



## Expression and function of *spineless* orthologs correlate with distal deutocerebral appendage morphology across Arthropoda

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### ABSTRACT

The deutocerebral (second) head segment is putatively homologous across Arthropoda, in spite of remarkable disparity of form and function of deutocerebral appendages. In Mandibulata this segment bears a pair of sensory antennae, whereas in Chelicerata the same segment bears a pair of feeding appendages called chelicerae. Part of the evidence for the homology of deutocerebral appendages is the conserved function of *homothorax* (*hth*), which has been shown to specify antennal or cheliceral fate in the absence of Hox signaling, in both mandibulate and chelicerate exemplars. However, the genetic basis for the morphological disparity of antenna and chelicera is not understood. To test whether downstream targets of *hth* have diverged in a lineage-specific manner, we examined the evolution of the function and expression of *spineless* (*ss*), which in two holometabolous insects is known to act as a *hth* target and distal antennal determinant. Toward expanding phylogenetic representation of gene expression data, here we show that strong expression of *ss* is observed in developing antennae of a hemimetabolous insect, a centipede, and an amphipod crustacean. By contrast, *ss* orthologs are not expressed throughout the cheliceral limb buds of spiders or harvestmen during developmental stages when appendage fate is specified. RNA interference-mediated knockdown of *ss* in *Oncopeltus fasciatus*, which bears a simple plesiomorphic antenna, resulted in homeotic distal antenna-to-leg transformation, comparable to data from holometabolous insect counterparts. Knockdown of *hth* in *Oncopeltus fasciatus* abrogated *ss* expression, suggesting conservation of upstream regulation. These data suggest that *ss* may be a flagellar (distal antennal) determinant more broadly, and that this function was acquired at the base of Mandibulata.

### 1. Introduction

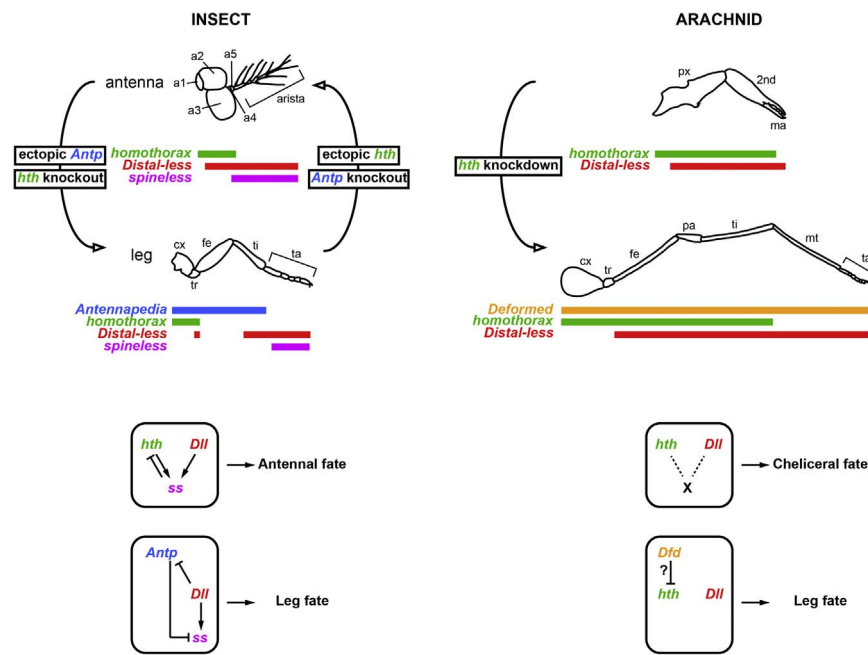
Homology, a shared correspondence or similarity as a result of common ancestry, is a key element of evolutionary inference. Historically, one of the grand challenges in comparative anatomy is the arthropod head problem, or the establishment of homologies for the segments and structures comprising the heads of arthropods (reviewed by Scholtz and Edgecombe (2006)). After over a century of debate, the positional homology of the deutocerebral (i.e., second head) segment of arthropods is generally accepted, based upon evidence from neuroanatomy (the innervation of the deutocerebral appendage pair by the deutocerebrum) and the boundaries of Hox gene expression, which is absent from the deutocerebral segment (Telford and Thomas, 1998; Hughes and Kaufman, 2002; Jager et al., 2006; Brenneis et al., 2008).

Acceptance of this hypothesis was previously interpreted to mean that chelicerae are highly modified antennae or vice versa, but the markedly different architectures of antennae and chelicerae have historically hindered their direct comparison (Boxshall, 2004). We recently showed that RNA interference (RNAi)-mediated knockdown of *homothorax* (*hth*) in the harvestman *Phalangium opilio* results in homeotic chelicera-to-leg transformation (Sharma et al., 2015a), comparable to *hth* knockdown experiments in insects that result in antenna-to-leg transformations (Dong et al., 2001, 2002; Ronco et al., 2008). Therefore, homology of antennae and chelicerae is additionally substantiated by a shared fate specification program that involves (a) the absence of Hox signaling, and (b) a requirement for *hth* to confer appendage identity (Fig. 1).

