Development 111, 1087–1095 (1991)
Printed in Great Britain © The Company of Biologists Limited 1991

The emergence of sense organs in the wing disc of *Drosophila*

FRANÇOISE HUANG, CHRISTINE DAMBLY-CHAUDIÈRE and ALAIN GHYSEN

Laboratoire de Génétique, Université Libre de Bruxelles, 67 rue des Chevaux, B-1640 Rhode-St-Genèse, Belgium

Summary

We have examined the origin of a set of precisely located sense organs in the notum and wing of *Drosophila*, in transformant flies where *lacZ* is expressed in the progenitor cells of the sense organs (the sensory mother cells) and in their progeny. Here we describe the temporal pattern of appearance and divisions of the sensory mother cells that will form the eleven macrochaetes and the two trichoid sensilla of the notum, and five campaniform sensilla on the wing blade. The complete pattern of sensory mother cells develops in a strict sequence that extends over most of the third larval instar and the first 10 h after puparium formation. The delay between the onset of *lacZ* expression and the first differentiative division ranges from 30 h, in the case of the earliest mother cells, to 2 h for the latest mother cells. The first division shows a preferential orientation which is also specific for each sensory mother cell. Up to this stage, there is no marked difference between the three types of mechanosensory organs.

Abbreviations: APF, after puparium formation; AS-C, *achaete-scute* complex; BPF, before puparium formation; SMC, sensory mother cell. Abbreviations of the sense organs examined in this paper are explained in the legend of Fig. 1.

Key words: peripheral nervous system, neurogenesis, imaginal disc, *Drosophila*, pattern formation, sensory mother cell.

Introduction

The sense organs of insects are often arranged in reproducible patterns, where each element of the pattern occupies a fixed position on the surface of the body. In *Drosophila*, this is shown by the eleven large bristles (macrochaetes) and two trichoid sensilla present on the notum, and most of the campaniform sensilla on the wing blade (Fig. 1). Each sense organ is formed by the progeny of a single precursor cell, the sensory mother cell (SMC). In the case of mechanosensory organs, such as bristles and campaniform sensilla, the SMC divides twice to generate four cells (reviewed in Bate, 1978). Two cells will differentiate into the external structures of the sense organ, a third will become the sensory neuron and the fourth will form a sheath around the dendrite. These four cells are often accompanied by a fifth cell, which forms a sheath around the soma and axon (Zacharuk, 1985; Hartenstein, 1988; Hartenstein and Posakony, 1989). This 'soma sheath cell' is of unknown origin.

The spatial pattern of the thoracic and head macrochaetes and their genetic determinants have been extensively studied during the past 40 years (e.g. Stern, 1954; Maynard-Smith and Sondi, 1961; Garcia-Bellido, 1981). However, little is known of the temporal pattern of sense organ formation, in part because it is generally not possible to visualize any of the steps that precede the final differentiation of the sense organ. Thus previous studies had to resort to indirect means to assess the temporal dimension of pattern formation.

One method used to time different aspects of the formation of sensory bristles is X-ray-induced mitotic recombination. The analysis of clones induced at different developmental times defines the latest time after which the progeny of the irradiated cell will be confined to either an epidermal or a sensory fate (lineage segregation). Mitotic recombination also makes it possible to define the latest time at which the wild-type copy of a given gene is required (perdurance). The results suggest that the formation of SMCs takes place as early as 40 h before puparium formation (Garcia-Bellido and Santamaria, 1978), and that lineage segregation occurs even before that time in the case of the macrochaetes (Garcia-Bellido and Merriam, 1971).

A second method is to subject flies to extensive irradiation so as to kill dividing cells (Poodry, 1975), while postmitotic cells will be relatively unaffected. The results revealed that the different macrochaetes enter their terminal division in a fixed sequence that extends over the first 15 h after puparium formation (APF).

The available data, however, tell us nothing about the sequence of appearance of the macrochaete SMCs, or the time course of the differential divisions, or whether other types of sense organs are also formed in a fixed sequence. Recently some of these questions have been addressed in the case of the microchaetes of the

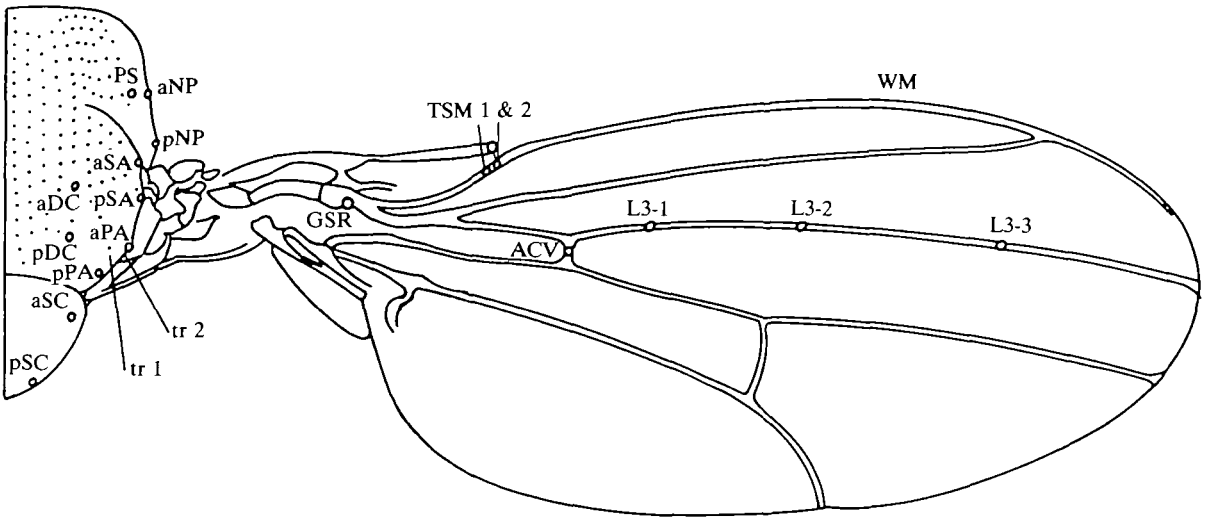

Fig. 1. Right side of the thorax and wing of *Drosophila*. ACV, campaniform sensillum of the anterior crossvein; aDC, anterior dorsocentral bristle; *a*NP, anterior notopleural bristle; aPA, anterior postalar bristle; aSA, anterior supraalar bristle; aSC, anterior scutellar bristle; GSR, giant sensillum of the radius; L3-1, L3-2, L3-3, campaniform sensilla of the third wing vein; pDC, posterior dorsocentral bristle; pNP, posterior notopleural bristle; pPA, posterior postalar bristle; PS, presutural bristle; pSA, posterior supraalar bristle; pSC, posterior scutellar bristle; tr1, tr2, trichoid sensilla 1 and 2; TSM 1 and 2, twin campaniform sensilla 1 and 2 of the margin; WM, chemosensory bristles of the anterior wing margin.

