

Temporal Pattern of Bristle Development on *Drosophila melanogaster* Sternites

J. H. Claxton

Department of Animal Science, University of New England, Armidale, N.S.W. 2351.

Abstract

Bristle development in *D. melanogaster* can be prevented with X-rays administered prior to the final differentiative divisions of bristle-committed epidermal cells. The epidermal cells are radiation-insensitive if the same dose is given after cell division. By subjecting variously aged early pupae to X-radiation and subsequently scoring bristle numbers in adults, a temporal pattern of radiation insensitivity was established on the abdominal sternites. The pattern is a simple gradient extending anteriorly and medially from the posterior lateral sternite 'corners'. The possible significance of this to the origin of bristle spatial patterns is discussed.

Introduction

The problems of pattern formation have attracted renewed interest over the last 15 years or so. In insects generally, and *Drosophila melanogaster* particularly, many diverse and some quite elegant experimental approaches have been made. Despite such innovations the precise mechanisms by which cellular patterns are created remain obscure, and they are still '... couched in terms of abstract formalisms rather than known molecular events' (Bryant 1978).

An enduring feature of the mechanics of pattern determination is the apparent requirement for two systems: one that imparts spatial information to cells, and a second which dictates if and how cells will respond to the information. This duality was initially introduced by Stern (1954*a*, 1954*b*) to accommodate the discovery of mutant bristle-pattern genes which behaved autonomously in genetic mosaics of *D. melanogaster*. The original terminology of prepattern and competence, which described the cellular information and response systems respectively, seems now to be largely replaced by 'positional information' (e.g. Postlethwait and Schneiderman 1971; Lawrence *et al.* 1972; French *et al.* 1976; Bryant 1978; Held 1979), a concept which was first proposed by Wolpert (1969). Along with this change in terminology there has developed the general view that the spatial information system is probably more simple, and the cellular response system correspondingly more complex, than was envisaged with prepattern and competence. For example, it has been common to model positional information schemes in insects on the simple monotonic segmental gradients described by Locke (1959, 1960) and Lawrence (1966), and a recent suggestion has been that the system might be based on interactions between adjacent cells (Bryant *et al.* 1981).

Little attention has so far been given to the temporal aspects of pattern formation and this is especially true when it comes to possible timing differences and timing

sequences in the development of individual pattern elements. An exception here is the work of Poodry (1975). By administering large doses of X-rays to early *D. melanogaster* pupae, bristle development is prevented in bristle-determined epidermal cells that have not already completed their final differentiative divisions. Thus if variously aged pupae are X-radiated, and subsequently, as adults, they are scored for presence-absence of bristles, then it is possible to judge the age when particular differentiative divisions occur. The relative timing of these events between bristles can also be determined. With this technique Poodry established a temporal sequence of cell division for the head and thorax macrochaetes of *D. melanogaster*. In this case, however, the sequence gave no obvious clues about the developmental origin of bristle spatial patterns.

The current study extends the same technique to bristles on the sternite segments of *D. melanogaster*, again with the object of (1) determining the nature of any temporal pattern of division among bristle-committed cells, and (2) examining if the temporal pattern has any significance for the origin of the spatial pattern of bristles. Indeed a temporal pattern was found and an attempt is made to interpret this in the context of positional information.

Methods

The fly stock examined was a randomly bred Oregon-RC strain of *D. melanogaster*, raised on standard food at $25 \pm 2^\circ\text{C}$.

So that X-radiation could be administered at specific times, the ages of pupae were determined relative to pupariation. When pupariation was proceeding rapidly in cultures, each bottle was cleared of pupae and the pupae discarded. Collections of pupae were then made by continually clearing each bottle every 15 min. The age in hours after pupariation (AP) was calculated from the mid-point of each collection and so AP had an error of ± 7.5 min. After collection, pupae were stored in fresh culture bottles to avoid dessication. Later at specific times between 19 and 30 h AP they were radiated, and then returned to the culture bottles. The flies failed to emerge following this treatment, so after a further 7 days they were removed from their pupal cases. The sternites were then dissected from the flies and immersed in aqueous mountant on a glass slide beneath a coverslip. With a projection microscope, drawings of sternites and of the positions of all bristle sockets were made.

