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REVIEW ARTICLE

The development and evolution of bristle patterns in Diptera

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SUMMARY

The spatial distribution of sensory bristles on the notum of different species of Diptera is compared. Species displaying ancestral features have a simple organization of randomly distributed, but uniformly spaced, bristles, whereas species thought to be more derived bear patterns in which the bristles are aligned into longitudinal rows. The number of rows of large bristles on the scutum was probably restricted to four early on in the evolution of cyclorraphous Brachyceran flies. Most species have stereotyped patterns based on modifications of these four rows. The possible constraints placed upon the patterning mechanisms due to growth and moulting within the Diptera are discussed, as well as within hemimetabolous insects. The holometabolic life cycle and the setting aside of groups of imaginal cells whose function is not required during the growth period, may have provided the freedom necessary for the evolution of elaborate bristle patterns. We briefly review the current state of knowledge concerning the complex genetic pathways regulating *achaete-scute* gene expression and bristle pattern in *Drosophila melanogaster*, and consider mechanisms for the genetic regulation of the bristle patterns of other species of Diptera.

Key words: Evolution, Bristle, Diptera, *Drosophila*

INTRODUCTION

Most insects bear sensory organs, such as bristles, over the cuticle of the body. With very few exceptions, bristles are always found in a spaced pattern, i. e. they are not, as a general rule, situated adjacent to one another but are always separated by intervening epidermal cells. This is achieved by a mechanism called lateral inhibition, whereby nascent bristle precursors prevent neighbouring cells from developing into bristles (Wigglesworth, 1940; Simpson, 1990). In many insects, the bristles are distributed randomly, but in others, they are organized into stereotyped spatial arrays. The bristles of the Diptera are a case in point and bristles at defined positions are used in taxonomy (chaetotaxy). In this paper, we will concentrate on the Dipteran dorsal mesothorax (notum), a much enlarged carapace that houses the powerful flight muscles.

A single species, *Drosophila melanogaster*, has been the focus of investigation into the genetic control of the arrangement of sensory bristles. In *D. melanogaster*, there are eleven large bristles, or macrochaetes, on each heminotum and these occupy stereotyped positions that rarely vary between individuals (Fig. 1). This pattern is widespread throughout most of the 2000 or so species of the family Drosophilidae and is also extremely old: specimens preserved in amber have been described that date from up to 40 million years ago (Grimaldi, 1987; Fig. 1). Amongst these, some macrochaetes are found in almost exactly the same positions as they are in extant species. It is known that, in *D. melanogaster*, the neuronal specificity of the bristle organ is dependent upon the site within the epithelium at which the bristle precursor cell arises (Ghysen, 1980). The positions of bristles are therefore likely to be of importance to the fly's behaviour which could explain why the patterns have been maintained over such long periods of time. Other features of the peripheral nervous system, such as the number and position of campaniform sensilla on the wing blade, have been similarly conserved (Dickinson et al., 1997).

There are, however, many thousands of species of Diptera and many of these, in particular those of the Schizophora that include *Drosophila*, have different, but equally stereotyped bristle patterns. Furthermore, some of these patterns, too, can be traced a long way back in evolutionary time (McAlpine, 1981b). The question therefore arises as to how all of these different patterns are made and to what extent the basic genetic mechanisms described in *D. melanogaster* have been conserved. In this essay, we will first explore the different bristle arrangements found throughout the Diptera, in order to define underlying subpatterns common to many species that may reflect a similar genetic regulation. We will then examine the constraints that may operate on the spatial arrangements of bristles with respect to growth, moulting and the life cycles of different insects. Finally we will discuss the possible genetic control of bristle patterns in Dipteran species other than *Drosophila*.

BRISTLE PATTERNS THROUGHOUT THE DIPTERA

Much work has been done on the phylogeny of the Diptera and different investigators have used different criteria to construct relationships. While they do not agree in detail, there is a general consensus concerning the outline of Dipteran evolution (Fig. 2; McAlpine, 1981b). It is not our purpose to infer phylogeny from the different bristle patterns. Rather, we wish to compare the bristle patterns seen in different groups of flies using a phylogenetic framework established by other means. We will describe the bristle patterns in a range of Diptera from those thought to display more ancestral characteristics to those thought to be more derived.

Nematocera (randomly distributed bristles)

Flies of the suborder Nematocera are thought to display many ancestral features (Wood and Borkent, 1981; see legend to Fig. 2). Most species belonging to three quarters of the 26 Nematoceran families bear a pattern of randomly distributed bristles on the notum; an example, *Simulium variegatum*, is given in Fig. 3. [Note that the bristles are nevertheless always spaced apart from one another; throughout this paper a 'random pattern' refers to spaced bristles whose position varies from individual to individual]. Some families display simple patterns of longitudinal bands of bristles separated by bristle-free interbands (e.g. *Chironomous thumii)*. In a very few species, such as in the mosquito *Anopheles gambiae*, the bristles show a tendency to line up into longitudinal rows on the scutum parallel to the dorsal midline. For the most part these rows are rather irregular, but when present they are often in similar positions to some of the stereotyped rows found in many Brachyceran flies (see below). In those Nematocera that do display bristle rows, the number of bristles in a row is variable between different individuals of the same species (e.g. the dorsocentral row of *Anopheles gambiae* (Fig. 3) displays between 15 and 21 bristles with an average of 18 (n=12)). This means that the precise position of any given bristle within a row is not defined.

In most Nematocera, the bristles are all of uniform size. Frequently they are long and thin. In those species with rows, the bristles that are aligned are bigger and longer than those that are randomly distributed. This is seen in only a few Nematocera, however, and, when present, the large bristles are only poorly distinguishable from the remaining ones. Just occasionally, strong, distinct, large bristles can be seen, particularly in the Anisopodidae (Fig. 3).

Orthorraphous Brachycera (bristles organized into bands or rows)

The Brachycera are thought to be monophyletic and to have arisen from one group of Nematocera but the relevant nematocerous sister group has not been identified (Woodley, 1981; see legend to Fig. 2). Interestingly however, a number of

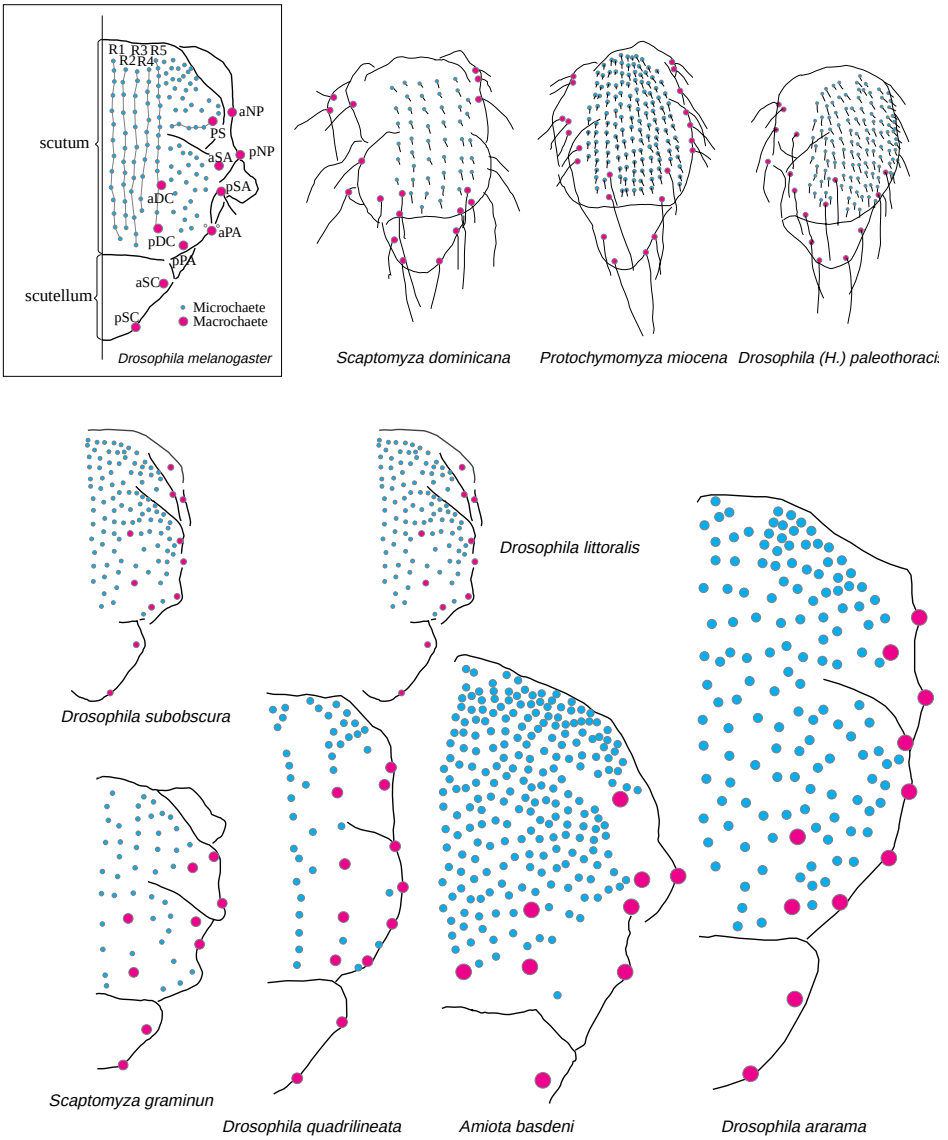


Fig. 1. Notal bristle patterns in species of the family Drosophilidae. Macrochaetes are shown in pink, microchaetes in blue. The inset shows a schematic drawing of the arrangement of bristles in *Drosophila melanogaster*. Note the large stereotyped macrochaetes and the alignment of microchaetes into five rows labelled R1 to R5. The three fossil species, *Scaptomyza dominicana*, *Protochymomyza miocena* and *Drosophila (H.) paleothoracis* have been reproduced from Grimaldi (1990; not to scale). They were found in amber dating from 23 to 40 million years. The remaining six extant species are represented by camera-lucida drawings and are shown to scale. Note that, while the positions of the macrochaetes remain similar, the number and arrangement of microchaetes is variable between species.