notum and the margin bristles of the wing blade (Hartenstein and Posakony, 1989). Contrary to the precisely located sense organs considered so far, each of which can be uniquely identified, the microchaetes and wing bristles are populations of morphologically identical bristles that have a fairly uniform distribution. For these sense organs, it appears that the formation of the SMCs and the subsequent divisions are largely synchronous for the entire population.

Here, we use an 'enhancer-trap' transformant line (O'Kane and Gehring, 1987), which expresses the *E.coli lacZ* gene specifically in sense organ precursors and in their progeny, to describe the formation of most of the precisely located sense organs on the notum and wing blade. We show that the final pattern emerges progressively over a period of about 40 h.

Materials and methods

Fly strains

The A101 transformant was generated and kindly provided by H. Bellen and coworkers in W. Gehring's laboratory. It contains a PlarB construct (Bellen *et al.* 1989) inserted at 85C (H. Bellen, personal communication). This construct contains a gene fusion that puts the *lacZ* gene in phase with the P element transposase gene. The resulting transposase–galactosidase fusion protein apparently retained the nuclear targeting domain of the transposase, since the antigen is localized in the nucleus (Ghysen and O'Kane, 1989).

Timing

We attempted to time larvae by several methods (Maroni and Stamey, 1983), none of which proved reliable in our hands. We therefore relied on the second moult (second instar to third instar) to calculate the age of each larva. Individuals were collected as late second instar, incubated for two hours at 25 °C, third instars were then selected and kept at 25 °C on standard medium. Initially we labelled the cuticle of the second instars with a waterproof marker, so that moulted larvae having shed their cuticle could be recognized at a glance. With a little practice, however, third instars are just as easily detected on the basis of their very distinct anterior spiracles. Each set of moulted larvae was divided into two groups, one for dissection and the other as control for the duration of the third instar and the dispersion of puparium formation. Maximum dispersion was six hours; that is, puparium formation occurred after 48±3 h at 25 °C. The age of the larvae 'after second moult' have been converted to the more usual 'before puparium formation' (BPF) scale by substraction from 48. For example, 30 h BPF refers to larvae that had undergone the second moult 18–20 h earlier.

Staining

Discs were dissected from larvae 18 h, 24 h, 30 h, 36 h, and 42 h after the second moult (recorded as 30 h, 24 h, 18 h, 12 h, and 6 h BPF), and 0 h, 1 h, 3 h, 5 h, 11 h, 13 h, and 15 h after puparium formation (APF). The discs were fixed in formaldehyde-phosphate buffer, rinsed and immunostained with monoclonal anti-β-galactosidase (Promega, diluted 1:1000) followed by biotin–avidin–peroxidase treatment (Vectastain, Vector labs).

Results

(a) Temporal sequence of SMCs in the notum primordium

The identity of the different SMCs was assessed by following the development of the pattern up to 15 h after puparium formation (APF), at which time the notum and wing have essentially assumed the adult shape so that the individual sense organs are easily identified. The resulting map of the presumptive sites in

Table 1. *Mean numbers and extremes of* lacZ-*expressing cells at the different sites where sense organs will form*

	30 h BPF	24 h BPF	18 h BPF	12 h BPF	6 h BPF	PF	1 h APF	3 h APF	5 h APF
Notum									
pSC	0.25 (0–1)	0.55 (0–1)	0.69 (0–1)	1.0 (1)	1.0 (1)	2.4 (2–4)	3.1 (2–5)	4.2 (3–5)	3.9 (3–5)
aPA	0	0.44 (0–1)	0.69 (0–1)	1.0 (1)	1.4 (1–3)	3.1 (2–5)	3.7 (3–5)	3.8 (3–5)	4.1 (4–5)
pDC	0	0.44 (0–1)	0.61 (0–1)	1.0 (1)	1.0 (1)	2.2 (2–3)	2.7 (2–4)	4.3 (2–4)	4.4 (4–5)
pNP	0	0	0.23 (0–1)	0.94 (0–1)	1.0 (1)	2.0 (2)	2.2 (2–4)	2.6 (2–4)	4.1 (3–5)
aNP	0	0	0	0.57 (0–1)	1.0 (1)	1.2 (1–2)	1.1 (1–2)	1.8 (1–2)	3.6 (2–5)
pSA	0	0	0	0.4 (0–1)	0.9 (0–1)	1.9 (1–2)	2.1 (2–3)	2.2 (2–3)	3.5 (3–5)
tr1	0	0	0	0.37 (0–1*)	1.0 (1)	1.5 (1–2)	1.8 (1–3)	1.9 (1–4)	3.0 (2–5)
aSC	0	0	0	0	0.5 (0–1*)	1.0 (1)	1.0 (1*)	1.6 (1–2)	2.8 (1–5)
aDC	0	0	0	0.05 (0–1)	0.2 (0–1)	1.0 (1)	1.0 (1*)	1.1 (1–2)	1.8 (1–2)
PS	0	0	0	0	0	1.0 (1*)	1.0 (1*)	1.0 (1)	2.0 (2)
aSA	0	0	0	0	0	0	0.76 (0–1*)	0.8 (0–1*)	1.0 (1)
pPA	0	0	0	0	0	0	0.62 (0–1*)	0.7 (0–1)	1.0 (1)
tr2	0	0	0	0	0.1 (0–1)	0.55 (0–1*)	0.53 (0–1*)	0.55 (0–1*)	1.0 (1)
N	8	9	13	19	10	9	22	10	14
Wing									
L3-2	0.125 (0–1)	0.44 (0–1*)	1.0 (1)	1.0 (1)	1.0 (1)	3.5 (3–4)	3.7 (3–5)	3.3 (3–4)	4.1 (3–5)
GSR	0.125 (0–1)	0.55 (0–1)	0.93 (0–1)	1.0 (1)	1.1 (0–1)	3.8 (3–4)	3.6 (2–4)	3.8 (3–5)	3.7 (3–5)
ACV	0	0.33 (0–1)	0.38 (0–1)	1.0 (1)	1.1 (1–2)	3.4 (3–4)	– (2–5)	– (2–5)	4.1 (3–5)
L3-3	0	0	0	0	0.22 (0–1)	1.0 (1)	1.0 (1–2)	1.0 (1)	1.6 (1–2)
L3-1	0	0	0	0	0	1.0 (1)	1.0 (1–2)	1.1 (1–2)	1.5 (1–3)
W.M.	0	0	0	0.47 (0–1)	1.0 (1)	1.0 (1)	1.0 (1)	– (2)	– (2–3)
N	8	9	13	18	9	9	21	9	15

