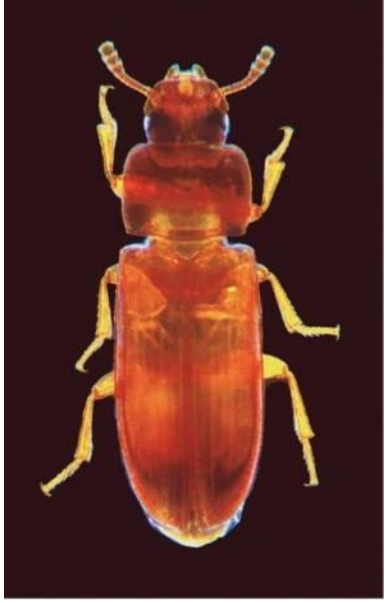


State of the art



Gene functions and gene regulatory networks (GRNs) diversify within species. In *Tribolium*, about 6% of the genes show strain specific RNAi phenotypes [1,2]. We observed two cases with respect to the aGRN: *Tc-hbn* leads to axis formation defects in one strain but to head defects in another and the sensitivity to *Tc-axin* RNAi differs [3,4].

The possibility for titrated gene-knock-down via RNAi makes *Tribolium* an excellent model system to study the microevolutionary diversification of GRNs as a first step in macroevolutionary morphological diversification.

However, bioinformatics reconstruction of GRNs from omics data has limitations as it is mainly based on correlation of gene expression. Therefore, additional types of input data and enhanced bioinformatics tools for GRN reconstruction would increase the resolution of evolutionary GRN comparisons.

Further, a more specific analysis of RNAi phenotypes by spatial restriction of RNAi would be advantageous.

Primary questions

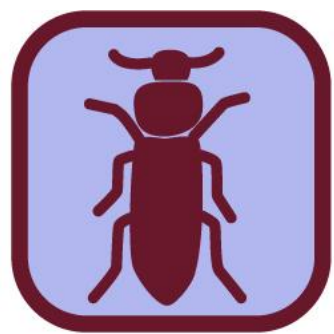
We want to understand the micro-evolutionary changes of a developmental GRN:

- What are the differences of the aGRN between strains?
- What are the aGRN modifications that explain the different phenotypes resulting from the same RNAi treatment?
- How can the reconstruction of GRNs be enhanced with data available in emerging model organisms?

Objectives

- Determine the degree of phenotypic variability of the aGRN in different strains of *Tribolium castaneum*.
- Compare the diverging architecture and dynamics of the aGRN from different strains.
- Enhance GRN reconstruction by adding information on its reaction to gradual challenges via titrated RNAi.
- Develop a tool for spatial restriction of RNAi.

Workplan



A) Determining gene function divergence:

To determine the degree of gene function divergence in the aGRN, we will use titrated knock-down of *Tc-hbn* and *Tc-axin* to quantify the phenotypic differences in several strains of *T. castaneum* and a closely related species (*Gnathocerus cornutus*). We will test additional key genes of the aGRN to see, how many gene functions show diverging phenotypic effects.

B) Reconstruction of GRN divergence with new tools:

For a comprehensive view on aGRN divergence, we will generate RNA-seq data from two *Tribolium* strains that show different RNAi phenotypes (one dataset taken from Collaborative Project 2). In addition, we will add data from *Tc-hbn* RNAi embryos from both strains with titrated knock-down (Fig. 1). We think that this provides additional information for GRN reconstruction. This unique dataset will be used for developing novel tools for GRN reconstruction. sGRN comparison with novel bioinformatics tools will reveal divergent and conserved nodes in the aGRN and the different compensatory dynamics leading to different phenotypes. Emerging hypotheses on the evolutionary divergence will be tested by additional experiments.

