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A study of *shaggy* reveals spatial domains of expression of *achaete–scute* alleles on the thorax of *Drosophila*

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Summary

A study of *shaggy* mutant clones on the notum reveals that a greater number of cells are diverted into the bristle pathway of differentiation and fewer cells remain to produce the epidermis. *shaggy* clones differentiate supernumerary microchaetae and macrochaetae but these are found in the correct spatial locations, e.g. clusters of macrochaetae are formed round the position of the extant macrochaetae. The *shaggy* mutant phenotype requires the functioning of the genes of the *achaete–scute* (AS–C) complex but a dosage study shows that it is unlikely that the AS–C is overexpressed in *shaggy* cells. Data are presented that argue, also, for a correct spatial expression of the AS–C in *shaggy* mutants. A study of clones doubly mutant for *shaggy* and different *achaete* and *scute* alleles is consistent with the hypothesis that the clusters of macrochaetae formed by *shaggy* represent the restricted spatial domains of expression of the AS–C. The results can be reconciled with the known role for the AS–C, in determining which bristle types differentiate where, and a role for *shaggy* in the cell interactions, within domains of the AS–C expression, leading to the definition of only one bristle mother cell.

Key words: *Drosophila*, development, pattern, mutant, *scute*, *shaggy*.

Introduction

A rather precise pattern of sensory bristles develops on the notum of *Drosophila*. Exactly eleven large bristles, or macrochaetae, each of which has been named, are found at defined positions on each heminotum. They are thought to develop as a response of the cells to their position within the field of positional information. The small bristles, or microchaetae, on the other hand, are indistinguishable from one another and their number varies slightly from fly to fly. They are, however, regularly spaced out and the spacing is thought to result from a phenomenon of lateral inhibition (Wigglesworth, 1940; Richelle & Ghysen, 1979; Held & Bryant, 1984).

The development of both macro- and microchaetae is known to be under the control of genes of the *achaete–scute* complex (AS–C). *achaete* is required mainly for the formation of the microchaetae, whereas *scute* is required mainly for the formation of the macrochaetae (Garcia-Bellido, 1979). The functions of these two genes, however, overlap considerably and the full pattern requires both. Different alleles of these genes result in the loss of specific bristles and there exist also hypermorphic alleles, *Hairy wing*, that result in overexpression and cause the production of supernumerary bristles (Campuzano *et al.* 1986; Garcia-Alonso & Garcia-Bellido, 1986; Balcells *et al.* 1988). Two other, unlinked, genes, *extra macrochaetae* and *hairy*, are thought to act as repressors of *scute* and *achaete*, and loss of function of either of these genes also results in the production of either supernumerary macro- or microchaetae (Moscoso del Prado & Garcia-Bellido, 1984). It is thus thought that the AS–C is involved in the decision to differentiate bristles in defined places (Garcia-Bellido & Santamaria, 1978). The spatial restriction of the AS–C expression would thus be the main factor that controls the basic pattern of sensory bristles [as well as other sensory organs and also some central neurones (Dambly-Chaudière & Ghysen, 1987; Garcia-Bellido & Santamaria, 1978; Cabrera *et al.* 1987; Romani *et al.* 1987)].

A molecular analysis of the AS–C (Carramolino *et al.* 1982) led to the description of several transcripts only two of which correspond to *achaete* and *scute*, respectively (Campuzano *et al.* 1985). Many *achaete* and *scute* alleles are rearrangements with break points scattered throughout the complex and mapping some distance away from the coding regions. A comparison of the position on the map of a number of terminal deficiencies and the mutant phenotype led Ruiz-Gomez & Modolell (1987) to suggest that the *scute* transcript is regulated by *cis*-acting site-specific elements that respond to topological cues. Therefore, different control sites would activate *scute* in each precise area where a bristle will form. Presumably this area will involve a

small region of the epidermis that is nevertheless considerably bigger than simply the one cell destined to become the macrochaete in question (Ghysen & Dambly-Chaudière, 1988; Stern, 1954*a*,*b*) and, accordingly, Romani, Campuzano, Macagno & Modolell (in preparation) observed by *in situ* hybridization, expression of *achaete* and *scute* in groups of cells in the imaginal wing disc.

Here we make use of the mutation *shaggy* to explore the spatial domains of expression of some *scute* alleles on the notum. Clones of cells mutant for *shaggy* transform epidermal cells into bristles and thus produce vast numbers of both macro- and microchaetae with little or no spacing between them. Different types of bristles, however, are produced in different locations and it has been shown that, although *shaggy* cells are unable to make a correct decision between an epidermal cell pathway and that of a sensory bristle, they are nevertheless able to respond correctly to positional cues (Simpson *et al.* 1988). On the notum, *shaggy* cells produce clusters of macrochaetae around the position of the extant ones. Here we show that the AS–C is required for this mutant phenotype but that *achaete* and *scute* are probably not overexpressed and that the spatial expression of these genes remains normal. A study of double mutant clones reveals that the clusters of macrochaetae formed by *shaggy* clones correspond to the domains of expression of different AS–C alleles. We also show that *shaggy* acts synergistically with *extra macrochaetae*, and may indirectly influence the expression of the AS–C via *extra macrochaetae*.

Materials and methods

Flies were raised on standard medium and maintained at 25 °C.

Lethal mutations at the *zw3* locus were described by Judd *et al.* (1972) and map to 3B1. We have called this gene *l(1)zw3*^{sgg} (*shaggy*) (Simpson *et al.* 1988). Throughout this report we have used the amorphic allele *sgg*^{D127} (isolated by P. Ripoll), which we will refer to simply as *sgg*. In one single experiment, another allele, *sgg*^{b12} (caused by an inversion, Judd *et al.* 1972), was also used and this is specified in the text (Table 5). For a description of other mutations and rearrangements see Lindsley & Grell (1968) and Lindsley & Zimm (1985), DIS vols 62, 64 and 65.

Clonal analysis

Clones mutant for *sgg* were produced in various genetic backgrounds by X-ray-induced mitotic recombination. Unless otherwise specified in the text, 24 h egg collections were made and flies were irradiated between 48 and 72 h AEL. The clones resulting from mitotic recombination were marked with *yellow* (*y*), *forked* (*f*^{36a}), *javelin* (*jv*), *multiple wing hair* (*mwh*) or *stubbly chaete* (*stc*). Flies were irradiated with 1000 R of X-rays (100 kV, 4 mA given for 3 min 18 s, 1·5 mm aluminium filter, Philips MG102 constant potential X-ray system, beryllium window). Thoraces were heated in 10% KOH and mounted between coverslips in Euparal. Clones were drawn onto standard diagrams of the notum. Clones were induced in flies of the following genotypes:

(1) *sgg f*^{36a}/*y*
(2) *y sgg f*^{36a}/+
(3) *Df(1)sc*^{19}, *y*$^{-}$ *sgg f*^{36a}/+; *mwh jv*/+
(4) *sc*^{1} *sgg f*^{36a}/*y sc*^{1} *v*
(5) *sc*^{1} *sgg f*^{36a}/*y*
(6) *y ac*^{1} *sgg w f*^{36a}/*ac*^{3}; *stc*/+
(7) *y ac*^{1} *sgg w*/*f*^{36a}
(8) *y sgg f*^{36a}/+; *emc*^{1}/*TM2, emc*
(9) *y sgg f*^{36a}/+; *emc*^{1}/+
(10) *y sgg f*^{36a}/+; +/+
(11) *y sgg f*^{36a}/+; *h*^{1}/*h*^{1}
(12) *y sgg f*^{36a}/+; *h*^{1}/+
(13) *Df(1)sc*^{19}, *y*$^{-}$ *sgg f*^{36a}/+; *Dp(1; 2)sc*^{19}, *y*$^{+}$/+
(14) *y sgg*/+; *CyO*/+
(15) *y sgg f*^{36a}/+; *CyO*/+
(16) *y sgg f*^{36a}/+; *Dp(1; 2)sc*^{19}, *y*$^{+}$/+
(17) *Dp(3; Y; 1)M2, emc*$^{+}$*y sgg w v*/+
(18) *sgg f*^{36a}/+

Flies of genoptypes (8), (9) and (10) were obtained from the cross ♀ *y sgg f*^{36a}/*FM6*; *TM2, emc*/+ × ♂ *emc*^{1}/+. *TM2* carries a weak allele of *emc* that is viable over *emc*^{1} (Moscoso del Prado & Garcia-Bellido, 1984). *emc*^{1}/*TM2* flies therefore have an *emc* phenotype. Flies of genotype (9) can be distinguished from those of genotype (10) by virtue of the synergism between *sgg* and *emc* (see Results); they invariably display at least one extra macrochaete on the thorax. Flies of genotypes (11) and (12) were obtained from the cross ♀ *FM6*/*y sgg f*^{36a}; *h*^{1}/+ × ♂ *h*^{1}/*h*^{1}. Flies of genotypes (13) and (14) were obtained from ♀ *Df(1)sc*^{19}, *y*$^{-}$ *sgg f*^{36a}/*FM6* and *y sgg*/*FM6* that were both crossed to *Dp(1; 2)sc*^{19}, *y*$^{+}$/*CyO* males and placed in the same bottles. Flies of genotypes (15) and (16) were obtained from ♀ *y sgg f*^{36a}/*FM6* × ♂ *Dp(1; 2)sc*^{19}, *y*$^{+}$/*CyO*. Flies of genotypes (17) and (18) were obtained from ♀ *FM7*/*Dp(3; Y; 1)M2, emc*$^{+}$ *y sgg w v* and ♀ *sgg f*^{36a}/*FM6* that were both crossed to Ore R males and grown in the same bottles.

Gene dosage studies

For the study of the phenotype of *emc*^{1}/*TM2, emc* flies bearing different doses of *sgg*$^{+}$ (Table 6), the following cross: ♀ *sgg w*/*y w*; *TM2*/+ × ♂ *y w*/Y; *Dp(1; 2)w*$^{+70}$, *sgg*$^{+}$/+; *emc*^{1}/+, yielded flies of genotypes (19) *sgg w*/*y w*; +/+; *emc*^{1}/*TM2, emc*, (20) *sgg w*/*y w*; *Dp(1; 2)w*$^{+70}$, *sgg*$^{+}$; *emc*^{1}/*TM2, emc*, and (21) *y w*/*y w*; *Dp(1; 2)w*$^{+70}$, *sgg*$^{+}$/+; *emc*^{1}/*TM2, emc*. These were all distinguishable, flies of genotype (19) are *w*, those of genotype (20) are *w*$^{+}$, those of genotype (21) are *y*. The frequency of recombination between *y* and *sgg* is of the order of only 1%.

For the study of the phenotype of flies heterozygous for both *sgg* and *emc* and bearing different doses of *achaete–scute* (Table 7), two crosses were performed. From ♀ *Df(1)260-1*, *y*$^{-}$/+; *emc*^{1}/*TM2, emc* × ♂ *y sgg f*^{36a}/*Dp(1; Y)w*$^{+}$*sgg*$^{+}$, flies of genotypes (22) and (23) were generated: (22) *Df(1)260-1*, *y*$^{-}$/*y sgg f*^{36a}; *emc*^{1}/+, (23) *y sgg f*^{36a}/+; *emc*^{1}/+. From ♀ *FM6*/*y sgg f*^{36a}; *Dp(1; 2)sc*^{19}/*CyO* × ♂ *emc*^{1}/*TM2*, flies of genotypes (24), (25) and (26) were obtained: (24) *y sgg f*^{36a}/+; *CyO*/+; *emc*^{1}/+, (25) *y sgg f*^{36a}/+; *Dp(1; 2)sc*^{19}/+; *emc*^{1}/+, (26) *FM6*/+; *Dp(1; 2)sc*^{19}, *y*$^{+}$/+; *emc*^{1}/+.

Results and discussion

shaggy *mutant clones reveal a precise pattern of bristles on the notum*

Clones of cells mutant for *shaggy* (*sgg*) were produced on the dorsal thorax by mitotic recombination. These produce dense clusters of extra bristles (see Figs 1, 2). A study of *sgg f*^{36a} clones and y control clones issued

