Heredity (2006) 97, 148–156

www.nature.com/hdy

SHORT REVIEW

The origin and evolution of stereotyped patterns of macrochaetes on the nota of cyclorraphous Diptera

P Simpson and S Marcellini[1]
Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, UK

A long-standing problem in evolutionary biology is how genetic variation arises within populations and evolves to make species anatomically different. Many of the morphological differences in body plans between animal groups are thought to result from changes in gene expression during development. The rules governing the structure and evolution of *cis*-regulatory gene sequences are unknown, however, and the evolution of traits between closely related species remains relatively unexplored at a molecular level. To study the evolution of gene regulation, it is necessary to find a tractable trait that varies between species and for which the genetic regulation is well known in at least one of the species. The stereotyped, two-dimensional pattern of bristles on the thorax of *Drosophila* has been intensively investigated and is due to a precise spatial expression of proneural genes. Other species of flies have different bristle patterns and so comparisons between them provide a good paradigm for the study of changes in gene regulation. Here, we review the current state of understanding of these changes.
Heredity (2006) **97,** 148–156. doi:10.1038/sj.hdy.6800874; published online 19 July 2006

Keywords: Diptera; *Drosophila*; *Calliphora*; *Anopheles*; bristle; *achaete-scute*

Introduction

Most invertebrates have sense organs distributed over the body surface. Adults (imagos) of the true flies or Diptera generally bear a large number of sensory bristles. These arise from single precursor cells, sensory organ precursors (SOPs) that are born in the imaginal epithelium and develop into a bristle organ complete with its cuticular shaft, socket and underlying neuron and sheath cell, at the time of metamorphosis (Hartenstein and Posakony, 1989). Movement of the shaft in its socket causes excitation of the underlying neuron. The precursors do not move from their site of origin and so the adult array of bristles indicates their spatial arrangement at birth. Development of the bristle pattern on the thorax of *Drosophila* is well described. There are two classes of bristles, large (macrochaetes) and small (microchaetes) that arise at two distinct temporal phases (Simpson *et al*, 1999) (Figures 1 and 3). Precursors of the macrochaetes are born early during the larval period. As a result of the long period of intervening growth before metamorphosis these bristles are widely spaced apart. Precursors of the microchaetes are born later during the pupal period and as a result are more closely spaced than macrochaetes. Macrochaetes differ from microchaetes in both structure and probable function. They are longer, thicker and stouter. They are arranged into a stereotyped array. Each macrochaete has a specific axonal projection pattern in the thoracic ganglion that depends upon the position at which the bristle precursor is born (Ghysen, 1980). The notal macrochaetes of *Drosophila* have also been shown to have directional sensitivity (their neurons respond to movement of the shaft in a preferential direction) (Walker *et al*, 2000). Although they display some regional specificity, microchaetes are variable in number and position and do not appear to have individual defined functions (Usui-Ishihara and Simpson, 2005).

The true flies or Diptera are a very large insect order with a huge number of species. A simplified phylogenetic tree of the Diptera is shown in Figure 2. Among Brachycera a distinction between macro- and microchaetes can be made in many taxa. This is particularly clear in the Cyclorrapha where macrochaetes are consistently found in patterned arrangements and microchaetes are mostly present ubiquitously (McAlpine, 1981; Simpson *et al*, 1999). In addition, macrochaetes have been shown to have position-specific axonal projection patterns in some of these species (Murphey *et al*, 1989; Usui-Ishihara and Simpson, 2005). In all cyclorraphous species examined so far, macrochaete SOPs are born earlier in the development of the notum than microchaete SOPs (Huang *et al*, 1991; Simpson *et al*, 1999; Wülbeck and Simpson, 2000; Pistillo *et al*, 2002). Using criteria of spacing and size it appears that in some orthorraphous Brachyceran lineages there is a clear distinction between the two types of bristles, but in others and indeed in some basal Cyclorraphan taxa the distinction is blurred and a continuum of bristle sizes can be seen. The macrochaetes are thought to represent a derived feature the origins of which may be traced back to a common ancestor with the Nematocera (McAlpine, 1981). Flies of the Nematocera, considered to be basal,

Correspondence: P Simpson, Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, UK.
E-mail: pas49@cam.ac.uk
[1]*Current address: Departamento de Bioquimica y Biologia Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción, Casilla 160-C, Concepción, Chile*
Received 12 October 2005; accepted 5 June 2006; published online 19 July 2006

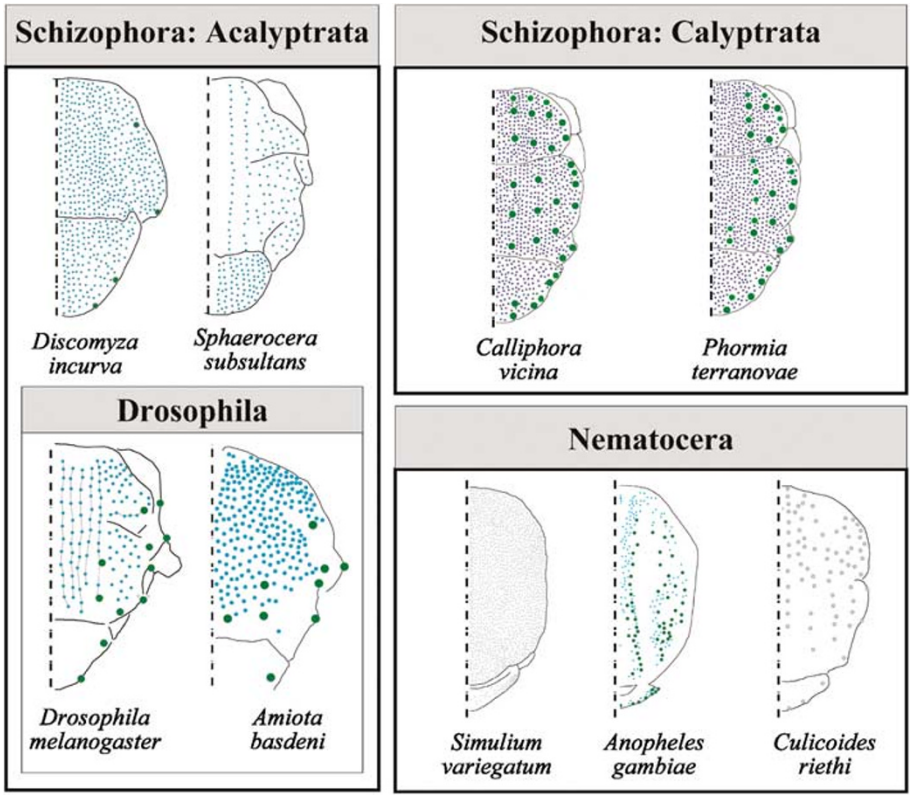

Figure 1 Schematic drawings of the thorax of species belonging to different groups of Diptera. Large green dots represent the macrochaetes and small blue dots the microchaetes. Species belonging to the Schizophora (see Figure 2) frequently bear stereotyped arrangements of macrochaetes. Of those mentioned in the text, *D. melanogaster* and *Ceratitis capitata* belong to the Acalyptrata, whereas *Calliphora vicina* and *Phormia terranovae* belong to the Calyptrata. All of these species have stereotyped bristle patterns. Nematoceran flies, in contrast, generally bear variable numbers of bristles rarely arranged into patterns. There are no clear macrochaetes in many members of this taxa (grey dots). Species of Nematocera mentioned in the text are *Anopheles gambiae*, *Culex pipiens*, *Clunio marinus*, *Toxorhynchites utilus*, *Chironomus thummi*, *Aedes aegypti* and *Chaoborus critillinus*. None of these have macrochaetes nor stereotyped bristle patterns.