The extremes (in parenthesis) give the smallest and largest numbers of cells that were observed in the sample. 1* means that sometimes a pair of faintly labelled cells is present; these have been counted as one because it is obvious that they do not result from the division of a SMC (see text). The ages of the discs are given as BPF (before puparium formation) or APF (after puparium formation). Ages BPF were calculated as described in Material and methods. Abbreviations of the sites are explained in the legend of Fig. 1. N, number of discs examined.

the disc corresponds amazingly well to the fate map constructed by Bryant in 1975.

Table 1 gives the mean number (in parenthesis, the extremes) of *lacZ*-expressing cells at each presumptive site, at different developmental times. Some of the stages are illustrated Fig. 2. The first SMC forms in the presumptive dorsal radius (dR, Fig. 2A). Next appear the SMC of the giant sensillum of the radius (GSR) and one in the ventral radius (vR, Fig. 2B). Among the SMC of the notum, the pSC usually appears first (Fig. 2B). However, in some discs, the aPA may come first (Fig. 2C), reflecting the indeterminacy in the time of onset of *lacZ* expression for the early SMCs (see below). Fig. 2B also illustrates the marked homology between the wing and haltere patterns at this stage (asterisks). Fig. 2D shows a 24 h BPF wing disc, and

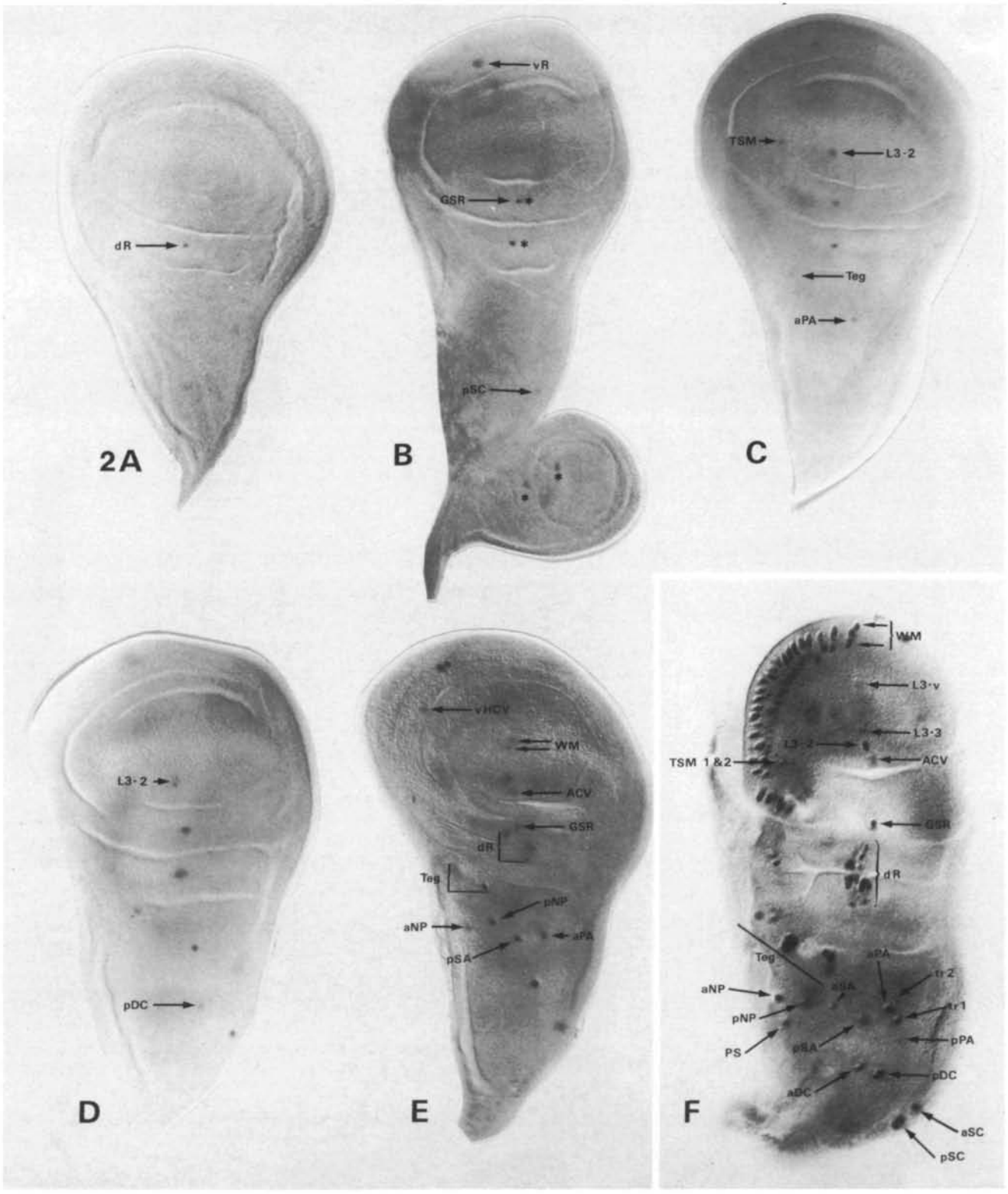

Fig. 2. Wing imaginal disc at different stages. (A) 30 h BPF; (B–D) 24 h BPF; (E) 12 h BPF; (F) 1 h APF. (B) The haltere disc has remained attached to the wing disc; asterisks indicate SMCs that are clearly homologous in the wing and the haltere. (D) Two cells are weakly labelled at the L3-2 site. (E) All the early and intermediate sensilla are present except for tr2. (F) An everted disc, where all SMCs studied in this paper are present (L3-1 is out of focus). Abbreviations as in Fig. 1 except dR, sensilla of the dorsal radius; L3-v, ventral sensillum of the third wing vein; Teg, sensilla and bristles of the tegula; vHCV, sensillum of the ventral humeral crossvein; vR: sensilla of the ventral radius.

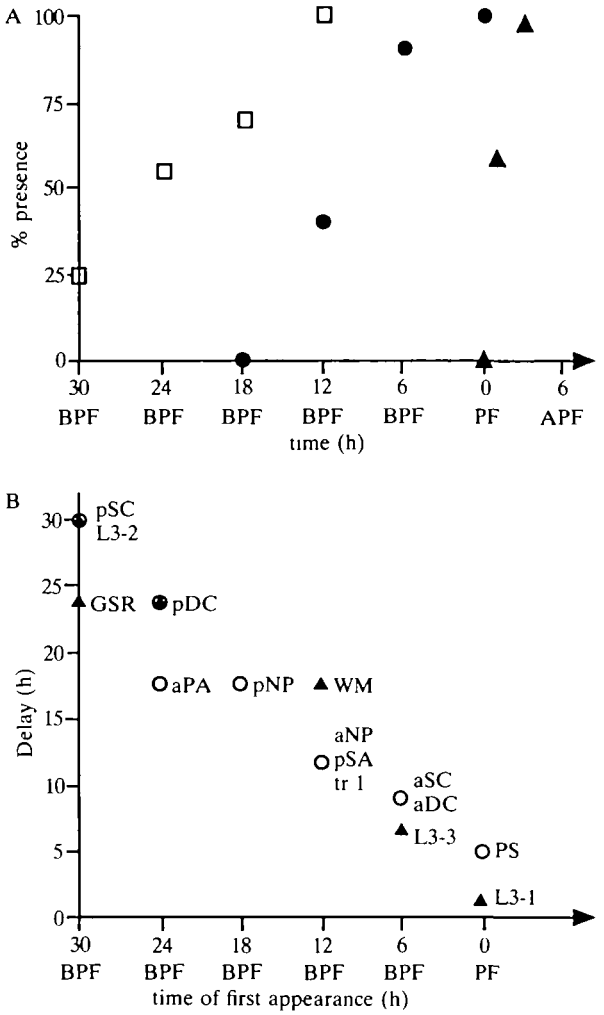

Fig. 3. Parameters of the onset of *lacZ* expression. (A) Onset of *lacZ* expression in an early, an intermediate and a late SMC. The ordinate gives the proportion of discs where *lacZ* expression is detected at the site of the posterior scutellar (pSC, squares), the posterior supraalar (pSA, circles) and the posterior postalar (pPA, triangles) macrochaete. The abscissa gives the age of the discs. (B) Delay between onset of *lacZ* expression and first division for all the SMCs considered in this paper. The ordinate gives the latency between the first time at which *lacZ* expression is detected at a given site, and the first time at which the corresponding SMC has divided. The abscissa gives the age at which *lacZ* expression is first detected. Circles, macrochaetes and trichoid sensilla on the notum; triangles, campaniform sensilla on the wing blade.

