero-anterior and latero-medial gradients of increasing density, of decreasing distance between nearest neighbours and of decreasing bristle length. A large bristle tends to be constantly positioned in each posterior-lateral 'corner' of the sternite.

Except for bristles that are aligned parallel with and near to the lateral sternite edges there is no evidence for bristles to be generally arranged in rows that are straight and parallel and that have a standard direction relative to the body axes. Thus, although developmental pattern-determining mechanisms are apparently necessary to accommodate the non-random arrangement of sternite bristles, there seems to be no requirement for these mechanisms to organize the bristles in rows.

Introduction

The bristles growing from the *Drosophila* epidermis are arranged in characteristic patterns whose features differ for different bristles and bristle groups. For example, pattern features include constant position, either of individual bristles (e.g. scutellars and dorsocentrals) or of bristle groups on specific regions (e.g. sternopleural bristles), regular directional density gradients (e.g. tergital microchaetes), evenness of spacing, either in one dimension (e.g. the microchaetes of one acrostichal row) or in two dimensions (e.g. tergital microchaetes), and arrangement in rows (e.g. acrostichal microchaetes).

Pattern development has been viewed in alternative ways that embrace the concepts of prepattern and competence (Stern 1954), positional information (Wolpert 1971), phase-shift (Goodwin and Cohen 1969), inhibition (Wigglesworth 1959) or simply bristle 'make' (Rendel 1963). The types of processes that might be incorporated in these concepts include biochemical gradients (Lawrence 1973), simple diffusion (Crick 1970), periodic diffusion (Goodwin and Cohen 1969), diffusion and reaction (Turing 1952) and cell lineage regularities (Garcia-Bellido 1966, 1968). As yet no unifying paradigm of pattern formation has emerged (Waddington 1972).

Despite numerous studies that have involved *Drosophila* bristle patterns, the characteristics of many remain unknown, incomplete or described only in subjective terms. Yet if our understanding of the development of pattern formation is ever to progress beyond a conceptual framework it seems essential that the exact nature of patterns be analysed. This need can be illustrated with the following example. All the pattern features of the acrostichal rows of bristles have not been determined quantitatively, yet these rows are sufficiently regular and standard on different flies of a wild-type stock for the feature of the arrangement in rows itself to demand an explanation in terms of a positive developmental determination. Much the same argument has been extended to the sternite bristles. Maynard Smith and Sondhi (1961) have proposed that these bristles may be determined developmentally in rows by a Turing-like process. Some or even most of the sternite bristles occasionally appear to be arranged in rows, but this is by no means always the case on every fly and every sternite. So the question arises as to whether the appearance of 'good' bristle rows on some sternites is a result of chance or whether a specific developmental process determines the rows, albeit with variable, or perhaps imperfect, precision. In other words, it is quite illogical to search for developmental determinants unless their end result positively demands their existence.

Materials

A randomly bred stock of Oregon-R *D. melanogaster* provided a small sample of females where the pattern of bristles on the third abdominal sternite was examined. The head, thorax and tergites of each fly were dissected away from the ventral abdominal surface, the latter then being set in aqueous mountant on a glass slide beneath a coverslip. A projection microscope was utilized to make drawings of the sternite boundaries, and the locations of all bristle socket cells, and to establish the lengths of individual bristles. The magnification of the projection microscope was adjusted so that the maximum width of sternite images was always standardized before drawing.

Table 1. Frequency distribution of bristle numbers and of average bristle length on the third sternite of 21 female flies

The results for right and left halves of each sternite have been combined. No bristles were scored in the elements of rows 1, 2 and 12. Values in parentheses are average bristle lengths expressed in micrometres

	Columns:					
Rows	1/12	2/11	3/10	4/9	5/8	6/7
3	0 (—)	3 (46)	1 (41)	3 (36)	2 (45)	0 (—)
4	1 (65)	13 (52)	7 (41)	9 (40)	8 (34)	8 (31)
5	3 (54)	14 (52)	7 (39)	8 (39)	10 (40)	10 (33)
6	2 (54)	15 (62)	5 (41)	8 (38)	11 (42)	23 (37)
7	2 (87)	10 (69)	4 (51)	13 (41)	14 (44)	12 (41)
8	1 (85)	9 (97)	3 (48)	12 (43)	12 (44)	19 (47)
9	4 (105)	15 (106)	2 (99)	6 (49)	9 (70)	17 (52)
10	0 (—)	13 (105)	4 (82)	3 (86)	13 (69)	9 (63)
11	0 (—)	0 (—)	5 (80)	14 (81)	15 (77)	2 (53)

