Developmental Biology 386 (2014) 440–447

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/locate/developmentalbiology

Evolution of Developmental Control Mechanisms

Sex-specific repression of *dachshund* is required for *Drosophila* sex comb development

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ARTICLE INFO

Article history:
Received 11 August 2013
Received in revised form
7 December 2013
Accepted 11 December 2013
Available online 19 December 2013

Keywords:
Drosophila
Sex comb
dachshund
Distalless
Sex determination
Novelty

ABSTRACT

The origin of new morphological structures requires the establishment of new genetic regulatory circuits to control their development, from initial specification to terminal differentiation. The upstream regulatory genes are usually the first to be identified, while the mechanisms that translate novel regulatory information into phenotypic diversity often remain obscure. In particular, elaborate sex-specific structures that have evolved in many animal lineages are inevitably controlled by sex-determining genes, but the genetic basis of sexually dimorphic cell differentiation is rarely understood. In this report, we examine the role of *dachshund* (*dac*), a gene with a deeply conserved function in sensory organ and appendage development, in the sex comb, a recently evolved male-specific structure found in some *Drosophila* species. We show that *dac* acts during metamorphosis to restrict sex comb development to the appropriate leg region. Localized repression of *dac* by the sex determination pathway is necessary for male-specific morphogenesis of sex comb bristles. This pupal function of *dac* is separate from its earlier role in leg patterning, and Dac at this stage is not dependent on the pupal expression of *Distalless* (*Dll*), the main regulator of *dac* during the larval period. *Dll* acts in the epithelial cells surrounding the sex comb during pupal development to promote sex comb rotation, a complex cellular process driven by coordinated cell rearrangement. Our results show that genes with well-conserved developmental functions can be re-used at later stages in development to regulate more recently evolved traits. This mode of gene co-option may be an important driver of evolutionary innovations.

Introduction

New morphological structures often evolve through the spatial and temporal redeployment of pre-existing genes (Carroll, 2008). For example, male-specific wing spots found in some *Drosophila* species are associated with novel expression patterns of *wingless* (Werner et al., 2010) and *Dll* (Arnoult et al., 2013) in pupal wings. In addition to this new and lineage-specific function, both genes play deeply conserved roles in wing patterning during earlier developmental stages (Held, 2002). One of the questions raised by these findings is how a gene can be co-opted to regulate novel phenotypes without pleiotropic effects on its ancestral functions. A related question is how complex, integrated phenotypes arise in the course of evolution (Monteiro and Podlaha, 2009; Salazar-Ciudad and Marín-Riera, 2013). Novel characters frequently consist of a suite of simpler traits that work together to perform a specific function (Wagner et al., 2008). While it is often relatively easy to identify a co-opted "master control gene" (Gehring, 1996) at a high level in the genetic hierarchy, determining how this gene regulates each of the many component traits can be more difficult.

The sex comb, a male-specific array of modified bristles that develops on the front legs of *Drosophila melanogaster* and many of its close relatives, is a powerful system for addressing questions about the origin and diversification of complex novel traits (Kopp, 2011; Seher et al., 2012). The sex comb evolved in the *Sophophora* subgenus within the genus *Drosophila* (Barmina and Kopp, 2007). The "teeth" (bristle shafts) that make up the sex comb show a number of structural modifications compared to the ancestral bristle morphology (Fig. 1). In *D. melanogaster* and some other species, sex combs also rotate by up to 90° during pupal development, from a transverse to an oblique or longitudinal orientation (Atallah et al., 2012, 2009a, 2009b; Tanaka et al., 2009; Tokunaga, 1962) (Fig. 1E).

Sex comb evolution involved the deployment of *doublesex (dsx)*, a transcription factor that mediates sex-specific differentiation in *Drosophila* and other insects, in a novel spatial pattern in the prothoracic leg (Tanaka et al., 2011). Subsequent changes in *dsx* expression are associated with the evolution of the remarkably diverse sex comb structures seen in different *Drosophila* species. *dsx* acts in concert with *Sex combs reduced* (*Scr*), the HOX gene responsible for the development of the prothoracic segment (Held, 2010), to induce sex comb formation. Sex comb development is restricted to a specific region within the foreleg by the action of genes that control proximo-distal, anterior–posterior, and dorso-ventral leg patterning (Kopp, 2011).

