Toward the Understanding of the Molecular Mechanism of Horn Development in *Trypoxylus dichotomus* (Insecta: Coleoptera, Scarabaeidae)*

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In Coleoptera, an evolutionary novel structure "horn" was evolved multiple times with tremendous range of diversity. However, the molecular mechanisms involved in horn formation are yet unknown. To investigate the developmental mechanisms underlying horn formation, we focus on the Japanese rhinoceros beetle *Trypoxylus dichotomus* (Coleoptera), which exhibits remarkable sexual dimorphisms in head and thoracic horns. The male-specific horns of *T. dichotomus* are one of the best models to study how a sexually dimorphic exaggerated horn is formed. To further understanding of molecular mechanisms underlying horn formation, we have employed for larval RNA interference (RNAi) method in *T. dichotomus*.

To understand how sexually dimorphic exaggerated horns are formed, we are currently employing two approaches. The first approach is a candidate gene approach. We focused on a highly conserved sex-determination gene *doublesex* (*dsx*) which produces both male- and female-specific isoforms by sex-specific alternative splicing. Both Dsx isoforms act as a transcription factor to control all aspects of sex-specific morphologies. First, we investigated the function of dsx in *T. dichotomus* using larval RNAi method. We show that sex-specific *T. dichotomus dsx* isoforms have an antagonistic function for head horn formation and only the male isoform plays a role in thoracic horn formation. These results suggested that the novel sex-specific regulation of dsx on horn morphogenesis may be the key evolutionary developmental event at the transition from sexually monomorphic to sexually dimorphic horns.

Our second approach is a high through-put approach. To identify novel genes involved in the sexually dimorphic horn development in *T. dichotomus*, mRNA of the developing horn discs has been assessed by deep-sequencing transcriptome analysis. Current progress in RNA-seq analysis by a next-generation sequencer will be presented.

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