

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

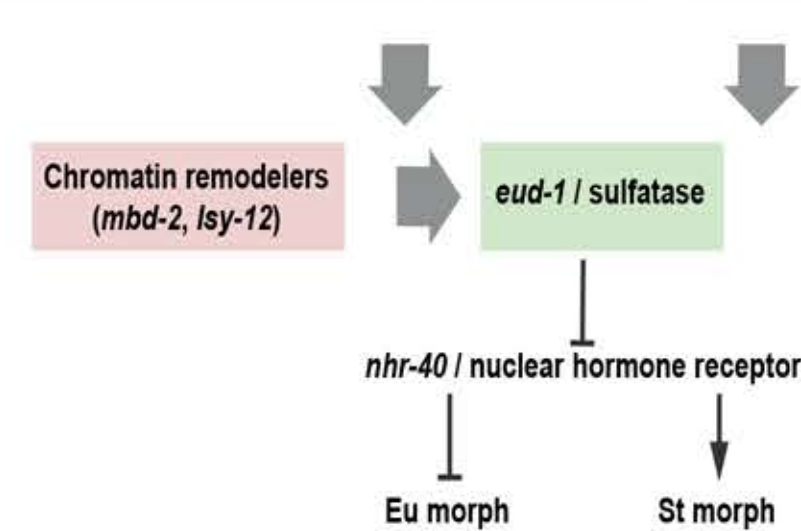
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## 1. *Pristionchus pacificus* plastic mouth form

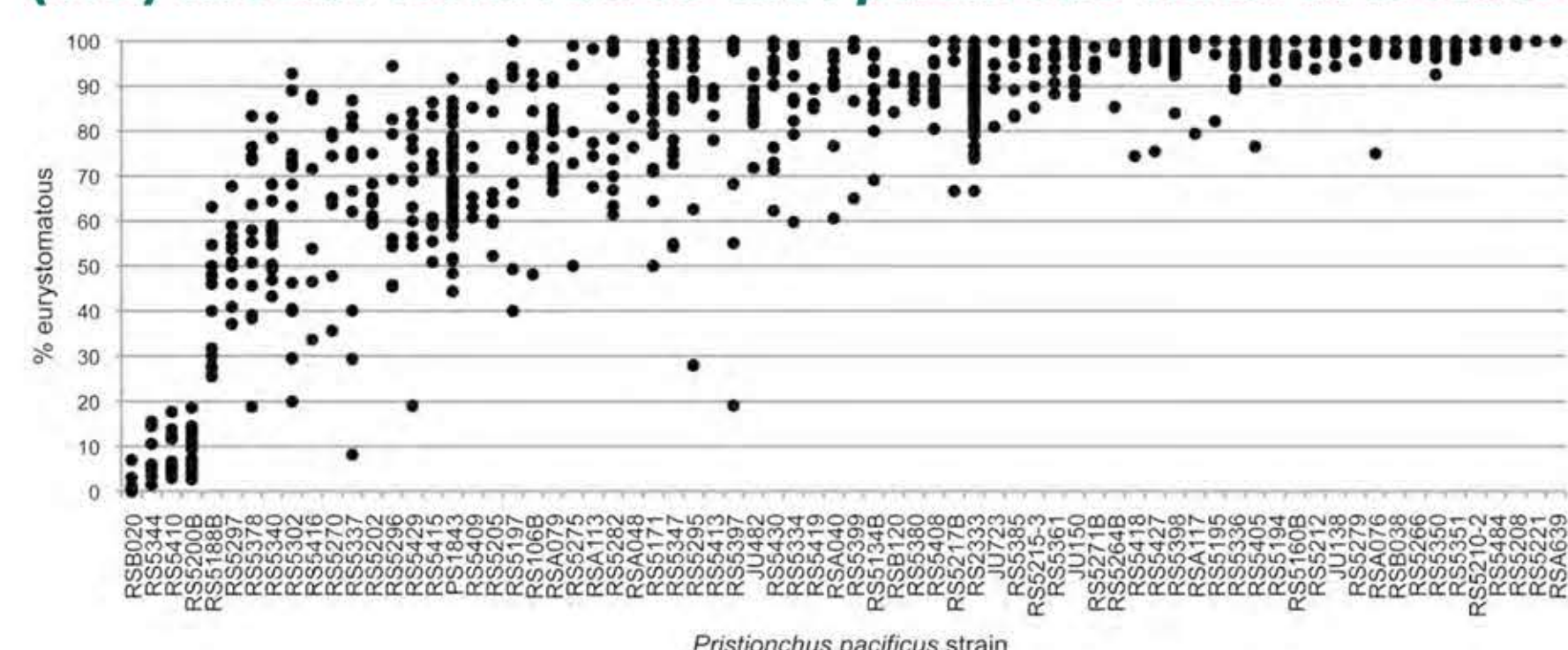
Phenotypic plasticity describes the property of a genotype to respond to environmental variation by producing distinct phenotypes. In the free-living nematode *Pristionchus pacificus*, the mouth form is developmentally plastic, resulting in two alternative mouth forms: the eury stomatous (Eu) predatory form has two strong teeth, whereas the alternative stenostomatous (St) form has a single tooth and is bacterivorous (Fig 1.A).

