Dissecting the genetic architecture underlying mouth dimorphism in Pristionchus pacificus identifies cis-regulatory variation in a supergene locus

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1. Pristionchus pacificus plastic mouth form

Phenotypic plasticity describes the property of a genotype to respond to environmental variation by producing distinct phenotypes. In the free-living nematode Pristionchus pacificus, the mouth form is developmentally plastic, resulting in two alternative mouth forms: the eurytomatus (Eu) predatory form has two strong teeth, whereas the alternative stenotomatus (St) form has a single tooth and is bacteriovorous (Fig 1.1A). The eud-1 first intron is a switch control (Fig 1.1B).

1.2 P. pacificus phylogeny

The switch between the two forms is environmentally sensitive, and a previous forward genetics approach showed a key switch function for the sulfatase-coding gene eud-1, mutations in which result in all-St worms (Fig 1.1B). In this study we used P. pacificus natural isolates with different Eu/St ratios (Fig 1.1C) to generate Recombinant Inbred Lines (RILs), and perform Quantitative Trait Locus (QTL) analysis to dissect the genetic architecture underlying mouth dimorphism in P. pacificus.

3. Cis-regulation of the switch gene

Copy number differences in a potential forkhead transcription factor binding site (PFB) within the promoter/enhancer region, besides a SNP in the eud-1 first intron between the parental lines caused differences in mouth-form ratios phenotype. Mutant lines showed an additive effect of these cis-regulatory elements, with a systematic change in the mouth form phenotype and downregulation of eud-1 expression (Fig 3.1A, 3.1B, 3.1C). Currently, we are using CRISPR-Cas9 technology to examine the potential involvement of various forkhead genes in controlling eud-1 expression, while also expanding our analysis to test variations in the causative region within 30 more P. pacificus natural isolates (PfNS say = 0.1444@T).

3.2 Parental cis-structural variation upstream eud-1

3.3 Cis-regulatory mutants phenotype

A supergene is a group of chromosomally linked genes, functionally essential to a complex trait, and inherited as a single unit. CRISPR-Cas9 knockout of the four genes influenced mouth form phenotype to various degrees (Fig 3.2D). And transcriptional reporters showed non overlapping expression of these genes in different sensory neurons (Fig 3.2E).

4. Additional Intrinsic regulatory elements

In eud-1 first intron, a region of 36 bp displayed high similarity to the potential binding site upstream eud-1 (Fig 4.1A). This region has shown to be highly conserved within P. pacificus natural isolates, therefore, not involved in natural variation. However, the deletion results in all-St worms (Fig 4.1B).

4.2 eud-1 first intron mutants phenotypes

Highlights (Polymorphism behind Polymorphism)

- Pristionchus pacificus has a developmentally plastic mouth, which can either develop into a predatory or bacteriovorous mouth form.
- QTL mapping identifies a supergene locus with a major effect on mouth dimorphism.
- Dimorphism in natural isolates is controlled through variation in cis-regulatory elements that, in turn, govern the expression of the switch gene (eud-1).
- A potential forkhead binding site variation in the promoter/enhancer region of eud-1, and a SNP in its first intron, additively regulate the expression of the switch.
- eud-1 first intron harbour a conserved regulatory element, when deleted, leads to a complete switch in the mouth form.

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References