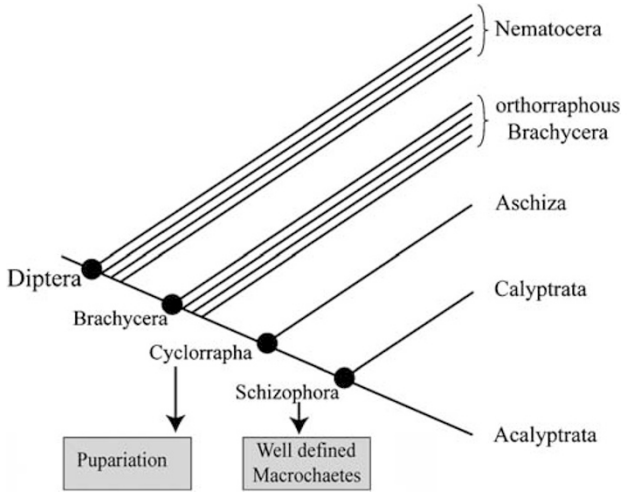

Figure 2 Phylogeny of the Diptera. The tree represents the phylogenetic relationships between the major taxa of Diptera. The Nematocera and the orthorraphous Brachycera are probably polyphyletic. The names of monophyletic groups are shown at the point of their emergence (filled black circles). The origin of the evolutionary novelties discussed in the text are indicated (grey boxes).

do not appear to bear macrochaetes (McAlpine, 1981; Simpson *et al*, 1999) (Figures 1 and 2). Firstly, although some taxa of Nematocera have long bristles these are generally thin and not thick and stout like the macrochaetes of the Cyclorrapha. Secondly the bristles in Nematocera are not arranged into stereotyped patterns but are often randomly positioned over the dorsal thorax. Thirdly the bristles, of whatever size, of many Nematocera are equally spaced from one another suggesting that their precursors arise simultaneously. Indeed the precursors for all sensory organs are born in a single short phase in the mosquito *Anopheles gambiae* (Wülbeck and Simpson, 2002). Thus, macrochaetes may have arisen in the lineage that led to the Brachycera. In this article, we discuss the possible origin of the macrochaetes themselves and of the stereotyped patterns.

Origin of macrochaetes

Heterochronic shift in proneural gene expression

In flies sensory bristles result from activity of the proneural genes *achaete (ac)* and *scute (sc)*. These encode transcription factors of the basic-helix-loop-helix family that, together with daughterless, provide neural potential to cells (Villares and Cabrera, 1987; Alonso and Cabrera, 1988; Ghysen and Dambly-Chaudiere, 1988; Gonzalez *et al*, 1989). Two temporally separate phases of *ac-sc* expression precede the formation of bristle precursors in *Calliphora vicina* (Calliphoridae), *Ceratitis*

capitata (Tephritidae) and *Drosophila* (Drosophilidae), an early one for macrochaetes and a later one for microchaetes (Cubas *et al*, 1991; Skeath and Carroll, 1991; Simpson *et al*, 1999; Wülbeck and Simpson, 2000; Pistillo *et al*, 2002). In the single species of Nematocera examined, *Anopheles gambiae*, there is only one phase of *AgASH (Achaete-Scute-Homologue)* expression, correlating with the simultaneous birth of all SOPs (Wülbeck and Simpson, 2002). Although there are two types of sense organs in *Anopheles*, bristles and scales, SOPs for both arise at the same time. Thus, the origin of macrochaetes may be linked to an additional, earlier phase of proneural gene expression. How could such a heterochronic shift result in two different types of sensory bristles? Studies in *Drosophila* indicate that an excess of scute before puparium formation (BPF) always results in additional macrochaetes, whereas an excess of scute after puparium formation (APF), results in only additional microchaetes (Rodriguez *et al*, 1990). So there is a distinct response within the epithelium to the same proneural protein at different times. On the other hand, although there is a small correlation between time of birth and bristle size (Murphey *et al*, 1980; Skaer *et al*, 2002b; Usui-Ishihara and Simpson, 2005), the earlier birth of macrochaete SOPs is not sufficient to account for their significantly larger size. Macrochaete SOPs are born over a period of about 30 h, the last forming at 3–4 h APF (Huang *et al*, 1991). The earliest microchaete SOPs already appear at 8 h APF (Usui and Kimura, 1993). So the question is: a shift in timing relative to what?

Additional pulses of 20-H ecdysone

In cyclorraphous flies pupariation heralds the beginning of metamorphosis. During this process, the larval skin is chemically modified to form the puparium, inside of which the pupal and then adult moults take place. Important hormonal changes occur. Moulting is regulated by pulses of 20-hydroxy-ecdysone (20E); for review see (Riddiford, 1993). In *Drosophila* there is a peak of 20E at each larval moult and during the last larval instar there are three small peaks (Warren *et al*, 2006) (Figure 3). One of these commits to pupariation and a second initiates wandering of the larva and synthesis of glue from the salivary glands. These are followed by a much larger peak, which correlates with pupariation. A further small peak causes head eversion and pupal cuticle deposition, and then a very large prolonged peak is associated with adult development. The role of 20E for moulting is very ancient. However, a role in the regulation of patterning and metamorphosis has evolved more recently (Truman and Riddiford, 1999). The effects of 20E are transduced by the heterodimeric receptors Ultraspiracle (Usp) and tissue-specific isoforms of Ecdysone Receptor (EcR), for review see Thummel (2001). Primary response genes such as *BR-C* and *E74* are initiated at all stages in response to 20E to regulate appropriate patterns of target gene activity (Ashburner *et al*, 1974). However, other transcription factors are stage-specific. For example *bFTZ-F1*, *BHR3* and *E75B* function at metamorphosis defining the outcome of the prepupal pulse of 20E, for review see Thummel (2001). Expression of stage-specific factors may define temporal states that will then ensure that responses to the next pulse of 20E will be distinct. EcR/Usp can also function as repressors in the absence of ligand. For example it has been shown that in the absence of Usp the SOPs form but differentiate prematurely (Schubiger and Truman, 2000). So the activity of 20E lifts the repressive activity of Usp and regulates the time of differentiation.

