A gradient of affinities for sensory bristles across the wing blade of
Drosophila melanogaster

P. RIPOLL1, M. EL MESSAL2, E. LARAN1 and P. SIMPSON2
1Centro de Biología Molecular, CSIC-UAM, Facultad de Ciencias, Universidad Autonoma, Madrid, Spain
2Laboratoire de Génétique Moléculaire des Eucaryotes du CNRS, Faculté de Médecine, Strasbourg, France

Summary

The wing blade of Drosophila melanogaster is composed of dorsal and ventral surfaces covered with hairs and rows of morphologically distinct bristles round the margin. The mutant shaggy causes a complete transformation of hairs into bristles over the entire wing surfaces. Clones of mutant bristles have a tendency to line up into straight bristle rows. Clones are straight and orderly near the wing margin but form bundles and vesicles when a long distance from the margin. Furthermore the bristle cells move distally along the future wing blade in the general direction of the margin. From these studies, we postulate the existence of a gradient of cell affinities for bristle cells that is maximal at the dorsoventral wing margin and decreases with distance away from it. The bristle clones also move onto the wing veins and often induce the formation of new veins in the surrounding shaggy+ cells. These new veins run from the clone and join up to existing veins. We conclude that there is a close relationship between bristles and veins.

Key words: cell affinity, sensory bristle, wing blade, Drosophila melanogaster, positional information, pattern formation.

Introduction

The theory of positional information (Wolpert, 1969) states that spatial patterns in animal development are generated via two steps: (1) the specification of position within a tissue via a coordinate system and (2) a cell’s interpretation of its position leading to the differentiation of a specific structure. Several mechanisms have been invoked that might be capable of generating positional information and a popular idea has been via the establishment of a morphogenetic gradient (Child, 1941) that could be composed of a diffusible chemical (Crick, 1970). If positional information is specified by means of the concentration of a chemical substance then Wolpert points out that the cells can respond in two different ways. The continuous set of positional values can be interpreted by the cells in a discontinuous way, if the cells are sensitive to different thresholds in the concentration (Wolpert, 1971). This could lead, for instance, to the subdivision of a primary anteroposterior embryonic field into a series of repeated elements. Alternatively the continuous gradient of positional information could lead directly to a continuous graded response on behalf of the cells. If this were the case then one should be able to demonstrate gradients of some cell characteristics, such as cell adhesivity, for example. In fact, quite a number of examples of graded cell properties have been described (Wolpert & Stein, 1984).

The highly stereotyped two-dimensional patterns on the insect integument have made insects favourite organisms for the study of pattern formation. Early experiments of transplanting pieces of epithelium from one site to another have revealed that the same set of positional values is repeated in every segment and this led to the concept of a reiterated gradient system for insect segments (Locke, 1959; Stumpf, 1966; Lawrence, 1966b). Similarly it was shown that the same set of positional values is also repeated in the different imaginal discs of Drosophila (Bryant et al. 1978). In accordance with the idea of a graded cell response to the positional values, graded cell properties have been described. From her results of the rotation of epithelial transplants in Dysdercus, Nübler-Jung (1977) describes a gradient of differential cell adhesiveness. Similarly from their experiments of grafting in the wing of Manduca, Nardi & Kafatos (1976a) describe a proximodistal gradient of
adhesivity.

We describe experiments on the imaginal wing disc of *Drosophila*. The wing is composed of dorsal and ventral surfaces each bearing three pattern elements: sensory organs (bristles and campaniform sensilla), veins and hairs. The bristles or macrochaetae only form along the wing margin and presumably do so as a response to a specific positional cue in this position. Elsewhere hairs are differentiated. We make use of the mutation *shaggy* which causes all the hairs of the two wing surfaces to be transformed into bristles. We describe a gradient of cell affinities for bristle cells across the wing blade decreasing with distance from the margin.

