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Identification of Sex-specific Transcripts of the *doublesex* Gene in the Sawfly, *Athalia rosae ruficornis* (Hymenoptera)*[1,*2]

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Homologs of the *doublesex* (*dsx*) gene of *Drosophila melanogaster* are highly conserved elements that regulate genetic sex determination in a wide range of organisms (Kopp, 2012). The *dsx* pre-mRNA is alternatively spliced by *trans*-acting proteins, resulting in sex-specific variants. Distinctive *dsx* isoforms in males and females are known to induce somatic sexual differentiation in a variety of insects (Oliveira *et al.*, 2009). However, functional assays of *dsx* gene are restricted to Diptera (Baker and Wolfner, 1988; Raymond *et al*., 1998) and Lepidoptera (Suzuki *et al*., 2005). Hymenoptera is a unique order in which arrhenotokous parthenogenesis (development of haploid males from unfertilized eggs) is the general mode of reproduction. The sawfly, *Athalia rosae*, is a new model species in this order that has been used to conduct studies in genetics and developmental biology with tools such as germline transformation by the *piggyBac* transposon-derived vector and gene knockdown by RNAi (Sumitani *et al*., 2003; Yoshiyama *et al.*, 2013). In addition, its genomic sequence is publicly available (http://www.ncbi.nlm.nih.gov/nuccore/AOFN00000000.1/). To investigate the regulatory mechanisms of sex determination in *A. rosae*, we identified its *dsx* homolog (*Ardsx*) using whole transcriptome shotgun sequencing (RNA-Seq) (Hatakeyama *et al.,* 2017). In this report, we describe the sex-specific transcripts of *A. rosae*.

The complete *Ardsx* coding sequence was confirmed using reverse transcription-polymerase chain reaction (RT-PCR) with cDNA constructed from adult females and males as templates. The *Ardsx* gene corresponded to a region spanning approximately 12 kb of the genome. The deduced amino acid sequence showed the typical Dsx structure, with a *doublesex* and *mab-3* (DM) DNA-binding domain (which is characteristic of the zinc-finger motif in the N-terminal region) and a Dsx dimerization domain in the C-terminal region. In males, exons 1, 2, and 3 joined to form a transcript encoding 234 amino acids. In females, specific splicing of 119 nucleotides within exon 3 resulted in the female-specific variant. The resultant female-specific transcript encoded 338 amino acids and had a longer C-terminal region than the male variant. In addition, we identified the *transformer 2* (*tra2*) homolog, which is known to regulate sex-specific alternative splicing of *dsx* pre-mRNA in *D. melanogaster* (Ryner and Baker 1991). In contrast, in silkworm (*Bombyx mori*), the *tra2* homolog is not required for sex-specific splicing of *B. mori dsx* pre-mRNA (Suzuki *et al.*, 2012). It would be interesting to determine whether *tra2* is involved in *dsx* splicing in *A. rosae*.

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