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Roles of Homeobox Genes in Proleg Development in the Sawfly, *Athalia rosae ruficornis* (Hymenoptera)*

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Abdominal appendages of insects have received less attention than thoracic legs and mouthparts, since the absence of appendages in abdomens is one of the features of insects (Snodgrass, 1935; Gullan and Cranston, 2010). Nevertheless, some holometabolous species have "unusual" abdominal appendages in their larval stages (Matsuda, 1976; Nagy and Grbic, 1999; Bitsch, 2012). The larvae of primitive Hymenoptera (sawflies) and Lepidoptera (moths and butterflies) have remarkable abdominal appendages (prolegs); however, their arrangement varies. For example, the sawfly (*Athalia rosae ruficornis*) has seven pairs of prolegs from the second abdominal segment (A2) to A8, and a pair of anal prolegs in the last segment, A11 (Oka *et al.*, 2010). On the other hand, the silkworm (*Bombyx mori*) has four pair of prolegs in A3-A6, and anal prolegs in A11 (Scoble, 1995; Tomita and Kikuchi, 2009). The following questions thus arise: what is the origin of the prolegs (serial homologues of cephalic and thoracic appendages)? and what is the underlying molecular regulation of proleg formation? Prolegs of *A. rosae ruficornis* were characterized by morphological and gene expression analyses, and proposed that they are coxopodal endites (outgrowths of ventral plates), but not the main shaft of appendages (Oka *et al.*, 2010). It is considered that the homeobox genes belonging to the Bithorax Complex (BXC), *Ultrabithorax* (*Ubx*), *abdominal-A* (*abd-A*), and *Abdominal-B* (*Abd-B*), are involved in the primary regulation of abdominal appendage development (Hughes and Kaufman, 2002; Robertson and Mahaffey, 2009). In this study, the functions of these three genes were analyzed by knocking down their transcripts during embryonic development.

Ubx, *abd-A* and *Abd-B* of *A. rosae ruficornis* have been cloned and characterized (Hatakeyama *et al.*, 2013). As RNA interference (RNAi) induced by the injection of double-stranded RNA (dsRNA) works very effectively in *A. rosae ruficornis* (Yoshiyama *et al.*, 2013), a short dsRNA corresponding to each gene transcript was synthesized and injected into hemocoel of the parental mother pupae. After adult emergence, eggs were taken from the dsRNA-injected mothers and allowed to develop parthenogenetically. The gene expression and phenotypes were examined during embryogenesis. The transcripts of each gene were specifically knocked down in embryos when body segments were formed and appendage primordia appeared. The larval phenotypes upon gene knockdown in comparison to normal phenotype were as follows:

Normal phenotype:
- No appendages were formed in A1.
- Seven pairs of prolegs were formed in A2-A8.
- No appendages were formed in A9, and in A10 which is usually reduced with only a small dorsal area remaining.
- A pair of anal prolegs was formed in A11.

Ubx knockdown phenotype:
- A pair of basal thoracic appendage-like structures appeared in A1, where appendages are not originally formed.
- Seven pairs of prolegs were formed normally in A2-A8.
- A pair of anal prolegs was formed normally in A11.

abd-A knockdown:
- No appendages were formed in A1.
- All prolegs in A2-A8 were absent and only rudimentary structures remained.
- A pair of anal prolegs was formed normally in A11.

Abd-B knockdown:
- No appendages were formed in A1.
- Seven pairs of prolegs were formed normally in A2-A8.
- A pair of prolegs appeared in A9, and in A10 which is formed similar in appearance to the other abdominal segments.
- A pair of anal prolegs was formed normally in A11.

These results suggest the following functions of homeobox genes in *A. rosae ruficornis* abdominal appendage development: *Ubx* suppresses appendages in A1, *abd-A* promotes prolegs in A2-A8, and *Abd-B* specifies the posterior abdominal segments and suppresses prolegs in A9 and A10.

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