### (1.B) *eud-1* as a switch control

Environment (nematode-derived metabolites, diet composition, liquid vs solid culture medium)



### (1.C) Mouth form ratios in *P. pacificus* natural isolates

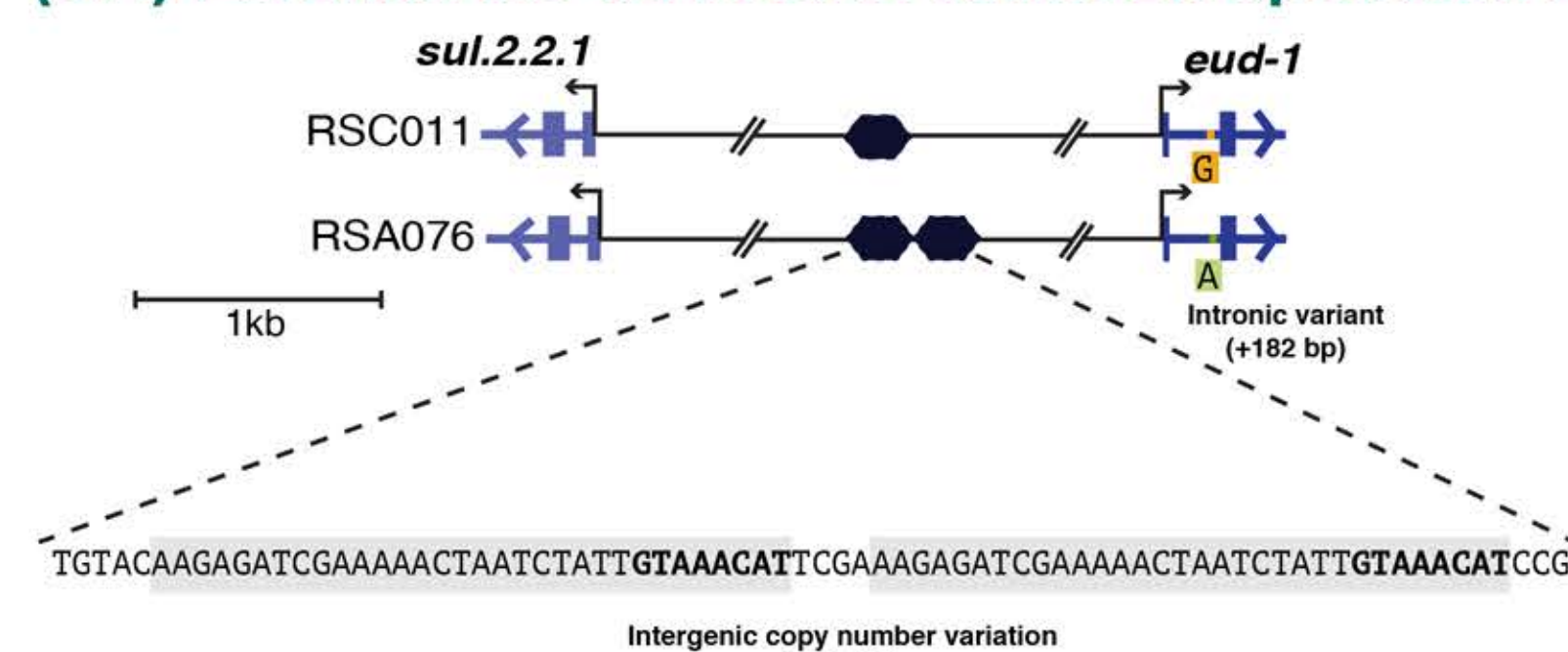


The switch between the two forms is environmentally sensitive, and a previous forward genetics approach showed a key switch function for the sulfatase-coding gene *eud-1*, mutations in which result in all-St worms (Fig 