Differently aged groups of pupae were subjected to radiation. This yielded a series where the youngest group to be radiated was aged 19.25 h AP, the oldest group was 30.25 h AP, and other groups represented most of the intervening ages at 15-min intervals between these extremes. In all, 187 females and 265 male flies were included in the study. Earlier work (Early 1977) had established that an age span of approximately 20–30 h AP would give an adequate cover of the transition from radiation-sensitivity to complete insensitivity.

Relatively few of the sternite bristles occupy constant positions and therefore they cannot be individually recognized and named as can the thoracic macrochaetes for example. Thus the timing of radiation insensitivity (RI) could not be judged by presence-absence scores for individual bristles on adult sternites. Instead, the left and right sides of sternites were divided into six specific regions (see Fig. 1), and the beginning of RI was gauged in two different ways: (1) by the earliest age of radiation when at least one bristle was subsequently scored in a particular region; and (2) by (i) counting the number of bristles per region (averaged over right and left sternite sides and also over same-sexed flies that were radiated at the same age), (ii) linearly regressing these numbers on age-at-radiation, and (iii) establishing the point of intersection between the regression line and the age (x) axis. For the regression method it was standard practice to include in each analysis, only the data at and upward of the earliest radiation age when a non-zero bristle number was scored.

It seemed initially that the second technique would be much more precise if only because the beginning of RI could be estimated by utilizing most of the data. Subsequently a weakness in this approach appeared; this will be raised in later discussion.

X-radiation (150 kV, 4 mA, 500 rad/min, total dose 10 000 rad) was administered over 20 min. This lengthy period was dictated by the capacity of the X-ray equipment and could not be shortened if the total dose of 10 000 rad was to be maintained. The ages when pupae were radiated were calculated from pupariation to the beginning of radiation. Thus assuming that 10 000 rad would be necessary to prevent bristle development in *all* histoblast cells that had not completed their final differentiative divisions, then the beginning of RI might be underestimated by up to 20 min. Together with the error in pupal aging ($\pm 7\cdot25$ min) this brings the total possible underestimate to nearly 30 min.

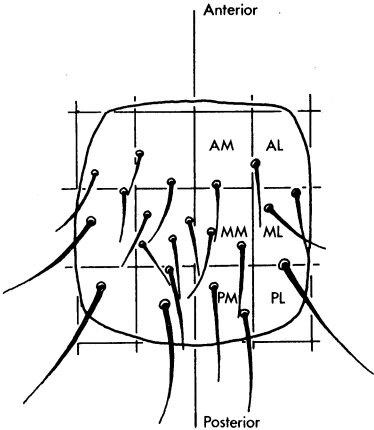


Fig. 1. Typical sternite illustrating subdivision into, and labelling of, six regions of equal size for each of the left and right sternite halves:
AM, anterior medial; *AL*, anterior lateral;
MM, middle medial; *ML*, middle lateral;
PM, posterior medial; *PL* posterior lateral.

Results

The earliest ages of radiation which were followed by non-zero bristle scores are listed in Table 1, separately for sexes, sternites, and regions. Only a small fraction of the total data was used to obtain these values and consequently they were expected

Table 1. Earliest ages (hours after pupariation) when non-zero bristle numbers were scored