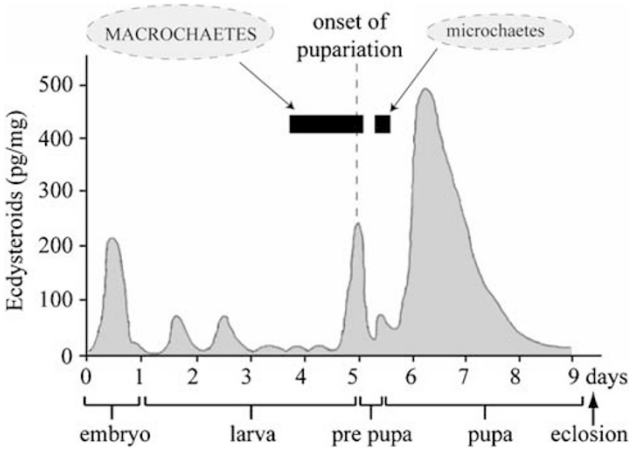

Figure 3 A correlation between peaks of ecdysteroid activity and the formation of macrochaete precursors. The graph shows the pulses of 20E experienced by *D. melanogaster* during larval and pupal life. Adapted from (Riddiford, 1993) and (Warren *et al*, 2006). A small pulse during the third and last larval instar causes wandering of the larva and glue synthesis. Pupariation is initiated at five days of development during a peak of 20E (dotted line). The temporal windows during which macrochaete or microchaete precursors are specified are shown as black rectangles. Note that macrochaete precursors form before, and microchaete precursors after, the 20E pulse corresponding to pupariation.

It is not completely understood what initiates the expression of *ac-sc* for macrochaete SOPs on the notum of *Drosophila*. The upstream activators Pannier and Iroquois are present in the notal epithelium before expression of *ac-sc*, suggesting that other permissive factors are required. It has been shown that the *atonal*-dependent sense organs, eyes, chordotonal organs and Johnston's organ, which arise in the early third instar, are dependent on the presence of 20E at that time (Niwa *et al*, 2004). Whatever the genetic regulation of expression, it is possible that the timing of SOP formation, relative to the successive pulses of 20E, determines whether a macro- or a micro-chaete will form. Formation of macrochaete SOPs in *Drosophila* is completed shortly before the peak corresponding to pupation, but microchaete SOPs are born after this pulse (Figure 3). A recent comparison between two species of blowfly suggests that the time of SOP formation is critical for the formation of macrochaetes. *Calliphora vicina* and *Phormia terranovae* (Calliphoridae) are two closely related flies with differing macrochaete patterns (Figure 1). They display two phases of *sc* expression on the developing notum, an early patterned one that is identical for both species and a later (ubiquitous) one. In *Calliphora* these are separated by about 24 h and macrochaete SOPs arise from the first phase and microchaetes SOPs from the second. In *Phormia*, however, the first phase is slightly delayed and the second phase greatly accelerated so that there is a small overlap between the two. As a result the last SOPs to form from the first patterned phase arise at the same time as the first SOPs from the second phase and

they display a morphology that is indistinguishable from the 'true' microchaetes (Skaer *et al*, 2002b). Interestingly the period of overlap occurs soon after pupariation, consistent with a hypothetical link with 20E levels.

So what about the lower flies that do not display macrochaetes? Very few families of Nematocera undergo pupariation. A few families do pupariate and carry out the pupal moult inside in the last larval cuticle: Perissommatidae, Scatopsidae, some Bibionidae, some Cecidomiidae (McAlpine, 1981). However, the pupae in all these cases are obtect, an ancestral feature where the appendages are fastened down to the body wall (see below). Note also that in these cases the puparium is little modified from the larva, unlike that of cyclorraphous flies. Obtect pupae are also characteristic of the Orthorrapha that similarly do not pupariate. Flies of a single superfamily of orthorraphous Brachycera, the Stratiomyoidea, do pupariate, but like the Nematocera do not have imaginal discs that are free from the body wall. In pupae of the Cyclorrapha, the appendages are free from the body wall, a state derived from the obtect condition. Pupariation must, therefore, have arisen several times independently (McAlpine, 1981).

Flies that do not pupariate moult directly from larva to pupa (McAlpine, 1981). Measurements of ecdysteroid titres have been made in a few such species: *Clunio marinus* and *Chironomus thummi* (Chironomidae), and *Toxorhynchites rutilus* (Culicidae) (Westbrook and Russo, 1985; Laufer *et al*, 1986; Neumann and Spindler, 1991). There is a probable commitment peak in the last (fourth) larval instar and then about a 10-fold increase corresponding to pupation. Therefore, the pulses that initiate larval wandering/glue synthesis and, in particular, pupariation in *Drosophila* are absent. Interestingly this difference is during the critical period that separates the formation of macro- and micro-chaete SOPs in *Drosophila*. We postulate that exposure to different pulses of 20E in the Cyclorrapha may predispose the epithelium to respond to *ac-sc* expression by making either macro- or micro-chaetes.

Early formation of imaginal discs that include the trunk

Many macrochaete precursors in *Drosophila* are born BPF. Furthermore they occupy precise stereotyped positions, as is the case for many other cyclorraphous species. The genetic control of the pattern in *Drosophila* has been shown to result from a cascade of activity of regulatory genes culminating in a precise spatial expression of *ac-sc* (Modolell and Campuzano, 1998; Calleja *et al*, 2002), see below. Sufficient time is required for these patterning events. In cyclorraphous flies the adult head and thorax develop from imaginal discs: invaginations of the epithelium that are continuous with the larval epithelium (Figure 4). The Cyclorrapha are the only holometabolous insects in which the discs form in the embryo. Thus, they can grow and be patterned during the entire larval period. A loss of sensitivity to juvenile hormone in the Cyclorrapha may allow this early growth (Truman and Riddiford, 1999). Furthermore the discs comprise not only the appendages but also the trunk. Thus, the notum is included in the wing imaginal disc. The wing disc is not associated with any sense organs present at the surface of the larva, although Keilin's organs and the dorsal organ remain associated with the leg and antennal

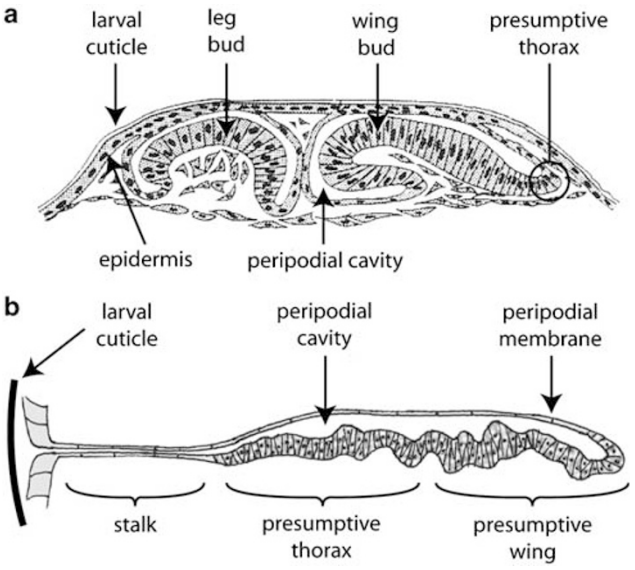

Figure 4 The evolution of imaginal discs. The drawings show cross-sections through the body of the larvae. (**a**) In the basal species *Anopheles gambiae* the future appendages are present as pouches budding from the larval epidermis. The future dorsal notum develops from a group of cells at the border between the larval and wing bud epithelia. Reproduced from (Clements, 1992). (**b**) In cyclorraphous Brachycera, as shown here for *D. melanogaster*, the developing imaginal discs are embedded well inside the laval body and remain connected to the larval epithelium through long thin stalks. The thorax and the wing both originate from the imaginal disc.

discs, respectively. The appendages are free from the body wall and in *Drosophila* are deeply embedded in the larval body. In *Phormia* the leg discs become deeply invaginated only after the final larval moult (Lakes-Harlan *et al*, 1991). Although the imaginal discs probably secrete a thin endocuticle at each larval moult (Svacha, 1992; Fristrom and Fristrom, 1993), they develop relatively free of the larval moult cycles.