Our results also provide further insight into the relationship between veins and bristles. Like bristles, veins occupy specific positions in the wing. They first appear in the pupal wing as lacunae, when the dorsal and ventral wing surfaces first come into contact, which progressively contract to be reduced to veins at the time of fusion of dorsal and ventral epithelia (Waddington, 1940). Garcia-Bellido (1977) studied vein development in various mutants that bear extra veins or have missing veins and his results led to the suggestion that vein determination results from a genetic decision that occurs autonomously in the wing epithelium and precedes bristle determination. The relationship between bristles and veins is not clear. The sensory nerves from the bristles (and campaniform sensilla) grow within the veins on their way to the central nervous system and it has been suggested that the veins may serve to guide the growing axons (Murray et al. 1984). Veins are present before the growth of nerve axons. Not all veins, however, carry nerves, although all nerves grow through veins (Murray et al. 1984). The innervated macrochaetae of the anterior wing margin actually develop on a vein. A study of mutants that develop ectopic bristles, elsewhere on the wing blade, such as *hairy* and *Hairy wing*, shows that these mutants are cell autonomous for bristle development (Garcia-Bellido & Merriam, 1971) and that axons from the ectopic bristles grow through an intervein area until they reach a vein (Palka et al. 1983). Our results point to a very close relationship between bristles and veins in that ectopic bristle clusters resulting from the mutation *shaggy* are able to induce new veins.

**Materials and methods**

Flies were raised on standard food medium and maintained at 25°C. Lethal mutations at the *zw3* locus were described by Judd et al. (1972) and mapped to 3B1. We have called this gene *shaggy* (*shg*) and will refer to it as *sgg* throughout this report. All alleles studied show a similar phenotype in clones. Here we have used *sgg*<sup>Y127</sup>, an EMS-induced allele isolated by P.R. For a description of other mutations and rearrangements, see Lindsley & Grell (1968). The cell marker *pawn* (*pwn*) is described in Garcia-Bellido & Dapena (1974).

Flies of the following genotypes were used:

1. *y sgg f<sup>E</sup>6<sup>e</sup>/+; pwn/+, (2) *y sgg f<sup>E</sup>6<sup>e</sup>/+, mwh jv/+,*
2. *y sgg f<sup>E</sup>6<sup>e</sup>, Ly+/+; 4, (4) *y sgg cf/f<sup>E</sup>6<sup>e</sup>, mwh jv/+, *
3. *y sgg f<sup>E</sup>6<sup>e</sup>/+, Lw<sup>i</sup>/+; 5, (6) *y sgg f<sup>E</sup>6<sup>e</sup>+/+, mwh jv/+;*
4. *y sgg f<sup>E</sup>6<sup>e</sup>/+, mwh ve h/mwh.*

In general, 24 h egg collections were made and flies were irradiated between 48 and 72 h after egg laying (AEL). For genotype (1) flies were irradiated at approximately 72 h and pupae were collected every 8 h in order to define precisely the time of irradiation with respect to pupariation. Results for this cross are given as hours before puparium formation (BPF).

Flies were irradiated with 1000 R of X rays, either by means of a Philips MG 151 Be, 100 kV, 15 mA, 2 mAl filter or a Philips MG 102 Be, 100 kV, 3 mA, 2 mAl filter, in order to induce mitotic recombination. The resulting clones were marked with *yellow, forked f<sup>E</sup>6<sup>e</sup>, pawn or multiple wing hairs* except for flies of genotype (5) in which the *sgg* mutation itself acts as its own marker (bristles in place of hairs). Wings were mounted between coverslips in Euparal. Clones and veins were drawn onto standard wing diagrams. Surface areas were measured by means of a planimeter.

**Results**

The differentiation of bristles and veins in *shaggy* clones

The wild-type wing of *Drosophila* displays a precise pattern of bristles, hairs, veins and a few campaniform sensilla (see Fig. 1). The blade is composed of dorsal and ventral wing surfaces covered almost uniformly by fine hairs. A series of morphologically distinct sensory bristles and large hairs is formed round the margin of the wing. The anterior bristles of the triple and double rows bear sockets and are innervated, whereas the long hairs of the posterior row and alula have no sockets and no axons. Clones of cells mutant for *shaggy* (*sgg*) induced by mitotic recombination later than 36 h BPF differentiate hairs, whereas clones induced before this time differentiate bristles anywhere on the surface of the wing blade. This is presumably due to the well-documented phenomenon of perdurance (Garcia-Bellido & Merriam, 1971), the later clones being composed of cells enclosing sufficient quantities of wild-type *sgg*<sup>+</sup> product, made prior to the recombination event, to differentiate normally. In Table 1, late-induced *sgg* clones have been grouped together according to their phenotype. Clones of up to about 10 cells are wild type (see Fig. 2A), clones of about 10 to 21 cells also develop hairs but these are spaced very close together (Fig. 2B). In a number of cases, clones made small vein-like structures composed of hairs spaced even
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Fig. 1. Morphology of the wild-type wing. The dorsal surface is shown. Small circles and dots represent the innervated bristles of the anterior margin. Co, costa bearing large bristles, TR, triple row of bristles, DR, double row of bristles. The posterior margin bears a double row of non-innervated hairs: PR (posterior row) and the alula (Af) bears a single row of very long hairs. All bristles and hairs point away from the thorax and toward the tip of the wing. I, II, III, IV and V represent the main wing veins. The boundary between anterior and posterior compartments is marked with a dotted line.