Sternite region	2	Age (h) for sternite number:					Average age (h)
		3	4	5	6	7	
Males							
PL	22·6	22·1	22·6	22·6			22·5
PM	22·1	20·1	22·1	22·6			21·8
ML	23·6	23·1	23·1	23·9			23·4
MM	22·6	21·1	23·1	23·4			22·6
AL	24·1	24·1	24·1	23·6			24·0
AM	24·1	24·1	24·1	24·1			24·1
Females							
PL	21·1	21·1	21·1	22·1	22·1	22·1	21·6
PM	22·6	21·1	21·1	21·1	22·1	22·1	21·7
ML	23·9	22·1	22·1	21·1	21·1	23·1	22·3
MM	22·6	22·1	22·1	22·1	22·1	22·1	22·2
AL	23·9	24·6	22·6	24·1	23·4	23·9	23·8
AM	24·6	24·6	23·9	23·9	23·9	23·1	24·0

to give a very approximate guide to the time when RI begins. Tentatively they suggest (1) that RI begins everywhere before about 25 h AP, (2) that RI may begin earlier on the posterior sternite regions (PL, PM) than the anterior ones (AL, AM), and (3) the earliest age when RI begins could be in the vicinity of 20 h AP.

For the plots of bristle number on age of X-radiation the correlation and regression coefficients were all positive. Of the 60 regression coefficients (6 sternite regions $\times$ 6 female and 4 male segments), 12 did not differ significantly ($P > 0.05$) from zero, and 9 of these were associated with regions PL and AL where adult numbers of bristles are smallest (Claxton 1974).

Table 2. Ages (hours after pupariation) when X-ray insensitivity begins

Ages were determined by regressing adult bristle number (ordinate) with age of X-radiation, and extrapolating the regression line onto the age axis

Sternite region	Age (h) for sternite number:						Average age (h)
	2	3	4	5	6	7	
Males							
PL	13.32	14.11	14.34	20.28			15.51
PM	22.70	20.85	20.23	22.30			21.52
ML	21.90	20.40	21.42	21.61			21.36
MM	23.49	23.10	23.70	23.62			23.48
AL	20.62 ^A	21.86 ^A	23.37	10.11 ^A			23.37
AM	23.90	24.07	24.14	22.74			23.71
PL+PM	22.03	19.82	18.98	21.56			20.60
ML+MM	23.23	22.56	23.10	23.12			23.00
AL+AM	23.26	23.41	23.90	21.36			22.98
PL+ML+AL	20.69	19.45	20.32	21.30			20.44
PM+MM+AM	23.17	22.26	23.66	23.01			23.03
Total	22.70	21.63	22.02	22.39			22.19
Females							
PL	8.92 ^A	8.60 ^A	14.41	9.58 ^A	18.48	20.77	17.89
PM	19.75	22.17	21.46	22.50	22.76	19.81	21.41
ML	13.80 ^A	17.76	19.51	20.28	21.42	18.66	19.53
MM	19.79 ^A	23.65	23.66	23.67	23.82	23.63	23.69
AL	23.84	16.02 ^A	22.92	22.38	18.15 ^A	13.60 ^A	23.05
AM	25.07	25.23	24.66	23.97	24.12	−6.62 ^A	24.59
PL+PM	20.69	20.56	19.89	20.53	21.36	20.47 ^A	20.58
ML+MM	21.30	22.32	22.56	22.59	22.84	22.22	22.31
AL+AM	24.37	23.65	23.65	23.51	22.51	12.23 ^A	23.54
PL+ML+AL	21.77	18.54	19.82	19.07	20.45	20.16	19.97
PM+MM+AM	22.45	23.25	23.00	23.18	23.45	22.56	22.98
Total	21.21	21.91	21.99	22.08	22.30	20.93	21.74

^A The regression lines from which these age values were estimated were not significantly ($P > 0.05$) different from zero and the values are not included in averages.

In Table 2, listed separately for the two sexes, for the different sternites, and for sternite regions, are the regression-estimated ages when RI begins. Those estimates based on regression lines with coefficients not significantly ($P > 0.05$) different from zero are not included in the sternite averages.

For each total sternite, the estimated age when RI begins more nearly matches the mean of the corresponding regional values than it does the smallest regional value.