Appendages that are free of the body wall and develop during early larval stages represent a derived condition (McAlpine, 1981). In contrast in Nematocera, imaginal discs only form at late larval stages. For example in *Aedes aegypti* and *Anopheles gambiae* (Culicidae) epithelial cells for the imaginal legs appear at the second larval instar (Clements, 1992). They are found as long folded structures at the fourth larval instar. In *Clunio marinus* (Chironomidae) and *Chaoborus cristallinus* (Chaoboridae) the discs only develop at the last larval instar (Neumann and Spindler, 1991; Svacha, 1992; Melzer *et al*, 1999). In Nematocera, as well as in the Orthorrapha, the appendages are fastened down to the body wall, an ancestral condition. Another characteristic of the discs of Nematocera is that they include the appendages but not the trunk. A fate map of the leg disc of *Culex pipiens* (Culicidae) indicated only the presence of the leg itself (Spinner, 1969). In *Anopheles gambiae* the dorsal notum of the adult was found to derive from a group of epithelial cells situated at the border between the wing appendage and the larval epithelium that undergo division and replace the larval epithelium during the last larval instar (Wülbeck and Simpson, 2002). Therefore, while the imaginal discs of cyclorrphous flies have an extended

period of time for patterning and development, those of Nematocera develop over a rather short time period.

Origin of stereotyped patterns

The four row bauplan

Macrochaetes are almost invariably arranged into species-specific patterns that, particularly in acalyptrate cyclorraphous flies, may be stereotyped (McAlpine, 1981; Simpson *et al*, 1999). Nearly all patterns, however, appear to be variations on a theme of four longitudinal rows on the scutum (Figure 5) (McAlpine, 1981; Simpson *et al*, 1999). This postulated ancestral pattern is independent of size and flies may display a complete or partial loss of rows but never additional rows. What is the basis for this apparent constraint in the bristle pattern? One possibility is that this trait is favoured by selection. Artificial selection experiments so far do not appear to support this view (Macdowell, 1915; Dominguez *et al*, 1987; Pineiro, 1992a, b; Dominguez *et al*, 1993). Although a 'wild type' pattern of 11 macrochaetes per heminotum is assumed to be fixed in *Drosophila melanogaster*, natural variants of this pattern can be found and enough genetic variation exists in nature to select flies for an increased number of notal bristles (Macdowell, 1915; Plunkett, 1926; Sheldon and Milton, 1972; Pineiro, 1992a, b; Dominguez *et al*, 1993). Selection experiments result in the presence of additional dorsocentral bristles, scutellar bristles and some bristles on the lateral scutum (Macdowell, 1915; Plunkett, 1926; Vreezen and Veldkamp, 1969; Pineiro, 1992a, b; Dominguez *et al*, 1993). The dorsocentral bristles may become numerous with continued selection, but they are either positioned close to the two extant ones or extend anteriorly to form a longitudinal row reminiscent of the ancestral pattern. Alternatively, developmental constraints could restrict the emergence of macrochaetes to particular sites. Indeed the additional bristles resulting from selection are not located over the sites of attachment of the flight muscles (Usui *et al*, 2004). There appears to be a constraint in the form of the indirect flight muscles that lie just below the surface of the scutum (Figure 6). These attach to the cuticle through tendons whose precursors are selected from the same epithelium as the bristle precursors (Lee *et al*, 1995; Fernandes *et al*, 1996; Frommer *et al*, 1996; Volk, 1999). In contrast to the bristles, the pattern of flight muscles and their attachment sites is highly conserved throughout the Diptera (Tiegs, 1955; Usui *et al*, 2004). Remarkably the flight muscles and the leg jump muscle attach in longitudinal domains that are between the rows of macrochaetes (Usui *et al*, 2004). From an examination of several hundred species, none were found in which the macrochaetes were located over the sites of muscle attachment. Bristle precursors develop in areas of *ac-sc* expression; tendon precursors develop in areas of expression of *stripe (sr),* a gene encoding a transcription factor with zinc finger motifs (Lee *et al*, 1995; Frommer *et al*, 1996). The domains of expression of *ac-sc* and *sr* are spatially segregated in *Drosophila* and *Calliphora vicina* (Usui *et al*, 2004; Richardson and Simpson, 2006). Experimentally contrived mis-expression of *sr* at the sites where bristle precursors develop, results in a loss of bristles in *Drosophila*; similarly mis-expression of *ac-sc* at the sites of tendon development interferes with muscle attachment (Usui *et al*, 2004). The mutual antagonism