Results and Discussion

Distributions of Bristle Length and Bristle Number

Frequency distributions of bristle length and bristle number were obtained by superimposing a 12- by 12-square grid system on each of the sternite drawings, scoring the numbers and lengths of bristles within each of the 144 grid elements, and summing the results in corresponding elements of the sample of 21 flies. The grid was a standard size and regularly positioned so that its sides corresponded exactly with the edges of each sternite at the latter's maximum width, and it extended anteriorly from the posterior edge of each sternite (Fig. 1). There was no reason to suspect any consistent asymmetry between the right and left halves of sternites, and the resulting frequency distributions were therefore condensed to 12 by 6 arrays by combining results for corresponding elements of the original columns 1 and 12, 2 and 11, etc.

The distribution of numbers and average bristle lengths are given in Table 1, which shows that:

1. Relatively few bristles occur in the elements of columns 1 and 12, and none in the elements of rows 1, 2 and 12. This indicates that bristles seldom develop very close to the sternite edges, and the anterior regions of each sternite particularly are devoid of bristles.

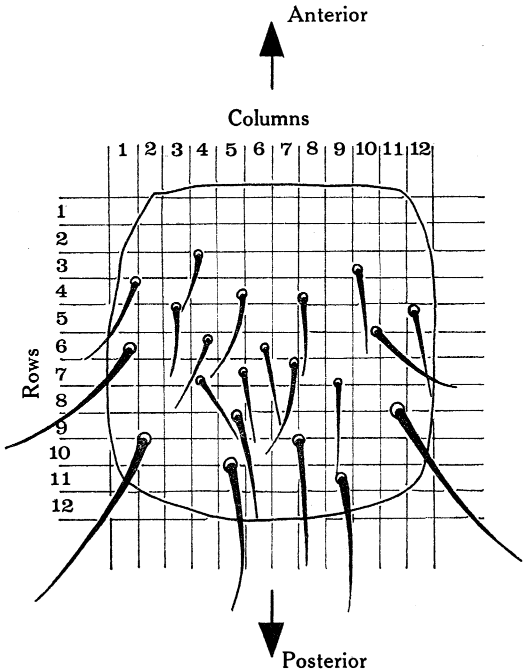

Fig. 1. Drawing of a third sternite to show the placement of a 12- by 12-square grid system subdividing the surface into 144 elements.

2. Sternite bristles generally have no obvious constancy of location comparable to the scutellars and dorsocentrals, for example. This is supported by the absence of numerous marked discontinuities in the bristle numbers of adjacent elements. In this regard a notable exception occurs in the posterior-lateral sternite 'corners' where the 28 bristles scored in elements 10,2/11 and 9,2/11 occur in relative isolation. Evidently there is a strong tendency for a bristle to be regularly positioned in each of these corner sites. For similar reasons there appears to be some constancy at two other locations represented in Table 1 by the six elements 10,5/8; 11,5/8; 11,4/9, where a total of 42 bristles were scored and where other elements adjacent to these generally contained much smaller numbers of bristles.
3. Many more bristles were scored in the elements of columns 2 and 11 than in the adjacent columns 1 and 12, and 3 and 10. This suggests that there is a row of bristles constantly located near to and parallel with each lateral edge of the sternite. The data in Table 1 give no other evidence of bristles occurring in rows that have constant position and direction on the sternite, though this issue is examined with broader reference, and in more detail, later.
4. The bristle numbers per element tend to increase from columns 3/10 toward columns 6/7, which straddle the mid-ventral line. This suggests that the spacing

between bristles diminishes generally from the lateral to the medial regions of each sternite. Comparable postero-anterior gradients in spacing also exist, but these are best demonstrated with nearest neighbour measurements. They are not apparent in the data in Table 1 because the boundaries to which bristles occur anteriorly on different sternites are highly variable in location. As a result, fewer flies contribute positively to the bristle scores in the third and fourth row elements, for example, than the fifth and sixth row elements.

5. Generally, average bristle length declines both medially from columns 2 and 11 and anteriorly from row 10. Thus the larger bristles are located near to, and parallel with, the lateral and posterior sternite edges, and the very largest bristles are usually those positioned constantly in the posterior-lateral sternite 'corners'.