The same is true for the compounded regional combinations PL + PM, ML + MM, etc. Presumably this principle also applies at the regional level. Thus for regions that ultimately support several bristles (e.g. MM), the regional age when RI begins would be an approximate average across the region with the possibility that each bristle may have a different, and perhaps characteristic, age when it becomes insensitive to radiation.

Because the slopes of the regression lines as well as their intercepts on the age axis differed for different regions, it seemed possible that plots of the compounded data in the various regional combinations would tend towards curvilinearity. However, the correlations for the totals, for example, were high (characteristically around 90%) so any tendency toward curvilinearity has apparently been more than offset by the increased precision in the compounded data points. For the sternite totals these points were based on averages of bristle number across regions.

The Table 2 results suggest that on different sternites and sternite regions, the age when RI begins may vary by as much as 12 h, ranging from the extremes of about 13 to 25 h AP. The bottom end of this range is much lower, and the span of the range much broader, than was indicated by the earliest non-zero bristle number scores given previously (Table 1). Despite the fact that the regression method was expected to be the more accurate in estimating the beginning of RI, it seems to be unreliable for region PL from which the lowest estimates came. The reasons for this will be elaborated later in the discussion.

Between-sex comparisons in Table 2 show differences in the beginning of RI to be generally small and inconsistently directed. Regardless of whether such comparisons are subjectively based or whether they are supported with simple statistical tests (e.g. a paired *t*-test for individual sternites and regions), there is no convincing evidence for meaningful sex differences in the beginning of RI.

As in the comparison between sexes, differences between sternites in the beginning of RI are also generally small. However, the Table 2 results do support the possibility that the beginning of RI follows a simple gradient which increases antero-posteriorly. This is most clear in the results for total sternites although sternite 2 of males and sternite 7 of females do not conform with the trend. One reason why sternite 7 may appear deviant in this regard is that it has relatively few bristles in regions AL and AM, and whereas for other sternites, estimates for the beginning of RI based upon total sternites have all been weighted upwards by the inclusion of AL and AM bristles, the same inclusion for sternite 7 has not had this effect.

Regional differences in the beginning of RI are larger than those in the between-sternites and between-sexes comparisons. In line with earlier indications, the age values seem to follow a simple gradient scheme with lowest values recorded in region PL, and increasing anteriorly and medially to the highest values on region AM. In general terms this pattern of change is evident on all sternites of both sexes and it is particularly consistent between sexes when the sternite averages are compared.

For the various regional combinations, the estimated ages when RI begins also support this simple gradient pattern. With only two exceptions (sternites 5 of males and 6 of females) the estimates increase regularly from the posterior, through the middle, to the anterior combinations, and from the lateral to the medial combinations. In this regard, the corresponding averages across sternites are only inconsistent for the antero-posterior sequence in males: the average for AL + AM has obviously been affected by sternite 5 where the regression determining 21.36 h AP as the age

when RI begins had a coefficient which differed significantly ($P \leq 0.05$) from zero by the barest of margins.

Discussion

Efficacy of the Method

There were two major concerns in utilizing the regression technique for estimating the beginning of RI. First, it was expected that slopes of regression lines would relate closely to adult bristle number in the sternite regions. Thus it seemed important that the regression line intercepts on the age axis be substantially independent of line slope; otherwise the intercepts could be a reflection of regional variation in adult bristle number without necessarily indicating any meaningful regional variation in the beginning of RI. To check this, regression coefficients were averaged for sternites and for sexes and then correlated across regions with the corresponding average intercepts on the age axis; the resulting correlation coefficient was 0.58. Thus with less than 35% (i.e. r^2) of the regional variance in the beginning of RI being accountable by slopes of regression lines, this establishes a fair independence of these variables.