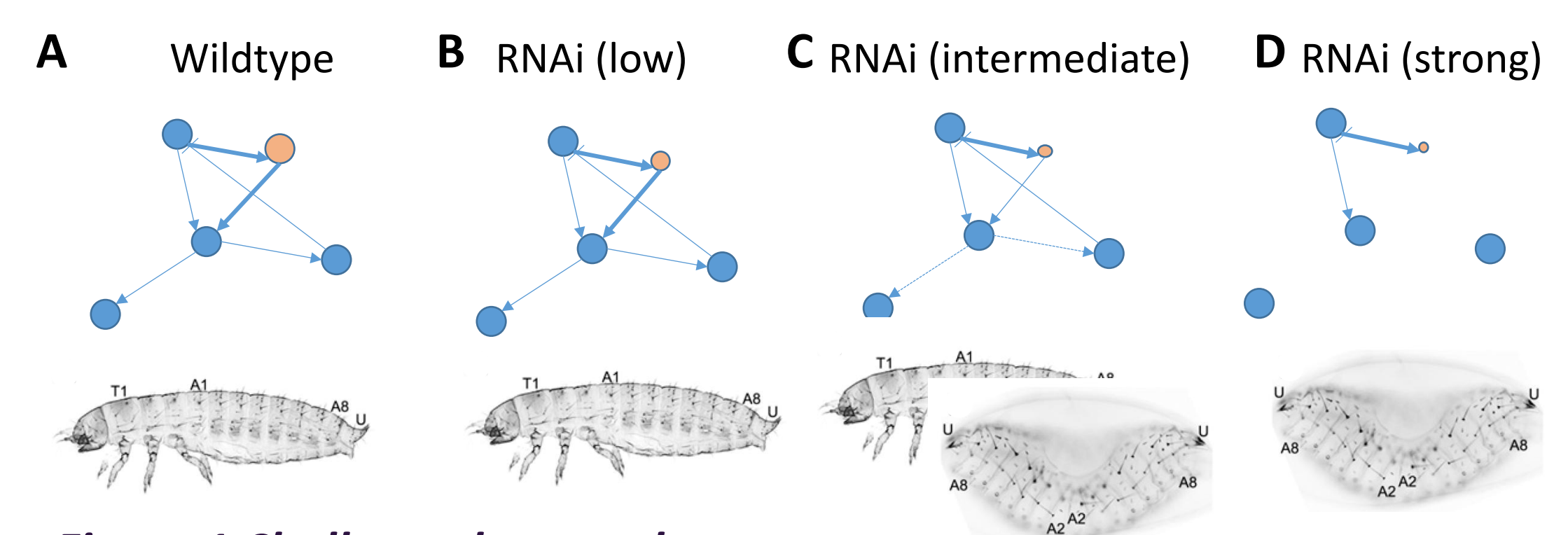


Figure 1 Challenged networks

(A) A wildtype GRN consists of genes (nodes) and interactions (edges). Minor reduction of gene function of one component (smaller circle in B) is compensated for, resulting in wildtype morphology. (C,D) Intermediate and high levels of gene knockdown challenge the GRN and lead to breakdown and a morphological phenotype. The compensatory reaction of the GRN and the changes after breakdown contain information on direction and strength of gene interactions.

C) New tool for spatial restriction of RNAi:

We have established temporal control on RNAi by heat-shock-mediated expression of a viral suppressor of RNAi (VSR) [5] and have managed to partially overcome sterility with that tool.

Now, we want to develop a tool for spatial restriction of RNAi. The ubiquitously expressed VSR will be flipped-out locally using the Cre-Lox system, allowing RNAi in those tissues.

Synergy and collaborations

• Collaborative Project 2: Reconstructing evolving GRNs

The embryonic stages used for the second strain are identical with the ones used for other protostomes (PL, MA, DJ, JR). We add the microevolutionary view on aGRN evolution.

• Collaborative Project 3: Novel bioinformatics and genetic tools

Provision of RNA-seq and phenotypic data for bioinformatics tool development by TB. Provision of data for species-agnostic transfer learning by ACH. New tool for spatial restriction of RNAi

- **TB:** Novel bioinformatics tools for reconstruction and comparison
- **NP:** Use of *Gnathocerus* for RNAi experiments

Technical innovation

- Titrated RNAi and quantification of phenotypes
- Using GRN challenge as additional information for GRN reconstruction
- New tool for spatial restriction of RNAi

Specific qualification

- Design and testing of transgenic constructs
- Cutting edge RNAi approaches (titration, spatial restriction)
- Bioinformatics GRN reconstruction and comparison



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