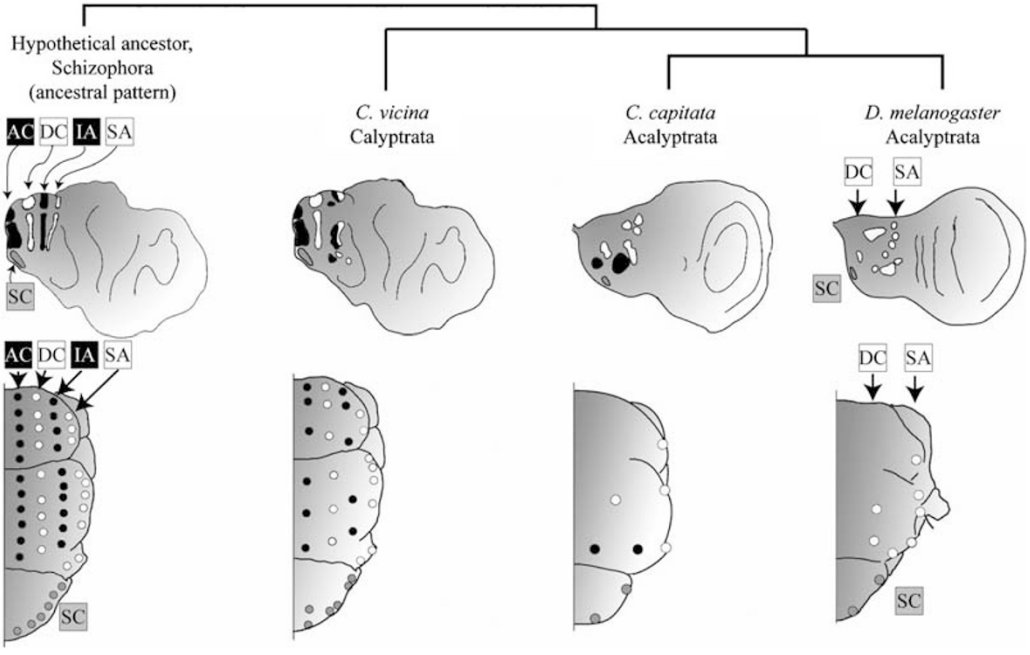

Figure 5 Proneural clusters may be derived from stripes of *sc* expression. Pistillo *et al* (2002) suggested that the proneural genes were expressed in an pattern of four longitudinal rows on the scutum of the ancestor leading to the Cyclorrapha (top left). These rows, and the bristles to which they give rise in the adult (bottom left) are named and represented with alternate black and white colour code. It is proposed that in the lineage leading to the Acalyptrata, different rows were entirely or partially lost, which correlates with a proneural gene expression pattern of reduced proneural clusters. As a result these flies have fewer bristles, but frequently in stereotyped arrangements on the notum. Abbreviations: AC, acrostichal; DC, dorsocentral; IA, intraalar; SA, supraalar; SC, scutellar. Adapted from Pistillo *et al* (2002).

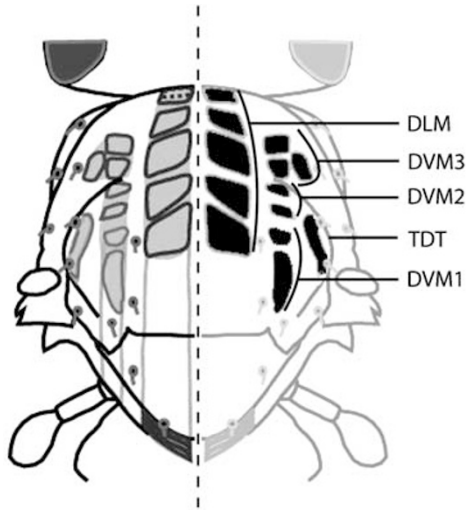

Figure 6 The muscle attachment sites on the thorax of Diptera. The indirect flight muscles of Diptera attach to tendons whose precursors arise in the same imaginal epithelium as the bristle precursors, shown for *Drosophila* on the left. Note that the macrochaetes form between the muscle attachment sites (small circles). On the right, the muscle attachment sites are named and shown in black. Abbreviations: DLM, dorso-longitudinal; DVM, dorso-ventral; TDT, trochanter depressor. Adapted from Levine and Hughes (1973).

between the products of these genes and their spatially separate expression domains in wild type *Drosophila*, would account for the spatial segregation of tendons and bristles. This molecular mechanism, if conserved, would account for the four row bauplan.

A conserved *trans*-regulatory landscape for *sc* expression in cyclorraphous flies

Among the cyclorraphous flies examined so far there is a correlation between the spatial expression of *sc* and bristle patterns. *sc* is expressed in discrete domains, either in stripes or in small clusters of cells called proneural clusters, at the sites of formation of the future bristle precursors (Cubas *et al*, 1991; Skeath and Carroll, 1991; Wülbeck and Simpson, 2000; Pistillo *et al*, 2002). Thus, changes in *sc* expression have accompanied evolution of the bristle patterns. Are these changes due to evolution of *cis*-regulatory sequences at the *sc* locus or to evolution of the upstream *trans*-regulatory factors, or both?

Studies in *D. melanogaster* have uncovered some of the transcriptional regulators of *ac-sc*. Pannier, a member of the GATA protein family, has been shown to directly activate *ac-sc* for development of the bristles on the medial half of the notum (Garcia-Garcia *et al*, 1999). The genes of the *Iroquois (iro)* complex are required for the bristles of the lateral half of the notum (Gomez-Skarmeta *et al*, 1996). Consistent with this *pannier (pnr)* is expressed in the dorso-medial notum and the *iro* genes *araucan* and *caupolican (caup)* in the dorso-lateral notum (Ramain *et al*, 1993; Gomez-Skarmeta *et al*, 1996). Downstream of Pnr and Iro, other transcription factors progressively subdivide the notum into smaller developmental units (Aldaz *et al*, 2003). Other factors act either downstream of Pnr and Iro, or modify their transcriptional activity through protein association. Thus, U-shaped (Ush) associates with Pnr modulating its activity, Wingless (Wg) is required for *ac-sc* expression in the medial notum, while Stripe (Sr) has been shown to negatively regulate bristle development (Phillips and Whittle, 1993; Cubadda *et al*, 1997; Haenlin *et al*, 1997; Ramain *et al*, 2000; Usui *et al*, 2004). *ac-sc*, *sr* and *wg* are all targets of Pnr and are expressed in discrete subsets of cells within the broad medial domain of *pnr* expression (Garcia-Garcia *et al*, 1999; Ghazi *et al*, 2003).

Expression of *pnr* in the dorso-medial notum is conserved between *Drosophila*, *Ceratitis* and *Calliphora* (Ramain *et al*, 1993; Wülbeck and Simpson, 2000; Pistillo *et al*, 2002). Furthermore, with only minor differences, expression of *caup*, *ush*, *sr* and *wg* is conserved between *Drosophila* and *Calliphora* (Richardson and Simpson, 2006). This suggests the existence of a system of interacting components that has been conserved across about 100 Myear of evolution. These components are also required for the development of many other structures that together make up the detailed morphology of the notum (Calleja *et al*, 2002). Expression of these genes may have evolved in concert since altering one component may affect others and modify many thoracic structures.

In contrast to the cyclorraphous flies, spatial expression of *trans*-regulatory factors in the basal species *Anopheles* reveals important differences. Expression of *Ag-pnr* is restricted to the medial notum but it does not occupy a single broad domain. Instead there are four separate domains of expression. Furthermore *Ag-ASH* (the *Anopheles* proneural gene) is expressed in domains that appear co-incident with those of *Ag-pnr*, rather than in subsets of cells within the *Ag-pnr* domains (Wülbeck and Simpson, 2002). Precursors for the sensory scales arise everywhere within each expression domain of *Ag-ASH*, but patterning of the bristles is quite different from anything seen in cyclorraphous flies. Bristles arise along the borders of *Ag-pnr*/*Ag-ASH* expression domains. It is not known how the bristle pattern is controlled but clearly there are no proneural stripes or clusters, and one does not observe a spatial correlation between the expression of the proneural gene and the positioning of bristles. The expression domains of *Ag-ASH* being co-incident with those of *Ag-pnr*, it is likely that *Ag-pnr* is involved in the regulation of the bristle pattern. This suggests a conserved role for a function of *Ag-pnr* in patterning the notum.

