

Global evolution of *nubbin* expression patterns in arthropods: emerging view

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INTRODUCTION

In our recent study, we focused on analyzing *nubbin* (*nub*) expression in several insect species (Li and Popadić 2004). As a part of our analysis, we used previously reported spider data (Abzhanov and Kaufman 2000) to infer the global patterns of *nubbin* evolution in arthropod embryos. The insight obtained suggested a progressive gain of *nub* expression in gnathal appendages, leg segments, and CNS. In the previous issue of *Evolution & Development*, Prpic and Damen (2005) provided new valuable data from another spider species. The new data raise a question as to whether two of our previously reported gains of expression (in gnathal appendages and in leg segments) were correctly inferred. More specifically, Prpic and Damen suggested a loss of expression as an alternative explanation. Here, I discuss the issue of gain vs. loss in the light of the observed extraordinary diversity and dynamics of *nubbin* expression in arthropods.

MAPPING OF NEW DATA

In order to make any inference about the patterns and direction of *nubbin* evolution, it is critical to consider all available data. At present, *nub* expression has been studied in chelicerates, crustaceans, and insects. Insight from the fourth major arthropod lineage, myriapods, is currently not available. The summary of *nub* expression patterns is depicted in Fig. 1.

In chelicerates, *nub* patterns were described for three species: a horseshoe crab *Limulus polyphemus* (Damen et al. 2002) and two spiders, *Steatoda triangulosa* (Abzhanov and Kaufman 2000) and *Cupiennius salei* (Prpic and Damen 2005). *Limulus* represents an ancient chelicerate lineage and as such, can provide an important insight into the ancestral pattern of *nubbin* expression in arthropods. Unfortunately, this report includes only a description of expression in opisthosomal appendages at later stages of embryonic development (Damen et al. 2002). There is no available information with regard to prosomal appendages, encompassing chelic-

erae, pedipalps, and legs. Thus, at present, the *Limulus* data cannot provide any additional insight and are not included in Fig. 1. The original spider report in *Steatoda* showed that *nub* is absent from the two distal-most appendages (chelicerae and pedipalps) and its expression is localized to a single band in the legs (Fig. 1A). The new spider study described by Prpic and Damen (2005) depicts a very different pattern. First, in *Cupiennius*, *nubbin* is expressed in *all* of the prosomal appendages. Second, its expression can be associated with *all* of the segments in pedipalps and legs (Fig. 1B).

In crustaceans, *nub* has also been studied in three species: *Artemia*, brine shrimp; *Pacifastacus*, crayfish; and *Porcelio*, woodlouse (Averof and Cohen 1997; Abzhanov and Kaufman 2000). The original *Artemia* report describes a *nubbin* pattern only in multibranching thoracic appendages, and no information is available with regard to the situation in the head region (Averof and Cohen 1997). In those multibranching appendages, *nub* expression is localized exclusively in an epipod (dorsal lobe). In endopods, ventral lobes corresponding to the walking-portion of multibranching legs in other crustaceans, *nubbin* is completely absent. Note that in this species, endopods do not have joints and function as swimming paddles. Thus, the *Artemia* data is of a limited value for the scope of present study and is not included in the discussion. The information regarding the situation in *Pacifastacus* is also limited. The two studies that describe *nub* expression in a crayfish are both focused on thoracic appendages (Averof and Cohen 1997; Damen et al. 2002), and do not include any references to the head region (hence the question mark in Fig. 1C). In their current report, Prpic and Damen refer to *Pacifastacus* data as being associated with all leg segments. However, the re-examination of the stainings in the original studies, Fig. 3I–J in Averof and Cohen (1997) and Fig. 2A in Damen et al. (2002), clearly shows that at 70% development there are only three rings of expression. This would correspond to some, but not all of the leg segments (Fig. 1C). More detailed studies will be necessary to determine whether *nub* expression indeed expands into additional leg segments at later stages. At present, the most information in crustaceans is provided by *Porcelio* data (Abzhanov and