Table 1. Size and morphology of clones found on 100 wings resulting from irradiation at 16–48 h BPF of y sgg f36a/+; pwn/+ animals

<table>
<thead>
<tr>
<th></th>
<th>Trichomes: normal spacing n</th>
<th>Trichomes: reduced spacing n</th>
<th>Trichomes: vein-like* n</th>
<th>Bristles plus trichomes n</th>
<th>Bristles n</th>
</tr>
</thead>
<tbody>
<tr>
<td>y sgg f36a</td>
<td>10-1 ±1-8</td>
<td>23</td>
<td>21-1 ±2-3</td>
<td>28</td>
<td>30</td>
</tr>
<tr>
<td>pwn</td>
<td>32-2 ±5-9</td>
<td>83</td>
<td>25-6 ±2-7</td>
<td>26</td>
<td>12-5 ±1-8</td>
</tr>
</tbody>
</table>

Figures represent average numbers of marked trichomes.
* These are not always clearly marked with f36a.
† Size for this class is presented as number of trichomes plus number of bristles × 4.

Table 2. Mean size of y sgg clones (number of bristles) and f36a twin control clones (number of hairs) resulting from X-irradiation at 48–72 h AEL of y sgg/f36a, mwh jv/+ flies

<table>
<thead>
<tr>
<th></th>
<th>Anterior bristle clones</th>
<th>Posterior bristle clones</th>
</tr>
</thead>
<tbody>
<tr>
<td>y sgg f36a</td>
<td>56 ± 6</td>
<td>198 ± 24</td>
</tr>
<tr>
<td>y sgg f36a</td>
<td>54 ± 7</td>
<td>211 ± 27</td>
</tr>
</tbody>
</table>

more closely together (Fig. 2C). These are found in the expected frequency for clones but are not always clearly marked with the f36a cell marker. We conclude that the sgg cells are as yet unable to produce bristles but are able either to make veins or to induce other sgg+ cells to make veins. Slightly larger clones are composed of bristles (Fig. 2D) or a mixture of bristles and hairs. Clones induced prior to 48 h BPF make marginal bristles that may or may not be associated with veins, but never any hairs. The extra veins are never marked with f36a. The transformation to bristles is thus complete. Therefore, as the wild-type products are depleted, sgg cells undergo a transition from hairs to bristles and are able to induce the formation of extra veins.

Table 2 shows the mean size of y sgg and control f36a twin spots induced from 48 to 72 h AEL. In the anterior part of the wing sgg clones make anterior bristles and clone size (measured as number of bristles) is four times smaller than the size of f36a clones (measured as number of hairs). This confirms previous morphological studies that indicated a bristle organ to be composed of four cells: nerve cell, nerve sheath, bristle shaft and bristle socket (Bate, 1978; Lawrence, 1966a). Hairs, on the other hand, are each secreted by a single cell (Dhobzhansky, 1929). In the posterior part of the wing, sgg clones make large hairs typical of the posterior row and alula. Surprisingly, these clones too are four times smaller than control f36a clones in the same area, in spite of the fact that they are not innervated. Either these large hairs are also the product of bristle mother cell divisions, three of the cells either dying or not producing any structure, or else the sgg clones producing posterior marginal structures stop dividing earlier. A zone of non-proliferating cells has been described in the wing disc along the future wing
Fig. 2. y sgg f^{36a} clones induced late in development in flies of the genotype y sgg f^{36a}/+; pwn/+ . (A) Clone induced between 8 and 16 h BPF. The f^{36a} hairs can be seen to have normal spacing. (B) Clone induced between 16 and 24 h BPF. The hairs have now become more closely spaced. (C) Two clones, close to an extant vein, are shown, induced between 24 and 32 h BPF. Few if any hairs appear f^{36a} but the hairs are densely packed and have formed round vein-like structures. (D) Clone induced between 32 and 40 h BPF, marginal bristles and no hairs have been differentiated. The clones in A, B and C have been outlined.