A second concern was that regions where the beginning of RI was indeed early could be given an exaggeratedly low estimate with the current regression method. This was possible because these regions would likely be the ones where complete RI is reached first, where bristle numbers plateau to the adult levels earliest, and where the early parts of this plateau might be included in the regression analysis, thus lowering both the regression coefficient and the intercept on the age axis. The problem was checked by eliminating the top data points one by one in succession and calculating a new regression and age intercept with each elimination. As a result of this exercise there was no evidence of upward (or downward) trends occurring in regression coefficients or age intercepts for five of the six sternite regions. But region PL was exceptional in this regard with clear indication that the age intercept (and thus the estimated beginning of RI) increased with successive elimination of top data points. The increase did not go above age-intercept values for other regions, and consequently it seems reasonably clear that although the regression-estimated beginning of RI for region PL is too low, the true value is still lower than the estimates for other sternite regions.

The foregoing discussion gives a basis for rationalizing the results of Tables 1 and 2. As noted earlier, the major discrepancy between these tables concerns region PL—and the problem is removed knowing that the beginning of RI for PL is underestimated in Table 2.

Radiation Insensitivity

The present observations do not establish the period over which sternite bristles acquire RI but they are not inconsistent with the range of up to 12 h suggested by Poodry (1975) for all abdominal bristles. Poodry also reported that radiation at 24 h AP prevented the development of all abdominal bristles. The present results are not in agreement here and indicate rather that radiation would have to be administered 4 and possibly 5 or more hours earlier to prevent all bristle development on the sternites.

If real differences in the beginning of RI exist between sexes, these appear to be small relative to regional differences across sternites. Differences between sternites are also relatively small but there is some indication of a simple antero-posterior increase. If substantiated, this gradient may parallel those of gene repressor concentrations, gene activity, and morphological expression mediated through the bithorax complex and described recently by Lewis (1978).

Regional differences across individual sternites seem to be reasonably standard for all sternites: the beginning of RI is lowest in the posterior-lateral 'corners' of sternites (region PL) and it increases from here both anteriorly and medially to the highest values in region AM. This gradient matches those of decreasing bristle length and of decreasing distance between neighbouring bristles (Claxton 1974); the bristles developing in region PL are not only the longest but also the most constant in their positioning. There is some evidence (Claxton 1974) that this constancy decreases gradually, at least medially and possibly anteriorly as well.

Pattern Formation

The acquisition of RI probably signals the completion of division in histoblast cells that are committed to bristle development (Poodry 1975). Thus if different cells begin to show RI at different ages this is presumably indicative of timing differences in the final divisions. In addition, but much more tenuous, is the possibility that such differences correlate with the earlier determinative events: regions where the beginning of RI is earliest, for example, may contain cells whose commitment to bristle formation is decided before cells of other regions where the beginning of RI occurs later. Poodry also suggested this connection but it was unhelpful to an understanding of pattern formation among the thoracic macrochaetes despite their having wide variation in the acquisition of RI. Making the same assumptions and asking similar questions of the sternite bristles does, however, yield a plausible model of pattern formation.

For patterns whose elements tend toward even spacing (including many if not all of the *D. melanogaster* microchaetes) the competitive model proposed by Wigglesworth (1953) offers a simple determinative scheme (e.g. Claxton 1964; Lawrence 1969; Held 1979). An added attraction is that competition is not necessarily an alternative to positional information; indeed, acting together, they offer an explanation for the pattern of sternite bristles.

Suppose the competitive scheme imparts evenness of spacing, i.e. once an epidermal cell is finally committed to bristle development it suppresses the same potential in nearby cells. Suppose also that a system of positional information tells cells *when*, rather than *whether*, to be determined as bristles, and more precisely, that the information specifies a monotonic sequence of determination beginning in the posterior-lateral sternite 'corners' and proceeding anteriorly and medially.

The operation of this scheme would (1) ensure determination first in the posterior-lateral sternite 'corners' with the extent of constancy in location here dependent upon the repeatability and stability of the positional information system; (2) direct further determination anteriorly and medially from these 'corners' in a progression wherein constancy of location would rapidly diminish; (3) impart a regularity in spacing between bristles through the function of competition. A secondary consequence of positional information in this context may be that it affects the intensity of competition as well as the ultimate size of bristles. In this way bristle spacing and bristle length may both come to be correlated with the spatial order in which determination proceeds.