Fig. 2E shows a 12 h BPF wing disc. Finally, Fig. 2F shows an everted disc 1 h after puparium formation, where all SMCs studied in this paper are present.

The period over which a *lacZ*-expressing SMC first becomes detectable at a given site is surprisingly large for the earliest SMCs (Fig. 3A). For example, in the case of the pSC, the SMC can already be detected before 30 h BPF in some discs, while, in other discs, it is still not detected at 18 h BPF. This 'open' period gets progressively reduced at later times, as illustrated in Fig. 3A in the case of an early, an intermediate and a late SMC.

(b) Multiple precursors and heterochronic pairs

At the time and position where the SMCs of tr1, aSC, aDC, PS, aSA, pPA and tr2 are about to appear, sometimes more than one cell expresses *lacZ* at a low level (Fig. 4,A–D). The most usual case is two adjacent cells (Fig. 4A, B), but sometimes three cells are lightly stained (Fig. 4C). Occasionally one of the two cells is strongly stained while the other is very lightly stained (Fig. 4D). In slightly older discs, one cell shows a high level of *lacZ* expression while none of the surrounding cells express *lacZ* at a detectable level. This suggests that occasionally two or more epidermal cells may begin to develop as SMCs and that competition between the two (presumably mediated by lateral inhibition, reviewed in Simpson, 1990) results in only one of them becoming fully differentiated as a SMC.

We have observed several cases where a late SMC appears a few cell diameters away from an early SMC, forming what we call 'heterochronic pairs'. For example, each of the three early SMCs of the notum has a 'follower': tr1 appears near the aPA, while the aSC and aDC form close to the pSC and pDC, respectively. Other cases of late SMC that might be 'followers' are tr2 with respect to the aPA, pPA with respect to tr1, and in the wing, L3-1 and L3-3 on either side of L3-2.

(c) Lineage

The delay between the beginning of *lacZ* expression and the first mitosis varies from a few hours up to about 30 h. This delay becomes progressively smaller for later SMCs, regardless of the type of sense organ that a SMC will later form (Fig. 3B). Thus both the period over which a given SMC first becomes detectable, and the lag between *lacZ* expression and mitosis, are inversely proportional to the time of appearance, as if the pace of the process was progressively accelerating. The two effects do not cancel out, however, and the order in which the SMCs undergo their first division is the same as the order in which they express *lacZ*.