1.B). In this study we used *P. pacificus* natural isolates with different Eu/St ratios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

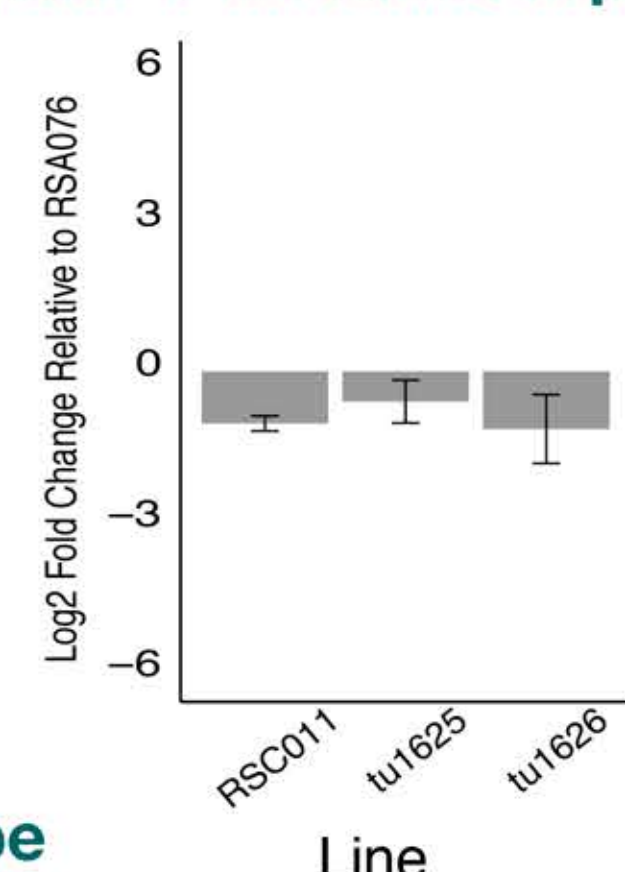
## 3. *Cis*-regulation of the switch gene

Copy number differences in a potential forkhead transcription factor binding site (PBS) 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.A, Fig 3.B, Fig 3.C). 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. (PBS seq = GTAAACT)