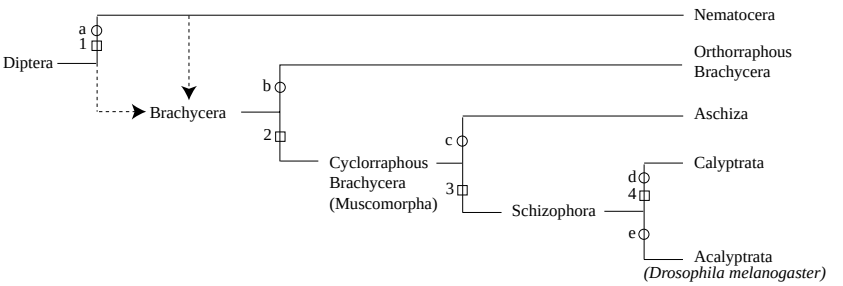


Fig. 2. Simplified phylogenetic tree of the Diptera. For general details of phylogenetic groupings see McAlpine (1981b). The Diptera are accepted to be a monophyletic taxon, the most striking synapomorphy being the modification of hindwings to halteres and associated changes in thoracic structure. These include enlargement of the mesothorax and reduction of the prothorax and metathorax. The suborder Nematocera [1] is probably paraphyletic and includes flies with many ancestral features such as antennae with many freely articulating flagellomeres. The Brachycera are monophyletic and are presumed to have arisen from some part of the Nematocera. The Brachycera have reduced antennal segments, fewer maxillary palp segments and altered wing venation. They are subdivided into orthorraphous and cyclorraphous [2] flies, the difference relating to the pupa, which in the cyclorraphous Brachycera is enclosed in a puparium (a modified form of the last larval cuticle). The cyclorraphous Brachycera are monophyletic and these flies are further subdivided into the Aschiza and the Schizophora [3], based on the presence of a ptilinum in the Schizophora (a sac-like organ arising from a modified frons that helps the imago to free itself from the pupa). The two groups of Schizophora, the Calyptrata [4] and Acalyptrata differ in the presence or absence of the calypter, a lobe at the base of the posterior part of the wing. The letters denoted by circles represent the bristle arrangments characteristic of the different groups. (a) Bristles are of uniform size, not aligned into rows. (b) Most species bear bristles of uniform size, not aligned into rows. A few have distinct macrochaetes and microchaetes; the macrochaetes are in irregular rows with a variable number of bristles per row. (c) Some species have bristles of uniform size, not aligned into rows. Some have distinct macrochaetes and microchaetes; the macrochaetes are in defined rows. (d) The macrochaetes and microchaetes are distinct; the macrochaetes are aligned into four rows on the scutum. In most species the number of macrochaetes per row is stereotyped. In some species the microchaetes are aligned into rows. (e) The macrochaetes and microchaetes are distinct; the macrochaetes form stereotyped species-specific patterns. In some species, the microchaetes are aligned into rows.

characters seen in the Anisopodidae are similar to those of the Brachycera with the most ancestral features (Woodley, 1981). The Brachycera probably date from the Triassic, and have been found in the lower Jurassic (Kovalev, 1981, 1982). The bristle patterns of nine of the 19 families of the orthorraphous Brachycera (see legend to Fig. 2) differ little from those of the Nematocera. These include species in which the bristles are of uniform size and are generally randomly distributed. Species from the remaining 10 families display a mixture of phenotypes, some bearing randomly distributed bristles, whereas others possess one or several bands or irregular rows of large bristles. In some families (e.g. the Bombylidae), the bristles are very long and thin, but other families include species that bear two distinct classes of stout bristles (e.g. the Empididae and the Dolichopodidae, not shown). These are the large bristles, variably called macrochaetae, macrosetae, bristles or setae, and small bristles, variably called microchaetae, setulae or hairs (Fig. 4). In fact, both types of bristles are sensory organs and are distinct from the trichomes or microtrichia that are merely cuticular processes secreted by the epidermal cells. When present the large bristles are organized into rows and, in some families, there are some distinct rows that occupy similar positions to those seen in the cyclorraphous Brachycera, see below. The rows of large bristles are usually complete, i. e. they cover the entire length of the scutum.

Cyclorraphous Brachycera (bristle patterns stereotyped)

The cyclorraphous Brachycera (also called the Muscomorpha) are the most highly derived Dipterans and are presumed to have arisen from a subgroup of the orthorraphous Brachycera (McAlpine, 1981a; see legend to Fig. 2). They are undoubtedly monophyletic and probably date from the late Cretaceous: specimens 70 to 73 million years old have been described (McAlpine, 1981b). Cyclorraphous Brachycera are divided into two sections, of which the Aschiza display the most ancestral features (see legend to Fig. 2). In all seven families of Aschiza, species with random bristle patterns are observed, while others bear bristles of equal size but whose distribution is non-uniform and important taxonomically. Long, thin bristles (pilose) are thought, from cladistic analysis, to be an ancestral feature for the Muscomorpha and are the most evident in densely haired species such as the Syrphidae (McAlpine, 1981b; see Fig. 4). They occur in all families of the Aschiza. In some species macrochaetes are found, organized into rows, although they are only weakly distinguishable from the remaining bristles. In other cases, however, specific rows of macrochaetes occupy defined sites, that appear to be homologous to those seen in the Schizophora (see below). Large characteristic macrochaetes at some of these locations are also seen in the Anisopodidae (Nematocera) and some orthorraphous Brachycera and were probably already present in the ancestors of the Muscomorpha and incorporated into their basic organization (McAlpine, 1981b; Garcia-Bellido, 1981).

The second section, the Schizophora, thought to be the most derived, is well represented by fossils, and most of the 79 families were well differentiated by the Oligocene (McAlpine, 1981b). Species with long thin hairs are rarely seen in the Schizophora. Here macrochaetes and microchaetes are nearly always clearly distinguishable (Figs 4, 5; McAlpine, 1981b). Whereas macrochaetes in the orthorraphous Brachycera are often only poorly distinguishable from microchaetes, throughout the Muscomorpha they are generally thick and stout.

The basic organization or ‘ground plan’ of the Schizophora is indicated in Fig. 5 (McAlpine, 1981b). We will concentrate on the macrochaetes of the scutum and scutellum. There is a basic arrangement of four rows of bristles on the scutum, the acrostichal (AC), dorsocentral (DC), intra-alar (IA) and supra-alar (SA) rows. In the Aschiza, these four rows, when present,

are poorly defined, whereas in the Schizophora they are very constant. There is also a single line of scutellar (SC) bristles round the lateral edge of the scutellum. (Postalar and notopleural bristles are found on more lateral sclerites). The pattern of most of the thousands of species of Schizophora can be superimposed upon this basic organization, even though some species display all rows and others have only a subset; examples are given in Fig. 5. This restriction to only four macrochaete rows on the scutum applies regardless of the size of the notum, which varies considerably between species.

The Schizophora are subdivided into two subordinate groups, the Calyptrata and the Acalyptrata (see legend to Fig. 2). There are 14 families of Calyptrata and these bear more macrochaetes than the Acalyptrata. The rows of macrochaetes generally extend the full length of the scutum, both above (presutural) and below (postsutural) the transverse suture (Fig. 5). Throughout the 65 families of Acalyptrata, the rows are incomplete and this is thought to be a derived feature (McAlpine, 1981a; Fig. 5). Bristles are missing preferentially from the anterior part of the scutum (Sturtevant, 1970; Garcia-Bellido, 1981). Thus the DC row, often includes only postsutural bristles, the AC row is usually absent, and when present generally comprises a single, posteriorly situated bristle, called the prescutellar. Furthermore there are generally only two SC bristles.

An additional point of some importance accompanying the rows of macrochaetes is, that, in many species of Schizophora, the number of bristles in each row is constant and furthermore the precise position of each macrochaete is often stereotyped. This is the reason why these bristles are so useful for taxonomical purposes.

A PATTERN OF RANDOMLY DISTRIBUTED BRISTLES MAY HAVE PRECEDED THE ALIGNMENT OF BRISTLES INTO ROWS

From the phylogeny presented in Fig. 2, it can be seen that the Nematocera show, in general, the most ancient characteristics and that, among the Brachycera, the Schizophora display the most derived features. The preceding description reveals that more precise and often stereotyped bristle patterns are a characteristic of the more derived Dipteran families (Fig. 2). Nematoceran flies generally bear randomly distributed bristles that are of equal size, but a few have rows of large bristles that are poorly aligned. Amongst the Brachycera, flies of those families with the most ancestral features often have bristle patterns similar to the Nematocera, whereas in flies of the more derived families, large bristles are nearly always present and are aligned into rows. In the cyclorraphous Brachycera, where macrochaetes are clearly distinguishable, they are very distinct features and the number of rows on the scutum is constant between species. Since a random arrangement appears to be ancestral, we hypothesize that the alignment of bristles into rows is a derived feature, which was preceded by an earlier state in which the bristles were simply randomly distributed.

It is intriguing that in species where the bristles are all of similar size (most Nematocera) they are not aligned into rows, whereas in species bearing two classes of bristles of different size (many Brachycera), the macrochaetes are invariably aligned into rows (or are in a stereotyped pattern) and the microchaetes may or may not be arranged in rows. Thus the lining up of bristles into rows seems to be a characteristic of