In all cases, the first division appears parallel to the epithelial surface, as judged by the position of the daughter nuclei. Hartenstein and Posakony (1989) also observed that the mitotic spindle is parallel to the epidermal surface in the case of the microchaetes. On the other hand, the alignment of the daughter nuclei shows a preferred orientation, as shown in Fig. 5. This suggests that the plane of the first division relative to the anteroposterior and dorsoventral axes of the disc is not oriented at random and, therefore, that the mother cell has an intrinsic polarity before mitosis begins (see also Bate, 1978).

The transgene present in A101 codes for a transposase-galactosidase fusion protein (see Material and methods) which is normally concentrated in the nucleus. Occasionally, however, the protein occupies a much larger volume. We believe that this expansion is due to nuclear breakdown at the onset of mitosis, and to the subsequent dispersion of the antigen in the

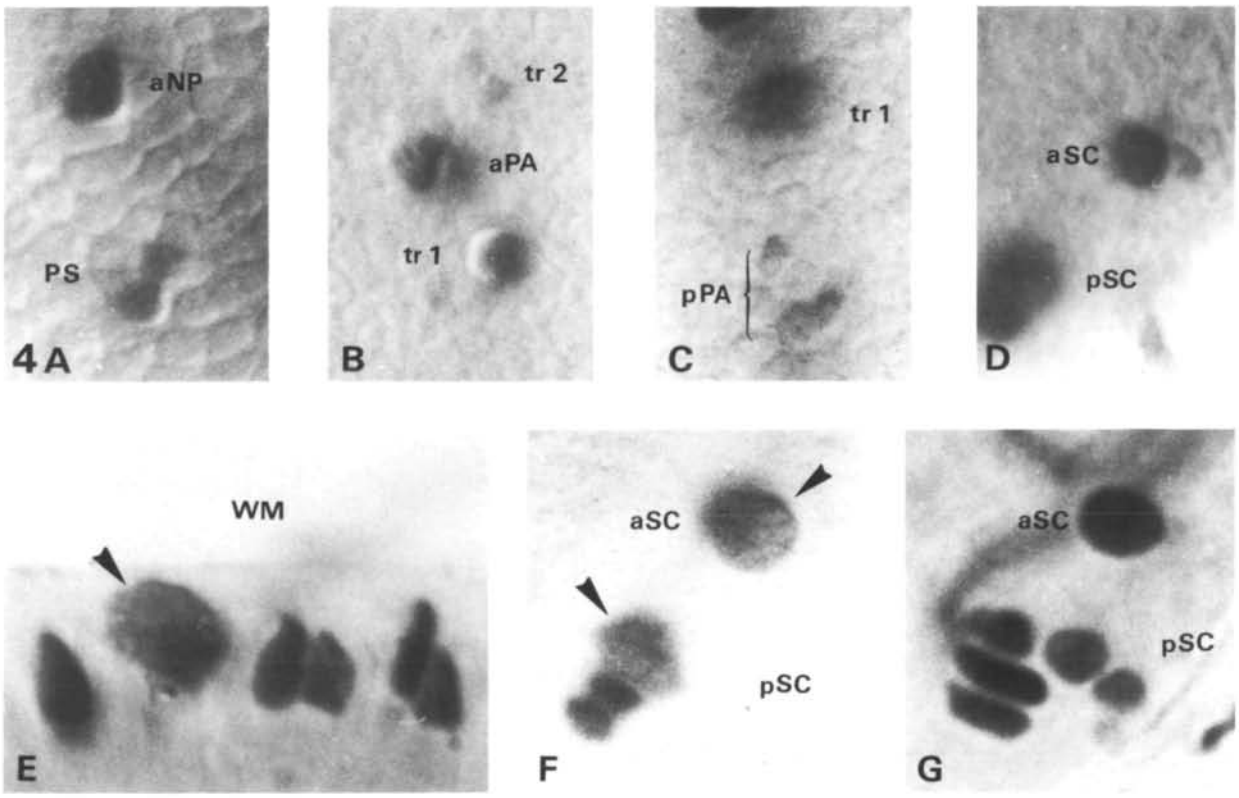

Fig. 4. Multiple precursors and lineage. (A–D) At least two neighbouring cells stain very slightly at a site where only one SMC will eventually be found. (A) PS site; (B) tr2 site; (C) pPA site; (D) aSC site. In D, one of the cells has a dark and enlarged nucleus typical of a mature SMC, while the other has retained the immature features shown in A–C. (E–G) Premitotic figures. Arrowhead shows a premitotic cell. (E) A small section of the anterior margin at 7 h APF. From proximal (left) to distal (right): a SMC, a SMC entering mitosis (arrowhead) and two pairs of cells resulting from the division of two SMCs. (F) The SMC of the aSC bristle as well as one of the daughters of the pSC SMC are entering mitosis. The other pSC daughter cell has already divided. A fourth cell (out of focus) expresses *lacZ*, possibly as a result of recruitment (see text). (G) The five cells that will form the pSC sense organ, at 3 h APF. In this disc, the aSC has already divided once but the two daughter nuclei cannot be distinguished at this focal plane.