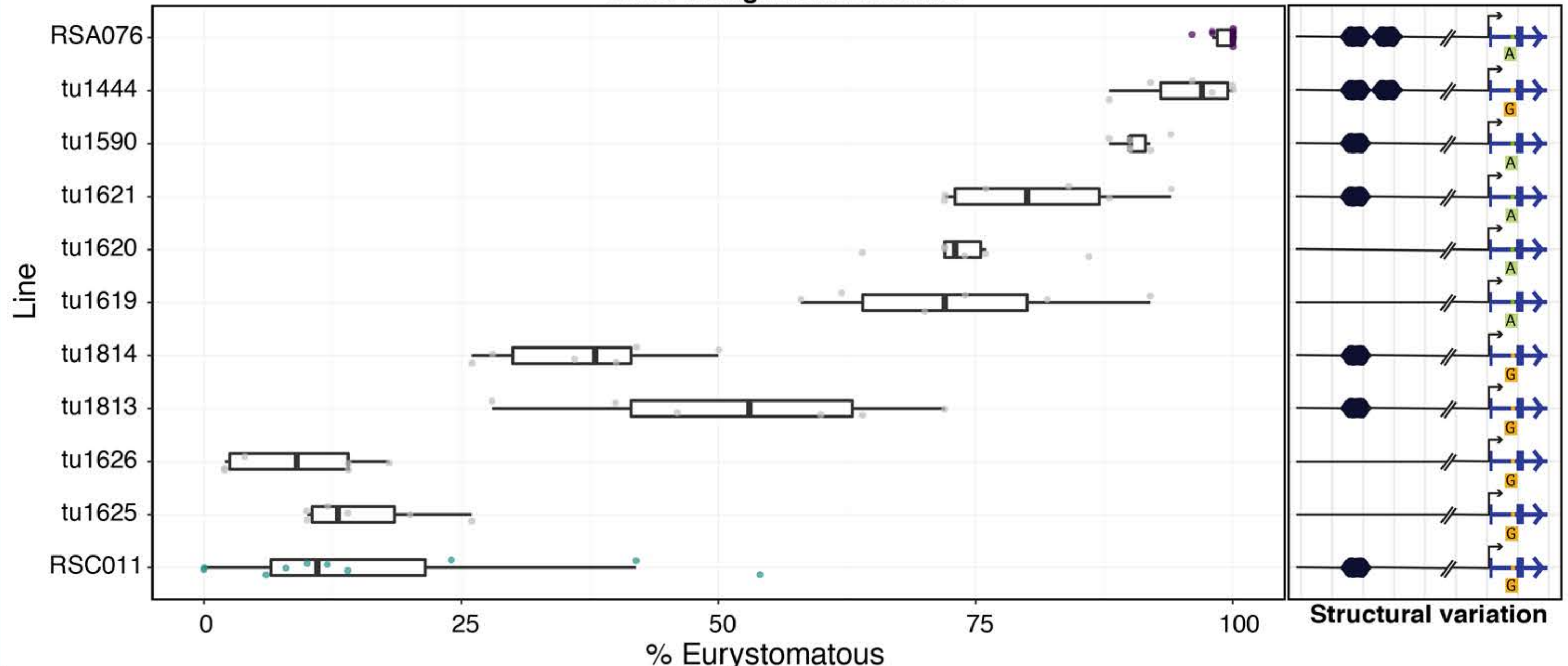
### (3.A) Parental *cis*-structural variation upstream *eud-1*