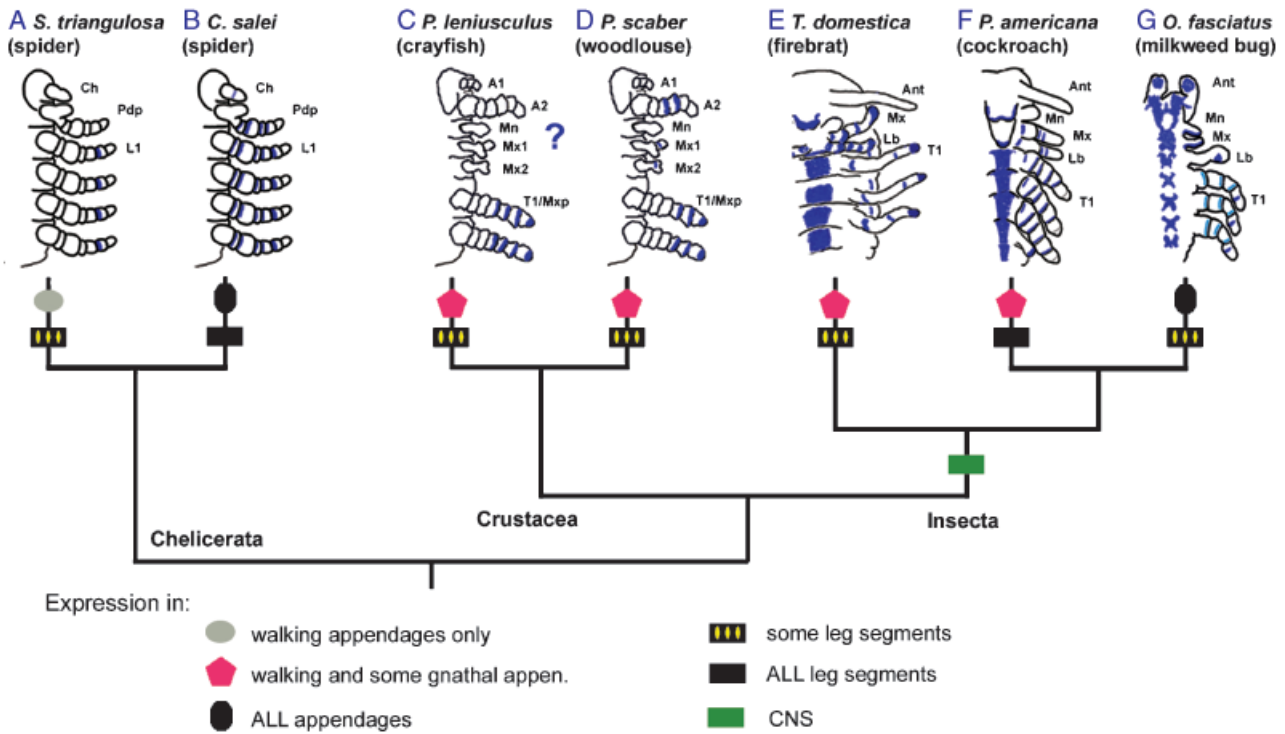


Fig. 1. A cladogram summarizing evolution of *nub* expression in arthropods. (A, B) Spiders: *Steatoda triangulosa* and *Cupiennius salei*. (C, D) Crustaceans: *Pacifastacus leniusculus* and *Porcelio scaber*. (E–G) Insects: *Thermobia domestica*, *Periplaneta americana*, and *Oncopeltus fasciatus*. Light blue depicts early expression in the *Oncopeltus* legs. Spiders: Ch, chelicerae; Pdp, pedipalps; L1, legs 1. Crustaceans: A1, first antenna; A2, second antenna; Mx1, maxillae 1; Mx2, maxillae 2; T1/mxp, first trunk limb/maxilliped. Insects: Ant, antenna; Mn, mandibles; Mx, maxillae; Lb, labium; T1, first thoracic leg.

Kaufman 2000). In this species, *nub* is expressed in some, but not all, gnathal appendages and in the legs (Fig. 1D). Significantly, the leg pattern can be associated with only two segments.