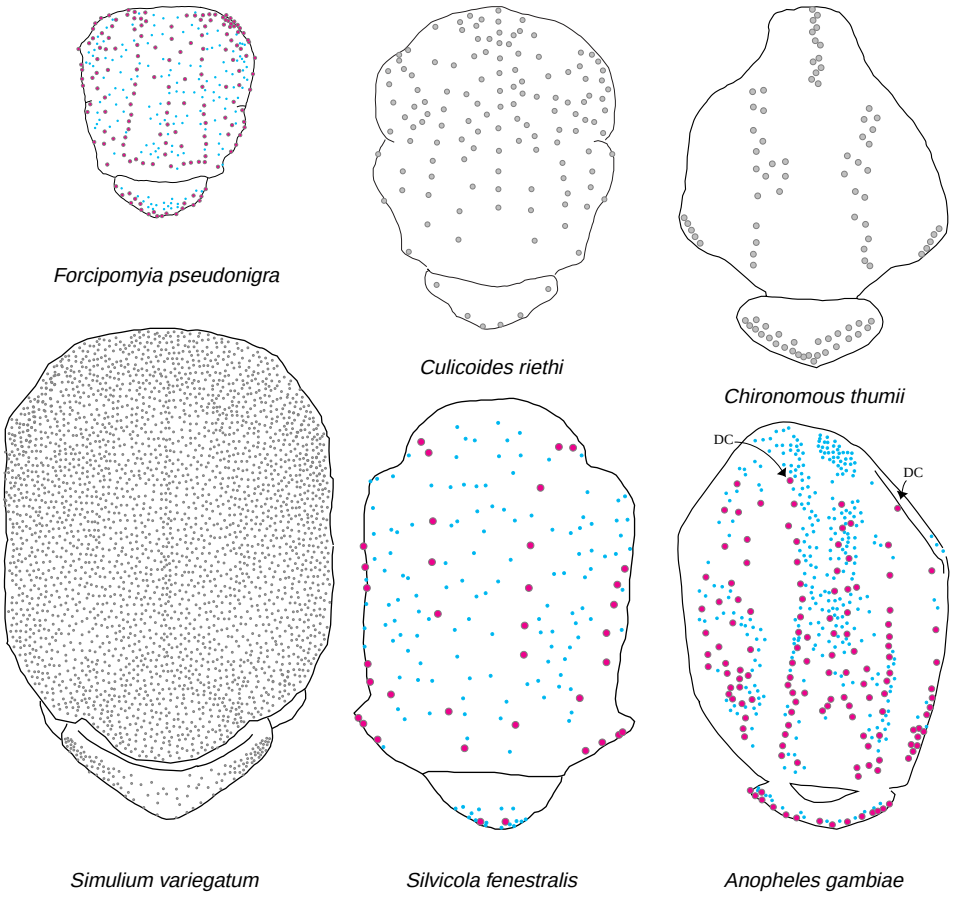


Fig. 3. Camera-lucida drawings of notal bristle patterns of different species of Nematocera shown to scale. *Anopheles gambiae* (Culicidae), note the presence of two rows of large bristles, one close to the midline and one at a position corresponding to the dorsocentral (DC) bristles of Brachycera. *Chironomus thumii* (Chironomidae), two band of bristles of uniform size can be seen on each heminotum. *Culicoides riethi* (Ceratopogonidae), the bristles are of uniform size and, although some are roughly aligned, rows are not readily apparent. *Forcipomyia pseudonigra* (Ceratopogonidae), rows of large bristles interspersed with smaller ones are present. *Silvicola fenestralis* (Anisopodidae), two rows of large bristles are present on each heminotum together with randomly positioned small bristles. *Simulium variegatum* (Simulidae), the notum is covered with uniformly spaced bristles.

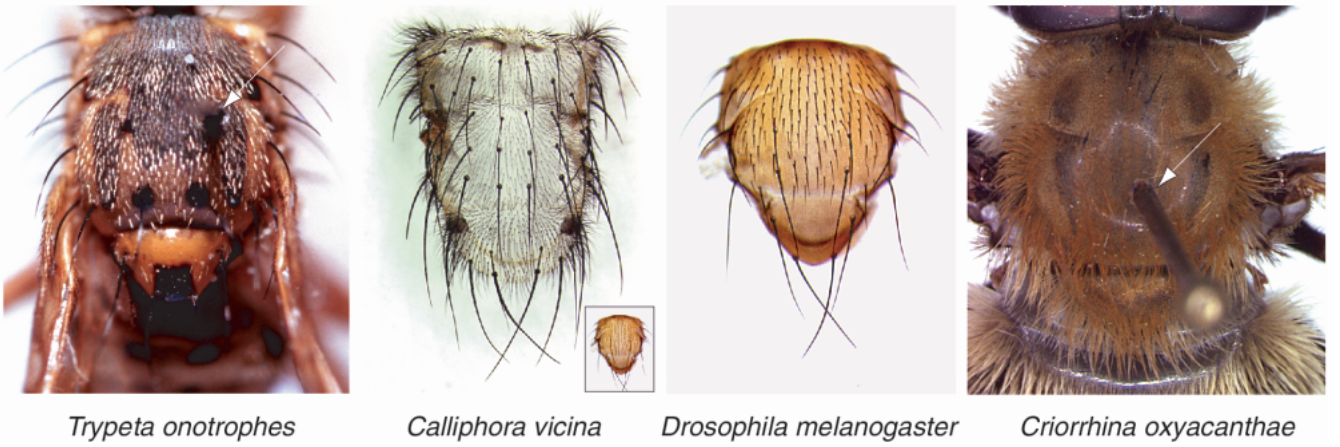
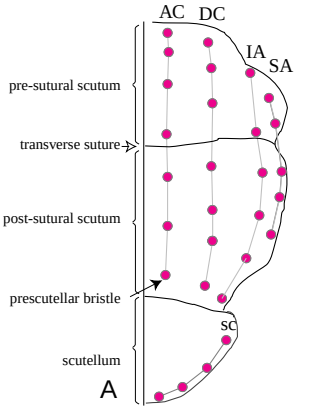


Fig. 4. Photographs of the thorax of some Diptera. *Trypeta onotrophes* (Tephritidae): note the difference in size and colour between the macrochaetes (long, stout and black) and the microchaetes (small, short, closer together and golden yellow). This specimen is pinned as indicated by the arrow (specimen courtesy of the Musée Zoologique de Strasbourg). *Calliphora vicina* (Calliphoridae): note the many rows of microchaetes intercalated between rows of macrochaetes on the scutum and between any two macrochaetes within a row. The inset shows *D. melanogaster* at the same scale, note the difference in size. *D. melanogaster* (Drosophilidae): a single microchaete is found between the two dorsocentral bristles. *Criorrhina oxyacanthae* (Syrphidae): the dense, long haired appearance represents the ancestral Muscomorphan state. This specimen is pinned as indicated by the arrow (specimen courtesy of the Musée Zoologique de Strasbourg).

flies carrying the two classes of bristles, and is a consistent feature of macrochaetes. It is not clear whether macrochaetes arose more than once in Diptera (McAlpine, 1981b). However, if the Brachycera had an ancestor in common with the Anisopodidae then the two classes of bristles may have been present in early Brachycera. Amongst Brachycera members of the family Rhagionidae represent the oldest fossils and the extant Rhagionidae probably display the most ancestral features of the Brachycera of today (Kovalev, 1981, 1982; Woodley, 1981). Some extant species of Rhagionidae display macrochaetes (Woodley, 1981). If macrochaetes arose only once then they have been lost many times since. Alternatively



B

	AC		DC		SC				
	pre sutural	post sutural	pre sutural	post sutural	0	1	2	>2	n
Calyptrata	56	79	83	90	4	0	31	65	52
Acalyptrata	0	39*	54	82	2	11	86	<1	57

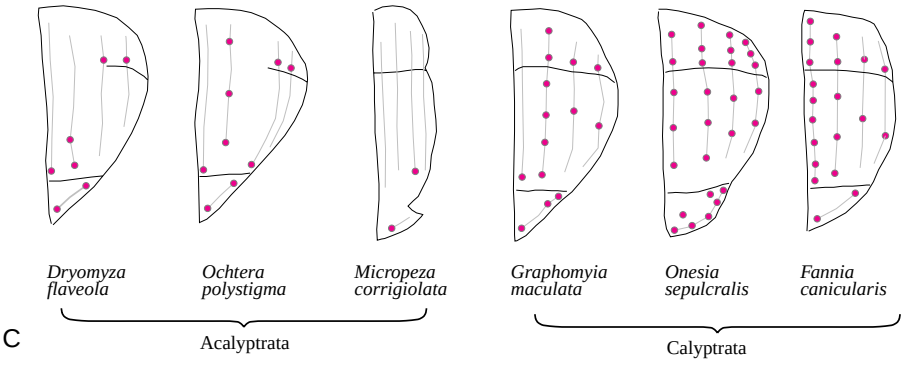


Fig. 5. Frequency of presutural and postsutural acrostichal and dorsocentral macrochaetes in species of Calyptrata and Acalyptrata (Brachycera: Schizophora). (A) A schematic representation of the ground plan of macrochaetes on the scutum and scutellum of the Muscomorpha (adapted from McAlpine, 1981b). There are four rows of macrochaetes on the scutum: acrostichal (AC), dorsocentral (DC), intra-alar (IA) and supra-alar (SA). They are labelled presutural or postsutural with reference to the transverse suture. Note that this suture is incomplete in many species. There is also a row of scutellar bristles (SC) along the edge of the scutellum. Other bristles on the notum are not shown. (B) The percentage of species bearing AC, DC and SC bristles (the numbers 0, 1, 2, >2 refer to the number of scutellar bristles). The data were collected from 9 families of Calyptrata and 17 families of Acalyptrata. Note that the Calyptrata have many more macrochaetes. *In the Acalyptrata, there is usually only a single postsutural AC bristle called the prescutellar. Drawings of six selected species are shown in C; the bristles present are denoted in pink. Note that each pattern can be superimposed over the groundplan.

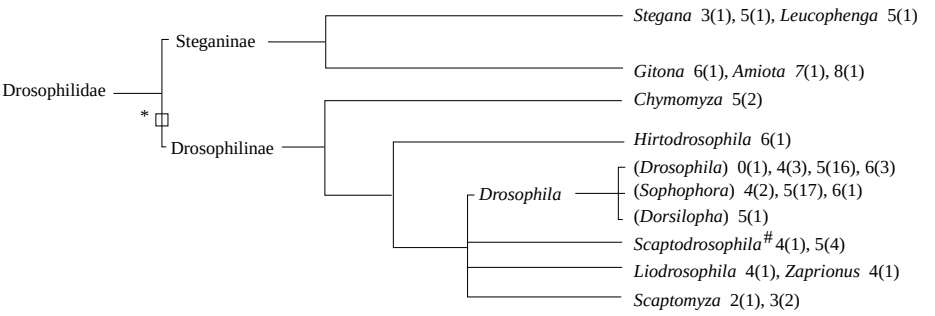


Fig. 6. The number of rows of microchaetes found on the acrostichal area of the scutum between the dorsocentral bristles and the dorsal midline (see Fig. 7A) in different species of the family Drosophilidae (Schizophora: Acalyptrata). The phylogenetic groupings are from Grimaldi (1990). The number of rows observed on each heminotum varies between 0 and 8 (these numbers include the dorsocentral row). The number of species examined is shown in brackets. The number of rows is not related to body size: 36 out of 58 species had five acrostichal rows; they displayed the full size range for the length of the notum (from 70 to 210 arbritary units). The variation in size was from 82 to 164 in the five species with four rows and 102 to 136 in the three species with six rows; the length was 176 in a single species with no rows. *Denotes the loss of the prescutellar macrochaete in the subfamily Drosophilinae; #In the genus *Scaptodrosophila*, this bristle is present.

they may have arisen independently in several Brachyceran lineages.

Another interesting point is that the number of macrochaete rows on the scutum does not vary: nearly all species display four rows (the AC, DC, IA and SA); they may have fewer, but never more, than four. Furthermore the four rows appear to be in homologous positions in different species of cyclorraphous Brachycera. This observation suggests that an ancestor, common to most of today's species already possessed these four rows. Thus, very early on in the evolution of the cyclorraphous Brachycera, a pattern consisting of a defined number of macrochaete rows was probably established. Indeed there is no apparent evidence that, in the ancestors of these flies, the number of macrochaete rows may have been more variable.