### (3.B) *eud-1* mutants expression



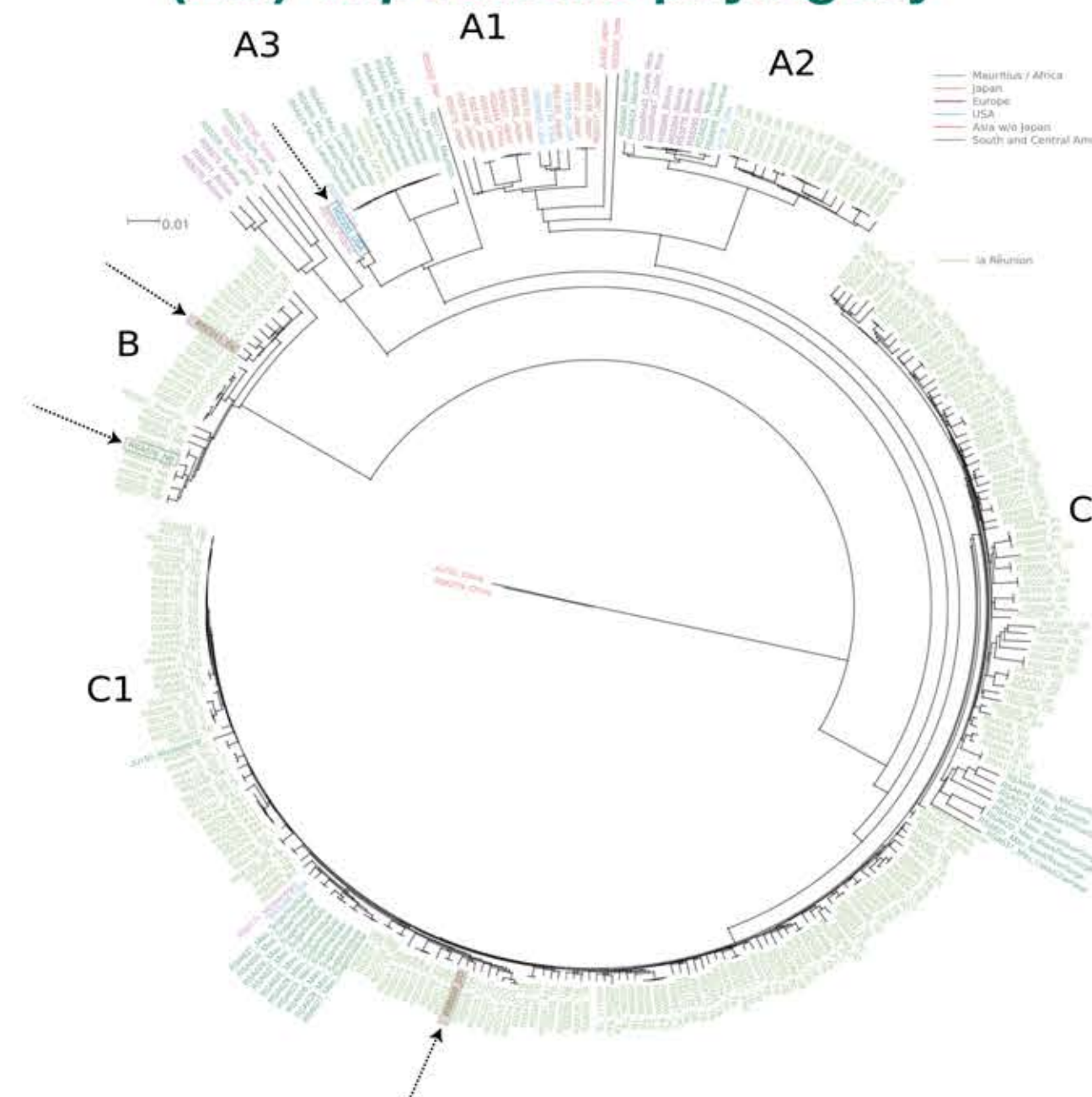
### (3.C) *Cis*-regulatory mutants phenotype