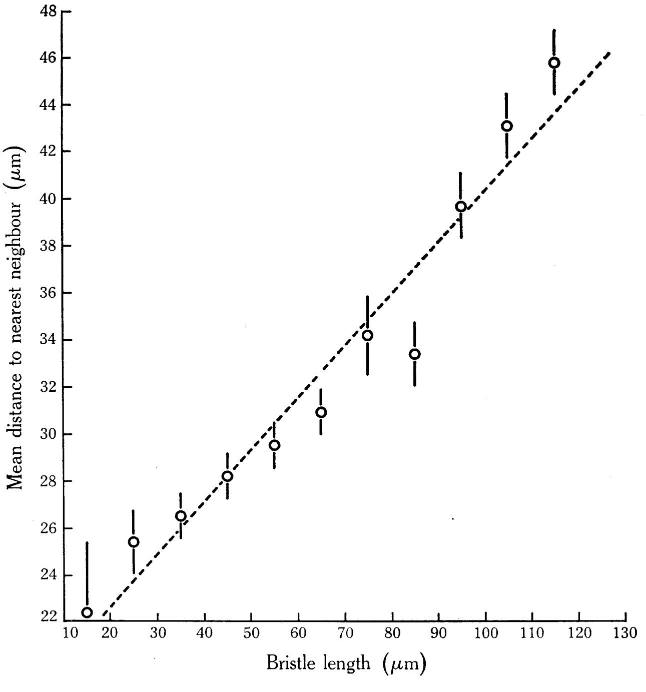

Fig. 2. Average values of distance to nearest neighbour plotted against the mid-points of 11 ranges of bristle length. Vertical lines represent standard errors and the dotted line the linear regression. Results are for 21 third sternites with a total of 420 bristles.

Nearest Neighbours

The spacing between bristles is related to bristle length such that the largest bristles are the ones most isolated from their neighbours. This is illustrated in Fig. 2, where mean distance from any bristle to its nearest neighbour is plotted for eleven ranges of bristle length. On the basis of a linear relationship, nearly 95% of the variation in mean distance to nearest neighbour is accounted for by variation in length. Also, while the smallest bristles may be shorter than the distance to their nearest neighbours, the largest ones are on average about $2\frac{1}{2}$ times as long as the distance to their nearest neighbours. Thus, like bristle length, nearest neighbour distances are generally greatest in the posterior-lateral 'corners' of the sternite and from there they diminish along postero-anterior and latero-medial gradients.

The constancy of some bristle sites, and the regular gradients of nearest neighbour distances over the sternite, show that bristles are not positioned randomly on the sternite. A more specific demonstration of non-randomness is given by combining mean distance to nearest neighbour ($\bar{r}$) and bristle density (ρ) in a measure of spatial distribution $R = 2\rho^{\frac{1}{2}}\bar{r}$. As shown by Clark and Evans (1954), R takes the value of unity for a random distribution, and it moves either in the direction of zero for distributions departing from randomness toward aggregation, or in the direction of 2·1419 for distributions departing from randomness toward uniformity.

Mean distance to nearest neighbour was calculated for each sternite and a corresponding density value was obtained from total bristle number expressed as a fraction of total sternite area. For the 21 sternites, R averaged about 1·37 with a standard error of about 0·02. It is thus clear that R differs statistically from unity and thus the distribution of sternite bristles departs from randomness in the direction of uniformity.

There is however one substantial difficulty in obtaining true values of R for the sternite bristles. The problem arises in estimating bristle density; to be meaningful, density should relate only to those regions where bristles develop and not to those regions near the sternite edges (particularly the anterior one) where bristles do not occur. So meaningful density values really rely on establishing meaningful bristle boundaries within sternites. No simple method of meeting this requirement objectively has been found so that appropriate density and true R values have both been underestimated. Thus, although there is no doubt that sternite bristles tend toward uniform spacing, the degree of this uniformity is certainly greater than is specified by $R = 1·37$.

Arrangement in Rows

It seems unlikely that any single statistical test or procedure would detect either the absence, or the presence and extent, of a tendency for points of a two-dimensional distribution to be arranged in rows. After all, rows may be straight or curved, parallel or intersecting, uniformly or irregularly spaced, and constantly or variably positioned with reference to the edges of the distribution. Also a series of points can either lie in perfect rows or they can display a variable tendency towards an arrangement in rows only in the statistical sense. So in combination all these possibilities allow arrangement in rows to occur in a great variety of different ways.