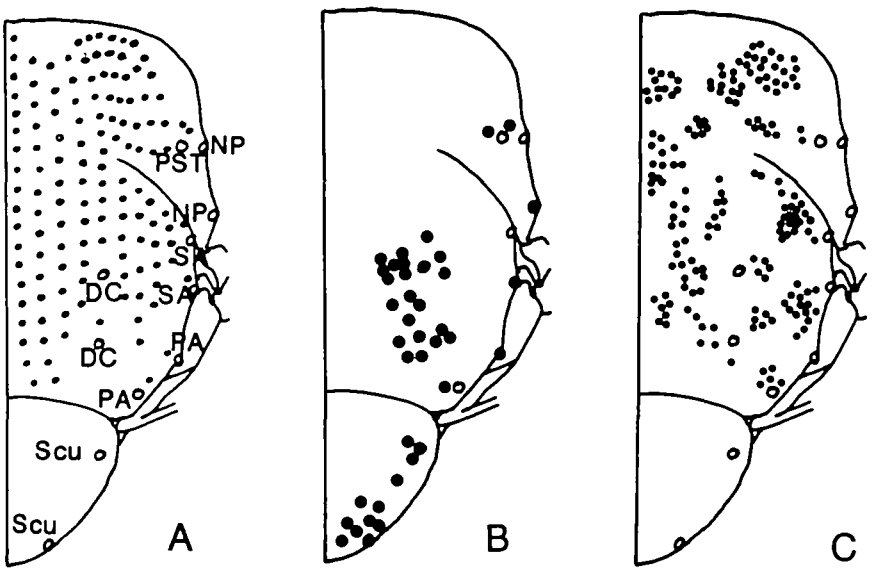

Fig. 1. (A) Standard diagram of the wild-type heminotum of *Drosophila* showing the positions of macrochaetae, large circles, and microchaetae, small circles. The macrochaetae are named as follows: DC, dorsocentral; Scu, scutellar; PA, postalar; SA, supraalar; NP, notopleural; PST, presutural. (B) The distribution of macrochaetae, large dots, observed in a study of 100 *y sgg* f^{36a} clones induced in *y sgg* f^{36a}/+ flies. The flies were irradiated between 48 and 72 h AEL, the average clone size was 16·4 ± 2·0 bristles, the largest clone comprised 69 bristles. Clones are a mixture of micro- and macrochaetae, only the macrochaetae are shown. These can be seen to be clustered around the positions of the extant macrochaetae. More bristles are found in the positions of the dorsocentral and scutellar bristles than in the positions of the other macrochaetae. It is thought that some clones in these regions may be lost, see text. Examination of a much larger sample of clones, however, shows that macrochaetae also arise in clusters around the postalar, supraalar, notopleural and presutural bristles (results not shown). (C) The distribution of microchaetae, small dots, observed from a study of the same 100 *y sgg* f^{36a} clones as in B. 15 clones are shown. The bristles, which develop at a high density, can be seen to differentiate in areas normally covered by microchaetae. No microchaetae are found on the scutellum or in the vicinity of the posterior postalar bristle.

Table 1. *Size of twin clones resulting from irradiation-induced mitotic recombination at 72 ± 2 h AEL in* sgg f^{36a}/y *flies*

Average number of bristles ± S.E.		
sgg f^{36a}	*y*	Number of clones
7·9 ± 0·5*	4·2 ± 0·3	61

* $P < 0{\cdot}01$ when compared with controls.

Table 2. *The spacing between bristles of* y sgg f^{36a} *clones induced in* y sgg f^{36a}/+ *flies measured by counting the number of intervening trichomes. As a control, spacing was measured between wild-type bristles in the same position as the clone on the other hemithorax*

sgg	Control wild-type bristles	Number of clones
2·7 ± 0·23*	4·9 ± 0·12	30

* $P < 0{\cdot}01$ when compared with controls.

from the same recombination event (twin spots) at 72 h AEL shows that about twice as many bristles are made by the mutant cells (Table 1). The surface area occupied by the *sgg* clones is not enlarged, however. Rather, the spacing between the bristles is reduced and fewer trichome-producing epidermal cells are present (Table 2). Therefore a greater number of cells are diverted into the pathway for sensory bristles, and fewer cells are left to make the epidermis. In the area surrounding the *sgg* clones, as on the remainder of the thorax, the pattern of bristles remains normal. Control clones produced marked bristles in normal positions and caused no disruption of the pattern.

Clones of *sgg* cells produced earlier than 72 h AEL show a tendency to round up and contract. Sometimes vesicles of cuticle bearing marked bristles are found inside the thoraces that carry *sgg* clones. This is perhaps due to different cell affinities such as those described for some areas of the wing (Ripoll *et al.* 1988). Differential cell affinities may also be the reason why many *sgg* clones are found in the centre of the thorax and fewer clones are found in the region of the postalar, supraalar and notopleural bristles (see Fig. 1B). Cell proliferation is probably unaffected in *sgg* mutant cells: a study of twin spots on the wing blade where accurate cell measurements can be made revealed clones of expected sizes (Simpson *et al.* 1988).

Both extra microchaetae and macrochaetae are formed in *shaggy* clones. They develop, however, at fairly precise locations. The positions of micro- and macrochaetae from 100 *y sgg* f^{36a} clones were drawn onto standard diagrams. On areas of the notum where microchaetae normally differentiate, *sgg* clones too produce microchaetae (Figs 1C, 2B). Microchaetae, therefore, although formed at a greater density, develop in those parts of the thorax that are normally covered with them. Microchaetae are not formed in ectopic positions, e.g. no microchaetae differentiate on the scutellum. Therefore, the basic spatial distribution is unaltered. In the regions surrounding the positions of the extant macrochaetae *sgg* clones produce both macrochaetae and microchaetae. Macrochaetae, however,