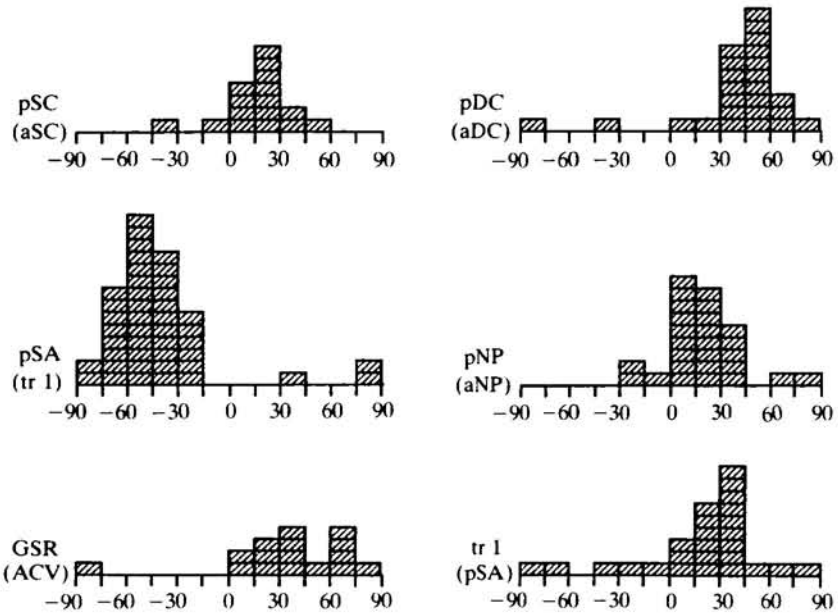

Fig. 5. Orientation of the first division of the SMC (in bold type) in the case of two early macrochaetes (pSC and pDC), two intermediate macrochaetes (pNP and pSA), a trichoid sensillum (tr 1) and an early campaniform sensillum (GSR). In all cases, the orientation was expressed as the angle in degrees between the line connecting the centers of the daughter nuclei, and the line connecting the reference point (in parenthesis) to the daughter nucleus closest to it. The reference point was usually the closest SMC, so as to reduce the error due to disc distortion during manipulation. '+' and '−', respectively, correspond to angles on the clockwise and counter-clockwise side of this axis. Since the two discs of a larva are mirror-symetrical, the values for right discs were inverted and pooled with those for left discs. Each dashed rectangle (■) represents the value obtained in one disc.

cytoplasm. Because of the large number of nearly synchronous divisions, the row of SMCs on the wing margin illustrates well the appearance of such premitotic cells (Fig. 4E). Premitotic cells are observed at other sites as well, and then, always at the time when a mitosis is expected (Fig. 4F in the case of the scutellar bristles). The fact that only one of the three cells is undergoing mitosis in the case of the pSC, Fig. 4F, indicates that the two daughters of the first division do not divide simultaneously, as already noted by other workers (Bodmer *et al.* 1989; Hartenstein and Posakony, 1989).

The subsequent divisions closely follow the first one in all cases where they can be unambiguously detected. The detail of these divisions is more difficult to follow, and we could not decide whether they are also polarized. The end result is that a cluster of five cells express *lacZ* (Fig. 4G), except in the case of the multiply-innervated chemosensory bristles of the wing margin where larger clusters are formed. In the case of the aPA, it seems that just after the SMC has undergone its first mitosis a nearby cell begins to express *lacZ* and joins the cluster. This would indicate that the fifth cell is recruited, rather than generated from the SMC, in agreement with the results of the lineage analysis of microchaetes (Hartenstein and Posakony, 1989).

Discussion

(a) A101, a transformant fly that gives a faithful representation of SMC formation

An important question in the interpretation of the A101 pattern is the relation between *lacZ* expression and cell fate in this transformant. Does *lacZ* expression coincide with the commitment of an ectodermal cell to become SMC, or is it a distant consequence of that decision? Two lines of evidence suggest that *lacZ* expression is a very early step in the development of the SMC. First, the genes *ac* and *sc*, which are probably essential for the determination of ectodermal cells into SMC, are expressed in clusters of cells shortly before one of these cells begins to express *lacZ* in A101, indicating that *lacZ* expression may be nearly simultaneous with SMC determination (Gonzalez, Campuzano and Modolell, personal communication). Second and most crucial, the finding that in some cases two (or rarely more) cells show weak *lacZ* expression in places where only one SMC will eventually be found suggests that *lacZ* expression reveals a 'pre-SMC' stage when cells can still be diverted from the SMC pathway. This 'pre-SMC' stage may represent the beginning of SMC differentiation programme, when cells are already singled out from the surrounding proneural cluster but are still subject to lateral inhibition by another pre-SMC. This (reversible) pre-SMC stage might be called 'will' as opposed to the (irreversible) 'commitment'. It is not clear whether 'willing' cells represent a discrete stage in a stepwise process, between 'competent' and 'committed', or whether all these words single out intermediate stages in a continuous transformation that leads from ectodermal cell to SMC.

Weakly stained pairs of cells have been very rarely observed in the case of early SMCs (see, however, two L3-2 cells in Fig. 2D). This may be because less discs of that group were analysed. Alternatively, it may be that at early times, when the disc is small, the size of the proneural clusters is correspondingly smaller than at later stages and, therefore, the probability of two cells beginning to differentiate as SMCs at the same time is accordingly reduced.

(b) Comparison with earlier results

Our sequence of appearance of macrochaete SMCs is remarkably consistent with that obtained by Poodry (1975) for the terminal cell divisions. He found that in some flies the final mitosis had already taken place around 0 h APF for the pSC and to a lesser extent of the pDC, as observed by radioresistance data, in excellent agreement with our results (Table 1).

The order of terminal divisions also correlates well with the order of bristle sizes, supporting the view that bristle size simply depends on the period over which the bristle-forming cell can differentiate (Mglinetz and Kostina, 1978). The sequence of expression of *lacZ* agrees also with the seriation inferred from a recent perdurance analysis in *Abruptex* and *Notch* mutants (de Celis *et al.* 1991). Besides this general agreement on the sequence of events, there is some uncertainty about the time of determination. The results of perdurance analysis suggest that determination occurs 10–20 h before the time when we first see *lacZ* expression. The segregation of lineages between sense organ and epidermis would occur even earlier. However, the change in genetic make-up that is used to infer perdurance or lineage segregation occurs only in the daughters of the irradiated cell, so that any local variation of mitotic activity will affect substantially the interpretation of the results. Thus a simple explanation for the 10–20 h discrepancy between our results and those based on clonal analysis is a lower-than-average mitotic activity at the position where the SMC will form.