The following analysis is restricted to detecting a tendency for sternite bristles to be arranged in rows that are straight and parallel, that have some minimum distance separating the closest adjacent rows, and that have, on different flies, a constant direction relative to the long axis of the body. Further, the analysis does not have the support of a rigorous mathematical derivation; rather it was devised from intuitive reasoning and relies on a limited series of trials to establish its utility. Therefore the analysis has no claim other than being a first attempt to employ meaningful parameters in the detection of specified row types.

The position of each bristle was specified by assigning X (along a laterally directed axis) and Y (along a longitudinally directed axis) coordinates to the centre of each bristle socket cell. A grid of parallel and equally spaced lines was superimposed on the bristle distribution of each sternite, and then each bristle was assigned a group according to which line it was nearest. (In cases where a bristle was equidistant from two lines, it was associated arbitrarily, but consistently, with the line intersecting the X axis at the smaller X value). For each group, deviations (d_i) of bristles from the appropriate line were listed, and from these a corrected sum of squares $[\Sigma(d_i-\bar{d})^2]$ was calculated. The sums for different lines were pooled, and the total pooled sum divided by the number of bristles in the distribution. For convenience the latter values are subsequently referred to as parameter A. For each value of A, another parameter, B, was also recorded—the number of grid lines to which at least one point was nearest. Provided the spacing between grid lines is not greater than that between bristle rows, then parameters A and B should take their smallest values when the grid lines are parallel to bristle rows. The object then was to evaluate A and B for the grid directed at various angles over the sternite and judge whether the smallest values of A and B are merely chance extremes of a continuous distribution of values, or whether they reflect some spatial property of the bristle pattern that exists only for certain directions.

The utility of B for the present purposes is more restricted than that of A. For example, it is obvious that B is sensitive to changes in width of a two-dimensional distribution of points, and because the sternites are approximately rectangular in shape the width of the bristle distribution will be smallest (on average) along either the X or the Y axis. Consequently B values may be lower when the grid is directed at 0° (parallel with the X axis) and 90° (parallel with the Y axis) than at other angles. Clearly then, unusually low values of B obtained for some particular placement angle(s) of the grid need to be accompanied by correspondingly low values of A to have meaningful significance.

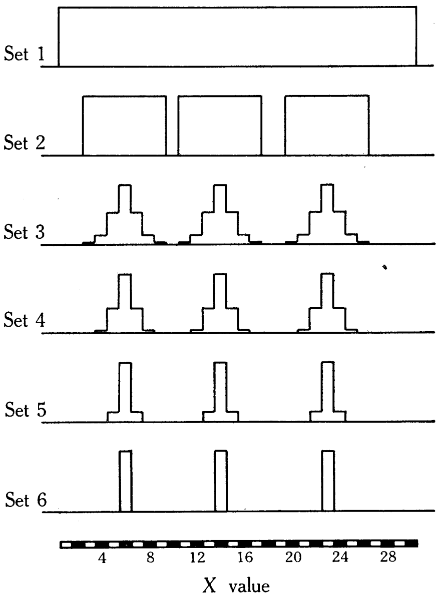

Fig. 3. Six frequency distributions of values (1–30) that were used to generate the X coordinates specifying the location of 'bristles' on artificial sternites.

To test the methodology, and also for use in subsequent comparisons, A and B values were obtained for six sets of 21 artificial sternites whose size and 'bristle' numbers were made to correspond with those of the 21 real sternites. The Y coordinates of bristles were fixed randomly for 21 artificial sternites and the same coordinate values repeated over the six sets. The X coordinates were generated randomly from the six X-value sets shown in Fig. 3. The first set is uniform, whereas the others consist of three uniform (set 2) or discontinuous normal (sets 3–6) distributions with means at three specific locations and with variance about each mean diminishing progressively from set 2 to set 6. The X coordinates generated from these sets are comparable to those of points distributed randomly along the X axis (set 1) or else clustered with varying degrees about three specific locations. The artificial sternites of set 1 thus have bristles randomly arranged in two dimensions, whereas the others have bristles arranged in three rows parallel to the Y axis, the rows becoming progressively more defined from set 2 to set 6. Some of the A and B parameters corresponding with these artificial sternites are given in Table 2; they are derived for grid lines directed parallel to the Y axis (90°) and for six different line spacings. Each listed number is a sum of 21 values obtained independently for the 21 sternites of each set. In general, both parameters behave as expected and diminish in value from set 2 to set 6, though it is obvious that the changes in A become more pronounced, and those in B less pronounced, as the spacing of grid lines increases and approaches the smaller of the two mean distances (8, 9 units) separating bristle rows (see Fig. 3). It is equally obvious that the A values fail to discriminate between the sternites of set 1 and set 2. But the rows of set 2 are poorly defined and presumably a much larger sample size would be required to establish consistently lower A values for set 2 than for set 1.