Independently of genetic evidence, paleontological descriptions of

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**Fig. 1.** Developmental dynamics of *hth* expression in deutocerebral and locomotory appendages of insects and arachnids (based on Duncan et al. (1998, 2010), Dong et al. (2001), Shippy et al. (2008), Toegel et al. (2009), Smith et al. (2014), and Sharma et al. (2015a)). Top left: Expression domains of *Antp*, *hth*, *Dll* and *ss* in the antenna and walking leg of *Drosophila melanogaster*. In *Drosophila*, *ss* is expressed in A2 through the arista. Labeled arrows indicate direction of homeotic transformation in misexpression experiments. Bottom left: Elements of the appendage fate specification pathway in *Drosophila*. Top right: Expression domains of *Dfd*, *hth*, and *Dll* in the chelicera and walking leg of *Phalangium opilio*. Note the absence of *Antp* in the leg bearing segments of arachnids. Bottom right: Elements of the appendage fate specification pathway in *Parasteatoda* and *Phalangium*. “X” denotes unknown cheliceral determinant/s.

Cambrian stem-group arthropods, concomitantly with improved techniques for fossil reconstruction and densely sampled phylogenies, have recorded early anterior appendages with multiple chelae (pincer-like claws) and multiple flagella (slender, articulated appendage termini corresponding to distal antennae). Such deutocerebral appendages are exemplified by leanchoiliids (an “antennate” megacheiran *sensu* Legg et al., 2013), which are part of the sister group lineage of extant Arthropoda (Megacheira; Chen et al., 2004; Legg et al., 2013; Siveter et al., 2014; Aria et al., 2015). These fossil appendages resemble neither modern chelicerae (which typically bear chelate terminal or subterminal segments, and dentition) nor modern antennae (which typically bear one or more flagella with numerous articles), but rather, a union of both appendage morphologies. Paleontologists have supported the deutocerebral origin of such appendages based on structural comparisons (Haug et al., 2012) and neuroanatomy in exceptionally preserved fossils (Ma et al., 2012; Tanaka et al., 2013; Yang et al., 2013; reviewed by Edgecombe and Legg (2014)). Given the phylogenetic placement of “Megacheira” in the arthropod tree of life as the paraphyletic sister group of crown-group Arthropoda (Daley et al., 2009; Kühn et al., 2009; Legg et al., 2013), reconstruction of deutocerebral appendage evolution is consistent with differential, lineage-specific retention of morphological features in Mandibulata and Chelicerata. However, other workers have inferred Megacheira to be more closely related to Chelicerata (Haug et al., 2012; Tanaka et al., 2013; Chipman, 2015); under this interpretation, the antenna would alternatively be constructed as a symplesiomorphic character.

The developmental genetic corollary of the hypothetical homology of antenna and chelicera is that downstream targets of *hth* may have also been retained in a lineage-specific manner, with modern mandibulates bearing determinants of flagellar identity, and chelicerates retaining the determinants of chela identity. To test the hypothesis that downstream targets of *hth* are lineage-specific, we examined the evolutionary dynamics of *spineless* (*ss*), a member of the bHLH-PAS family of transcription factors and homolog of the mammalian dioxin receptor (Struhl, 1982). In the larval antenna of the fruit fly *Drosophila melanogaster*, *ss* is initially co-activated by the proximo-distal (PD)

axis patterning genes *hth* and *Distal-less* (*Dll*) in the distal territory of the antennal disc. By the third larval instar, *ss* represses *hth* in the distal antenna (Duncan et al., 1998). *ss* loss-of-function mutants display distal antenna-to-leg transformations, whereas ectopic expression of *ss* results in transformations of the maxillary palp and distal leg to distal antenna, and ectopic antennae in the rostral membrane (Duncan et al., 1998; Emerald and Cohen, 2004; Emmons et al., 2007). These data suggest that *ss* is the primary determinant of distal antennal fate in *D. melanogaster*.

Separately, *ss* is also expressed transiently and early (late second through third larval instars) in the tarsus of the *D. melanogaster* walking legs, and is required for activation of *bric-a-brac* and repression of *bowl*, two distally acting transcription factors that pattern tarsomeres (Godt et al., 1993; de Celis Ibeas and Bray, 2003). Loss-of-function mutants of *ss* display fusions or deletion of medial tarsomeres, and it has been suggested that *ss* acts to establish the tarsal field, which is subsequently partitioned into tarsomeres by *bric-a-brac* and *bowl* (Duncan et al., 1998; de Celis Ibeas and Bray, 2003).

Comparative work on the *ss* ortholog of the flour beetle *Tribolium castaneum* has shown conserved function of *ss* in patterning distal antennal identity, with respect to *D. melanogaster*. Both parental and larval RNAi against the *T. castaneum* *ss* ortholog result in transformation of a large region of the distal antenna to leg identity (Shippy et al., 2008; Toegel et al., 2009); in the tarsus, larval RNAi additionally results in tarsomere-patterning defects and truncation of the tarsus (Toegel et al., 2009; Smith et al., 2014).

Beyond these two holometabolous insects, expression and function of *ss* orthologs have not been investigated. Furthermore, extrapolating evolutionary scenarios from holometabolous insect models is complicated by the derived condition of both the antenna and the tarsus in these species. Holometabolous insects specify antennal identity at two points in development (during embryogenesis and metamorphosis), whereas hemimetabolous insects and non-insect hexapods specify antennal identity only once during embryogenesis (Shippy et al., 2008; Smith et al., 2014). With respect to tarsal morphology, the condition of five tarsomeres on the walking legs (four in the metathor-

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