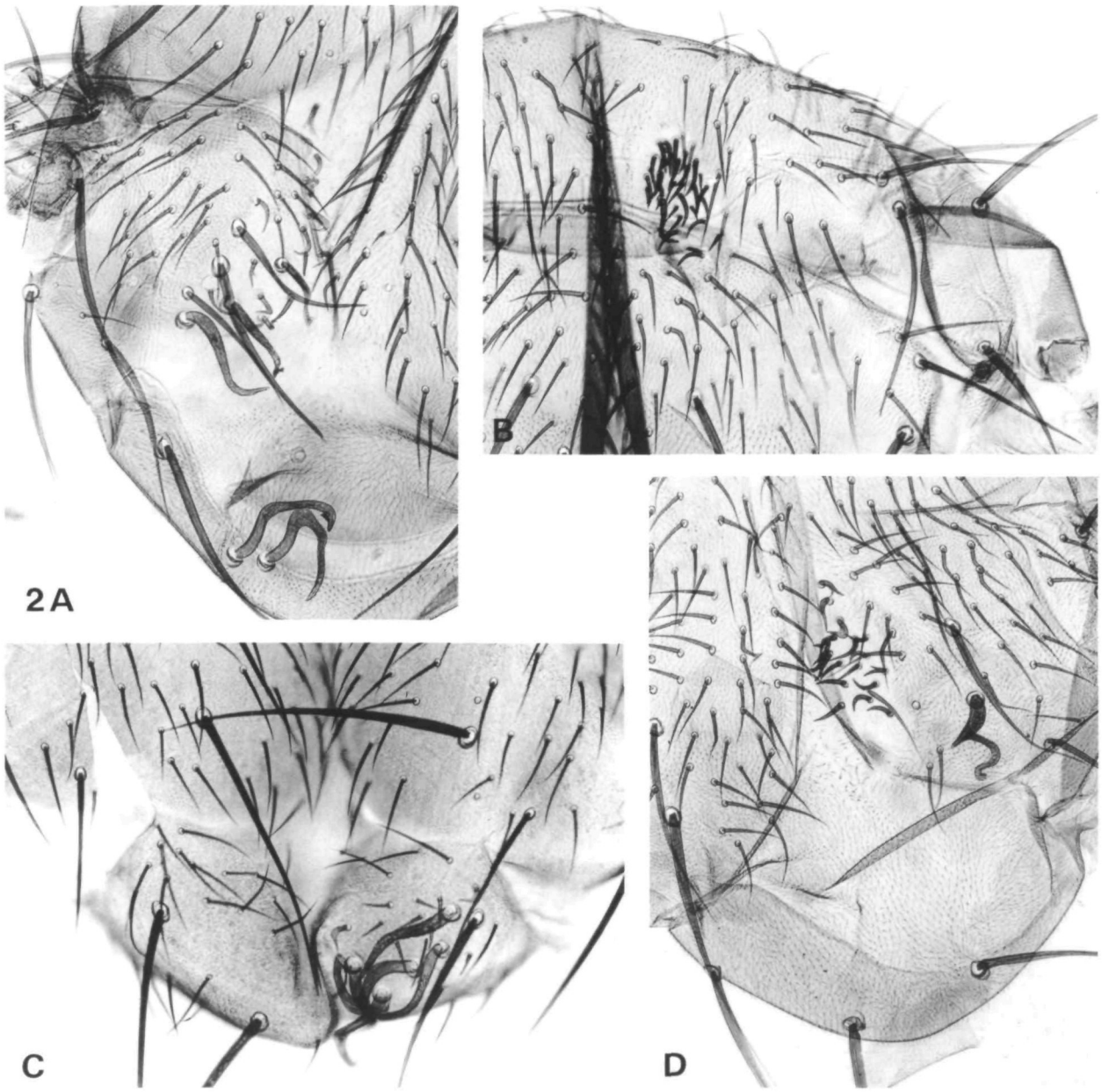

Fig. 2. (A) A *y sgg* f^{36a} clone induced in a *y sgg* $f^{36a}/+$ fly after irradiation between 48 and 72 h AEL. Both macrochaetae and microchaetae have been formed. The macrochaetae can be seen to be clustered around the dorsocentral and the posterior scutellar bristles. No microchaetae have been formed on the scutellum. (B) A *sgg* f^{36a} clone induced in a *sgg* f^{36a}/y fly after irradiation between 48 and 72 h AEL. A group of microchaetae are seen in the anterior part of the thorax. In this region *sgg* clones never differentiate macrochaetae. (C) A *y sgg* f^{36a} clone induced in a *y sgg* $f^{36a}/+$; h^1/h^1 fly after irradiation between 48 and 72 h AEL. In the mutant h^1 background, *sgg* clones are able to differentiate microchaetae on the scutellum. (D) A sc^1 *sgg* f^{36a} clone induced in a sc^1 *sgg* f^{36a}/y fly after irradiation between 48 and 72 h AEL. A marked posterior dorsocentral bristle and a cluster of microchaetae are found juxtaposed to a scutellum which is lacking the anterior scutellar bristle. The absence of this bristle is thought to be a consequence of cells of the mutant clone extending over the scutellum: the sc^1 mutant, which lacks scutellar bristles, is epistatic in phenotype over that of *sgg*.

are only found clustered around the positions of the extant macrochaetae (Figs 1B, 2A), they do not differentiate elsewhere. This is particularly clear for the dorsocentral and the scutellar bristles, to which we will confine the rest of our analysis. Therefore as with the microchaetae, the basic spatial distribution of macrochaetae is unaltered in the *sgg* mutant. These observations therefore suggest that, although extra bristles are formed by *sgg* cells, the basic spatial organization that dictates which type of bristle is to be placed where remains unaltered. It has in fact been shown that *sgg* cells on the wing blade know where they are and respond correctly to positional cues (Simpson *et al.* 1988).

The shaggy *phenotype requires the function of* achaete-scute *but is not attributable to an overexpression of* achaete-scute

The decisions governing which type of bristles are to be placed where on the notum are thought to fall under the control of the genes of the *achaete-scute* complex (AS-C). In the absence of the AS-C no bristles form (Garcia-Bellido & Santamaria, 1978). The *Hairy wing* (*Hw*) alleles of the AS-C represent a gain of function. They cause an overexpression of either *achaete* (*ac*) or *scute* (*sc*) and lead to the production of extra micro- or macrochaetae (Balcells *et al.* 1988). Their phenotype superficially, therefore, resembles that of *sgg*. (There are, however, important differences that will be discussed later). It was therefore of interest to determine whether or not the AS-C is expressed normally in *sgg* mutant cells or whether the *sgg* phenotype could be attributed to an overexpression of the AS-C. To address the question of whether the AS-C is required for the phenotype of *sgg*, clones doubly mutant for *sgg* and a deletion of the AS-C were made. Clones were induced in *y Df(1)sc*^{19} *sgg f*^{36a}/+; *mwh jv*/+; flies [*Df(1)260-1* was also used and gave identical results to those with *Df(1)sc*^{19}, data not shown]. In the first experiment, 50 thoraces from a late irradiation (72 ± 4 h AEL) were mounted and scored for the presence of clones. A total of 29 *mwh jv* clones were observed together with 25 naked patches presumed to be AS-C$^-$ *sgg* clones. In order to establish that this result pertains to all bristles of the notum, a further 34 naked patches, from flies irradiated between 48 and 72 h AEL, were selected under the dissecting microscope. Such presumed clones covered most of the area of the notum and no *y f*^{36a} bristles were ever seen. The AS-C is therefore epistatic over *sgg* for the thoracic phenotype and the supernumerary bristles seen in *sgg* clearly require the wild-type *achaete* and *scute* functions. In order to test the hypothesis that *ac* and *sc* might be overexpressed in *sgg* mutant cells, a gene dosage study was performed. Flies carrying only one dose of *sgg* and one, two, three or four copies of the AS-C$^+$ were constructed and found to display a wild-type appearance (results not shown). Furthermore, flies heterozygous for the AS-C and carrying one, two or three copies of *sgg*$^+$ are also wild-type (results not shown). Finally the phenotype of *Hw*^{1}, a gain-of-function allele of *ac*, remains unaltered in the presence of one, two or three doses of *sgg*$^+$ (results not shown). As a further test, clones mutant for *sgg* were made in flies bearing one, two or three copies of the AS-C and their phenotype was found to be unchanged (Table 3). From these observations, we conclude that *sgg* plays no regulatory role concerning the expression of the AS-C. It therefore seems unlikely that the *sgg* phenotype is due to a derepression of the AS-C.

Not all of the bristles found in the adult fly require the function of the AS-C. For example, many of the bristles of the wing margin, medial and dorsal triple row bristles and bristles of the double row differentiate in marginal clones that are AS-C$^-$ (Garcia-Bellido & Santamaria, 1978). *shaggy*, on the other hand, affects all bristle types found on the fly body including those of the wing margin, such that *sgg* clones on the wing blade transform epidermal hairs into marginal bristles (Simpson *et al.* 1988). AS-C$^-$ clones on the wing blade (away from the wing margin) differentiate trichomes. It was therefore possible to test the epistatic relationship between *sgg* and AS-C for this part of the body. Doubly mutant AS-C$^-$ *sgg* clones on the wing blade have a *sgg* phenotype, that is they differentiate bristles (results not shown). Therefore the AS-C appears to be epistatic over *sgg* only in those regions of the body in which the AS-C is required. In other words, the *sgg* phenotype does not result from a derepression of *ac* or *sc* such that these genes are ectopically expressed. These results argue again that the spatial expression of AS-C elements is not altered in the *sgg* mutant, and this would therefore explain why on the notum, although there are more bristles than is usual in *sgg* clones, the bristles are nevertheless formed in the correct general positions. *shaggy* is perhaps therefore involved in some other aspect of bristle differentiation, and plays a different role from that of proneural genes such as the AS-C for the notum [and other, perhaps related, genes (Villares & Cabrera, 1987; Alonso & Cabrera, 1988) for the wing] that govern the spatial distribution of morphologically distinct bristles.