Table 3. % Frequency with which y sgg clones fragment into separate pieces and move distally away from f^{36a} twin clone. Genotype 2: y sgg/f^{36a}, mwh jv/+ , irradiated at 48–72 h AEL. Genotype 6 (control twin spots): Dp(3;1)mwh+ f^{36a}/+; mwh ve h/mwh, irradiated at 72 ±2 h AEL.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Twin clones separated</th>
<th>Twin clones with y sgg distal to f^{36a} twin</th>
<th>Twin spots with y sgg clone in several fragments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>69</td>
<td>24</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>48*</td>
<td>3†</td>
</tr>
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<td></td>
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* The mwh clone was chosen arbitrarily and scored relative to the f^{36a} twin.
† The frequency of split f^{36a} and mwh clones was pooled and then divided by two.

margin (O’Brochta & Bryant, 1985). In this report, we shall refer to all marginal structures as bristles.

shaggy bristle clones move to be on veins
A study was made of the relative positions of y sgg and f^{36a} twin clones. The results are presented in Table 3. The two clones are frequently separated when compared to control non-sgg twin spots. Furthermore the sgg clone is more often distal to the control spot from which we conclude that the sgg cells
Table 4. Frequency with which clones are found on veins and are associated with extra vein material or with missing parts of veins. \(y^{sgg}/f^{36a}; mwh/j^v/+\) flies were irradiated at 48–72 h AEL.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>% clones on vein</th>
<th>% clones associated with extra veins</th>
<th>% clones associated with missing veins</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>(y^{sgg}f^{36a})</td>
<td>51</td>
<td>24</td>
<td>5</td>
<td>176</td>
</tr>
<tr>
<td>(y^{sgg})</td>
<td>44</td>
<td>0</td>
<td>0</td>
<td>133</td>
</tr>
</tbody>
</table>

move distally along the wing blade. Associated with this movement \(sgg\) clones frequently become split into two or occasionally three fragments. One place where these clones move to is onto veins because a greater proportion of them is found on veins (51%) when compared with control clones (44%), see Table 4. This difference taken by itself is not significant. However it becomes significant if we take into account the fact that the surface area of control clones, which are long and straight, is four times greater than that of \(sgg\) clones which are often rounded up (surface area in arbitrary units: \(sgg\) clones 0.40 ± 0.08, \(n = 176\), control clones 1.72 ± 0.04, \(n = 133\)). This means that for a total surface area of 70-4 units a vein was crossed 90 times in the case of \(sgg\) cells, whereas it was crossed only 58 times for a total surface area of 228.8 units in the case of \(f^{36a}\) control cells \((P < 0.01)\). Another indication of the affinity of bristles for veins is that, whereas \(sgg\) clones are straight near the wing border, they are round in more central positions, but occasional internal clones when on a vein line up to form bristle rows along the vein.

It also appears that \(sgg\) clones are clustered more distally on the veins: 10/30 clones on vein II were found on the distal portion, similarly 27/35 clones on vein III were distal, 8/14 on vein IV and 4/10 on vein V. This clustering can be seen in the clones represented in Fig. 3A. In order to verify that this apparent attraction for the veins is really due to the nature of a vein and not merely a function of those particular positions on the wing, \(sgg\) clones were studied in wings of animals mutant for \(fused\) \((fu^s)\) in which veins III and IV are fused together along much of their length. The results, shown in Fig. 3B, reveal that the clones now cluster on the fused III and IV vein.

shaggy clones induce new veins

Many \(sgg\) bristle clones cause the differentiation of extra veins (Table 4, Fig. 4A). Sometimes the bristles sit on the new vein but far more often the new vein extends away from the clone and in 95% of the cases \((n = 30)\) they join up to an existing vein (see Fig. 5). Like the majority of the normal veins, most of these new veins are roughly parallel to the proximodistal wing axis, and many of them extend from the clone in a proximal direction (Fig. 5). These extra veins may be associated with clones on either the dorsal or ventral wing surface. Mutant clones can also cause existing veins to become thickened (Fig. 4B) or to alter course (Fig. 4C). In the posterior part of the wing, extra veins are also formed from clones of posterior bristles which do not sit on a vein in the normal wing.