(c) Relation between the two SMCs of a heterochronic pair

A striking feature of the temporal pattern of SMC formation is the existence of pairs of nearby SMC formed at very different times, which we called 'heterochronic' pairs. We cannot exclude that these pairs are circumstantial. A more attractive hypothesis, however, is that in each heterochronic pair the late SMC forms at the edge of the zone of lateral inhibition (Wigglesworth, 1940) that is supposed to surround the early one (Richelle and Ghysen, 1979). This explanation is supported by several lines of evidence. First, each pair depends on a single control region of the AS-C (Ruiz-Gomez and Modolell, 1987, reviewed in Ghysen and Dambly-Chaudière, 1988) and corresponds to a single cluster of AS-C expression (Romani *et al.* 1989). Likewise L3-2 and its 2 followers, L3-1 and L3-3, depend on the same AS-C control region. Furthermore, in AS-C mutant where only one L3 sensillum develops,

it invariably forms at the L3-2 position; if two sensilla are present, one will be L3-2 and the second may be either L3-1 or L3-3 (Leyns *et al.* 1989). Second, in several cases such as the aDC-pDC or the aSC-pSC pairs, a bristle may form at an intermediate position when AS-C expression is reduced (Stern, 1956). Third, in an analysis of individual mutant flies Child (1935) observed a high correlation between the absence and the presence of the two bristles of an heterochronic pair, but no correlation between other bristles.

In each case, the late SMC of the pair appears three to four cells away from the early one. The relief from inhibition of the second SMC could simply result from the intercalary growth of the disc, leading to an extension of AS-C expression beyond the radius of inhibition, or from a temporal change in the pattern of expression of AS-C. Further separation of the SMCs, as for example in the case of the aSC-pSC or aDC-pDC, may involve additional intercalary growth, short-range migration or cell rearrangements or translocations similar to those that have been proposed to occur during evagination and elongation of the leg disc (Fristrom, 1976). In connection with the latter possibility it is worth mentioning that there is a drastic change in the shape of the wing disc around 5 h APF; whether this morphogenetic reshaping is achieved only by changes in cell shapes or involves cell movements is not known.

If our interpretation of the heterochronic pair is correct it indicates that, in the notum and wing veins, lateral inhibition plays a dual role in pattern formation: first, ensuring that only one SMC will form at each site; second, allowing late SMCs to form at regular distances from early ones. This patterning mechanism must obviously be silent in the case of tightly packed sense organs such as the campaniform sensilla on the dorsal radius, or the mechanosensory bristles on the wing margin.

(d) Lineage

SMCs formed early show a longer delay before dividing than SMCs formed later. This latency can last up to 30 h for the earliest SMCs. Once the first differentiative division has taken place, the subsequent steps seem to be very similar for all mechanosensory organs. The second differentiative divisions of the SMC follow the first after about 3 h, as has been reported to be the case of the chemosensory bristles on the wing margin and of the microchaetes on the notum (Hartenstein and Posakony, 1989). A comparison of our results with those of Murray *et al.* (1984) suggests that in the case of the campaniform sensilla of the wing, the terminal division is followed almost immediately by the onset of neural differentiation. This again is similar to the case of the notum microchaetes, where the onset of neuronal differentiation follows the terminal division by a few hours at the most (Hartenstein and Posakony, 1989).

The preferred orientation of the first division could be due to several factors. It might be part of the developmental programme specific to the SMC and be involved in the final polarity of the sense organ. This seems unlikely on several counts. First, this orientation (relative to the position of surrounding mother cells taken as landmarks) is not related to the direction of the bristle (relative to the position of surrounding bristles) in the adult. Second, the orientation preference is not absolute and divergences of up to 90° are occasionally observed, yet the direction of the bristle is invariant. A more likely explanation is that the orientation of the first differentiative division reflects an intrinsic polarity of the SMC resulting from the orientation of the mitosis that generated the mother cell (Hyman, 1989).

If this explanation is correct, it implies that the local orientation of mitosis is tightly controlled during the growth of the disc. Furthermore, since the orientation at a given site is the same for SMCs that may emerge over a period of 12 h or more, it appears that either the same direction is imposed locally for a relatively long time or mitosis is slowed down in the regions where mother cells will form.

A local decrease of mitotic activity has been observed along the dorsoventral boundary of the wing disc (O'Brochta and Bryant, 1985). Interestingly, this boundary corresponds to the presumptive wing margin, where one or more rows of tightly packed bristles will form. The long hairs of the posterior margin are not innervated, yet there is little doubt that they are structurally similar to the innervated bristles (Hartenstein and Posakony, 1989) and indeed mother cell markers such as A37 (Ghysen and O'Kane, 1989) or A101 are transiently expressed in a row of cells along that margin. Thus the zone of non-dividing cells that extends all along the wing margin defines a region where a continuous row of SMCs will form. Another case where mother cells seem to appear in regions of low mitotic activity is the microchaetes, in the dorsocentral region of the notum, where no cell divisions occur after puparium formation (Hartenstein and Posakony, 1989). Furthermore, the microchaete SMCs that form at the edge of the dorsocentral region arise within clusters of late replicating cells, suggesting that here again SMC formation is linked to a peculiar mitotic behavior.

These results raise the possibility that only mitotically quiescent cells can become SMCs. This prerequisite would readily explain the paradoxical result that, at least in some cases, lineage segregation seems to occur well before the SMC has been singled out. It would also account for the unexpected finding that ubiquitous expression of the gene *scute* in a sc^- fly leads to the formation of sense organs at roughly appropriate sites, even though the positional information normally provided by the localized expression of the *sc* gene is missing (Rodriguez *et al.* 1990).

We thank W. Gehring and his coworkers for making the A101 line available to us and J. Modolell for critical comments on the manuscript and for communicating results and ideas prior to publication. This work was supported by a contract between the Belgian government and the Université Libre de Bruxelles and by a grant from the Human Frontier Science Programme Organisation. A.G. is chercheur qualifié of the Fonds National de la Recherche Scientifique (Belgium).

References

BATE, C. M. (1978). Development of sensory systems in arthropods. In *Handbook of Sensory Physiology* vol. 9, pp. 1–53. (ed. M. Jacobson) Berlin, New-York: Springer Verlag.

BELLEN, H. J., O'KANE, C. J., WILSON, C., GROSSNIKLAUS, U., PEARSON, R. K. AND GEHRING, W. J. (1989). P-element mediated enhancer detection: a versatile method to study development in *Drosophila*. *Genes & Dev.* **3**, 1288–1300.