Table 2. Values of A and B for six sets of artificial sternites, for six different spacings of grid lines and for the grid directed at 90°

Each entry is a sum of individual values for 21 sternites

Set	Grid line spacing (X-axis units): 2	3	4	5	6	8
			Parameter A			
1	2·28	7·24	18·17	26·63	38·63	74·61
2	2·47	8·06	14·73	29·31	43·49	76·77
3	2·59	6·42	6·72	12·82	18·70	26·69
4	2·76	4·92	3·76	8·35	10·67	9·87
5	1·92	2·82	1·95	3·55	4·73	4·73
6	0	0	0	0	0	0
			Parameter B			
1	213	183	145	126	112	95
2	200	158	123	104	94	81
3	156	124	124	98	79	69
4	126	108	116	83	68	68
5	102	88	102	78	63	63
6	63	63	63	63	63	63

A more comprehensive analysis of the artificial sternites of sets 1, 2 and 3 is illustrated graphically in Fig. 4. Here the A and B parameters were evaluated for six different spacings of the grid lines, and for 18 different grid directions, specified by 5° intervals ranging from 0 to 175°. A prior analysis where A and B were evaluated at 1° intervals from 0 to 179° showed the precision of 5° intervals to be adequate for the present purposes. Again, each plot in the graphs in Fig. 4 represents a sum of 21 values obtained independently for the 21 sternites of each set.

The rows of bristles at 90° on the set 3 sternites are reflected in the markedly low values of A and B at and also near the 90° direction. These low values are more