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0012-1606/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ydbio.2013.12.017

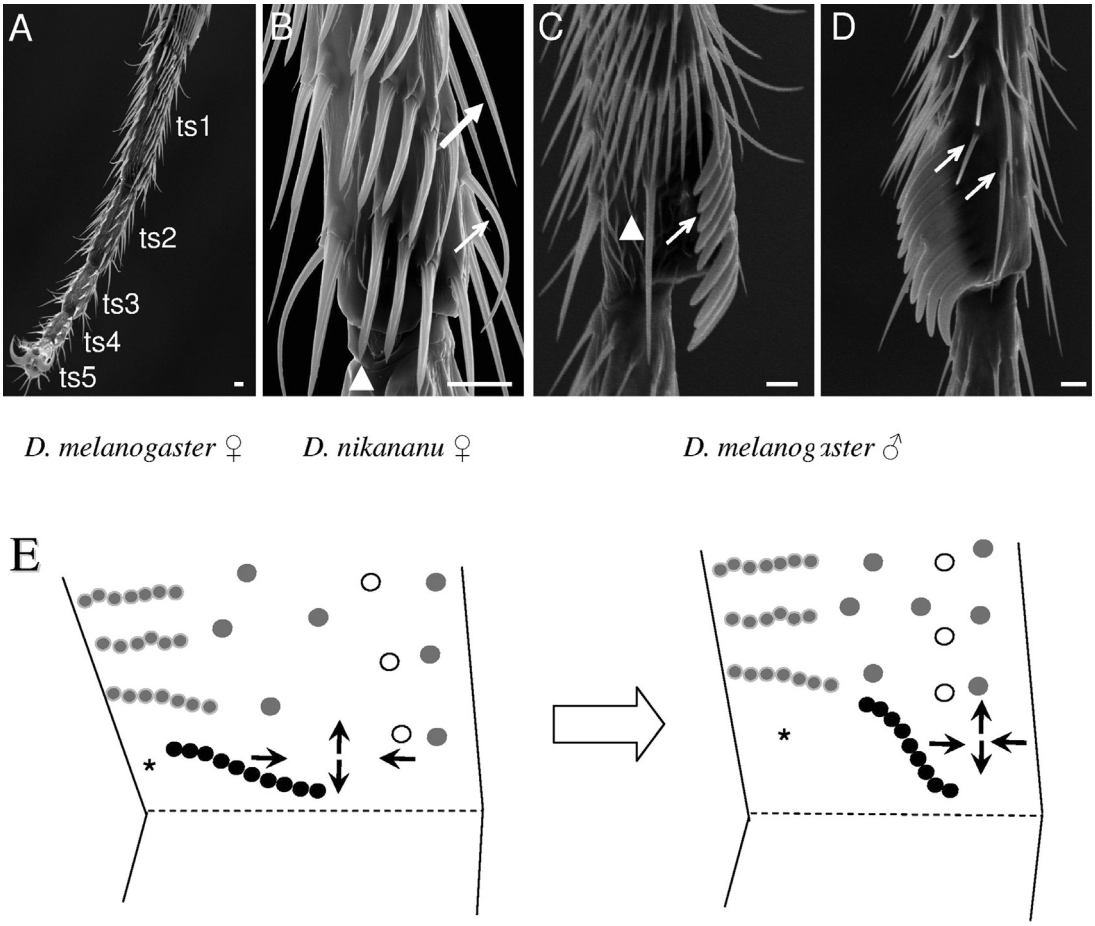

Fig. 1. The sex comb. (A) Scanning electron micrograph of a female *D. melanogaster* tarsus, showing all five segments (ts1-ts5). (B) Close-up view of the distal ts1 of female *D. nikananu*. The female bristle pattern is widely conserved in *Drosophila* and related genera. Sensory organs in both sexes include needle-shaped mechanosensory longitudinal row bristles (large arrow), curvilinear chemosensory bristles (small arrow) and pencil-shaped transverse row bristles (arrowhead). Note that transverse bristle rows (TBRs), and the bristles just anterior to them (right) continue to the distal tip of the segment. (C) In male *D. melanogaster*, the most distal TBR on ts1 is replaced by a rotated sex comb (arrow), consisting of enlarged bristles with curved tips. Arrowhead indicates the TBR just proximal to the sex comb. (D) Bristles just anterior and dorsal to the sex comb (arrows) are displaced proximally from the distal tip of the segment. (E) The sex comb is initially transverse and rotates as a consequence of male-specific tissue extension in the distal ts1 (black arrows). This extension also causes the bristles just anterior and dorsal to the comb to migrate proximally, displacing them from the tip of the segment. Sex comb bristles are depicted by black circles, longitudinal row bristles by large gray circles, transverse row bristles by small gray circles, chemosensory bristles by open circles, and the central bristle by an asterisk. Distal is down and anterior is to the right in all panels. Scale bars: 10 μm.

In *D. melanogaster* and its relatives, sex combs are homologous to the distal transverse bristle rows (TBRs) and to the morphologically similar bristles just anterior to these rows (longitudinal rows 6 and 7); the same precursors are found in conspecific females and in males of species that lack sex combs. In contrast to the other transverse row bristles, which are straight, pencil-shaped, and lightly pigmented, sex comb teeth are thicker, curved, blunt