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Cloning of Genes Expressed Differentially by Sex in the Sawfly, *Athalia rosae ruficornis* (Hymenoptera)*

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Hymenopteran insects are known as haplodiploid organisms: males develop from unfertilized eggs while females from fertilized eggs. It has been shown that the sex of the sawfly, *Athalia rosae ruficornis*, a member of Hymenoptera, is determined by the single-locus multiple-allele system (Naito and Suzuki, 1991), however, its underlying molecular mechanisms are not clear. As a first step toward elucidating the molecular basis of the sex determination in *A. rosae*, we cloned and examined the genes that expressed differentially by sex.

We screened cDNAs that expressed in a sex specific-manner by using the cDNA subtraction method. The respective cDNA libraries were constructed from females and males in the early developmental stage (the third day of embryogenesis). These libraries were subtracted from each other by selective PCR amplifications, and PCR products that included presumable female- and male-specific genes were separately subcloned into TOPO TA cloning vector. Ninety-six colonies randomly selected from each cDNA pool were sequenced. Finally, two clones that expressed in females specifically were obtained. Since these two clones lacked 5'- and 3'-terminal regions, the full-length cDNAs were obtained by the rapid amplification of cDNA ends (RACE) method, cloned and sequenced. Homology analyses revealed that the amino acid sequences deduced from each cDNA were similar to those in the *Drosophila melanogaster* genome with unknown functions. The results of reverse transcription PCR (RT-PCR) confirmed that these two genes expressed only in female embryos. Unfortunately however, no male-specific genes were obtained.

In the present study, two interesting cDNAs that did not express sex specifically were also obtained. One was the *mog* gene homologue and another was the cytochrome P450 monooxygenase gene homologue. The *mog* gene functions in germ cell differentiation in *Caenorhabditis elegans*. The cytochrome P450 monooxygenases are among the most important enzymes associated with insecticide detoxification and activation. These genes would be applicable to reproductive control of insects and to construction of useful insect strains.

In future studies, we plan to analyze the functions of these genes using the transposon (*piggyBac*)-mediated stable germline transformation system that we recently established (Sumitani *et al.*, 2003).

References

Naito, T. and H. Suzuki (1991) *J. Hered.*, **82**, 101–104.

Sumitani, M., D.S. Yamamoto, K. Oishi, J.M. Lee and M. Hatakeyama (2003) *Insect Biochem. Mol. Biol.*, **33**, 449–458.

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