The microchaetes of cyclorraphous Brachycera are often also aligned into rows but, in contrast to the macrochaetes, the number of rows varies widely between species. For example, throughout the Drosophilidae, while the macrochaete pattern changes little, the microchaete pattern is very variable (Fig. 1). The phylogeny of the family Drosophilidae has been quite extensively studied and cladistic analysis suggests that randomly distributed microchaetes represent the ancestral state, whereas bristle rows are a more recently acquired feature (Grimaldi, 1990). Interestingly, the variation within this family, when placed in the phylogenetic framework provided by Grimaldi (1990), suggests that rows can decrease or increase in number through evolutionary time (Fig. 6). In some cases, too, the arrangement of bristles into rows is replaced by an atavistic pattern of randomly distributed bristles. The number of rows is not a simple reflection of changes in body size, as discussed later.

Stereotyped bristle arrangements may have arisen from a simple pattern of rows

While the patterning of macrochaetes into rows appears to be a very ancient feature, the stereotyped nature of the specific patterns of macrochaetes seen in the Schizophora appears to be more recently derived. These patterns are clearly variations of the basic theme of four bristle rows on the scutum. In different species, complete rows, or subsets of bristles from within rows, are absent (Fig. 5; Garcia-Bellido, 1981). This is especially true of the Acalyptrata. In parallel to the bristle loss, the precise positions of the remaining macrochaetes are stereotyped in many species. It is noteworthy that, in those few Nematocera bearing macrochaetes, and in many orthorraphous Brachycera, the number of macrochaetes within a row is variable (as it is within the rows of microchaetes in all species) whereas, in many species of Schizophora, the number is invariant and each bristle occupies a defined site on the notum.

From these observations, it is tempting to speculate that, within the Diptera, the alignment of bristles into rows was preceded by

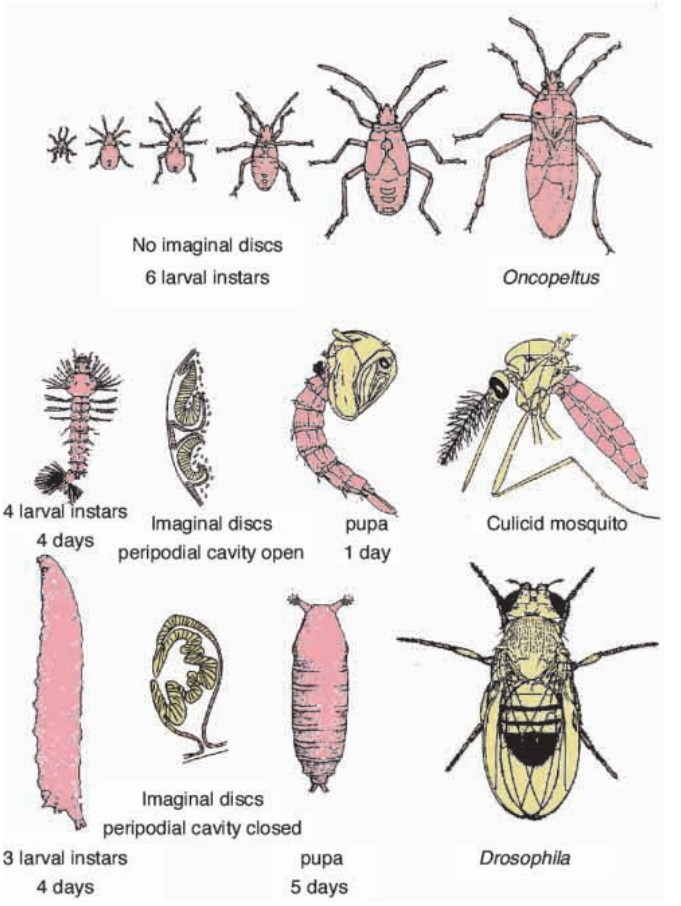


Fig. 7. Life cycles of different insects. Hemimetabolous insects, like *Oncopeltus fasciatus*, hatch from the egg as a small replica of the adult and grow by moulting. They retain the same epidermis throughout. Nematoceran flies such as Culicid mosquitos have a holometabolic life cycle but only the head and thorax of the imago is replaced at metamorphosis from simple imaginal discs. In cyclorraphous Brachyceran flies such as *Drosophila* the entire imaginal body is constructed from the imaginal discs at metamorphosis.

a random pattern and that an arrangement of four rows of macrochaetes on the scutum was present in an ancestor common to the Schizophora and has remained a constant feature of most of today's species. The stereotyped patterns of many species may thus be derived from this basic arrangement, different species bearing a subset of the rows or a subset of bristles from each row.

BRISTLE PATTERNS AND THE CYCLES OF GROWTH AND MOULTING IN INSECTS

Hemimetabolous insects

Hemimetabolous insects do not in general display stereotyped bristle arrangements and, with the exception of the Collembola, bristle patterns in these orders are rarely of taxonomical use. This may be a consequence of the mode of growth. Insects grow by moulting: during the intermoult period, the epidermis undergoes cell division (or cell growth) and at each moult the old cuticle is shed and a new one secreted. In hemimetabolous insects, the cuticle of each instar is secreted from the same epidermis throughout the entire life cycle (Fig. 7). As the epidermal cells divide body size increases at every moult. New features, such as additional bristles, can therefore be added at each instar. New bristles, however, can only arise at some distance from pre-existing ones because of the spacing mechanism. So how do complex stereotyped bristle patterns arise if they have to be constructed piecemeal with new elements being added at intervals?

The simplest possibility is for the animals to hatch from the egg with a complete set of bristles that remains unchanged at every instar. These patterns would be formed during embryogenesis before the cycle of moulting begins and no new elements would be added during subsequent growth. A number of Collembolan species do in fact display stereotyped patterns that do not change throughout postembryonic development, in spite of many moults (Hopkin, 1997). Amongst holometabolous insects (see below) this is also true for the cuticular patterns of many Dipteran and Lepidopteran larvae and indeed growth of the body wall of cyclorraphous Brachyceran larvae is exclusively due to an increase in cell size, not cell number.

Amongst insects that add new pattern elements at each instar, both stereotyped and random patterns can be found. Indeed some species have complex patterns that are built up gradually in a stereotyped fashion throughout different instars. This is the case for some Collembola, in which the arrangement of bristles at successive instars has been quite well studied (Hopkin, 1997). At hatching, the patterns are incomplete in the sense that the body surface is not covered uniformly with bristles, only part of the final pattern is present in the form of some bristles at some positions. The complete pattern is built up gradually during the next four instars by the addition of new bristles that appear at a maximum distance from the earlier bristles, but in spaces that were already provided at hatching, in the same way one would piece together a jigsaw puzzle. An example is given in Fig. 8, which shows the second thoracic segment of *Protaphorura armata* (Lobbes, 1992). Thus it appears that the positional information for the adult pattern may have been established entirely during embryogenesis. This hypothesis would account for the observation that individuals of the same species display the same pattern as adults, but achieve this by adding on different bristles at different instars (Bretfield, 1990).

The body surface of many hemimetabolous insects is covered with uniformly spaced bristles at hatching and new bristles are added at each moult so as to maintain the same relative density. New bristles only arise at a certain distance from pre-existing ones and they thus form by intercalation when growth of the epidermis provides sufficient space between bristles that were made at earlier instars (Wigglesworth, 1940; Lawrence and Hayward, 1971; Fig. 8). In the majority of these insects, the bristles are randomly

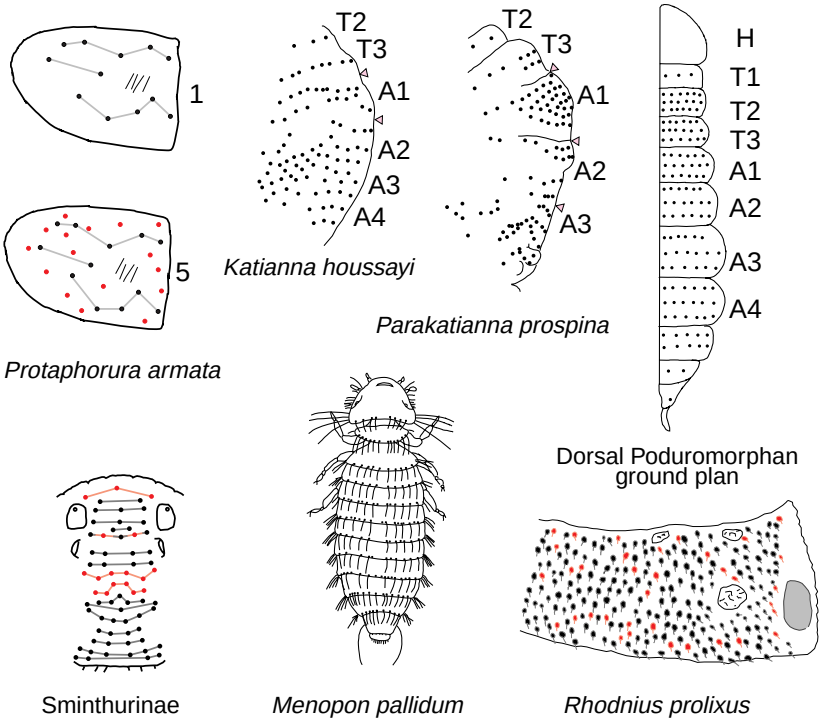


Fig. 8. Bristle patterns in hemimetabolous insects. The Collembola have a ground plan of three transverse rows of bristles on each segment, as shown for the Poduromorpha (adapted from Yosii, 1956). In some species, the number of rows may be reduced *(Katianna houssayi)* in others doubled *(Parakatianna prospina;* drawings adapted from Betsch et al., 1990). The second thoracic segment of *Protaphorura armata* is shown at the first and last (5th) instar (adapted from Lobbes, 1992). The rows of bristles are very irregular but the number of bristles within them is constant. The bristles formed during embryogenesis are shown in black, those formed at subsequent instars in colour. Note that new bristles are intercalated into spaces where none were found previously. The dashed lines represent probable muscle insertion sites. Species of Sminthurinae intercalate new rows of bristles, as well as new bristles within rows (shown in colour) as they grow and moult (adapted from Betsch et al., 1990). *Menopon pallidum*, the chicken louse (Mallophaga), displays a simple pattern of rows of large bristles aligned with the posterior margin of each segment. *Rhodnius prolixus* (Hemiptera) adds on new bristles (shown in colour) stochastically at each mount in spaces provided by growth of the epidermis (adapted from Wigglesworth, 1940).

distributed over most of the body (Fig. 8). The necessity to maintain a constant density and yet keep adding to the pattern, probably makes it difficult to construct stereotyped arrangements.

