

Do *doublesex*, *intersex*, and *fruitless* Homologs Regulate Sexual Differentiation in *Bombyx mori*?

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Recently the large-scale expressed sequence tag (EST) analysis and whole-genome shotgun (WGS) analysis have been performed for the *Bombyx mori* genome. We have been collaborating with the genome project, and trying to utilize the genome data for understanding of *Bombyx*-specific biological functions. Especially we are interested in the sex determination. We searched the *Bombyx* EST and WGS database using *Drosophila* sex determining genes as queries. First, *Bmdsx*, an ortholog of *doublesex*, the most downstream gene in the sex determination cascade in *Drosophila* somatic cells, was found. We analyzed the gene structure of *Bmdsx*, and revealed that it was transcribed into sex-specific mRNA isoforms. The mechanism of the sex-specific splicing was different from that in *dsx* in *Drosophila*. It depends on male-specific repression of splicing. To understand the function of *Bmdsx*, we introduced female- and male-type genes into the germ line of *Bombyx*. The transgenic females expressing male-type *Bmdsx* mRNA formed male-like abnormal structures in genitalia, pheromone gland, and internal sexual organs, indicating that *Bmdsx* affects the sexual differentiation. We also found two *intersex* (*ix*) homologs, *Bmix1* and *Bmix2*. It is known that *Drosophila* IX interacts with the female DSX protein. BmIX1 and BmIX2, however, did not interact with BmDSX-F in the yeast two-hybrid system. IXs may have different roles between *Drosophila* and *Bombyx*. Third, *Bmfri*, an ortholog of *fruitless*, the sex-determinant of the *Drosophila* nervous system, was also found in the *Bombyx* EST database. Our analysis of its genomic and mRNA structures revealed that it encoded at least four isoforms containing different zinc-finger domains. All of the four mRNA isoforms were expressed predominantly in female in the head of larvae and adults, suggesting a sex-specific function of *Bmfri*. We can tentatively conclude that the genetic system for sex determination has been partially conserved during evolution of insects.