Table 3. *Size of* shaggy *clones in flies bearing one, two or three doses of AS-C, irradiated between 48 and 72 h AEL*

Genotype*	Number of doses AS-C	Number of bristles per clone ± S.E.	Number of clones
(13)	1	18·6 ± 2·0	38
(14)	2	17·3 ± 2·5	49
(15)	2	18·9 ± 2·8	34
(16)	3	23·2 ± 2·0	52

* Flies of genotypes (13) and (14) were grown together in the same bottles and flies of genotype (15) and (16) were siblings, see Materials and methods.
(13) *Df(1)sc*^{19}, *y*$^-$ *sgg f*^{36a}/+; *Dp(1; 2)sc*^{19} *y*$^+$/+
(14) *y sgg*/+; *CyO*/+
(15) *y sgg f*^{36a}/+; *CyO*/+
(16) *y sgg f*^{36a}/+; *Dp(1; 2)sc*^{19} *y*$^+$/+

The shaggy *pattern reveals the spatial domains of expression of different* scute *alleles*

Stern (1954*a*,*b*) made a study of clones mutant for *ac* which removes the dorsocentral bristles. Such clones behave autonomously. When the mosaic border line runs through the dorsocentral region, however, occasionally a bristle will form at some distance from the normal site. This locally restricted nonautonomy led Stern to suggest that the macrochaete position is first defined as a region and later narrowed down to a single cell. Our observations on *sgg* clones are consistent with this idea; the clusters of extra macrochaetae seen in *sgg* suggest the presence of a small region around each extant bristle, the limits of which specify position for that bristle. This supports the notion that determination of a bristle results from a collective decision of a group

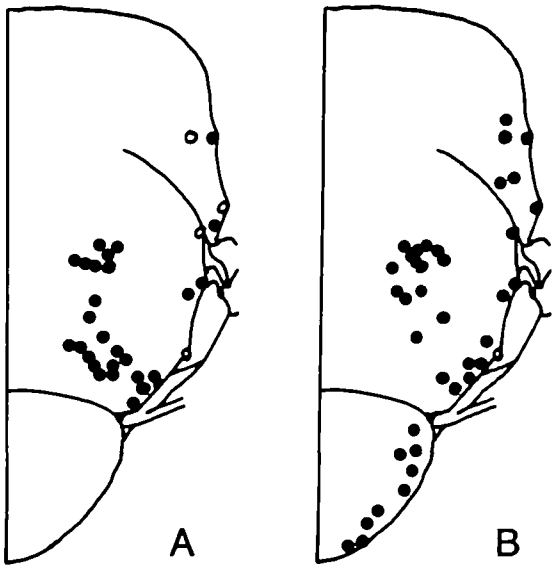

Fig. 3. (A) The distribution of macrochaetae (closed circles) from 90 *sgg* clones marked with f^{36a} and induced in sc^1 *sgg* f^{36a}/y sc^1 *v* flies. Flies were irradiated between 48 and 72 h AEL and the average clone size was 15·5 ± 2·6 ($n = 54$). In sc^1 flies, in which the scutellar bristles are absent, the *sgg* clones, which are also mutant for sc^1, fail to differentiate bristles anywhere on the scutellum unlike the *sgg* clones induced in sc^+ flies in Fig. 1B. Elsewhere the clones show the typical *sgg* phenotype. (B) The distribution of macrochaetae from 89 *sgg* clones marked with *y* f^{36a} and induced in *y* ac^1 *sgg w* f^{36a}/ac^3; *stc*/+ flies. Flies were irradiated between 48 and 72 h AEL and the average clone size was 15·1 ± 2·1 ($n = 58$). Few clones produce macrochaetae in the region of the posterior dorsocentral bristle when compared with those differentiated by *sgg* clones in an ac^+ background (Fig. 1B). The presence of occasional *sgg* bristles at or close to the site of the posterior dorsocentral bristle can be attributed to the hypomorphic nature of the ac^1 and ac^3 mutations; such bristles are often intermediate in size, see text.

of cells in a defined area of the epidermis. Such a group of cells can be called an equivalence group (Palka, 1986). It is then thought that cell interactions between members of the group will lead to the determination of only one bristle mother cell which then inhibits its neighbours from also becoming bristles by a mechanism of lateral inhibition (Wigglesworth, 1940). A similar mechanism appears to govern the segregation of neuroblasts in the embryo (Doe & Goodman, 1985).

A number of *ac* and *sc* alleles have been described that each remove a specific bristle or a subset of bristles on the thorax. Many of these alleles are due to chromosomal rearrangements with break points at some distance from the coding regions (Campuzano *et al.* 1985). The *sc* break points are thought to affect *cis*-acting site-specific control elements that govern the expression of the *sc* transcript (Ruiz-Gomez & Modolell, 1987). This would mean that each control site specifically activates *sc* in the area in which the bristle under its control is destined to be produced. It is unlikely that this activation of *sc* occurs precisely in the one cell that will become the bristle. Rather *sc* would be activated in all the cells of the equivalence group that is responsible for that bristle. In other words, the size of the equivalence group would correspond to the number of cells expressing *sc*. It has in fact been observed that *sc* transcripts are found in groups of cells in the areas of the imaginal disc where each bristle will later form (Romani, Campuzano, Macagno & Modolell, in preparation). It has also been observed in the embryo that *sc* transcripts are found in groups of cells from which one will segregate as a neural precursor (Cabrera *et al.* 1987).

If *sc* is thus expressed in small regions over the thorax and if, as we have argued, this regional control is unaltered in *sgg*, then the clustering of macrochaetae around the position of the extant bristles seen in *sgg* clones would represent the spatial domains of expression of *sc*. In order to explore this possibility, we have made clones simultaneously mutant for *sgg* and specific *ac* or *sc* alleles. We chose sc^1, which removes the two scutellar bristles and the anterior notopleural, and ac^1 and ac^3, which remove the posterior dorsocentral bristle.

shaggy clones in sc^+ flies can differentiate a cluster of macrochaetae on the scutellum as in Fig. 2A. The largest clone seen on the scutellum comprised 7 macrochaetae. In the first experiment, *sgg* clones were induced in animals mutant for sc^1. (sc^1*sgg* f^{36a}/y sc^1 *v* flies were irradiated.) The distribution of macrochaetae is shown in Fig. 3A. No *sgg* f^{36a} macrochaetae were found on the scutellum. *sgg* f^{36a} macrochaetae were still found clustered in the region of the dorsocentral bristles. In the second experiment, sc^1 *sgg* clones were induced in otherwise sc^+ flies (sc^1 *sgg* f^{36a}/y flies were irradiated). Fifteen sc^1 *sgg* f^{36a} clones were observed on the nonscutellar regions of the thorax; they had a *sgg* phenotype. Twelve normal control *y* clones were also seen. Four *y* clones were found on the scutellum. Six cases of naked patches on the scutellum were observed, four of which removed one scutellar bristle while the two others removed both. One of these naked patches was associated with a *y* twin spot and one with a *sgg* f^{36a} clone on the scutum (Fig. 2D). They thus presumably correspond to the *sc sgg* clones. sc^1 is therefore only epistatic over *sgg* in that region of the thorax where the mutant phenotype is seen. Elsewhere the AS-C is functioning normally and the *sgg* mutant phenotype is expressed. Therefore, the domain of expression of *scute* for the scutellar bristles, the spatial control of which is abolished in the sc^1 allele, extends only over the scutellum, but covers an area larger than that occupied by each of the two normal scutellar bristles.