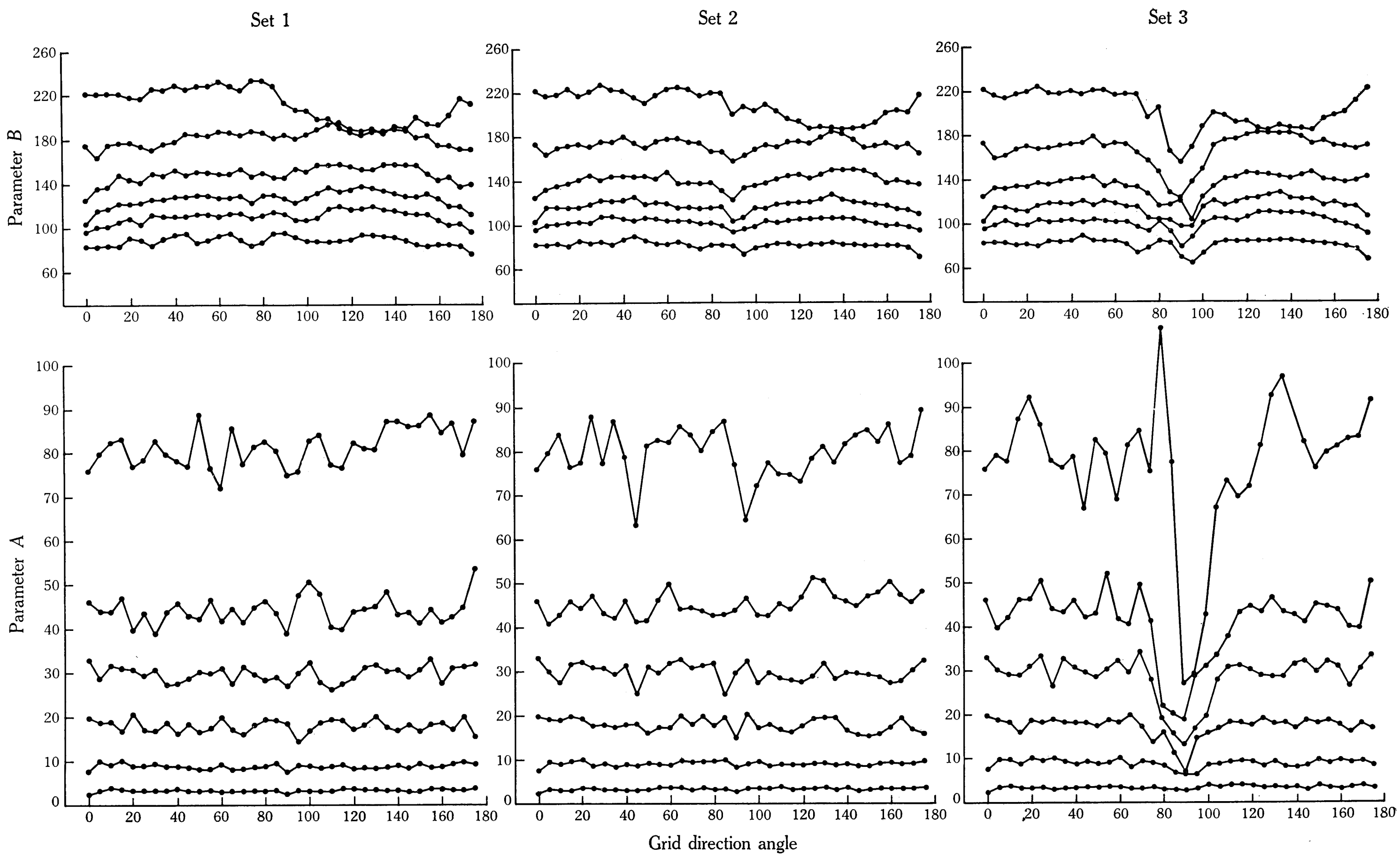

Fig. 4. *A* and *B* values for three sets of artificial sternites, for six different spacings of grid lines (2, 3, 4, 5, 6 and 8 *X* units), and for 18 different grid directions ranging from 0° (parallel with the *X* axis) through intervals of 5° to 175°. Each plot is a sum of individual values for 21 sternites. Plots for the same grid line spacing but different grid direction angles are connected by lines; except for one instance of overlapping (set 1, parameter *B*, spacings 2 and 3), the resulting curves are in sequence such that for parameter *B* the top curve corresponds with a spacing of 2 units and the bottom one with a spacing of 8 units. The sequence is reversed for parameter *A*.

prominent for the larger grid line spacings which more nearly match the spacing between bristle rows. Comparable but ungraphed analyses of the A and B values derived from the artificial sternites of sets 4, 5 and 6 showed a similar pattern to those from set 3, with the lower values at and near 90° being even more prominent. By contrast, the imperfect bristle rows of the set 2 sternites have not resulted in noticeably lower A values at 90°. For five of the six grid line spacings the B values at 90° are slightly lower than those at 85 and 95°, for example, but this is primarily a reflection of the varying width of the bristle distributions which, on average, are narrower at 90° than at most other angles. With the present method then, it is clear that rows without better definition than those typical of the set 2 sternites would escape detection whereas the rows of set 3 would be easily detectable.