Simple arrangements, such as a lining up of the bristles into rows, are found, however. In some species, such as the chicken louse *Menopon pallidum*, only the bristles along the edge of the segment borders (or the borders of other sclerites) are aligned (Fig. 8). Sometimes, too, these 'border' bristles are morphologically distinct from the other, more uniform bristles. The segment borders are known to be lineage restriction boundaries (Lawrence and Struhl, 1996), meaning that there is no mixing of cells across them and, in addition, they have special growth properties (Simpson and Morata, 1981). Therefore, as the animal grows, bristles of distinct morphology can remain at these boundaries and new ones of a similar type can be intercalated between them.

In yet other insects displaying rows of bristles, new rows are intercalated between pre-existing ones in much the same way as new bristles are intercalated between ones that formed earlier. In a number of Collembola, the bristles are organized into rows, not unlike those seen in the Diptera. The basic arrangement of bristles on the body segments is a pattern of three transverse rows of simple bristles, although this number may be reduced or doubled (Yosii, 1956; Betsch et al., 1990; Fig. 8). The pattern changes with growth through both the intercalation of new rows of bristles and the intercalation of new bristles within each row (Betsch et al., 1990; Fig. 8).

Holometabolous insects: Diptera

Holometabolous insects like the Diptera have evolved a unique strategy for development that involves a complete change of body form. The larval form that hatches from the egg grows through a classical cycle of moulting. The imago, in contrast, is formed from the imaginal discs that are set aside during embryogenesis, grow during the larval period and differentiate only during a complex process of metamorphosis at the pupal stage (Fig. 7). In many Brachycera, the entire imaginal body is reconstructed from imaginal discs and histoblasts and the pupal stage is correspondingly long. In the Nematocera, metamorphosis is less extreme and the life cycle shows a number of ancestral features. The number of larval instars, for instance, is greater and the imaginal discs are much simpler, the future appendages being fastened down to the body wall (the imaginal discs are free from the body wall in Brachycera, remaining attached by a thin stalk in most species; Fig. 7). In all cyclorraphous Brachycera, metamorphosis takes place inside a puparium (the hardened skin of the third instar larva) that provides a protective cocoon in which the adult body can be partially or entirely reconstructed from the epithelium of the imaginal discs; the larval body is absorbed. In contrast, in many Nematocera, the larval body wall grows by cell division during the larval and pupal periods and parts of it are passed on from larva to pupa to adult (Fig. 7). Finally, in many Nematocera, the pupal period is extremely short, sometimes lasting less than one day.

One consequence of holometabolic development is that the imaginal discs, tucked away inside the larva, are freed from the necessity to moult and differentiate prior to pupation. Studies in *D. melanogaster* have shown that the imaginal cells grow by cell division throughout the larval period but secrete cuticle for the first time only at pupation and for the second and final time at the pupal-imaginal moult (Ursprung and Nöthiger, 1972; Fristrom and Fristrom, 1993). This means that there are really only two 'instars' for the imaginal cells: one larval and one pupal. In *D. melanogaster*, at least, the macrochaete precursors arise during the larval period before the pupal moult, whereas the microchaete precursors form during the pupal period after secretion of the pupal cuticle (Huang et al., 1991; Usui and Kimura, 1993; Fristrom and Fristrom, 1993). In other Brachyceran species also, it is likely that the macrochaetes arise before the microchaetes, since the macrochaetes are generally spaced further apart from one another than are the microchaetes, suggesting that there has been a longer interval for division of the intervening epidermal cells (Lawrence and Hayward, 1971).

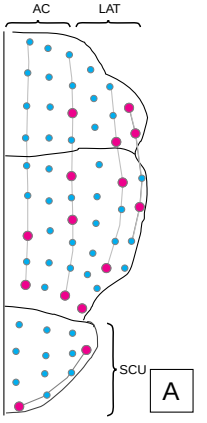
In many Brachycera, the notal macrochaetes are not only bigger, but also sometimes of a different colour and/or shape to the microchaetes (Fig. 4). The difference in morphology between the bristles is probably simply a reflection of the fact that they arise at different instars, separated by an intervening moult cycle. It is a characteristic of most insects that new and/or different morphological features appear at different moults, due to the different levels of juvenile hormone and repeated exposure of the cells to ecdysteroids (Riddiford, 1993; Fristrom and Fristrom, 1993). Indeed a study of campaniform sensilla on the wing blade of *D. melanogaster* has revealed the presence of two subpopulations with distinct physiological properties and central projections, that are born at different periods after pupariation when the precursors are subjected to different concentrations of ecdysteroids (Dickinson and Palka, 1987). The Nematocera, and some of the orthorraphous Brachycera, frequently bear only a single bristle type of uniform size. It is not known when the bristle precursors of the notum arise in any species of Nematocera so it remains to be seen whether they all develop at the same instar.

One potential advantage of holometabolic development is that there is an extended period of developmental time for the construction of the macrochaete pattern. In *D. melanogaster*, the macrochaete precursors appear over a period of 48 hours, a genetic 'prepattern' having been established earlier (see section on Genetic mechanisms). Indeed, the several layers of complex genetic circuitry involved in positioning of the macrochaete precursors may require this prolonged period of development to perform their function. The microchaete pattern, on the contrary, is constructed over a much shorter time period: the precursors form during approximately 4-6 hours (Usui and Kimura, 1993), and there is no evidence that it relies upon a genetic 'prepattern'.

The development of two distinct bristle patterns in the Schizophora thus probably occurs in two discrete time periods separated by an intervening moult. One consequence of this is, that, the pattern of microchaetes, that develops later, has to be superimposed over the existing pattern of macrochaetes. Each macrochaete precursor produces an inhibitory zone around itself preventing the surrounding cells from developing into bristle precursors themselves (see section on Lateral inhibition). The presence of macrochaetes at many sites therefore considerably restricts the number and positions of cells that can develop as microchaetes, in the same way that newly arising bristles have to be interspersed between ones that are already formed in Hemimetabola. In many species, microchaetes are simply randomly distributed and interspersed between the macrochaetes. Just as in the Collembola described earlier, they arise either in areas devoid of macrochaetes or by intercalation between the pre-existing macrochaete precursors when the

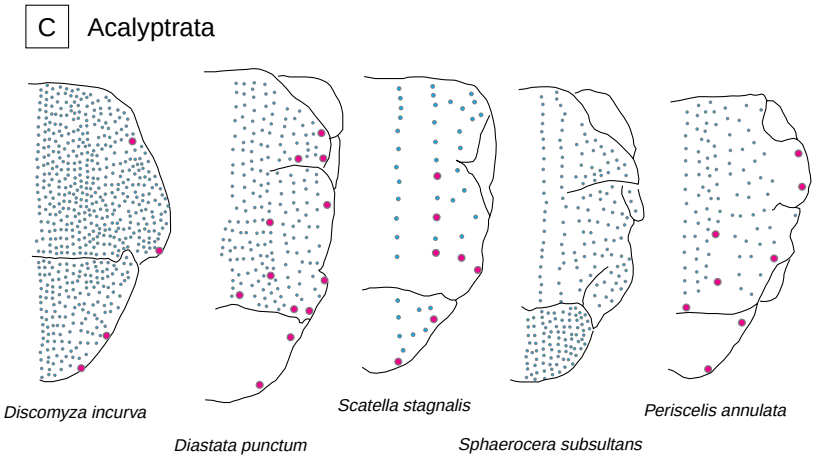
space provided by division of the epidermis is sufficient. Mutant *D. melanogaster* flies that differentiate additional, ectopic macrochaetes, but retain normal bristle spacing, bear fewer microchaetes simply because there is less room for them.

In all Diptera, the position of microchaetes is less precisely specified than that of the macrochaetes. In many species, however, the microchaetes are aligned into rows, although they only very rarely occupy individual, stereotyped sites. We examined the bristle patterns of 106 species of Schizophora to see how the rows of microchaetes are arranged in relation to the macrochaetes. Acalyptrata are either devoid of acrostichal macrochaetes or carry a single prescutellar bristle (Fig. 5). There is thus a fairly large macrochaete-free area between the DC bristles and the dorsal midline (each heminotum arises from an individual imaginal disc). It is in this area that the microchaetes most frequently form longitudinal rows aligned with the DC row (Fig. 9). Rows of microchaetes are much less frequently found elsewhere on the notum where there are



B

	AC	LAT	SCU	n
Calyptrata present	94	92	86	52
Calyptrata in rows	52	15*	4*	
Acalyptrata present	89	80	35	54
Acalyptrata in rows	72	20*	9*	