Occasionally \(sgg\) clones cause the removal of part of a regular vein (Table 4, Fig. 4D). The missing section in this case may be either distal or proximal to the clone (in 5 cases out of 9 it was proximal). All missing veins were found in the posterior compartment and were associated with clones on either surface.

shaggy bristles line up near the wing margins and are round in the central part of the wing blade

\(sgg^+\) clones of hairs, anywhere on the wing blade, have an aspect ratio of about 6 to 1 (they are never round) and are parallel to the proximodistal wing axis. \(y^{sgg}\) clones are normal in the wing margin itself.
Fig. 4. y sgg bristle clones from flies of the genotype y sgg/f36a; mwh jv/+ irradiated at 48–72 h AEL. (A) A clone that has induced the formation of an extra vein that runs from the clone and joins up to an existing vein. (B) A round sgg clone that is placed on a regular vein and has caused it to thicken greatly. (C) A clone that has caused a regular vein to alter course. (D) A posterior sgg clone that has caused part of a regular vein to fail to differentiate. The clones in A and C have been outlined.

Fig. 5. The orientation of extra veins induced by y sgg bristle clones on flies of the genotype y sgg/f36a; mwh jv/+ irradiated at 48–72 h AEL. The clones themselves are shown as shaded patches and are generally found at the open end of the new vein. It can be seen that the new veins join up with existing veins and that many of them run proximally from the clone.

The capacity of sgg bristles to line up into straight rows is particularly well illustrated for sgg clones that forming correct bristle rows. They are also ordered and show a lining up of the bristles into rows when close to the wing margin, see fig. 5 of Simpson et al. (1988). By contrast, clones further inside the wing blade round up as if trying to minimize contact with surrounding hairs (Fig. 6B). Sometimes the clones even form vesicles that are found between the two wing surfaces (Fig. 6C). (The distribution of round clones is shown in Fig. 3A.) In the very centre of the proximal part of the wing no clones are found. It is possible that they have moved elsewhere, or else that they have been lost by sloughing. Very few clones appear to be lost however; we found 10 f36a single clones and 37 y sgg single clones for 84 twin clones (from irradiation at 48 to 72 h AEL of y sgg/f36a; mwh jv/+ flies). The greater number of y clones is expected due to recombination between y and f36a.
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develop on the margins of wings mutant for Lyra (Ly). Ly wings have a large section of the anterior and posterior margin cut away and no bristles are formed on the new scalloped margin (Fig. 6D). y sgg marginal clones take advantage of this new edge to make straight single bristle rows (Fig. 6E; y sgg/f^{3oa}; Ly/+ flies were irradiated at 48 to 72 h AEL, n = 12 marginal clones). The mutation cut also causes scalloping of the wing margin. In a study of flies mosaic for cut, Santamaria & Garcia-Bellido (1975) found

Fig. 6. sgg bristle clones from flies of the genotype y sgg/f^{3oa}; mwh jw/+ (A–C), of the genotype y sgg/f^{3oa}; Ly/+ (E) or of the genotype y sgg cf/f^{3oa}; mwh jw/+ (F). (A) A straight, organized y sgg clone composed of posterior row bristles and running along a vein. (B) A rounded y sgg clone. (C) A clone of y sgg bristles that has formed a vesicle lying between the dorsal and ventral wing surfaces. (D) The anterior wing margin of a fly showing the scalloping due to the mutation Ly. (E) A y sgg clone on the scalloped Ly margin. The clone forms a straight single row of ventral bristles on the new margin. (F) A clone homozygous for y sgg and cf, no scalloping of the margin is apparent.
that 21% of clones homozygous for that mutant autonomously produced gaps in wing margin. We looked at clones of cells simultaneously homozygous for cf and sgg in flies heterozygous for these two mutants: $y$ sgg $CF^{\text{5fly}}$, $mwh$ jv/+; flies were irradiated between 48 and 72 h AEL. From a study of 23 marginal clones no scalloping was observed. Therefore sgg suppresses the scalloping effect by producing continuous marginal bristle rows (Fig. 6F).