Although the proposed model appears to give a simple and effective explanation of sternite bristle patterns, and while it is similar to one accommodating the spatial characteristics of bristles in the acrostichal rows of *D. melanogaster* (Claxton 1976), it of course remains highly speculative.

Acknowledgment

The author wishes to thank Mrs Carolyn Harper for expert technical help.

References

- Bryant, P. J. (1978). Pattern formation in imaginal discs. In 'The Genetics and Biology of *Drosophila*'. Vol. 2c. (Eds M. Ashburner and T. R. F. Wright.) p. 230. (Academic Press: New York.)
- Bryant, S. V., French, V., and Bryant, P. J. (1981). Distal regeneration and symmetry. *Science (Wash., D.C.)* **212**, 993–1002.
- 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**, 302–17.
- Claxton, J. H. (1974). Some quantitative features of *Drosophila* sternite bristle patterns. *Aust. J. Biol. Sci.* **27**, 533–43.
- Claxton, J. H. (1976). Developmental origin of even spacing between the microchaetes of *Drosophila melanogaster*. *Aust. J. Biol. Sci.* **29**, 131–5.
- Early, W. M. (1977). The temporal sequence of bristle pattern formation in *Drosophila*. Honours Thesis, University of New England, Armidale, N.S.W.
- French, V., Bryant, P. J., and Bryant, S. V. (1976). Pattern regulation in epimorphic fields. *Science (Wash., D.C.)* **193**, 969–81.
- Held, L. I. (1979). Pattern as a function of cell number and cell size on the second-leg basitarsus of *Drosophila*. *Wilhelm Roux's Arch. Dev. Biol.* **187**, 105–27.
- Lawrence, P. A. (1966). Gradients in the insect segment: The orientation of hairs in the milkweed bug *Oncopeltus fasciatus*. *J. Exp. Biol.* **44**, 607–20.
- Lawrence, P. A. (1969). Cellular differentiation and pattern formation during metamorphosis of the milkweed bug *Oncopeltus*. *Dev. Biol.* **19**, 12–40.
- Lawrence, P. A., Crick, F. H. C., Munro, M. (1972). A gradient of positional information in an insect *Rhodnius*. *J. Cell Sci.* **11**, 815–53.
- Lewis, E. B. (1978). A gene complex controlling segmentation in *Drosophila*. *Nature (London)* **276**, 565–70.
- Locke, M. (1959). The cuticular pattern in an insect, *Rhodnius prolixus* Stål. *J. Exp. Biol.* **36**, 459–77.
- Locke, M. (1960). The cuticular pattern in an insect—the intersegmental membranes. *J. Exp. Biol.* **37**, 398–406.
- Poodry, C. A. (1975). A temporal pattern in the development of sensory bristles in *Drosophila*. *Wilhelm Roux's Arch. Dev. Biol.* **178**, 203–13.
- Postlethwait, J. H., and Schneiderman, H. A. (1971). Pattern formation and determination in the antenna of the homoeotic mutant antennapedia of *Drosophila melanogaster*. *Dev. Biol.* **25**, 606–40.
- Roseland, C. R., and Schneiderman, H. A. (1979). Regulation and metamorphosis of the abdominal histoblasts of *Drosophila melanogaster*. *Wilhelm Roux's Arch. Dev. Biol.* **186**, 235–65.
- Stern, C. (1954a). Genes and developmental patterns. *Caryologia* VI. [Suppl.: Proc. 9th Int. Congr. Genet. Florence, Italy.] pp. 355–69.
- Stern, C. (1954b). Two or three bristles. *Am. Sci.* **42**, 213–47.
- Wigglesworth, V. B. (1953). Determination of cell function in an insect. *J. Embryol. Exp. Morph.* **1**, 269–77.
- Wolpert, L. (1969). Positional information and the spatial pattern of cellular differentiation. *J. Theoret. Biol.* **25**, 1–47.