

# **Evolutionary changes in gene expression profiles associated with coevolution of the male and female genitalia in a ground beetle group**

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## **Background**

Speciation increases biological diversity; a good understanding of speciation mechanisms is pivotal when engaging in evolutionary studies. Male and female genital morphologies often differ markedly among closely related species, but exhibit correlated or coevolutionary patterns between the sexes. Genital diversification may enhance speciation. The evolutionary processes/causes of male and female genital morphology have been explored in comparative studies of genital morphology and mating behaviors, and in mating experiments. However, few studies have sought to trace the relevant evolutionary processes using genomic, genetic, and developmental approaches toward an understanding of genital morphology.

Ground beetles of the subgenus *Ohomopterus* (genus *Carabus*) are remarkably diverse in terms of the size and shape of the male and female genital parts (thus the male copulatory piece and the female vaginal appendix). Matching and correlated evolution (coevolution) are in play between the sexes of each species. The genetic background of species-specific genital morphology has been studied via quantitative genetic and genomic analyses. However, the genetic mechanisms responsible for the sex- and species-specific genital morphologies associated with coevolution, and the interspecific divergences in male and female genital morphologies, remain poorly understood. In this thesis, I aimed to trace the genetic changes involved in coevolution of the copulatory piece and vaginal appendix. I studied the changes in gene expression profiles during genital morphogenesis in males and females of closely related *Ohomopterus* beetle species.

## **Methods**

Chapter 2 details the gene expression profiles revealed by transcriptome data of male and female *Carabus maiyasanus* third (last)-instar larvae and pupae. I clarified the timing of genital morphogenesis/formation by examining the expression profiles of key genes involved in organ size control across the developmental stages. Chapter 3 outlines the changes I identified in gene expression associated with the coevolution of extremely enlarged (“exaggerated”) genitalia in both sexes of *C. uenoi*. I compared the gene expression profile of that species to those of the closely related species *C. maiyasanus*

and *C. iwawakianus* (the genital part sizes of which differ from each other but are much smaller than those of *C. uenoi*). I identified gene networks involved in the exaggeration of genitalia from genes expressed differentially between species by weighted gene coexpression network analyses (WGCNA). I also compared gene expression changes between the sexes of the latter two species. Chapter 4 reports my comparison of the gene expression profiles of four species that differ in terms of genital size (*C. arrowianus* and *C. insulicola* [long genital parts]; *C. komiyai* and *C. esakii* [short genital parts]). I hypothesized that species exhibiting similar genital lengths would evidence similar changes in gene expression. I used clustering of genes expressed differentially among species and gene function (ontology) enrichment analyses to identify genes that influenced the genital length differences among the four species.

## **Results and Discussion**

As detailed in Chapter 2, I found that genital morphogenesis commenced in the early prepupal stage and that genital formation commenced in the interval between the late prepupal and early pupal stages. Chapter 3 reports that sex-discordant expression changes affected differences between the long and short genital parts of *C. maiyasanus* and *C. iwawakianus*, whereas sex-concordant expression changes played major roles in the coevolution of the exaggerated genitalia of *C. uenoi*. These results imply that genital length coevolution may be controlled by different genetic mechanisms in each sex, but that coevolution of the exaggerated genitalia of *C. uenoi* may be controlled by a genetic mechanism shared by both sexes. Thus, the coevolution of male and female genitalia can be driven by sex-discordant or -concordant selection.

As detailed in Chapter 4, I identified one gene cluster that plays a role in the differences in genital length evident in females and two gene clusters that play a role in males. One cluster in each sex was similarly expressed in species with similarly sized genital parts. However, the expression profiles of another male cluster differed among species with long genital parts. Neither male nor female clusters evidenced sex-concordant expression profiles in terms of genital size differences. The results outlined in Chapters 3 and 4 imply that genetic control of genital length differs between the sexes of species with short and long parts, possibly under sex-discordant selection. However, sex-concordant gene expression within a shared gene network under sex-concordant selection may explain the formation of the extremely elongated genital parts of *C. uenoi*.

## **Conclusions**

I found that coevolution of species-specific male and female genital part sizes among

*Ohomopterus* species reflected both sex-concordant and -discordant gene expression changes that varied by species, but also sex-specific gene expression changes that were common to the species with genital parts of similar sizes. The observed differences in the mechanisms that control genital size may reflect variations in the selection pressures experienced by male and female genitalia. The results of my study thus shed light on the complex genetic background and coevolutionary processes underlying the genital coevolution of the sexes.