

## 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 of 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 1A). *T. domestica* belongs to Thysanura, phylogenetically the closest extant relative of winged (pterygote) insects, making it ideal



Figure 1. The firebrat, *Thermobia domestica* (A), the two-spotted cricket, *Gryllus bimaculatus* (B).

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 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.

Interestingly, our previous work showed that *vg* expressing epidermal tissue forms lateral outgrowths in non-winged segments in a mealworm beetle (Ohde *et al.*, 2013). From these facts, we hypothesize that ancestral lateral body wall outgrowths evolved into functional wings. To test this hypothesis, we are now comparing the role of *vg* and other “wing genes” between wings and lateral outgrowths in a basal winged insect, *Gryllus bimaculatus*, and non-winged insect, *Thermobia domestica* (Figure 1).

### II. Wing color patterns and mimicry of ladybird beetles

Insects have evolved a tremendous range of diversity of wing color patterns which play various ecologically important roles such as intraspecific sexual signaling, mimesis, mimicry, and warning against predators. The molecular mechanisms responsible for generating such color patterns in most ladybird species, however, remain elusive. To investigate the developmental mechanisms of color pattern formation, we have been focusing on 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 signal to convey to predators that they are distasteful. At the same time, various other insect species are utilizing this ecological signal by mimicking the ladybirds’ wing color patterns. Mimicry provides an exciting opportunity to study how independent lineages of insects have evolved convergent color patterns. To explore color pattern formation mechanisms in mimicry, we are focusing on the leaf beetle, *Argopistes coccinelliformis*, which has color patterns similar to *Harmonia*, and which is thought to be a Batesian mimicry of ladybird beetles (Figure 2B). To elucidate the molecular mechanisms underlying these wing color patterns, we established a technique for germline

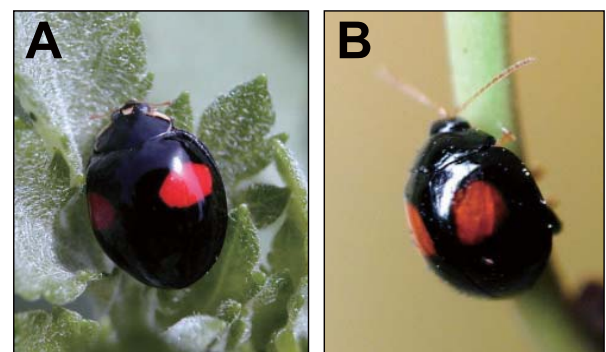


Figure 2. The ladybird beetle, *Harmonia axyridis* (A) and the leaf beetle, *Argopistes coccinelliformis* (B).

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2017. The former title is indicated by an asterisk (\*).

transformation using a *piggyBac* vector and RNAi in the ladybirds.

Recently, we identified several key regulatory genes associated with the color pattern formation of ladybird beetles using next generation sequencing technologies (RNA-seq and *de novo* genome assembly), and an RNAi-based screening method that we established. Many of these genes are expressed in specific domains of wing color patterns, suggesting that regulatory shift, such as change in enhancer activity, at these gene loci may be crucial for evolution of wing color patterns in ladybirds. Now, we are trying to elucidate the evolutionary origin of color patterns in ladybirds focusing on regulatory shifts at these genetic loci. To tackle this issue, we are establishing genome-editing technologies using TALEN and CRISPR/Cas9, and have already established an efficient method of gene disruption. At present, we are establishing more complicated genome editing techniques such as genomic insertion, inversion and duplication to identify the crucial regulatory shift that may have driven evolution of wing color patterns in ladybird beetles.

In the future, we are planning to analyze how the similar wing color patterns of model and mimic are generated based on the knowledge obtained from *H. axyridis*, – 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 3A). 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. In addition, we are employing a high throughput 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 (RNA-seq). We narrowed down the genes associated with horn formation to 40 genes, and performed RNAi-based knockdown screening to provide deep insights into where, when, and how the head and thoracic horns are formed during development. We successfully identified 11 transcription factors that contribute to horn formation. These 11 genes include larval head- and appendage-patterning genes, which are involved in *Onthophagus* horn formation, suggesting the early redeployment of this subset of genes during the scarab horn evolution.

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).

#### Publication List:

[Original paper]

- Matsuda, K., Gotoh, H., Tajika, Y., Sushida, T., Aonuma, H., Niimi, T., Akiyama, M., Inoue, Y., and Kondo, S. (2017). Complex furrows in a 2D epithelial sheet code the 3D structure of a beetle horn. *Sci. Rep.*, 7, 13939.

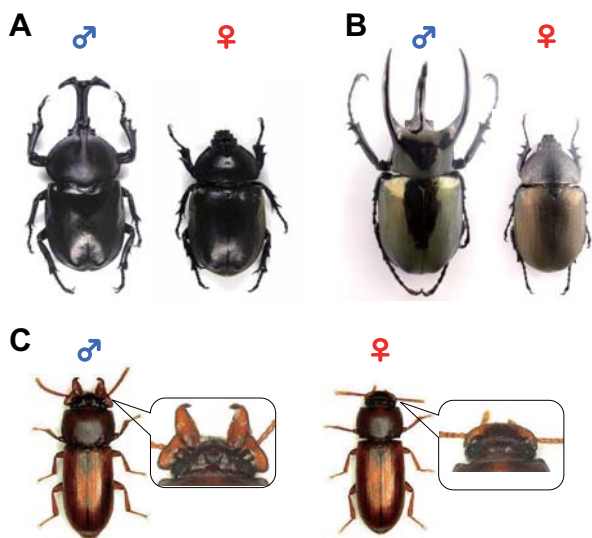


Figure 3. The Japanese rhinoceros beetle, *Trypoxylus dichotomus* (A), the Atlas beetle, *Chalcosoma atlas* (B) and the broad-horned flour beetle, *Gnatocerus cornutus* (C). Adult male (Left) and female (Right).