**Discussion**

**Gradient of cell affinities**

Here we shall argue for the existence of a gradient of cell affinities for bristle cells across the wing blade, the affinity being maximal in the wing margin, where bristles normally form, and decreasing with distance from the margin. This is based on the observations that the clones move distally in the general direction of the wing margin, and on a study of the shape of sgg clones. The integration of mutant cells is maximal in the margin where clones are normal. In the region close to the margin, the majority of clones also becomes organized into more or less long straight bristle rows suggesting that cell affinities between transformed and wild-type cells are similar and that transformed cells can move around freely. Further within the wing, mutant bristles usually appear in bundles and the clones are round in shape. Since these cells are capable of forming rows, presumably they fail to do so because they are sufficiently different from the surrounding cells for them to be secluded in the smallest possible area. Sometimes these clones form vesicles that separate off from the wing epithelium and remain trapped between the dorsal and ventral surfaces. Finally there is a region in the centre of the wing where the transformed clones are very rare suggesting that they have moved away from there. They may have moved closer to the margin or have separated off from the epithelium and been lost. Clone frequencies suggest that very few are in fact lost. Cell affinities also explain why during migration the clones are not split into many small fragments (even though they may become separated into two or three fragments): they migrate as a group because their affinities for one another maintain them together.

There are two alternative explanations for this movement. One is that the transformed cells actively move to an area which is more compatible, i.e. to an area where the difference in cell affinities is tolerable. As this difference becomes smaller the closer the cells are to the border, clones are able to form an organized pattern that resembles the wild-type bristle rows. Alternatively the mutant cells are 'pushed' outwards by the normal cells that do not recognize them as normal neighbours. It seems likely that the movement is essentially passive since the direction of movement (distally) does not always represent the nearest route to the margin. It does however follow the main orientation of growth, normal clones being long and thin and orientated parallel to the proximodistal wing axis. Not all clones end up in the margin of course. The gradient also provides an argument for this, as when they approach the border the 'pushing' would become less efficient. Also clones can presumably only move between the time when bristle cells become determined (about 40 h before pupariation, Garcia-Bellido & Merriam, 1971) and the time of differentiation at metamorphosis.

If the distal movement of sgg clones does occur passively, then it would seem unlikely that such movement is comparable to that which drives sorting out and reaggregation of cells of similar affinity from dissociated imaginal discs (Garcia-Bellido, 1966; Gauger et al. 1985). Similarly, sorting out and vesicle formation also appear to be different processes (Fehon & Schubiger, 1985). Wing and haltere cells do not sort out (Fehon & Schubiger, 1985), but bithorax clones in the haltere form vesicles (Morata & Garcia-Bellido, 1976). Also engrailed clones in the posterior compartment can form vesicles (Morata et al. 1983) but engrailed cells and posterior cells do not sort out (Garcia-Bellido & Santamaria, 1972). Nevertheless, the formation of vesicles and the distal movement of sgg clones is likely to be mediated by cell affinities.

The work of Steinberg on cell aggregates suggests that cells with high affinity come to lie in a central position while cells of lowest affinity are found round the edges (Steinberg, 1970). A similar situation prevails for cell monolayers (Nicol & Garrod, 1979). It is noteworthy that the wing margin occupies a central position in the imaginal disc as shown by the fate map of the wing disc constructed by Bryant (1975). It is probable that intercellular affinities play an important role in shaping an organism. For example, differential adhesion may stabilize the sequential partitioning of the embryo into separate parts. Lawrence & Morata (1976) suggest that compartment boundaries in *Drosophila* may be maintained by different cell affinities between anterior and posterior compartments, since when the mutation engrailed changes posterior cells to anterior ones, these are then able to intermingle freely with anterior cells. It is probable that cell affinities are also important for pattern regulation in insects where cell movements occur. For example, squares of epithelium when rotated 180° will often rotate back into their original orientation (Bohn, 1974; Lawrence, 1974; Nübler-Jung, 1974). The distortion in pattern and polarity that occurs in many insects after transplantation of pieces of epithelium...
from one site to another are those expected if graft and host tissues differ in adhesiveness. While little cell movement occurs during pattern regulation in the imaginal discs of Drosophila, the occurrence of intercalary regeneration (French et al. 1976) shows the importance of local cell interactions and it is likely that properties of the cell surface mediate this.