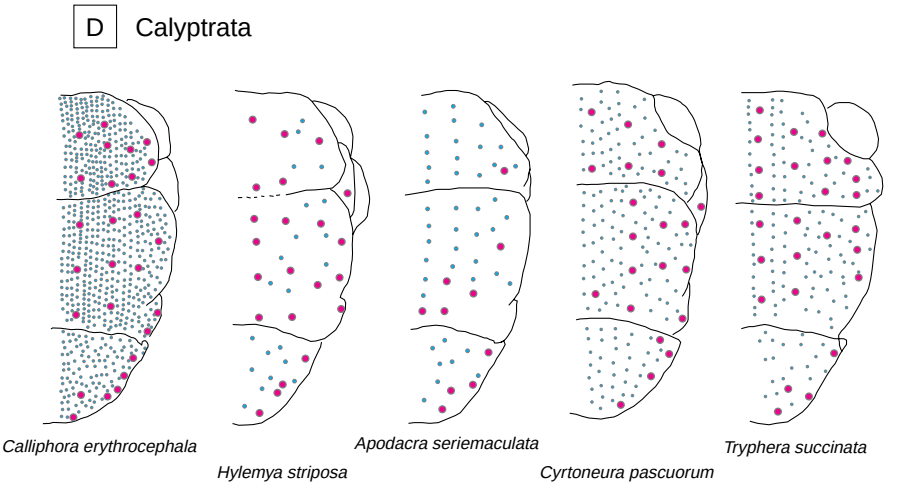


Fig. 9. The arrangement of microchaetes into longitudinal rows on the notum of species of Acalyptrata and Calyptrata (Brachycera: Schizophora). (A) The regions of the notum that were scored: AC, the acrostichal area, LAT, the lateral area, SCU, the scutellum. The presence or absence of microchaetes and whether they are arranged into rows is recorded as a percentage. *Rows on the lateral half of the scutum and on the scutellum are often irregular. The data was collected from 9 families of Calyptrata and 17 families of Acalyptrata. Selected examples are drawn in C and D (not to scale). *Discomyza incurva* (Ephydridae): note the presence of many rows of closely spaced (small) microchaetes and the near absence of macrochaetes. The rows continue to the base of the scutellum, which is enlarged in this species. *Diastata punctum* (Diastatidae): rows are seen only on the AC area where there is a single prescutellar macrochaete. *Scatella stagnalis* (Ephydridae) a single row of microchaetes are found at the position of the acrostichal macrochaetes and another is aligned with the dorsocentral bristles. *Sphaerocera subsultans* (Sphaeroceridae): this species is apparently devoid of macrochaetes and bears two rows of AC microchaetes. The scutellum has a squarer shape and bears rows of microchaetes. *Periscelis annulata* (Periscelidae): rows are only seen on the AC area. *Calliphora erythrocephala* (Calliphoridae): a large species with macrochaetes and microchaetes of very different size. There are three rows of microchaetes between the midline and the acrostichal macrochaetes and five to six rows between the acrostichal and dorsocentral macrochaetes. In addition, three rows are intercalated between the dorsocentral and intra-alar macrochaetes. *Hylemya striposa* (Anthomyidae): microchaetes are sparse and randomly distributed. *Apodacra seriemaculata* (Muscidae): three rows are aligned with the acrostichal, dorsocentral and intra-alar macrochaete rows. *Cyrtoneura pascuorum* (Muscidae): five rows of AC microchaetes and five to six rows on the scutellum. *Tryphera succinata* (Tachinidae): note a single row between the midline and the acrostochal macrochaetes, and two rows between the acrostichal and dorsocentral macrochaetes. Note that in some species microchaetes are also intercalated between macrochaetes within the same row.

generally more macrochaetes. Similarly on the scutellum, which is much smaller than the scutum, microchaetes, when present, are generally not organized into rows. The exceptions are found in species, such as *Discomyza incurva* and *Sphaerocera subsultans*, where the scutellum is either enlarged and/or devoid of macrochaetes (Fig. 9). Thus, from this small survey, it appears that an alignment of microchaetes into rows on some areas of the notum is correlated with an absence of macrochaetes.

When compared with the Acalyptrata, Calyptrata generally bear many more macrochaetes. These often include all four rows on the scutum and both presutural and postsutural bristles (Fig. 5). Although some display random microchaete patterns, other species do have rows of microchaetes in spite of a complete macrochaete pattern (Fig. 9). However, in our sample, the Calyptrata are notably larger than the Acalyptrata (the average length of the notum was 4.9 mm and 1.8 mm respectively). Fig. 4 shows an example: the blowfly *Calliphora vicina* is very much larger than *D. melanogaster*. In these larger species, the difference in size between the macrochaetes and microchaetes is even more pronounced and is accompanied by enormous differences in relative density, e.g. in *D. melanogaster*, there is space for one microchaete between the two DC macrochaetes whereas, in *Calliphora erythrocephala* and *Calliphora vicina*, there are 8 to 10 microchaetes between any two DC bristles (Figs 4, 9). In many of these large flies, rows of microchaetes are found in the large spaces available between the rows of macrochaetes (Fig. 9). Thus, as in the Collembola, rows of bristles have been intercalated between pre-existing rows that were made at an earlier moult.

WHY DID THE BRISTLE PATTERNS EVOLVE?

The conservation of stereotyped bristle patterns over long periods of evolutionary time in Diptera suggests that these patterns are important for behaviour and indeed the axonal projection pattern of each macrochaete organ is dependent on the site in the epithelium at which the precursor is born (Ghysen, 1980). Thus these spatial arrangements may have been maintained by natural selection (Garcia-Bellido, 1981). However, very little is known about the function of the notal bristles and therefore it is difficult to speculate on the nature of such selective pressures. Bristles on the notum are all mechanosensory and, in *D. melanogaster*, stimulation of some elicits a cleaning response (Vandervorst and Ghysen, 1980). Although the main function of the enlarged dipteran thorax is to provide space and attachment sites for the flight muscles, it does not seem likely that the complex patterns of macrochaetes are involved in the regulation of flight manoeuvers. Many of the Syrphidae or hoverflies, which are perhaps the champions of fly aerobatics, are devoid of macrochaetes and have random patterns of long thin uniformly sized bristles (Fig. 4). Furthermore, other orders of flying insects do not display stereotyped thoracic bristle patterns. The Odonata or dragonflies have randomly spaced fine duvetous bristles on the thorax, yet they are powerful fliers and some can attain speeds of 60 kilometers per hour (although their flight is regulated by a different mechanism from that of Diptera: they have no halteres and the two pairs of wings are inserted on top of the thorax, like helicopter blades, rather than laterally as in flies and airplanes). The thorax of bees and moths bears similar long fine uniformly spaced bristles. In order to understand the reason for the stereotyped patterns, we will need to learn more about the function of the macrochaetes in Diptera.

THE GENETIC MECHANISMS UNDERLYING BRISTLE PATTERNS

The genetic control of bristle patterning has been almost exclusively studied in *Drosophila melanogaster*, a species of Acalyptrata with a reduced, stereotyped macrochaete pattern and five rows of acrostichal microchaetes (Fig. 1). Two important regulatory gene networks have been uncovered. The first includes the genes of the *achaete-scute (ac-sc)* complex (AS-C) and their regulators (Fig. 10). The *ac-sc* genes encode related basic-helix-loop-helix (bHLH) proteins characteristic of a family of transcriptional regulators that work as heterodimers together with the product of the gene *daughterless* (Ghysen and Dambly-Chaudière, 1988; Cabrera and Alonso, 1991; Villares and Cabrera, 1987; Gonzalez et al., 1989). Expression of these genes provides cells with neural potential, allowing them to develop into nerve cells. Within domains of *ac-sc* expression, single, spaced cells are chosen to become sensory organ precursors. This is achieved by a phenomenon of lateral signalling that is mediated by a second network of genes that encode elements of the Notch signalling pathway (Artavanis-Tsakonas et al., 1995; Kimble and Simpson, 1997). Thus nascent neural precursors prevent neighbouring cells from also becoming neural precursors, by means of an inhibitory signal that represses *ac-sc* expression (Fig. 11).

Regulation of *achaete-scute*

There are four genes in the AS-C, but only two, *ac* and *sc*, are required for bristle development. The macrochaetes on the notum arise from small clusters of cells expressing *ac-sc* in the larval imaginal disc, called proneural clusters, that prefigure the sites of each of the future bristles (Cubas et al., 1991; Skeath and Carroll, 1991; Fig. 10). Expression is then progressively refined to single precursor cells. The *ac-sc* genes share *cis*-regulatory enhancer sequences that are scattered over about 100 kb of DNA (Ruiz-Gomez and Modolell, 1987; Gomez-Skarmeta et al., 1995; Fig. 10). The *cis*-acting elements respond to local positional cues, presumably conveyed by transacting factors that regulate the dynamic spatial and temporal expression patterns of these genes. Therefore the positions of the eleven stereotyped macrochaetes is the result of complex regulation of *ac-sc* expression. However, it has also been shown that experimentally contrived ubiquitous expression of *sc*, in the absence of endogenous *ac-sc* expression, results in the development of bristles that arise first at the normal wild-type locations (Rodriguez et al., 1990. Note, however, that, in the genotypes employed, enhancer-induced expression of *lethal of scute* and *asense* may contribute to bristle positioning). This demonstrates that there may be more than one genetic mechanism that ensures the correct positioning of the macrochaetes.

In contrast to the macrochaetes, the rows of acrostichal microchaetes arise in the pupal notum from longitudinal stripes of *ac-sc* expression that resolve to single, spaced precursors

(Fig. 10; K. U. and K. Kimura, unpublished data). The stripes arise sequentially; it is not known whether they are controlled by similar enhancer sequences (Fig. 10).

Lateral inhibition

Bristle spacing requires cell-cell communication mediated by the Notch signalling pathway. Cells within a proneural cluster or stripe that express *ac-sc* are probably initially equivalent in that it has been shown experimentally that they all have the capacity to develop into sensory bristle precursors (Hartenstein and Posakony, 1990; Heitzler and Simpson, 1991). Each cell expresses not only *ac-sc*, but also both the ligand, Delta, and the receptor, Notch, as well as the means to transduce the signal (Kooh et al., 1993). Therefore each cell can both send and receive the inhibitory signal. With time, however, only a single cell comes to express strongly the ligand and its neighbours have their receptors activated. This relies on a feedback loop within each cell linking high levels of activation of the receptor with reduced production of the ligand (Seydoux and Greenwald, 1989; Heitzler and Simpson, 1991; Wilkinson et al., 1994; Fig. 11). The regulatory loop is under transcriptional control via the *ac-sc* genes themselves that regulate the expression of *Delta* (Heitzler et al., 1996; Künisch et al., 1994). It is possible that any small difference between cells, in the level of any component of the loop, can be amplified via the loop itself such that eventually only a single cell will become a signalling cell (Seydoux and Greenwald, 1989; Heitzler and Simpson, 1991). This mechanism ensures that a spaced pattern of microchaete precursors emerges from a stripe of cells expressing *ac-sc* and one or two spaced macrochaete precursors from each of the proneural clusters.