sgg clones were also made in flies mutant for ac^1/ac^3. In such flies, the posterior dorsocentral bristles are lacking. ac^1 and ac^3 are hypomorphic mutations, however, and occasionally a posterior dorsocentral bristle will arise; very often this bristle is considerably smaller than normal. (*y* ac^1 *sgg w* f^{36a}/ac^3; *stc*/+ flies were irradiated.) The distribution of macrochaetae found in a study of 58 *sgg* clones is presented in Fig. 3B. Bristles are found around the positions of all macrochaetae

Table 4. *Numbers of macrochaetae and microchaetae found in* sgg *clones produced in flies carrying <1, 1, 2 or 4 copies of* emc$^+$ *by irradiation-induced mitotic recombination at 48–72 h AEL*

Genotype*	Dosage *emc*$^+$	Number of bristles per clone ± S.E.	Number of macrochaetae per clone	Number of microchaetae per clone	Number of clones
(8)	<1	21·4 ± 2·7	1·44	20·0	27
(9)	1	13·7 ± 2·0	0·95	12·2	40
(10)	2	16·4 ± 2·1	0·5	14·8	36
(18)	2	12·1 ± 2·2	0·45	11·7	49
(17)	4	11·0 ± 2·3	0·22	10·8	46

* Flies of genotypes (8), (9) and (10) were siblings. Flies of genotypes (17) and (18) were grown together in the same bottles.
(8) *y sgg* $f^{36a}/+$; *emc*^{1}/*TM2, emc*
(9) *y sgg* $f^{36a}/+$; *emc*$^1/+$
(10) *y sgg* $f^{36a}/+$; $+/+$
(17) *Dp(3; Y; 1)M2 emc*$^+$ *y sgg w v*/+; $+/+$
(18) *sgg* $f^{36a}/+$; $+/+$

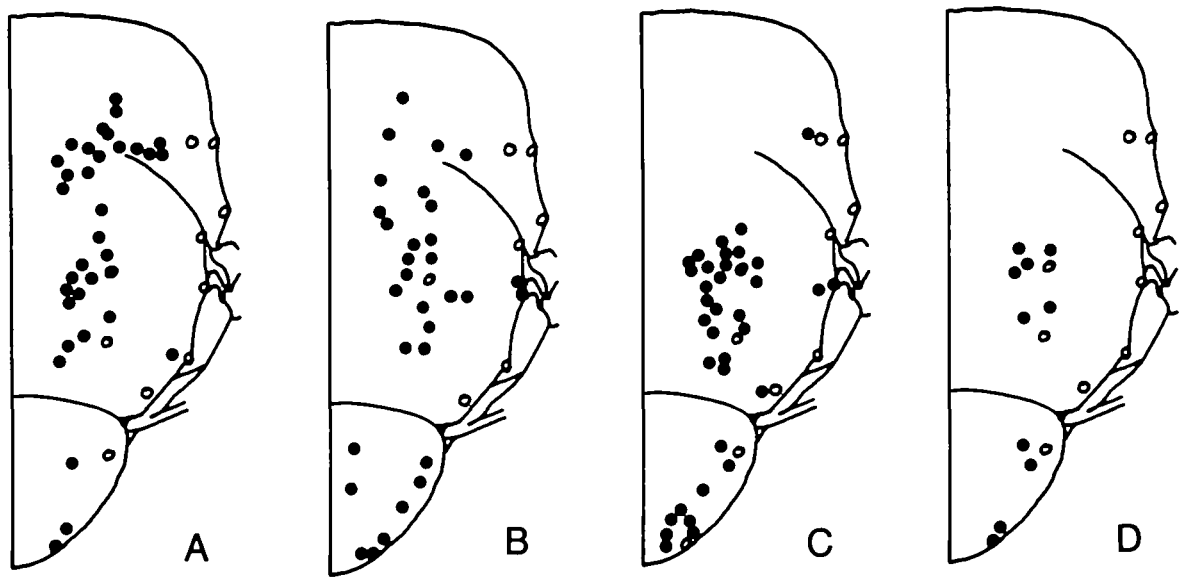

Fig. 4. Distribution of macrochaetae differentiated by *sgg* clones in which the cells were also carrying a variable number of copies of *emc*$^+$. The flies of the genotypes used in A, B and C were siblings. All flies were irradiated between 48 and 72 h AEL. Data pertaining to numbers of clones observed and clone sizes can be obtained from Table 4. (A) Flies were of the genotype *y sgg* $f^{36a}/+$; *emc*^{1}/*TM2, emc* and show an *emc* mutant phenotype; they carry less than one dose of *emc*$^+$. Macrochaetae are also seen on the anterior half of the thorax in an area that only bears microchaetae in *emc*$^+$ flies. (B) Flies were of the genotype *y sgg* $f^{36a}/+$; *emc*$^1/+$ and therefore only carry one copy of *emc*$^+$. Consistent with experiments revealing the synergism that exists between *sgg* and *emc*, in these flies, too, supernumerary macrochaetae are observed on the anterior thorax. (C) Control flies that were of the genotype *y sgg* $f^{36a}/+$; $+/+$ and were therefore diploid for *emc*$^+$. Macrochaetae are only found clustered around the positions of the extant bristles as in Fig. 1B. (D) Flies were of the genotype *Dp(1; Y; 3)M2 y emc*$^+$ *sgg w v*/+; $+/+$ and therefore the *y sgg* clones in this experiment carry 4 copies of *emc*$^+$. Fewer macrochaetae were formed by these clones, see Table 4, they are restricted to the dorsocentral and the scutellar bristle clusters. Control flies of the genotype *sgg* $f^{36a}/+$; $+/+$ and therefore diploid for *emc*$^+$ were grown in the same bottles as those carrying the duplication. The distribution of macrochaetae in these *sgg* clones was similar to that of Figs 1B and 4C, not shown.

except the posterior dorsocentrals where only one *sgg* bristle was observed. Interestingly this bristle was also intermediate in size. One control *stc* bristle was also found in this position from observations of 26 clones. *ac*^{1} *sgg* clones were also produced in flies that were otherwise *ac*$^+$ (*y ac*^{1} *sgg w*/f^{36a} flies were irradiated). The distribution of macrochaetae from 54 *sgg* clones was very similar to that of the preceding experiment (not shown). Amongst these flies eleven cases of a missing posterior dorsocentral bristle were recorded, five of which were associated with either *y* microchaetae or with f^{36a} control bristles. Seven cases of f^{36a} posterior dorsocentral bristles were found. We have shown, therefore, that the cluster of macrochaetae produced by *sgg* cells in the region around the posterior dorsocentral bristle is removed when these cells are also mutant for