and heavily melanized (Fig. 1). The distinctive shape of sex comb teeth depends on *dsx* (Tanaka et al., 2011), but the downstream mechanisms that mediate its effects have remained unknown. In this study, we investigate the role of *dachshund* (*dac*), a gene involved in proximo-distal leg patterning and sensory organ development, in establishing bristle diversity in the *Drosophila* foreleg. Our results show that *dac* acts downstream of *dsx* in the developmental pathway that controls the morphology of sex comb teeth, explaining one of the many aspects of sex-specific morphogenesis.

Materials and methods

Ectopic expression and RNAi

The GAL4/UAS system (reviewed in Phelps and Brand, 1998) was used to drive ectopic gene expression or RNAi. In cases where this proved lethal, or where an earlier phenotype masked a later one (e.g., by ablating leg segments), the TARGET system (McGuire et al., 2003) was used to limit the activity of the GAL4 to specific periods in development. Flies carrying *tub-GAL80^{ts}* and the desired GAL4 and UAS constructs were shifted from 18 °C to 30 °C at the desired timepoint. GAL80 binds to GAL4 at the colder temperature, preventing activation of UAS transgenes, but it is inactive at the warmer temperature, allowing GAL4 to bind to UAS and activate gene expression. The following constructs were used: *UAS-dac* (Shen and Mardon, 1997); *UAS-Dll* (Gorfinkiel et al., 1997); *UAS-DsxM* (Lee et al., 2002); *UAS-dac-RNAi* (Dietzl et al., 2007); *UAS-Dll-RNAi* (Dietzl et al., 2007); *UAS-Tra^{F}* (Ferveur et al., 1995); *sca-GAL4*; *rn-GAL4* (St. Pierre et al., 2002); *Dll-GAL4*.

Mitotic clones

The FLP/FRT system (Theodosiou and Xu, 1998) was used to generate *dac* mutant clones through mitotic recombination. We crossed *dac^{E462}*/CyO (Bloomington *Drosophila* Stock Center) and *dac^{4}*/CyO (Mardon et al., 1994) males to *hs-FLP*; *FRT 40A*::*GFP* females to generate hypomorphic and null mitotic clones, respectively. Larvae were heat-shocked for 1 h at 37 °C at 72 h after hatching to induce mitotic recombination. Null or hypomorphic *dac* clones were marked by the absence of GFP. In addition to being