BODMER, R., CARRETTO, R. AND JAN, Y. N. (1989). Neurogenesis of the peripheral nervous system in *Drosophila* embryos: DNA replication patterns and cell lineages. *Neuron* **3**, 21–32.

BRYANT, P. J. (1975). Pattern formation in the imaginal wing disc of *Drosophila melanogaster*: Fate map, regeneration and duplication. *J. exp. Zool.* **193**, 49–78.

CHILD, G. (1935). Phenogenetic studies on *scute-1* of *Drosophila melanogaster*. I. The associations between the bristles and the effects of genetic modifiers and temperature. *Genetics* **20**, 109–126.

DE CELIS, J. F., MARI-BEFFA, M. AND GARCIA-BELLIDO, A. (1991). Cell autonomous role of *Notch*, an epidermal growth factor homolog in sensory organ differentiation in *Drosophila*. *Proc. natn. Acad. Sci. U.S.A.* (in press).

FRISTROM, D. (1976). The mechanism of evagination of imaginal discs of *Drosophila melanogaster*. III. Evidence for cell rearrangement. *Devl Biol.* **54**, 163–171.

GARCIA-BELLIDO, A. (1981). From the gene to the pattern: chaeta differentiation. In *Cellular Controls in Differentiation*, pp. 281–304 (ed: C. W. Lloyd and D. A. Rees) London: Academic Press.

GARCIA-BELLIDO, A. AND MERRIAM, J. R. (1971). Parameters of the wing imaginal disc development of *Drosophila melanogaster*. *Devl Biol.* **24**, 61–87.

GARCIA-BELLIDO, A. AND SANTAMARIA, P. (1978). Developmental analysis of the *achaete-scute* system of *Drosophila melanogaster*. *Genetics* **88**, 469–486.

GHYSEN, A. AND DAMBLY-CHAUDIÈRE, C. (1988). From DNA to form: the *achaete-scute* gene complex. *Genes & Dev.* **2**, 495–501.

GHYSEN, A. AND O'KANE, C. (1989). Detection of neural enhancer-like elements in the genome of *Drosophila*. *Development* **105**, 35–52.

HARTENSTEIN, V. (1988). Development of *Drosophila* larval sensory organs: spatiotemporal pattern of sensory neurons, peripheral axonal pathways and sensilla differentiation. *Development* **102**, 869–886.

HARTENSTEIN, V. AND POSAKONY, J. W. (1989). Development of adult sensilla on the wing and notum of *Drosophila melanogaster*. *Development* **107**, 389–405.

HYMAN, A. A. (1989). Centrosome movement in the early division of *Caenorhabditis elegans*: A cortical site determining centrosome position. *J. Cell Biol.* **109**, 1185–1193.

LEYNS, L., DAMBLY-CHAUDIÈRE, C. AND GHYSEN, A. (1989). Two different sets of *cis* elements regulate *scute* to establish two different sensory patterns. *Roux's Arch Devl Biol.* **198**, 227–232.

MARONI, G. AND STAMEY, S. C. (1983). Use of blue food to select synchronous, late third-instar larvae. *Drosophila Information Service* **59**, 142–143.

MAYNARD-SMITH, J. AND SONDHI, K. C. (1961). The arrangement of bristles in *Drosophila*. *J. Embryol. exp. Morph.* **9**, 661–672.

MGLINETZ, V. A. AND KOSTINA, I. V. (1978). Genetic control of bristle length in *Drosophila*. *Genetika* **4**, 285–293.

MURRAY, M. A., SCHUBIGER, M. AND PALKA, J. (1984). Neuron differentiation and axon growth in the developing wing disc of *Drosophila melanogaster*. *Devl Biol.* **104**, 259–273.

O'BROCHTA, D. A. AND BRYANT, P. J. (1985). A zone of non-proliferating cells at a lineage restriction boundary in *Drosophila*. *Nature* **313**, 138–141.

O'KANE, C. J. AND GEHRING, W. J. (1987). Detection *in situ* of genomic regulatory elements in *Drosophila*. *Proc. natn. Acad. Sci. U.S.A.* **84**, 9123–9127.

POODRY, C. A. (1975). A temporal pattern in the development of sensory bristles in *Drosophila*. *Wilhelm Roux's Arch. EntwMech. Org.* **178**, 203–213.

RICHELLE, J. AND GHYSEN, A. (1979). Bristle determination and pattern formation in *Drosophila*. I. A model. *Devl Biol.* **70**, 418–437.

RODRIGUEZ, I., HERNANDEZ, R., MODOLELL, J. AND RUIZ-GOMEZ, M. (1990). Competence to develop sensory organs is temporally and spatially regulated in *Drosophila* epidermal primordia. *EMBO. J.* **9**, 3583–3592.

ROMANI, S., CAMPUZANO, S., MACAGNO, E. AND MODOLELL, J. (1989). Expression of *achaete* and *scute* genes in *Drosophila* imaginal discs and their function in sensory organ development. *Genes & Dev.* **3**, 997–1007.

RUIZ-GOMEZ, M. AND MODOLELL, J. (1987). Deletion analysis of the *achaete-scute* locus of *Drosophila melanogaster*. *Genes & Dev.* **1**, 1238–1246.

SIMPSON, P. (1990). Lateral inhibition and development of sensory bristles of the adult peripheral nervous system of *Drosophila*. *Development* **109**, 509–519.

STERN, C. (1954). Two or three bristles. *Am. Sci.* **42**, 213–247.

STERN, C. (1956). The genetic control of developmental competence and morphogenetic tissue interactions in genetic mosaics. *Wilhelm Roux's Arch. Entwicklmech. Org.* **149**, 1–25.

WIGGLESWORTH, V. B. (1940). Local and general factors in the development of 'pattern' in *Rhodnius prolixus* (Hemiptera). *J. exp. Biol.* **17**, 180–200.

ZACHARUK, R. Y. (1985). Antennae and sensilla. In *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, vol. 6, pp. 1–69 (ed L. I. Gilbert and G. A. Kerkut) New York: Pergamon Press.

(Accepted 18 December 1990)