Gene duplication and evolution of *cis*-regulatory sequences

The apparent conservation of upstream regulators may mean that much of the change is to be found in *cis*-regulatory sequences of the *ac-sc* genes themselves. The *ac-sc* complex of *Drosophila* has been dissected in some detail. Expression of *ac-sc* in proneural clusters is regulated by discrete independently acting *cis*-regulatory enhancer elements scattered over about 100 kb (Ruiz-Gomez and Modolell, 1987; Gomez-Skarmeta *et al*, 1995) (Figure 7). Interaction between some of these and their upstream regulators has been examined in detail (Gomez-Skarmeta and Modolell, 1996; Garcia-Garcia *et al*, 1999; Ramain *et al*, 2000). When did these sequences arise and to what extent are they conserved? If stereotyped patterns are derived from an ancestral

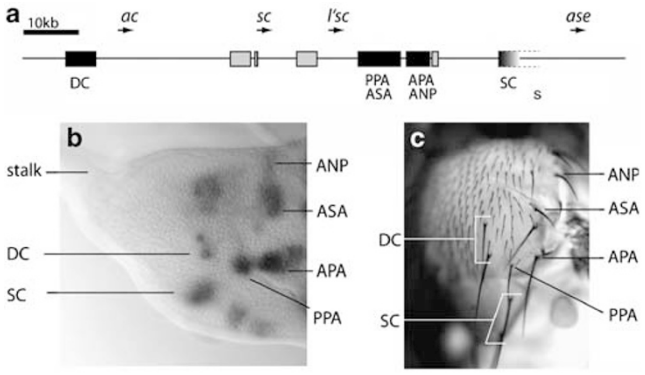

Figure 7 Genomic organisation and function of the *ac-sc* complex in *D. melanogaster*. (**a**) Genomic diagram showing the position of *ac*, *sc*, *lethal of scute (l'sc)* and *asense (ase)* as small arrows. Known enhancers driving expression of *ac* and *sc* in the thorax and wing are represented as black or grey boxes respectively. The name(s) of the proneural cluster(s) where the enhancer is active is indicated below. The downstream limit of the SC enhancer is still uncertain (fading black filling). Adapted from Gomez-Skarmeta *et al* (1995). (**b**) A photograph of the thoracic region of a wing disc, showing the expression of *sc* mRNA in proneural clusters. The clusters whose enhancers are known have been named. (**c**) A dorso-lateral view of an adult thorax. The macrochaetes arising from the proneural clusters described in (**b**) are named.

pattern of rows, then are proneural clusters derived from an ancestral expression pattern of longitudinal stripes like those seen in *Calliphora* (Figure 5)? Are such stripes of expression driven by homologous enhancers? The answers to these questions await the isolation of regulatory sequences from other species. In the meantime, however, there are some indications that enhancers for spatial expression may have arisen somewhere in the lineage leading to the cyclorraphous flies.

The four domains of *Ag-pnr* expression appear to be co-incident with the domains of expression of *Ag-ASH* (Wülbeck and Simpson, 2002). This suggests that regulation of *Ag-ASH* by Ag-Pnr may be much simpler and would not need recourse to numerous enhancer elements. Thus, *Anopheles Ag-ASH* may not have a complex modular promoter like that of *Drosophila*. If so, this implies an origin for these sequences after the divergence of *Anopheles* and *Drosophila*. Interestingly, the number of genes at the *ac-sc* complex has increased throughout the Diptera: *Anopheles* has only two genes whereas *Drosophila* has four. The four genes have arisen from three independent duplication events (Skaer *et al*, 2002a). The first duplication (giving *asense*, a neuronal precursor gene and a single proneural gene) occurred within the insect clade since the beetle *Tribolium castaneum* and probably the butterfly *Precis coenia* have these genes (Galant *et al*, 1998; Wheeler *et al*, 2003). The second two, however, took place within the Diptera. Phylogenetic analysis revealed that *Ag-ASH*, the proneural gene of *Anopheles* is homologous to three proneural genes in *Drosophila: lethal of sc* (essential for development of the central nervous system), *sc* and *ac* (essential for development of the peripheral nervous system) (Skaer *et al*, 2002a). If the duplications included not only coding sequences but also regulatory sequences, then this may have facilitated the emergence of numerous enhancer elements that would then be free to diverge. It is tempting to speculate that the duplication events are associated with the evolution of stereotyped patterns of the Cyclorrapha.

Conclusions and perspectives

We have discussed the possibility that macrochaetes may have arisen as a result of an additional, early phase of proneural gene expression before pupation. Two other factors may have contributed to their formation. First the Cyclorrapha is a monophyletic group characterised by the phenomenon of pupariation. This has probably incurred the introduction of an additional peak of 20E that regulates puparium formation prior to the peak that regulates pupation *per se*. The response of the epithelium to the presence of *ac-sc* before, at and after the extra 20E peak may allow a distinction between macro- and microchaetes. Second the early formation of imaginal discs free from the body wall that include the trunk, allows a prolonged period of time during larval life within which to pattern the macrochaetes. Have macrochaetes evolved more than once within Diptera? Macrochaetes are found throughout the Brachycera but many taxa are devoid of them. So either they have arisen more than once or they arose once and have been lost many times. The fact that most stereotyped patterns are variations on a theme of four longitudinal rows on the scutum, would seem to suggest an origin, in an ancestral species, of a four row bauplan that would be unlikely to have arisen multiple times. However, the discovery that macrochaete patterns are constrained by sites of attachment of an invariant pattern of flight muscles may indicate that early proneural gene expression in any lineage could have led to convergent patterns. Thus, perhaps macrochaetes have arisen independently a number of times.

The spatial expression pattern of *sc* correlates with the bristle patterns in the cyclorraphous species examined so far, but such a correlation is not seen for the *ac-sc* homologue of *Anopheles*, a basal Dipteran species. To better understand the steps leading to this transition, it is necessary to examine the expression of *ac-sc* homologues in phylogenetically intermediate families, such as those of the orthorraphous Brachycera (Figure 2). The differences in spatial expression patterns of *sc* between *Calliphora* and *Drosophila* appear to be mainly due to differences at the *sc* locus itself. There appears to be a conserved network of upstream regulators. Thus, the challenge for the future will be the isolation of *cis*-regulatory sequences of *sc* from other cyclorraphous species and their comparison with those of *Drosophila*. This may be a first step towards tracing the origins of these enhancers within the Dipteran lineage and their role in the evolution of stereotyped bristle patterns.

Acknowledgements

We thank the members of our group for stimulating discussions and the Wellcome Trust for their financial support.

References

Aldaz S, Morata G, Azpiazu N (2003). The Pax-homeobox gene *eyegone* is involved in the subdivision of the thorax of *Drosophila*. *Development* **130**: 4473–4482.

Alonso MC, Cabrera CV (1988). The *achaete-scute* gene complex of *Drosophila melanogaster* comprises four homologous genes. *EMBO J* **7**: 2585–2591.

Ashburner M, Chihara C, Meltzer P, Richards G (1974). Temporal control of puffing activity in polytene chromosomes. *Cold Spring Harb Symp Quant Biol* **38**: 655–662.