A proximodistal gradient of cell affinities has also been demonstrated in the wing of Manduca by Nardi & Kafatos (1976a). These authors suggest that a gradient of differential adhesion (Steinberg, 1963) could be a mechanism whereby pattern is specified (Nardi & Kafatos, 1976b). They propose this as an alternative to the more popular models of a gradient of diffusion of some chemical substance (Lawrence et al. 1972). While it is clear that a diffusion gradient cannot account for all the results of intercalary regeneration in insect segments (French et al. 1976; Wright & Lawrence, 1981), it would seem more likely that a gradient of cell affinities, such as the one we describe, is a consequence, rather than a cause, of an underlying gradient of positional information whatever the means by which the position is specified.

Although the establishment of patterns in Drosophila imaginal discs involves very little cell movement there are indications of local movement of bristles late in development at pupariation. A striking example is that of the sex comb bristles on the male foreleg which rotate 90° relative to the rest of the leg (Tokunaga, 1962). Several studies suggest that movement of bristle cells is necessary for the generation of straight bristle rows. For example, on the tarsus, bristles in row one can be embraced by clones belonging to either the anterior or the posterior compartment (Lawrence et al. 1979). Also Held (1979) presents evidence that suggests that bristles may initially form a wiggly line which is later straightened by cell movements. Our results not only show that bristle cells move but that sgg bristle clones have an autonomous capacity to line up into rows when at or near the margin (see also Simpson et al. 1988).

The lining up of sgg bristles near the margin appears to be related to the juxtaposition of dorsal and ventral cells since clones on a scalloped margin, as in the case of Ly and ct, also form single bristle rows. It is interesting to note that axons from all the innervated bristles of the double and triple rows run through vein I which forms at the dorsoventral boundary, as does the costal nerve (Murray et al. 1984). It is thought that cell recognition and selective adhesion are important in determining the paths of axon outgrowth (Thomas et al. 1984). It is tempting to speculate that the dorsoventral boundary plays a role in axon guidance. Hopefully a more detailed analysis will provide further insight into this phenomenon.

**Vein induction**

It appears from our results that the determination of bristles and the formation of veins are very closely linked and that bristles are able to induce veins to form. First, clones of sgg bristles have an affinity for veins since they will move onto existing veins and sometimes cause a rerouting of the latter. Similarly ectopic bristles formed in the case of the mutants hairy and Hairy wing are also mostly found on veins (Garcia-Bellido & Merriam, 1971) and, in the case of mwh hairy clones, the bristles will also move out of the clone and onto veins (P. Ripoll, unpublished observations). Even when the veins form in abnormal positions, as in the case of fused wings, they still attract the sgg bristles. There does not appear to be any relationship between the veins and the gradient of cell affinities described in the previous section. Possibly some other influence is involved in the attracting of the bristle cells towards the veins. Second, sgg clones on either the dorsal or the ventral wing surface will induce the formation of new veins. These veins are made by wild-type cells. They generally develop in approximately the same proximodistal orientation as the normal veins and they join up to existing veins. The apparent polarized development of the new veins may be related to the gradient of cell affinities that we have described in the previous section. During pupal development veins develop through the progressive obliteration of central lacunae, a process that also extends proximalwards from the margin (Waddington, 1940). In apparent contradiction with the induction of new veins, sgg clones are sometimes associated with missing veins. It is not clear why this should be the case.

Although we have not verified this, it is tempting to speculate that the new veins enable sensory axons from the bristles to reach an existing vein and hence find their way to the central nervous system as is the case for the ectopic bristles in wings mutant for Hairy wing (Palka et al. 1983). However, extra veins also form from clones of posterior bristles. In the normal wing, these are not found on a vein and they do not make nerves. The posterior row and alula structures, however, in spite of being without sockets and nerves, may be a sort of abortive bristle and may be composed of four cells; they occasionally make a socket. It is also interesting to note that late-induced sgg clones which have insufficient sgg + product to enable them to produce hairs but were unable to make bristles, are nevertheless able to induce the formation of veins. We assume that these cells, which may have formed smooth cuticle, were present and were sufficiently 'bristle-like' to induce veins.

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References


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