

Embryonic Appendage Development in the Sawfly, *Athalia rosae ruficornis* (Hymenoptera): Involvement of *decapentaplegic* in the Primary Determination*

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Appendage development in insects has received much attention due to the interests in the evolutionary aspects as well as the mechanisms of underlying gene regulation. The essential genes involved have been identified in several insect species across the orders (Morata and Sanchez-Herrero, 1999; Jockusch *et al.*, 2004). Expression of one of the essential genes, *Distal-less (Dll)*, has been used as the marker for appendage identity (Panganiban, 2000). However, molecular studies demonstrated some exceptions. The mandible, apparent appendage of a cephalic segment developed without *Dll* expression in myriapods, crustaceans and insects (Popadic *et al.*, 1998). *Dll* was expressed in all prolegs of lepidopteran embryos, while the expression was absent in the prolegs of two hymenopteran sawflies (Suzuki and Palopoli, 2001). These findings imply that *Dll* expression may not be a definitive marker for appendages and that there are other pathways to achieve appendage formation without *Dll* expression.

We have focused on the *decapentaplegic (dpp)* gene that is known as the upstream regulator of *Dll* in appendage development. The orthologous gene of the sawfly *Athalia rosae ruficornis* (*Ar dpp*) was cloned and examined for the expression pattern in the embryonic appendages (Yamamoto *et al.*, 2004). *Ar dpp* was expressed in all cephalic segments including the mandible, in the thoracic legs and in all proleg primordia including pleuropodia and anal prolegs. The results indicate that *Ar dpp* expression reflects the primary determination of embryonic appendages and is a good marker to identify appendages. The expression of *dpp* could also be used to identify the numbers of abdominal segments. The numbers of larval abdominal segments of sawflies were used as one of the morphological characters for the classification of species. It was reported that the larval abdominal segments of *A. rosae* were ten due to the fused appearance in the posterior proleg-less segments (Schulmeister, 2003). Examinations of both the expression pattern of *Ar dpp* and the morphology using longitudinal sections revealed that the embryonic abdomen of *A. rosae* consists of 11 segments, and the ninth and tenth segments are fused in later stage of embryogenesis.

The understanding of gene functions essential for the appendage development is the key to elucidate evolutionary diversification of insect appendages. Comparative molecular analyses are in progress in both serially homologous appendages of one species and directly homologous ones of different species (Jockusch *et al.*, 2004). Our findings will contribute to the analysis of gene function in the direct homologues in different species.

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