Calleja M, Renaud O, Usui K, Pistillo D, Morata G, Simpson P (2002). How to pattern an epithelium: lessons from *achaete-scute* regulation on the notum of *Drosophila*. *Gene* **292**: 1–12.

Clements AM (1992). *The biology of mosquitos. Volume 1: development, Nutrition and Reproduction*. Chapman and Hall: London.

Cubadda Y, Heitzler P, Ray RP, Bourouis M, Ramain P, Gelbart W *et al* (1997). *U-shaped* encodes a zinc finger protein that regulates the proneural genes *achaete* and *scute* during the formation of bristles in *Drosophila*. *Genes Dev* **11**: 3083–3095.

Cubas P, de Celis JF, Campuzano S, Modolell J (1991). Proneural clusters of *achaete-scute* expression and the generation of sensory organs in the *Drosophila* imaginal wing disc. *Genes Dev* **5**: 996–1008.

Dominguez A, Albornoz J, Santiago E (1987). Analysis of lethals in selected lines of *Drosophila melanogaster*. *Theoret Appl genet* **74**: 409–413.

Dominguez A, Albornez J, Santiago E, Gutierrez A (1993). Chromosomal analysis of *D. melanogaster* long term selected lines. *J Heredity* **84**: 63–66.

Fernandes JJ, Celniker SE, VijayRaghavan K (1996). Development of the indirect flight muscle attachment sites in *Drosophila*: role of the PS integrins and the stripe gene. *Dev Biol* **176**: 166–184.

Fristrom D, Fristrom JW (1993). The metamorphic development of the adult epidermis. In: Bate M. and Martinez-Arias A (eds) *The Development of Drosophila melanogaster*. Cold Spring harbor Laboratory Press: Cold Spring Harbor, NY. pp 843–897.

Frommer G, Vorbruggen G, Pasca G, Jackle H, Volk T (1996). Epidermal *egr*-like zinc finger protein of *Drosophila* participates in myotube guidance. *EMBO J* **15**: 1642–1649.

Galant R, Skeath JB, Paddock S, Lewis DL, Carroll SB (1998). Expression pattern of a butterfly *achaete-scute* homolog reveals the homology of butterfly wing scales and insect sensory bristles. *Curr Biol* **8**: 807–813.

Garcia-Garcia MJ, Ramain P, Simpson P, Modolell J (1999). Different contributions of *pannier* and *wingless* to the patterning of the dorsal mesothorax of *Drosophila*. *Development* **126**: 3523–3532.

Ghazi A, Paul L, VijayRaghavan K (2003). Prepattern genes and signalling molecules regulate stripe expression to specify *Drosophila* flight muscle attachment sites. *Mech Dev* **120**: 519–528.

Ghysen A (1980). The projection of sensory neurons in the central nervous system of *Drosophila*: choice of the appropriate pathway. *Dev Biol* **78**: 521–541.

Ghysen A, Dambly-Chaudiere C (1988). From DNA to form: the *achaete-scute* complex. *Genes Dev* **2**: 495–501.

Gomez-Skarmeta JL, Modolell J (1996). *araucan* and *caupolican* provide a link between compartment subdivisions and patterning of sensory organs and veins in the *Drosophila* wing. *Genes Dev* **10**: 2935–2945.

Gomez-Skarmeta JL, Rodriguez I, Martinez C, Culi J, Ferres-Marco D, Beamonte D *et al* (1995). Cis-regulation of *achaete* and *scute*: shared enhancer-like elements drive their coexpression in proneural clusters of the imaginal discs. *Genes Dev* **9**: 1869–1882.

Gomez-Skarmeta JL, del Corral RD, de la Calle-Mustienes E, Ferre-Marco D, Modolell J (1996). Araucan and caupolican, two members of the novel *iroquois* complex, encode homeoproteins that control proneural and vein-forming genes. *Cell* **85**: 95–105.

Gonzalez F, Romani S, Cubas P, Modolell J, Campuzano S (1989). Molecular analysis of the *asense* gene, a member of the *achaete-scute* complex of *Drosophila melanogaster*, and its novel role in optic lobe development. *EMBO J* **8**: 3553–3562.

Haenlin M, Cubadda Y, Blondeau F, Heitzler P, Lutz Y, Simpson P *et al* (1997). Transcriptional activity of *pannier* is regulated negatively by heterodimerization of the GATA DNA-binding domain with a cofactor encoded by the *u-shaped* gene of *Drosophila*. *Genes Dev* **11**: 3096–3108.

Hartenstein V, Posakony JW (1989). Development of adult sensilla on the wing and notum of *Drosophila melanogaster*. *Development* **107**: 389–405.

Huang F, Dambly-Chaudiere C, Ghysen A (1991). The emergence of sense organs in the wing disc of *Drosophila*. *Development* **111**: 1087–1095.

Lakes-Harlan R, Pollack GS, Merritt DJ (1991). From embryo to adult: anatomy and development of a leg sensory organ in *Phormia regina* Meigen (Insecta: Diptera). I. Anatomy and physiology of a larval 'leg' sensory organ. *J Comp Neurol* **308**: 188–199.

Laufer H, Vafopoulou-Mandalos X, Deak P (1986). Ecdysteroid titres in *Chironomus* and their relation to haemoglobins and vitellogenins. *Insect Biochem* **16**: 281–285.

Lee JC, VijayRaghavan K, Celniker SE, Tanouye MA (1995). Identification of a *Drosophila* muscle development gene with structural homology to mammalian early growth response transcription factors. *Proc Natl Acad Sci USA* **92**: 10344–10348.

Levine J, Hughes M (1973). Stereotaxic map of muscle fibres in indirect flight muscles of *Drosophila melanogaster*. *J Morphology* **140**: 153–158.

Macdowell ECH (1915). Bristle inheritance in *Drosophila*. I. Extra bristles. *J ExpZool* **19**: 61–97.

McAlpine JF (1981). *Manual of Nearctic Diptera*. Research Branch Agriculture: Canada.

Melzer RR, Sprenger J, Nicastro D, Smola U (1999). Larva-adult relationships in an ancestral dipteran: a re-examination of sensillar pathways across the antenna and leg anlagen of *Chaoborus crystallinus* (DeGeer, 1776; Chaoboridae). *Dev Genes Evol* **209**: 103–112.

Modolell J, Campuzano S (1998). The *achaete-scute* complex as an integrating device. *Int J Dev Biol* **42**: 275–282.

Murphey RK, Jacklet A, Schuster L (1980). A topographic map of sensory cell terminal arborizations in the cricket CNS; correlation with birthday and position in a sensory array. *J Comp Neurol* **191**: 53–64.

Murphey RK, Possidente D, Pollack G, Merritt DJ (1989). Modality-specific axonal projections in the CNS of the flies *Phormia* and *Drosophila*. *J Comp Neurol* **290**: 185–200.

Neumann D, Spindler KD (1991). Circasemilunar control of imaginal disc development in *Clunio marinus:* temporal switching point, temperature-compensated developmental time and ecdysteroid profile. *J Insect Physiol* **37**: 101–109.