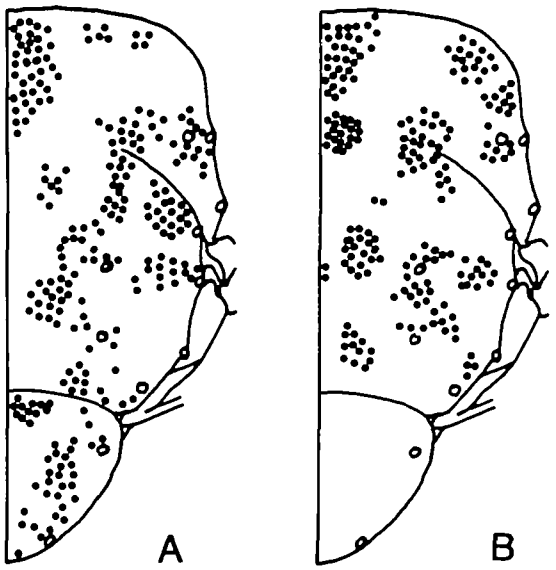

Fig. 5. The distribution of microchaetae in *sgg* clones produced in sibling flies homozygous or heterozygous for the mutant h^1. Flies were irradiated between 48 and 72 h AEL. (A) 28 clones from *y sgg* $f^{36a}/+$; h^1/h^1 flies are shown from a study totalling 68 clones. The clones were found to encompass ectopic microchaetae on the scutellum. (B) 21 clones from *y sgg* $f^{36a}/+$; $h^1/+$ sibling flies are represented from a total of 45 clones studied. The distribution of microchaetae resembles that seen in flies diploid for h^+ as in Fig. 1C.

Table 5. *Numbers of macrochaetae found on the thorax (including the humerus) of flies heterozygous for both* sgg *and* emc

Genotype	Number of macrochaetae per heminotum ± S.E.	Number of flies
†*FM6*/+; $emc^1/+$	13·4 ± 0·16	18
$sgg^{D127}/+$; *Sb*/+	13·0 ± 0·2	25
$sgg^{D127}/+$; $emc^1/+$	14·8 ± 0·2*	32
$sgg^{D127}/+$; $emc^{E12}/+$	14·0 ± 0·18*	25
$sgg^{b12}/+$; $emc^1/+$	14·6 ± 0·2*	25
$sgg^{b12}/+$; $emc^{E12}/+$	14·0 ± 0·16*	25

Flies of the first three genotypes were siblings obtained from the cross ♀ *FMC*/sgg^{D127} × ♂ emc^1/Sb.

* $P < 0·1$ when compared with $sgg^{D127}/+$; *Sb*/+ flies.

† *FM6* actually carries a rearrangement causing a slight *Hw* effect. This presumably accounts for the slight increase in bristle number in these flies.

ac^1 (or ac^1/ac^3) even though in *ac* flies only the single posterior dorsocentral bristle is missing. Occasionally in these flies, posterior dorsocentral bristles were observed at some distance from the normal site (one case of a *y* bristle, two of wild-type bristles and one case of a f^{36a} bristle; see also Stern, 1954*a*,*b*).

These results, therefore, provide further evidence for the idea that the AS–C transcripts are expressed in small domains on the thoracic epidermis, domains that cover an area considerably greater than the single cell

Table 6. *Numbers of macrochaetae found on the thorax of* emc^1/TM2, emc *flies bearing one, two or three doses of* sgg^+

Genotype†	Number of doses sgg^+	Number of macrochaetae per heminotum ± S.E.	Number of of flies
(19)	1	21·1 ± 0·3 } *	26
(20)	2	20·0 ± 0·15 } *	40
(21)	3	18·5 ± 0·2 } *	31

* $P < 0·01$.

† Flies of genotypes (19), (20) and (21) were siblings, see Materials and methods.

(19) *sgg w/y w*; +/+; $emc^1/TM2$, *emc*
(20) *sgg w/y w*; *Dp(1; 2)*w^{+70}, sgg^+; $emc^1/TM2$, *emc*
(21) *y w/y w*; *Dp(1; 2)*w^{+70}, sgg^+; $emc^1/TM2$, *emc*

Table 7. *The numbers of macrochaetae found on the thorax (including the humerus) of flies heterozygous for both* sgg *and* emc *and bearing one, two or three doses of AS–C*

Genotype*	Number of copies AS–C	Number of macrochaetae per heminotum ± S.E.	Number of flies
(22)	1	13·0 ± 0·1	23
(23)	2	15·2 ± 0·2	24
(24)	2	15·0 ± 0·2	25
(25)	3	18·2 ± 0·1	25
†(26) control sgg^+	3	14·8 ± 0·2	25

* Flies of genotypes (22) and (23) were siblings. Flies of genotypes (24), (25) and (26) were siblings, see Materials and methods.

† This genotype was included as *emc* interacts by itself with AS–C.

(22) *Df(1)260-1*, y^-/y *sgg* f^{36a}; $emc^1/+$
(23) *y sgg* $f^{36a}/+$; $emc^1/+$
(24) *y sgg* $f^{36a}/+$; *CyO*/+; $emc^1/+$
(25) *y sgg* $f^{36a}/+$, *Dp(1; 2)*$sc^{19}/+$; $emc^1/+$
(26) *FM6*/+, *Dp(1; 2)*$sc^{19}/+$; $emc^1/+$

that will finally produce a bristle. In the *sgg* mutant, the process that normally leads to the singling out of only one bristle mother cell is somehow defective such that a group of bristles are formed in each domain. Therefore *sgg* permits us to visualize the extent of the domains, within which the AS–C transcripts are effective.

Unlike shaggy, hairy *and* extra macrochaetae *may disrupt the spatial expression of* achaete *and* scute

The genes *hairy* (*h*) and *extramacrochaetae* (*emc*) are thought to act as repressors of *ac* and *sc* on the notum (Moscoso del Prado & Garcia-Bellido, 1984). The phenotype of *emc* is somewhat similar to that of *sgg*: mutant clones of lethal alleles produce extra macro- and microchaetae (Garcia-Alonso & Garcia-Bellido, 1988). Viable alleles of *emc* result in the formation of extra macrochaetae (Moscoso del Prado & Garcia-Bellido, 1984). Flies mutant for *h* carry extra microchaetae. Similarly the gain-of-function *Hw* alleles also cause additional bristles. Hw^{49c}, which causes an overproduction of *ac* and *sc*, leads to the differentiation of extra

micro- and macrochaetae, whereas Hw^1, which causes an overproduction of *ac*, leads to the development of extra microchaetae (Garcia-Alonso & Garcia-Bellido, 1986; Campuzano *et al.* 1986; Balcells *et al.* 1988). The phenotypes of all these mutants, however, differ fundamentally from that of *sgg* in that the supernumerary macrochaetae or microchaetae are found not only in the regions of notum where they would normally develop but also in ectopic positions. For example, in Hw^1 flies microchaetae are found on the wing blade and, in *h* flies, microchaetae develop on the scutellum. Similarly in the case of *emc*, macrochaetae are found in more anterior positions on the thorax with a new distribution, the pattern of which is reminiscent of other dipteran species (Moscoso del Prado & Garcia-Bellido, 1984; Garcia-Alonso & Garcia-Bellido, 1988). In other words, unlike *sgg*, the spatial distribution of the morphologically distinct bristle types is altered in these mutants. It has also been observed that, in Hw^1 and Hw^{49c} wing discs, *ac* and *sc* transcripts accumulate in ectopic locations (Balcells *et al.* 1988).