The microchaetes are variable in number and position within the rows, and it appears that, for these bristle precursors, the choice of signalling cell is random (Heitzler and Simpson, 1991). The choice of the macrochaete precursor cells is not random but is in some way biased since generally a cell at a specific position is chosen from the group (Cubas et al., 1991; Skeath and Carroll, 1991). This accounts for the stereotyped nature of the macrochaete pattern. Nevertheless, the singling out of the precursors still requires the Notch-Delta regulatory

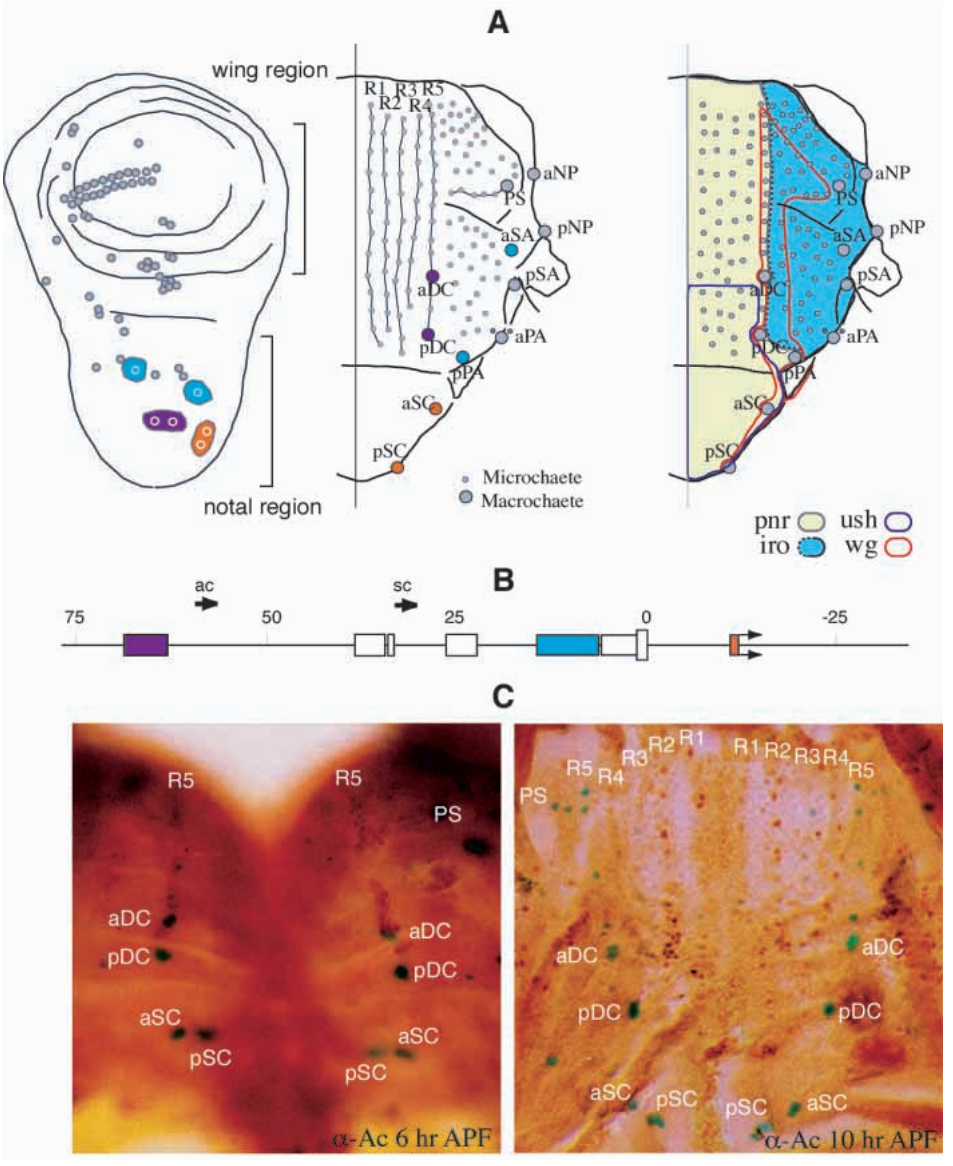


Fig. 10. Regulation of *achaete-scute* genes in *Drosophila melanogaster*. (A) The macrochaete precursors arise in the third instar imaginal discs from clusters of *achaete-scute*-expressing cells at specific sites (shown in colour together with the correponding bristles on the adult notum). The expression domains of known upstream regulators are shown on the right, *pnr (pannier), ush (u-shaped), wg (wingless)* and *iro (iroquois)*. Note that they are expressed in longitudinal bands that are aligned with the bristle rows. The dorsocentral bristles arise within the domain of expression of *pannier*, but outside that of *u-shaped*. (B) *achaete-scute* expression is driven by *cis*-regulatory elements distributed throughout this gene complex (schema adapted from Gomez-Skarmeta et al., 1995). (C) The expression of *achaete-scute* (revealed with an anti-Achaete antibody seen in brown) in the notum is shown at 6 and 10 hours after pupariation (APF). The preparations are also stained for *lacZ* (in blue) expressed from the enhancer trap line A101, which labels the sensory precursors. At 6 hours APF, the first *achaete-scute* stripe corresponding to microchaete row 5 can be seen. At 10 hours APF, bristle precursors from the first stripes to form are apparent and the expression of *ac-sc* in stripes 2 and 4 can be seen.

loop and, in the case of the DC precursors, the outcome may be biased by means of local variations in the levels of Ac-Sc (Cubadda et al., 1997; Haenlin et al., 1997).

Development of the proneural clusters relies on a prepattern

The spatiotemporal expression of *ac-sc* must rely on upstream transcriptional regulators that act through the enhancer elements. Some of these are known, they are expressed in distinct domains over the epithelium of the notal disc and are thought to define a 'prepattern' (Stern, 1954; Campuzano and Modolell, 1992; Simpson, 1996). It is interesting to note that the few upstream activators that have been described so far are all expressed in longitudinal bands parallel to the bristle rows characteristic of the ground plan of higher Diptera and seen in many Calyptrata (Fig. 10). Two transcriptional activators, Iroquois and Pannier, are required for the bristles of the lateral and medial halves of the notum, respectively (Gomez-Skarmeta et al., 1996; Leyns et al., 1996; Ramain et al., 1993; Cubadda et al., 1997; Haenlin et al., 1997). Pannier acts through the DC enhancer to control development of the two DC bristles, but is negatively regulated by the product of the *u-shaped* gene. Together the partially overlapping but distinct expression domains of *pannier* and *u-shaped* lead to the precise positioning of the DC bristle row (Cubadda et al., 1997; Haenlin et al., 1997; Fig. 10). It is not known how the discrete proneural clusters arise from the broad bands of expression of these genes. In addition, the signalling molecule, Wingless, is expressed in a longitudinal band in the centre of each heminotum and is also required for *ac-sc* expression; the two DC precursors arise on the edge of the Wingless stripe (Phillips and Whittle, 1993; Fig. 10).

Two negative regulators of *ac-sc*, *extramacrochaetae (emc)* and *hairy (h)*, have also been described (Moscoso del Prado and Garcia-Bellido, 1984a,b). *hairy* encodes a bHLH-containing transcription factor that represses *ac-sc*, while *emc* encodes a protein that sequesters the *ac-sc* proteins and prevents them from binding to DNA (Rushlow et al., 1989; Van Doren et al., 1991, 1992, 1994; Garell and Modolell, 1990; Ohsako et al., 1994). *hairy* and *emc* are expressed (independently of *ac-sc)* in complex spatial domains and are part of the prepattern that defines the positions at which bristles will arise (Cubas and Modolell, 1992; our unpublished observations). In general, high levels of Emc in the larval discs are found at sites of low levels of Ac-Sc. These regions may be refractory to Ac-Sc activity, which could account for the fact that bristles first arise at normal locations under experimental conditions of uniform, ubiquitous *sc* expression (Rodriguez et al., 1990; Cubas and Modolell, 1992). Conversely, loss of function of *emc* leads to the development of additional ectopic macrochaetes in atavistic patterns of rows closer to the ground plan that resemble those of other Diptera (Garcia-Bellido, 1981; Garcia-Alonso and Garcia-Bellido, 1988). Since Emc is a post-translational repressor of Ac-Sc, this observation suggests that *ac* and *sc* are expressed at low (basal?) levels in the imaginal disc, at sites other than the proneural clusters from which the extant bristles arise.

Loss of *h* leads to the appearance of microchaetes in ectopic positions, such as on the scutellum and the lateral notum (Moscoso del Prado and Garcia-Bellido, 1984a,b), patterns that are also characteristic of other Diptera. On the leg, *h* is expressed in longitudinal bands that alternate with stripes of *ac-sc* expression (Orenic et al., 1993). On the notum, however, *h* does not appear to play a role in defining the rows of acrostichal microchaetes (our unpublished results).

Conservation of genetic mechanisms in other Diptera

In the flies displaying in general the most ancestral features, the Nematocera, bristles are generally arranged in spaced but random patterns. This is possibly the most ancient state and is certainly the simplest to construct. Studies in the hemimetabolous insects have shown that in species with random patterns such as *Rhodnius*, the bristle precursors arise in a stochastic fashion after each instar (Wigglesworth, 1940). In thus it appears that most of the body wall cells have neural potential. Therefore one prediction is that *ac-sc* will be found to be expressed ubiquitously over the epithelium in these animals. If so, it is also likely that the organization of the *ac-sc* locus will be simpler with perhaps fewer genes and less requirement for multiple regulatory sequences (Galant et al., 1998). An ubiquitous expression of *ac-sc*, together with Notch-mediated lateral signalling, would theoretically be all that is needed to generate such patterns. Lateral inhibition itself is likely to be very ancient and to be used to generate a pattern of spaced bristles in nearly all insects.

We have argued that an arrangement of bristles into rows may have initially been derived from a random pattern. In many species, the rows are complete and extend the full length of the scutum. The microchaetes of *D. melanogaster* are organized in this manner and they are formed from longitudinal stripes of *ac-sc* expression. We therefore predict that, in other species of Diptera, rows of bristles are likely to result from stripes of Ac-Sc. This may also be the case for the four rows of macrochaetes. The basic genetic mechanism that allows microchaetes and macrochaetes to align into rows is likely to be common to both classes of bristles. It could simply be repeated at consecutive instars. If a stripe-like expression of *ac-sc* is derived from an earlier ubiquitous one, then how could the transition from ubiquitous *ac-sc* expression to one of stripes have arisen?

The following observations suggest that the mechanism that allows bristle rows to form is simple and dynamic. Firstly, a pattern of rows can change throughout development: new rows can be intercalated between pre-existing ones after division of the epidermis during the intermoult, as seen in both Diptera and Collembola (Figs 8, 9). Furthermore, the microchaete rows in *D. melanogaster* (which form within a single instar) arise sequentially: row 5 is followed by rows 1 and 3 and finally rows 2 and 4 intercalate between the others (Usui and Kimura, 1993; Fig. 10). The pattern of five rows can be modified experimentally: intermediate stages can be visualized in semi-starved flies which may have fewer rows and occasionally, exceptionally large individuals bear additional partial rows between the usual five (Fig. 11). Finally, while it is constant between individuals of any one species, it appears that, throughout the history of the Drosophilidae, the number of microchaete rows on the scutum has changed frequently (Fig. 6).

An arrangement of bristles into rows is a widespread characteristic of a number of insect orders. Although we know of no evidence for this, it seems possible that the transition

from randomly distributed bristles to the organization of straight rows may have occurred more than once throughout evolutionary time. If so, this suggests that the underlying genetic mechanism may depend on regulatory gene networks that were already present in species with randomly distributed bristles. The most obvious candidate to have been co-opted to generate spaced stripes of *ac-sc* expression is that of Notch-mediated lateral inhibition. A strong argument in favour of this hypothesis is that, in *D. melanogaster* flies mutant for *Notch*, the acrostichal microchaetes are no longer arranged into rows. An almost complete loss of *Notch* function results in adjacent bristles that cover the entire scutum rather than simply five bands of adjacent bristles, whereas partial loss leads to a disruption of the bristle rows (Fig. 11; Hartenstein and Posakony, 1990; Heitzler and Simpson, 1991). A further indication that the mechanism allowing spacing of bristles within a row is linked to that governing spacing of the rows themselves is afforded by a comparison of these two parameters in 58 species of Drosophilidae. It can be seen from Fig. 12 that the two are correlated, such that, as the spacing between rows increases, so does the spacing between bristles within a row. Bristle density decreases in larger flies so spacing between and within rows remains linked in spite of the fact that body size varies.