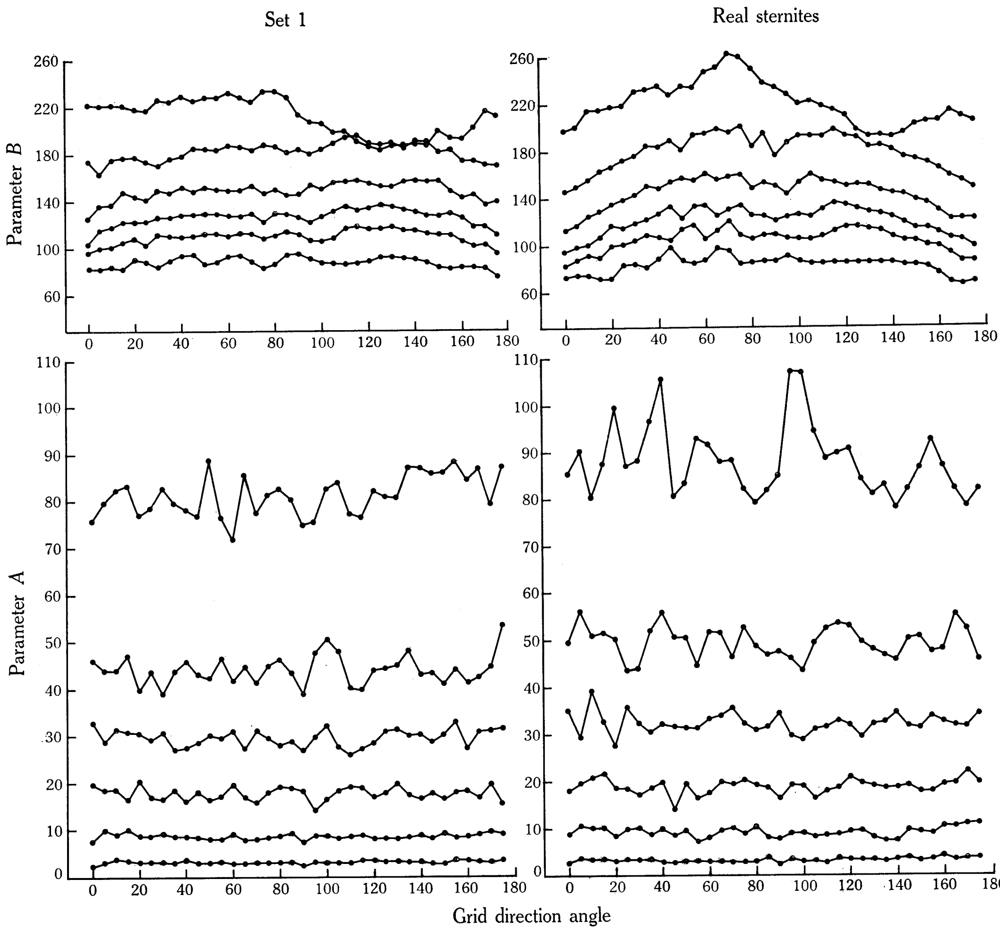

Fig. 5. A and B values for the real and set 1 artificial sternites, presented in a similar fashion to the data given in Fig. 4 (see caption to Fig. 4).

Fig. 5 compares the variation in A and B values for the real sternites with that of the set 1 artificial sternites. A subjective examination of the results for the real sternites reveals no unusually low values of A and B that tend to occur consistently for all or even most of the grid line spacings at the same grid angle. Certainly there are no relatively low values comparable to those derived from the set 3 sternites at 90° (Fig. 4). Thus if sternite bristles do tend to be arranged in rows then either the rows are less well defined than those of the set 3 sternites or else one or more of their characteristics is beyond the capacity of the current test to detect.

Two features of the results for the real and set 1 sternites require comment. The variation in B value over different grid angles is noticeably M-shaped, and this is more pronounced for the real than the random data. The explanation of this shape and also of the low B values that occur at or near 0° (or 180°) is related to the rectangular boundaries of the bristle distribution on the sternites. Only if the variation in A values had been M-shaped also, with particularly low values at and near 0 and 90°, could this feature possibly be connected with a tendency towards an arrangement in rows in the directions of 0 and 90°.

A contrast between the results for the real and artificial set 1 sternites is the wider fluctuation in A that occurs at different angles for the real sternites. The contrast is largest for the values corresponding with the largest grid line spacing. The significance of this most likely relates to the different type of spacing between bristles—a spacing that is random for the set 1 sternites but that has a strong tendency towards evenness for the real sternites (see nearest neighbour results).

The frequency distribution of bristle numbers presented in Table 1 suggested the existence of bristle rows near to and parallel with the lateral sternite edges. A probable reason that these rows have not resulted in significantly lower A and B values at 90° is because the remaining bristles located in the more medial regions of the sternites either are not arranged in rows parallel with the long body axis, or, if they are, row definition is poor.

It has not been the intention here to examine A and B values that correspond with some specific fractional area(s) or region(s) of the sternite. Similarly, individual sternites have not been kept separate in the final analysis. It would seem that these levels of discrimination could best be examined if the present subjective comparisons between the results for the set 1 and real sternites were put on a firm statistical basis.

Finally, the rows that run along inside the lateral sternite edges may simply approximate the position of two straight and parallel boundaries that restrict the appearance of bristles to the sternite region contained between them. Consequently the present study has found no evidence demanding the requirement for specific developmental mechanisms that deliberately place bristles of the third sternite in rows.

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