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Establishment of Larval RNAi in the Sawfly, *Athalia rosae ruficornis* Jakovlev (Hymenoptera) Aiming at Functional Analysis of Genes Involved in Appendage Development during Larval-adult Transition*

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In holometabolous insects, the larval appendages are formed during embryonic development and then adult appendages develop during metamorphosis. Adult appendage development has been extensively investigated in the fruit fly, *Drosophila melanogaster* and the molecular regulatory mechanisms are well understood (Kojima, 2004). The larval appendage formation, however, remains largely unelucidated in *Drosophila*, because larval appendages in this species are extremely degenerative structures. Embryonic appendage development has been studied in other insect species and the findings are compared with those obtained in *Drosophila* adult appendage development. To understand the whole process of insect appendage development, it is most desirable to compare the mechanisms of appendage development between larvae and adults in single species. The turnip sawfly, *Athalia rosae ruficornis* may serve as one such model species since both larvae and adults have well-developed appendages, particularly legs, and information about general biology and embryogenesis is already available (Sawa *et al*., 1989; Oka *et al*., 2007).

For gene functional analysis, induction of misexpression of the gene of interest is often used. RNA interference (RNAi) is an effective method to induce gene silencing, namely decomposition of mRNA mediated by the artificially introduced double-stranded RNA (dsRNA), and is utilized in many species (Tomoyasu *et al*., 2008). In *A. rosae ruficornis*, embryonic RNAi in which dsRNA is injected into eggs, to investigate gene function in the embryonic stage has already been established (Sumitani *et al*., 2005). In this study, we tested the feasibility of using larval RNAi, the method in which dsRNA is injected into the larvae to induce gene silencing at the stage of larval-adult transition (Tomoyasu and Denell, 2004), in *A. rosae ruficornis*.

First, we synthesized a 485-bp-long dsRNA for the *enhanced green fluorescence protein* (*EGFP*) gene. The dsRNA targeting *EGFP* gene transcript was injected into hemocoel of the last instar larvae of a transgenic strain carrying the *EGFP* gene in the genome (Sumitani *et al*., 2003). The EGFP fluorescence was examined at the stage of larval-adult transition. Fluorescence was dramatically decreased in dsRNA-injected individuals during that stage. The phenotype was caused by decomposition of the *EGFP* gene transcript as demonstrated by reverse-transcription PCR (RT-PCR). Larval RNAi was, therefore, effective in *A. rosae ruficornis*.

Next, we carried out an RNAi experiment to prevent the expression of *Distal-less* (*Dll*) gene, one of the essential genes involved in appendage development. A 491-bp-long dsRNA targeting *Dll* gene transcript was injected into the larvae as described above. Typical phenotypes observed in adult thoracic legs were fusion of the trochanter and femur or fusion of the tibia and tarsus, and peculiar swellings at the fused part between the tibia and tarsus. Also, some flagella and pedicellus were fused in adult antennae. In adult gnathal appendages, three segments (2nd– 4th proximal segments) of the maxillary palpi and all segments of the labial palpi were fused and peculiar swellings occurred at the fused part of the palpi as seen in the thoracic legs. All these effects appeared at the regions where *Dll* was originally expressed in each appendage primordium. These findings suggest that *Dll* is involved in the subdivision and directed growth of

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metameric appendages.

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