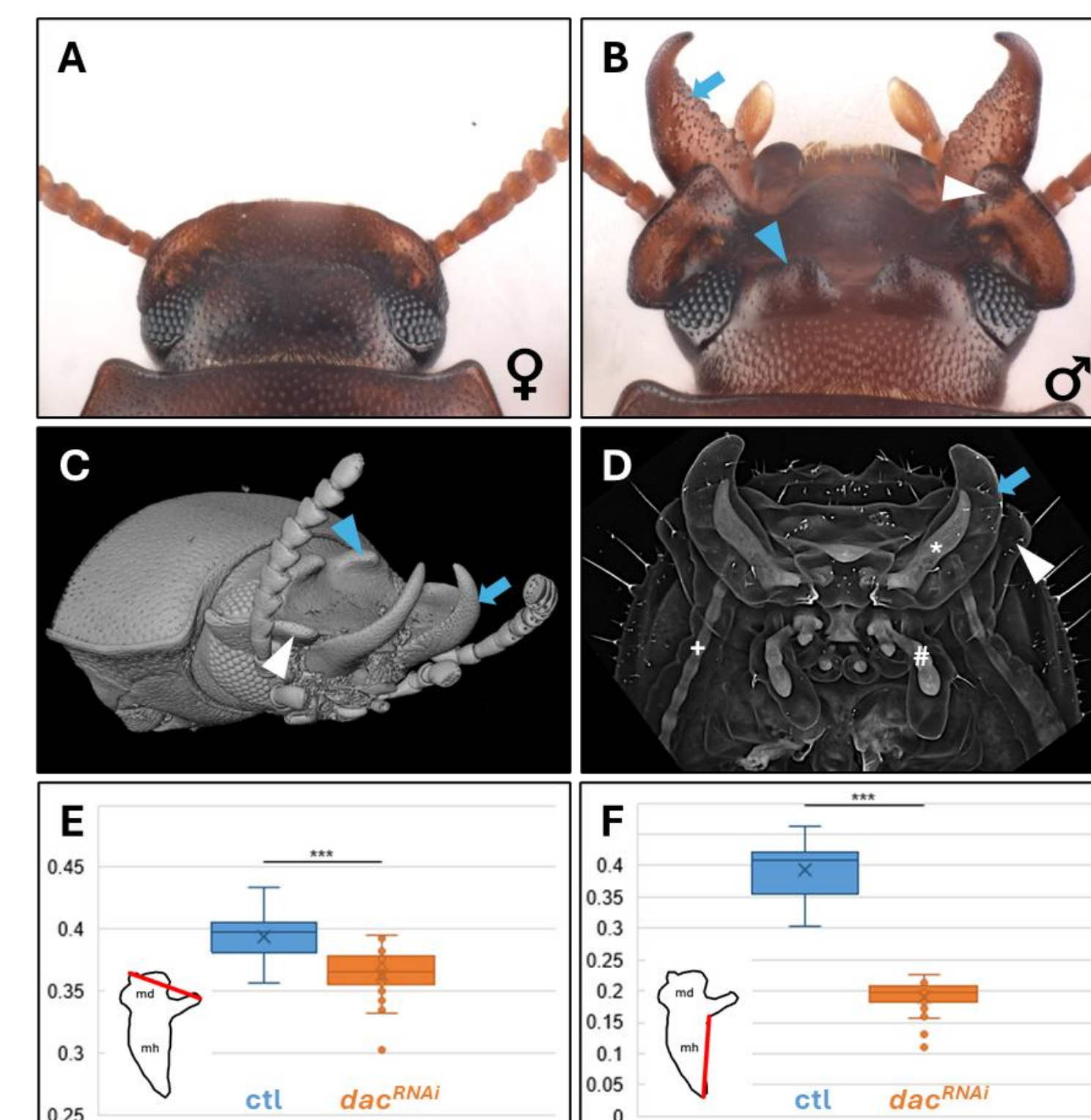


State of the art

Insects undergo remarkable transformations during metamorphosis. How are these morphological changes governed and how does evolution of post-embryonic gene regulatory networks (GRNs) contribute to morphological diversity in insects. We focus on head development in two closely related beetle species: *Gnatocerus cornutus* and *Tribolium castaneum*. Male *G. cornutus* develop exaggerated head structures (B, C), absent in females (A) and *T. castaneum*, providing a model to study the genetic basis of novel trait evolution.

Preliminary Data

Our preliminary work shows that mandibular horns develop during prepupal stages (D), and that the appendage patterning gene *dachshund* affects mandible horn size (E, F) but not the other exaggerated head structures in male *G. cornutus*. Gene expression analyses (RNAseq) highlight two candidate genes in the Wnt signaling pathway, which is a crucial member of the embryonic anterior GRN (aGRN)¹, and it is known to regulate head horn development in other beetles². We hypothesize that the embryonic aGRN is re-deployed in *G. cornutus* to guide the development of exaggerated head traits.



Primary Questions

- Is the embryonic aGRN re-used to pattern the anterior adult head?
- What GRN changes drive the formation of novel exaggerated head traits?

Objectives

- Describe head and mandibular horn formation on cellular and molecular basis.
- Identify candidate genes and GRN modules underlying head and mandibular horn formation.
- Functionally validate candidate genes.



Work Plan

A) Cellular analysis of head horn and mandible outgrowth development: Nano-CT data will be used to gain cellular resolution of the processes leading to horn formation.

B) GRN of pupal head formation in *G. cornutus* males and its relation to the embryonic aGRN: To identify major cell types, genes, signaling pathways and GRN modules involved in male head formation, snRNA-seq and snATAC-seq will be used. GRN dynamics will be analyzed and compared to the embryonic aGRN to test the hypothesis of re-use in post-embryonic development.

C) Molecular differences in pupal head development between horn vs. no-horn phenotypes:

To identify genes, pathways and cell types specifically involved in exaggerated head trait development, additional snRNA-seq and scATAC-seq data will be generated for the same developmental stages in *G. cornutus* females and *T. castaneum* males. Cell type composition, gene expression and GRNs will be compared between “horn” vs. “no-horn” phenotypes.

D) Functional validation of candidate genes applying RNA interference: Ten to fifteen candidate genes will be functionally validated for their role during larval/prepupal exaggerated trait development, as well as during embryonic development with a focus on aGRN genes applying gene knockdown by RNA interference (RNAi).

E) Development of live imaging lines to visualize exaggerated trait development *in vivo*: To study exaggerated head trait development at unprecedented detail and to assess the effect of candidate gene knock-down, transgenic establish fluorescent imaging lines³ will be established.

Synergy and collaborations

- **Collaborative Project 1: Wnt signaling in anterior development**
Comparison of the involvement of direct Wnt targets in postembryonic head patterning and during exaggerated head trait evolution.
- **Collaborative Project 2: Reconstructing evolving GRNs**
Involvement of embryonic aGRN genes in metamorphosis.
- **Collaborative Project 3: Novel bioinformatics and genetic tools**
Provision of omics data for the establishment and test of new bioinformatic tools for cross-species comparisons.
- **GB:** transgenesis, imaging line generation, embryonic aGRN
- **DJ:** single-cell omics
- **PL:** live imaging, quantitative image analyses

Technical innovations

- Integration of multi-omics data to study phenotypic novelties on cellular resolution.
- Innovative transgenic imaging lines to study post-embryonic development.

Specific qualification

- single-cell omics, integration of multi-omics data, GRN reconstruction
- RNAi, genome editing
- analysis of quantitative imaging data, live imaging



Nico Posnien
Fac. of Biology
University

References

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