Niwa N, Hiromi Y, Okabe M (2004). A conserved developmental program for sensory organ formation in *Drosophila melanogaster*. *Nat Genet* **36**: 293–297.

Phillips RG, Whittle JR (1993). *wingless* expression mediates determination of peripheral nervous system elements in late stages of *Drosophila* wing disc development. *Development* **118**: 427–438.

Pineiro R (1992a). Selection for canalization at extra dorsocentral and scutellar bristles in *Drosophila melanogaster*. *J Heredity* **83**: 445–448.

Piniero R (1992b). Selection for canalization at two extra dorsocentral bristles in *Drosophila melanogaster*. *J Heredity* **83**: 49–55.

Pistillo D, Skaer N, Simpson P (2002). *scute* expression in *Calliphora vicina* reveals an ancestral pattern of longitudinal stripes on the thorax of higher Diptera. *Development* **129**: 563–572.

Plunkett CR (1926). The inheritance of genetic and environmental factors in development. *J Exp Zool* **46**: 181–244.

Ramain P, Heitzler P, Haenlin M, Simpson P (1993). *pannier*, a negative regulator of *achaete* and *scute* in *Drosophila*, encodes a zinc finger protein with homology to the vertebrate transcription factor GATA-1. *Development* **119**: 1277–1291.

Ramain P, Khechumian R, Khechumian K, Arbogast N, Ackermann C, Heitzler P (2000). Interactions between chip and the *achaete/scute-daughterless* heterodimers are required for *pannier*-driven proneural patterning. *Mol Cell* **6**: 781–790.

Richardson J, Simpson P (2006). A conserved trans-regulatory landscape for *scute* expression on the notum of cyclorraphous Diptera. *Dev Genes Evolut* **216**: 29–38.

Riddiford L (1993). Hormones and *Drosophila* development. In: Bate M, Martinez-Arias A (eds) *The development of Drosophila melanogaster*. Cold Spring Harbor Laboratory Press: Cold Spring harbor, NY. pp 899–939.

Rodriguez I, Hernandez R, Modolell J, Ruiz-Gomez M (1990). Competence to develop sensory organs is temporally and spatially regulated in *Drosophila* epidermal primordia. *EMBO J* **9**: 3583–3592.

Ruiz-Gomez M, Modolell J (1987). Deletion analysis of the *achaete-scute* locus of *Drosophila melanogaster*. *Genes Dev* **1**: 1238–1246.

Schubiger M, Truman JW (2000). The RXR ortholog USP suppresses early metamorphic processes in *Drosophila* in the absence of ecdysteroids. *Development* **127**: 1151–1159.

Sheldon BL, Milton MK (1972). Studies on the scutellar bristles of *Drosophila melanogaster*. II. Long-term selection for high bristle number in the Oregon RC strain and correlated responses in abdominal chaetae. *Genetics* **71**: 567–595.

Simpson P, Woehl R, Usui K (1999). The development and evolution of bristle patterns in Diptera. *Development* **126**: 1349–1364.

Skaer N, Pistillo D, Simpson P (2002b). Transcriptional heterochrony of *scute* and changes in bristle pattern between two closely related species of blowfly. *Dev Biol* **252**: 31–45.

Skaer N, Pistillo D, Gibert J-M, Lio P, Wulbeck C, Simpson P (2002a). Gene duplication at the *achaete-scute* complex and morphological complexity of the peripheral nervous system in Diptera. *Trends Genet* **18**: 399–405.

Skeath JB, Carroll SB (1991). Regulation of *achaete-scute* gene expression and sensory organ pattern formation in the *Drosophila* wing. *Genes Dev* **5**: 984–995.

Spinner W (1969). Transplantationsversuche zur Blastemgliederung, Regenerations- und Differenzierungsleistung der Beinanlagan von *Culex pipiens*. *Wilhelm Roux' Archiv* **163**: 259–286.

Svacha P (1992). What are and what are not imaginal discs: reevaluation of some basic concepts (Insecta, Holometabola). *Dev Biol* **154**: 101–117.

Thummel CS (2001). Molecular mechanisms of developmental timing in *C. elegans* and *Drosophila*. *Dev Cell* **1**: 453–465.

Tiegs O (1955). The flight muscles of insects - their anatomy and histology; with some observations on the structure of striated muscle in general. *Phil Trans Royal Society of London* **238**: 221–359.

Truman JW, Riddiford LM (1999). The origins of insect metamorphosis. *Nature* **401**: 447–452.

Usui K, Kimura K (1993). Sequential emergence of evenly spaced microchaetes on the notum of *Drosophila*. *Roux Arch dev Biol* **203**: 151–158.

Usui K, Pistillo D, Simpson P (2004). Mutual exclusion of sensory bristles and tendons on the notum of Dipteran flies. *Curr Biol* **14**: 1047–1055.

Usui-Ishihara A, Simpson P (2005). Differences in sensory projections between macro- and microchaetes in Drosophilid flies. *Dev Biol* **277**: 170–183.

Villares R, Cabrera CV (1987). The achaete-scute gene complex of *D. melanogaster*: conserved domains in a subset of genes required for neurogenesis and their homology to *myc*. *Cell* **50**: 415–424.

Volk T (1999). Singling out *Drosophila* tendon cells: a dialogue between two distinct cell types. *Trends Genet* **15**: 448–453.

Vreezen WJ, Veldkamp JF (1969). Selection and temperature effects on extra dorsocentral bristles in *Drosophila melanogaster*. *Genetica* **40**: 19–39.

Walker RG, Willingham AT, Zuker CS (2000). A *Drosophila* mechanosensory transduction channel. *Science* **287**: 2229–2234.

Warren JT, Yerushalmi Y, Shimell MJ, O'Connor MB, Restifo LL, Gilbert LI (2006). Discrete pulses of molting hormone, 20-hydroxyecdysone, during late larval development of *Drosophila melanogaster*: correlations with changes in gene activity. *Dev Dyn* **235**: 315–326.

Westbrook A, Russo R (1985). Ecdysone titers during the fourth larval instar of three species of *Toxorhynchites*. *Proc New Jersey Mosq Control Assoc* **72**: 63–70.

Wheeler SR, Carrico ML, Wilson BA, Brown SJ, Skeath JB (2003). The expression and function of the *achaete-scute* genes in *Tribolium castaneum* reveals conservation and variation in neural pattern formation and cell fate specification. *Development* **130**: 4373–4381.

Wülbeck C, Simpson P (2002). The expression of *pannier* and *achaete-scute* homologues in a mosquito suggests an ancient role of *pannier* as a selector gene in the regulation of the dorsal body pattern. *Development* **129**: 3861–3871.

Wülbeck C, Simpson P (2000). Expression of *achaete-scute* homologues in discrete proneural clusters on the developing notum of the medfly *Ceratitis capitata*, suggests a common origin for the stereotyped bristle patterns of higher Diptera. *Development* **127**: 1411–1420.