In order to generate a spaced pattern of repeated stripes of *ac-sc* expression, it would be sufficient to create a longitudinal boundary (using a positional cue such as the dorsal midline, for example) along which the Ac-Sc proteins could accumulate to higher levels. This 'stripe' could then inhibit neighbouring cells and establish an Ac-Sc-free interstripe zone, which would be followed by the acumulation of Ac-Sc along a new boundary when the distance exceeded the range of the inhibitory signal. This would result in the creation of a new 'stripe'. Stripes could be built up consecutively in this manner. Furthermore, once the pattern is established, new stripes could be intercalated between existing ones as the animal grows, in much the same way that new bristles are intercalated between those that formed earlier (Wigglesworth, 1940). Intercalation of stripes is frequently seen during pattern formation in many animals (Kondo and Asai, 1995; Meinhardt, 1995).

Our hypothesis is that lateral inhibition may have been used to generate a spaced pattern of stripes of *ac-sc* expression when such a pattern of stripes initially arose. This does not mean that the establishment of stripe-like expression in extant species still relies on this process. In the case of the Ac-Sc stripes that give rise to the microchaetes in *D. melanogaster*, lateral signalling is clearly required but probably does not alone account for the entire pattern. Throughout the Muscomorpha, while the number of microchaete rows varies considerably between species, it is generally constant within any one species. In the Drosophilidae at least, this is not a simple consequence of a larger or smaller body size, since the number of rows is not correlated with size (Fig. 6). Therefore a stereotyped number of microchaete rows may depend upon a stable genetic control perhaps involving a prepattern such as that involved in the generation of the macrochaete pattern in *D. melanogaster*.

The macrochaetes in the Muscomorpha are arranged into four rows and this pattern was probably established quite early in the evolution of this group. It has been conserved in spite of large variations in body size. The reduced pattern of macrochaete rows seen in Acalyptrata such as *D. melanogaster* is probably derived from the Muscomorphan ground plan of four rows. In this species, the dynamic,

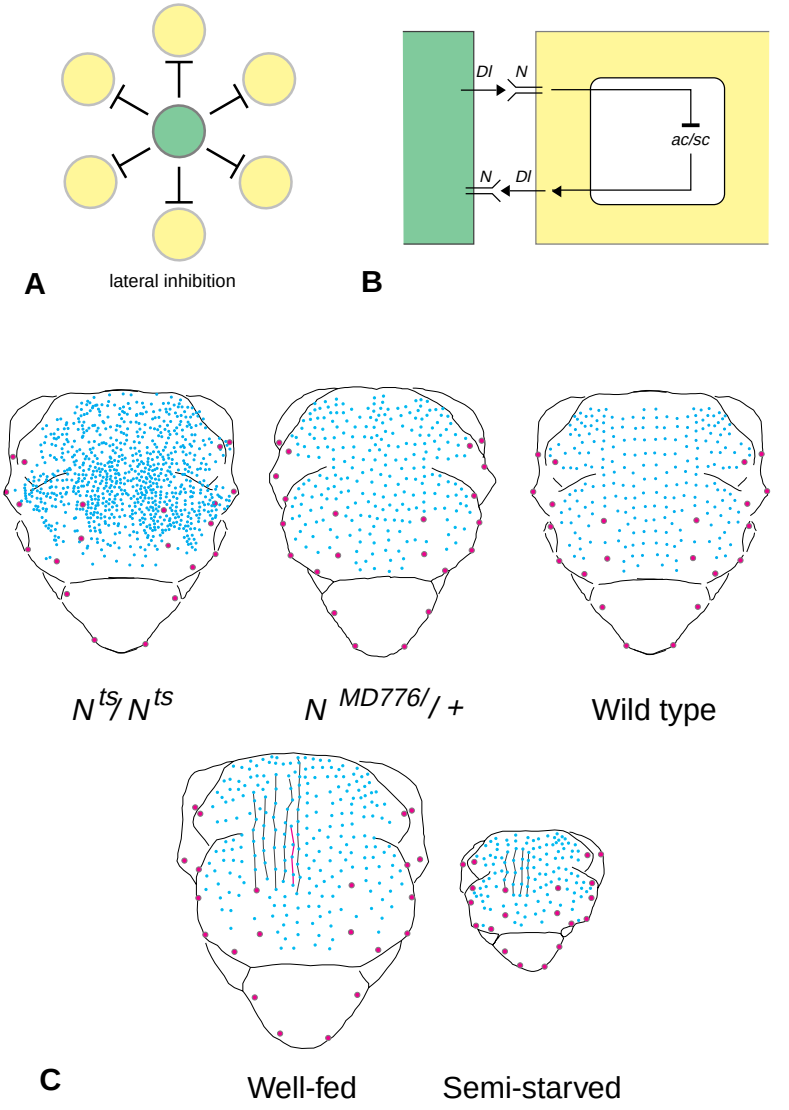


Fig. 11. Notch signalling and the spacing of bristles and bristle rows in *Drosophila melanogaster*. (A) Nascent bristle precursors inhibit neighbouring cells, preventing them from becoming bristles by means of the Notch signalling pathway that represses *achaete-scute*. (B) Notch and its ligand Delta are linked within each cell by a regulatory loop, such that activation of the receptor leads to downregulation of the ligand. (C) The density of bristles increases and the alignment of the acrostichal microchaetes into rows is lost, when the function of Notch is partially reduced ($N^{MD766}/+$). In the almost complete absence of Notch function (N^{ts} flies grown at 28°C), mutant flies bear a continuous field of adjacent bristles over the entire scutum. Semi-starved flies may display fewer microchaete rows and, in addition, the rows are irregular. In contrast, well-fed flies sometimes bear additional microchaetes between the rows.

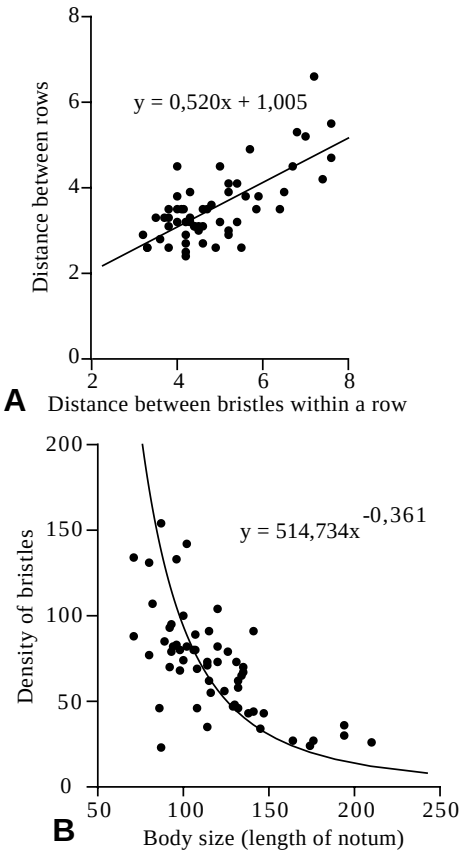


Fig. 12. The distance between microchaetes within a row and the distance between rows of microchaetes is correlated in species of the family Drosophilidae. (A) The distance (in arbitrary units) separating bristles within the same row as well as that separating bristles from adjacent rows is plotted for 58 species of Drosophilidae. An increase in distance is correlated for the two parameters. (B) The length of the notum (in arbitrary units) is plotted against the bristle density, which reflects the average distance between bristles (the number of bristles within a square of defined size at the same position was counted). It can be seen that, between Drosophilid species, the absolute number of bristles on the scutum increases rather little with size and so the overall spacing between bristles increases.

complex expression of *ac-sc* in the proneural clusters is regulated by a number of different upstream factors (Fig. 10). The prolonged period of development of the imaginal discs in the Acalyptrata may have allowed the accumulation, over evolutionary time, of this complex genetic circuitry. If so, it is likely that the generation of simpler patterns may be achieved with fewer regulatory networks. Some of the gene products used to regulate *ac-sc* in *D. melanogaster* may not be used for this purpose in Nematoceran species, for example, where organization of both the bristle patterns and the imaginal discs is less complex.

In *D. melanogaster*, the upstream regulators act through discrete *cis*-regulatory elements in the *ac-sc* locus and one interesting question concerns the conservation of these enhancer elements and whether perhaps they are conserved in flies bearing more ancestral patterns. They could, for example, direct a stripe-like gene expression for the complete macrochaete rows seen in other species. Such questions await the isolation in other Diptera of homologues of the *D. melanogaster ac-sc* genes and their transcriptional regulators.

CONCLUSIONS

It appears from the preceding survey that elaborate bristle patterns in flies are correlated with more complex life histories. The two are therefore likely to have evolved together. A decrease in the number of moults accomplished during development of the imaginal epithelium may have been an important factor. Diptera with more ancestral features display simple patterns of randomly distributed bristles whereas in more derived species the bristles are aligned into rows. Early on in the evolution of the Muscomorpha, the number of macrochaete rows on the scutum was restricted to four and most species have patterns based on modification of these four rows. Such a stepwise evolution of stereotyped patterns is likely to have been accompanied by increased complexity in the regulation of the *ac-sc* genes. In *Drosophila melanogaster*, there are clearly several discrete levels of regulation executed by different genetic pathways. These may have come into play at different times during the past, perhaps suddenly through the co-option of genetic circuits already employed for other developmental processes (Garcia-Bellido, 1983). Although small differences in function can be detected, the Ac-Sc proteins in *D. melanogaster* are functionally redundant (Hinz et al., 1994). The complex temporal and spatial regulation in this species may have been made possible through gene duplication. Insects with simpler patterns may well be found to have fewer *ac-sc* homologues (Galant et al., 1998) and less complex *cis*-regulatory regions. The structure and function of the *ac-sc* genes has been conserved in a number of animals and, in vertebrates, a regulatory loop involving Notch, similar to that described in *Drosophila*, is used to generate a spaced population of primary neurons often arranged into longitudinal rows (Chitnis et al., 1995). The isolation of these genes from other animals will provide a clearer picture of the evolutionary changes that underly their regulation.

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