In order to further explore the difference in phenotype between the aforementioned mutants and *sgg*, *sgg* clones were produced in flies mutant for either *emc* or *h*. 27 *sgg* clones resulting from irradiation of *y sgg* $f^{36a}/+$; $emc^1/TM2$, *emc* flies were studied, as well as 30 *sgg* clones resulting from irradiation of *y sgg* $f^{36a}/+$; $+/+$ sibling flies. The average clone sizes from these two genotypes were not significantly different (Table 4). However, the clones in *emc* flies were found to encompass a larger number of macrochaetae (Table 4). The most striking difference, however, is found in the distribution of these macrochaetae, see Fig. 4A. Ectopic macrochaetae are found clustered in a more anterior region of the notum, an area normally covered only by microchaetae. Therefore, in flies mutant for *emc*, the *sgg* phenotype is changed such that the spatial distribution of the additional macrochaetae is altered.

shaggy clones were also examined after irradiation of *y sgg* $f^{36a}/+$; h^1/h^1 flies and of *y sgg* $f^{36a}/+$; $h^1/+$ control siblings. 68 experimental and 45 control clones were studied. The distributions of microchaetae from these two genotypes are shown in Fig. 5. It can be seen that, in the *h* flies, *sgg* clones on the scutellum can include microchaetae (see also Fig. 2C). Therefore, in flies mutant for *h*, the *sgg* phenotype is altered such that the spatial distribution of the supernumerary microchaetae is changed.

These results, therefore, show that the spatial distribution of the supernumerary bristles found in *sgg*, both macrochaetae and microchaetae, is altered in flies mutant for *emc* or *h*. In other words, *emc* and *h* are both epistatic over *sgg*. We have previously argued that the regular spatial pattern normally seen in *sgg* reflects an accurate spatial expression of the AS–C. One explanation, therefore, for the altered distribution of macro- and microchaetae seen in *sgg emc* or *sgg h* clones would be that in these two cases the spatial distribution of the AS–C is altered. This would mean that *emc* and *h* not only cause an overexpression of *sc* or *ac* (Moscoso del Prado & Garcia-Bellido, 1984) but in addition an ectopic expression. It has already been suggested that the segmentation genes (Nüsslein-Volhard & Wieschaus, 1980), of which *h* is one, might be responsible for the correct spatial expression of AS–C (Cabrera *et al.* 1987; Ghysen & Dambly-Chaudière, 1988). A recent study reveals a normal spatial expression of *ac* and *sc* transcripts in *emc* and *h* wing discs so these genes may affect a very late expression of the AS–C (Romani, Campuzano, Macagno & Modolell, in preparation).

shaggy *acts synergistically with* extra macrochaetae

Because of the similarity of their phenotypes, we examined the relationship between *sgg* and *emc* and *h*. A dosage study revealed no relationship between *sgg* and *h* (results not shown). On the other hand, *sgg* and *emc* were found to act synergistically: the double heterozygotes display additional macrochaetae on the thorax (Table 5). The phenotype of *emc* was in fact found to vary with the number of doses of sgg^+: the number of additional macrochaetae on the thorax of $emc^1/TM2$, *emc* flies decreases with extra doses and increases with fewer doses of sgg^+ (Table 6). Similarly the phenotype of clones mutant for *sgg* is also altered in flies carrying one, two or four doses of emc^+. Although the overall clone size does not vary, a greater number of macrochaetae are found in *sgg* clones in which the dosage of emc^+ is reduced, whereas fewer macrochaetae were formed by *sgg* clones that also carried four copies of emc^+ (Table 4). Furthermore, the spatial distribution of macrochaetae differentiated by the *sgg* clones is not only altered in flies that were mutant for *emc*, but also in those that were heterozygous for *emc* (Fig. 4B). Therefore to some extent, through their respective roles on bristle differentiation, *sgg* and *emc* can compensate for one another.

The synergism between *sgg* and *emc* appears to be due to overexpression of the AS–C since it is suppressed by haploidy of the AS–C and enhanced by triploidy of the AS–C (Table 7).

Conclusions

We have shown that the production of supernumerary bristles caused by the *sgg* mutant does not appear to result from a spatial derepression of the genes of the AS–C. This is in opposition to the mutants *h* and *emc* for which a gene dosage study revealed regulatory roles on *ac* and *sc* (Moscoso del Prado & Garcia-Bellido, 1984). The results are consistent with the idea that, whereas the AS–C specifies the general position of each bristle through small regionally restricted zones of expression, *sgg* acts through an independent cellular phenomenon that is concerned with cell interactions leading to the sponsorship of only one bristle mother cell within each zone. Under this hypothesis, therefore, each cluster of macrochaetae produced by *sgg* mutant cells reflects the normal geographical extent of the zones of expression of the AS–C transcripts.

The following picture for the respective roles of the AS–C, *emc*, *h* and *sgg* is consistent with published data and with that presented here. The AS–C products are expressed in small discrete regions over the epidermis of the thorax as a result of the repressing effects of *emc* and *h*. These zones of expression lead to the formation of equivalence groups within which *sgg* and probably other genes such as those of the neurogenic class (Campos-Ortega, 1985; Dietrich & Campos-Ortega, 1984) play a role in the cell–cell interactions that lead to the definition of one bristle mother cell. This cell would then prevent other cells of the group from becoming bristles and the range of lateral inhibition would extend over the radius of each equivalence group. When the amount of emc^+ is reduced, the regional control of the AS–C is no longer so effective and the *sc* products appear in larger areas and also in some ectopic locations. Consequently the equivalence group becomes larger than the area over which the mechanism of lateral inhibition can function and extra bristles appear. The synergism between *sgg* and *emc* can therefore be explained as follows. A single dose of emc^+ leads to a relaxation of the regional control of the AS–C but in general the effect is insufficient to lead to an abnormal pattern and the mechanism of lateral inhibition is still effective. When, however, the amount of sgg^+ is simultaneously reduced in these cells, the cell interactions leading to the definition of only one bristle cell are also less effective and as a result of these two effects extra bristles are formed. Lowering the number of copies of the AS–C in such flies doubly heterozygous for *emc* and *sgg*, compensates for the less effective repression by *emc* and restores the normal bristle pattern.

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