With regard to phylogenetic sampling, insects represent the most studied arthropods. At present, *nub* expression has been examined in four species including: *Thermobia*, a basal wingless insect lineage; *Periplaneta* and *Oncopeltus*, two hemimetabolous insects; and *Drosophila*, a derived holometabolous model system (Billin et al. 1991; Ng et al. 1995; Ng et al. 1996; Mirth and Akam 2002; Li and Popadić 2004). As we have reported previously (Li and Popadić 2004), all insects share a novel expression in the CNS and exhibit an extraordinary diversity of patterns in gnathal appendages and legs. In *Thermobia*, *nub* is absent in two distal-most head appendages (antennae and mandibles), has distinct patterns in the other two mouthparts (maxillae and labium), and exhibits a patched expression in legs (Fig. 1E). In *Periplaneta*, *nub* continues to be absent in antennae and mandibles. However, there is a novel pattern in maxillae and labium, and leg expression now encompasses *all* of the leg segments (Fig. 1F). In *Oncopeltus*, there is another modulation of the expression pattern. For the first time in insects, *nub* is expressed in distal-

most appendages (antennae and mandibles). There is also a new pattern in maxillae and labium and the leg expression encompasses some, but not all of the segments (Fig. 1G). In *Drosophila*, *nub* has been studied extensively regarding its role in neurogenesis and wing development (Ng et al. 1995, 1996). What is relevant for this discussion though, is that *nub* is expressed in all of the leg segments except tarsus (Mirth and Akam 2002).

INFERRING THE ANCESTRAL PATTERN OF *NUB* EXPRESSION IN ARTHROPODS: INSIGHTS FROM SPIDERS AND INSECTS

In their article, Prpic and Damen (2005) raise a question as to whether two of the apomorphies in our original study (expression in all of the gnathal appendages and in all of the leg segments) were defined correctly. As shown by their new spider data, *nub* is expressed in both of these domains in *Cupiennius* (Fig. 1B). Consequently, Prpic and Damen argue that expression of *nub* in walking and all gnathal and procephalic appendages pre-dates the origin of the extant arthropod classes. In a similar way, their finding that *nub* is localized in

each pedipalpal and leg segment in *Cupiennius* suggests that this may be an ancestral feature as well. If correct, this conclusion would also be in contrast to our original view that *nub* was recruited for a novel role in leg segmentation during early stages of insect evolution (Li and Popadić 2004). The question is can we distinguish between these two alternative explanations with presently available data. In other words, was *nubbin* evolution in arthropods characterized by early gain and subsequent loss of expression or was *nubbin* independently recruited for new roles in particular lineages?

There are two key concerns with regard to the “early gain” view favored by Prpic and Damen. First, there is no *a priori* reason to argue that *Cupiennius* data are more reflective of an ancestral pattern than that of *Steatoda*. Essentially, we have a situation where two spider data sets are quite different, but neither is more informative than the other. This is because both species represent more derived spider families. Without an inclusion of basal spider orders such as Mesothelae and Mygalomorphae it is not possible to infer the ancestral pattern in arachnids. The extension of the studies in *Limulus* (horseshoe crab) would also be highly beneficial in providing an insight into the situation in chelicerates in general. Second, in light of extraordinary dynamics of *nub* expression in insects, the independent gain of novel features in *Cupiennius* appears to be more than likely. Insect leg expression ranges from patchy signal in some segments (*Thermobia*, Fig. 1E) to encompassing all of the segments (*Periplaneta*, Fig. 1F) to losing signal in some of segments (*Oncopeltus*, Fig. 1G) to regaining expression in all but the tarsal segment (*Drosophila*, Mirth and Akam 2002). In that sense, the situation in spiders is similar to one in *Periplaneta* and *Oncopeltus* (compare Fig. 1, A and B to Fig. 1, F and G). The difference is that in insects we can use a basal lineage (*Thermobia*) to infer the ancestral pattern.

In summary, the new data by Prpic and Damen (2005) present an important complement to previous studies that highlight how dynamic and diverse *nubbin* expression is in arthropods. We are also in complete agreement about the need for additional comparative studies of *nubbin* in arthropods. Only with a much more extensive phylogenetic sampling will we be able to distinguish between an “early gain” and an “independent recruitment” hypothesis.

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