

DIVISION OF EVOLUTIONARY DEVELOPMENTAL BIOLOGY



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The Division of Evolutionary Developmental Biology was started in June 2015. We focus on the evolutionary novelties acquired by insects through evolution, in order to elucidate the molecular and evolutionary mechanisms leading to the large variety of traits that they display. From this wealth of exciting traits, our lab currently focuses on promoting research into (1) the origin and diversification of insect wings, (2) wing color patterns and mimicry of ladybird beetles, and (3) acquisition and diversification of beetle horns.

I. Origin and diversification of insect wings

Of the various flying animals on the earth, insects have evolved a unique flight organ. Despite over two centuries' debate, the evolutionary origin of the insect wing is still an enigma. We try to approach this issue using evo-devo methods. In *Drosophila melanogaster*, the wing master gene *vestigial* (*vg*) and its interaction partner *scalloped* (*sd*) play pivotal roles in the formation of wing field identity. For this reason, these genes are ideal candidates for investigating wing origin and evolution.

One way to identify the structure from which insect wings first evolved is to explore the function of “wing” genes in ancestral wingless (apterygote) species. We chose the firebrat, *Thermobia domestica*, as a model (Figure 1). *T. domestica* belongs to Thysanura, phylogenetically the closest extant relative of winged (pterygote) insects, making it ideal for elucidating wing origin. We cloned *vg* and *sd* orthologs from *T. domestica* (*Td-vg* and *Td-sd*). To examine the functions of these genes, we developed RNA interference (RNAi) based methods for *T. domestica*. We are currently testing for



Figure 1. The firebrat, *Thermobia domestica*.

functional effects of altered transcription of each of these wing genes in the ancestrally wingless firebrats. In addition, we are performing comparative analyses of the function of these same genes in “primitively winged” (hemimetabolous) insects, to obtain additional clues relevant to understanding the origin and evolution of insect wings.

II. Wing color patterns and mimicry of ladybird beetles

Insect wing color patterns demonstrate a tremendous range of diversity and have evolved to fulfill various ecologically important functions such as intraspecific sexual signaling, mimesis, mimicry, and warning against predators. The molecular mechanisms responsible for generating such patterns, however, remain unknown for most species. To investigate the developmental mechanisms of color pattern formation, we chose the multicolored Asian ladybird beetle, *Harmonia axyridis*, which has conspicuous and variable wing color patterns consisting of black and red pigments (Figure 2A). Vivid wing color patterns of ladybirds function as a warning to predators that they are distasteful, and ladybird beetles are mimicked by various insect species. Mimicry provides an exciting opportunity to study how independent lineages of insect converge on similar color patterns. For exploring color pattern formation in a mimic, we use the leaf beetle, *Argopistes coccinelliformis*, which has color patterns similar to *Harmonia*, and which is thought to be a Batesian mimic of ladybird beetles (Figure 2B). To elucidate the molecular mechanisms underlying these wing color patterns, we have established a technique for germline transformation using a *piggyBac* vector and RNAi in the ladybirds. Recently, we also designed a TALEN-based method

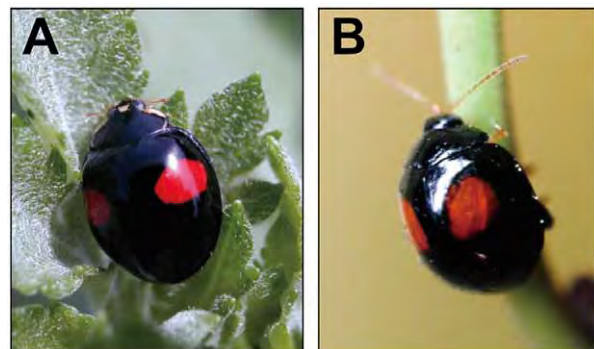


Figure 2. The ladybird beetle, *Harmonia axyridis* (A) and the leaf beetle, *Argopistes coccinelliformis* (B).

for genome editing in *H. axyridis*. Based on the knowledge obtained from *H. axyridis*, we are trying to understand how the similar wing-color patterns of model and mimic are generated – for example, do they use conserved or divergent mechanisms?

III. Acquisition and diversification of beetle horns

Insects show a tremendous range of diversity in “horns”, rigid body outgrowths that function as weapons. Horns are exciting for evo-devo studies because they have arisen multiple times *de novo*, as evolutionary “novelties”.

However, the molecular mechanisms involved in sexually dimorphic horn formation are still poorly understood. To investigate the developmental mechanisms of horn formation, we focus on the Japanese rhinoceros beetle, *Trypoxylus dichotomus* (Coleoptera), which exhibits remarkable sexual dimorphisms in head and thoracic horns (Figure 3). The male-specific horns of *T. dichotomus* are one of the best models to study how an extreme, sex-specific morphology is formed. We have developed a technique for larval RNAi in *T. dichotomus*, permitting us to rigorously and systematically test the functional roles of a large suite of candidate developmental genes, revealing for the first time the molecular mechanisms responsible for growth of male rhino beetle horns.

To understand how *sexual dimorphism* in exaggerated horn growth arises, we are currently employing two main approaches: a candidate gene approach and a high throughput approach. To identify novel genes involved in the sexually dimorphic horn development in *T. dichotomus*, we are assessing mRNA of the developing horn discs using deep-sequencing transcriptome analysis. Furthermore, to begin to understand how, molecularly, beetle horns have diversified, we are extending our analyses to include additional beetle species with different types of exaggerated horns, including rhinoceros beetles with diverse horn structures as well as horned beetles in other phylogenetic groups.



Figure 3. The Japanese rhinoceros beetle, *Trypoxylus dichotomus*. Adult male (Left) and female (Right).

Publication List:

[Original paper (E-publication ahead of print)]

- Hatakeyama, M., Yatomi, J., Sumitani, M., Takasu, Y., Sekiné, K., Niimi, T., and Sezutsu, H. Knockout of a transgene by transcription activator-like effector nucleases (TALENs) in the sawfly, *Athalia rosae* (Hymenoptera) and the ladybird beetle, *Harmonia axyridis* (Coleoptera). *Insect Mol. Biol.* 2015 Oct 26.