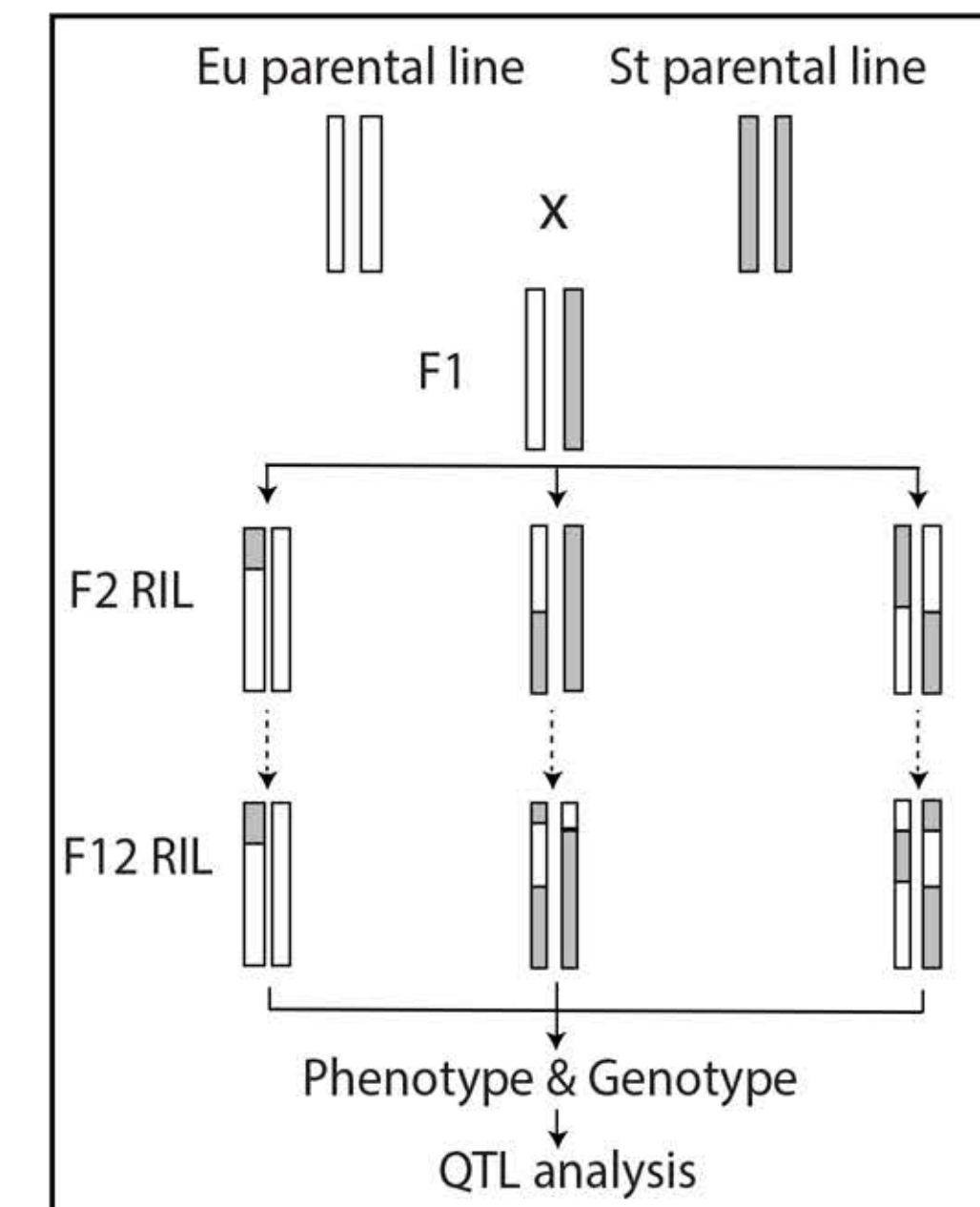
## 2. QTL mapping identifies a supergene locus

Two sets of RILs were generated; one between parents from the same clade (clade B), and the other between parents from different clades (clade A, clade C) (Fig 2.A). RILs were generated by crossing a highly Eu line with a highly St line, followed by inbreeding for at least 12 generations (Fig 2.B). The phenotype of inbred lines was scored, followed by sequencing of chosen lines and QTL analysis. The analysis revealed the involvement of one major locus on the X chromosome, spanning a recently described supergene locus containing *eud-1*, its paralog, and two more genes encoding  $\alpha$ -N-acetylglucosaminidases (*nag*) (Fig 2.C).

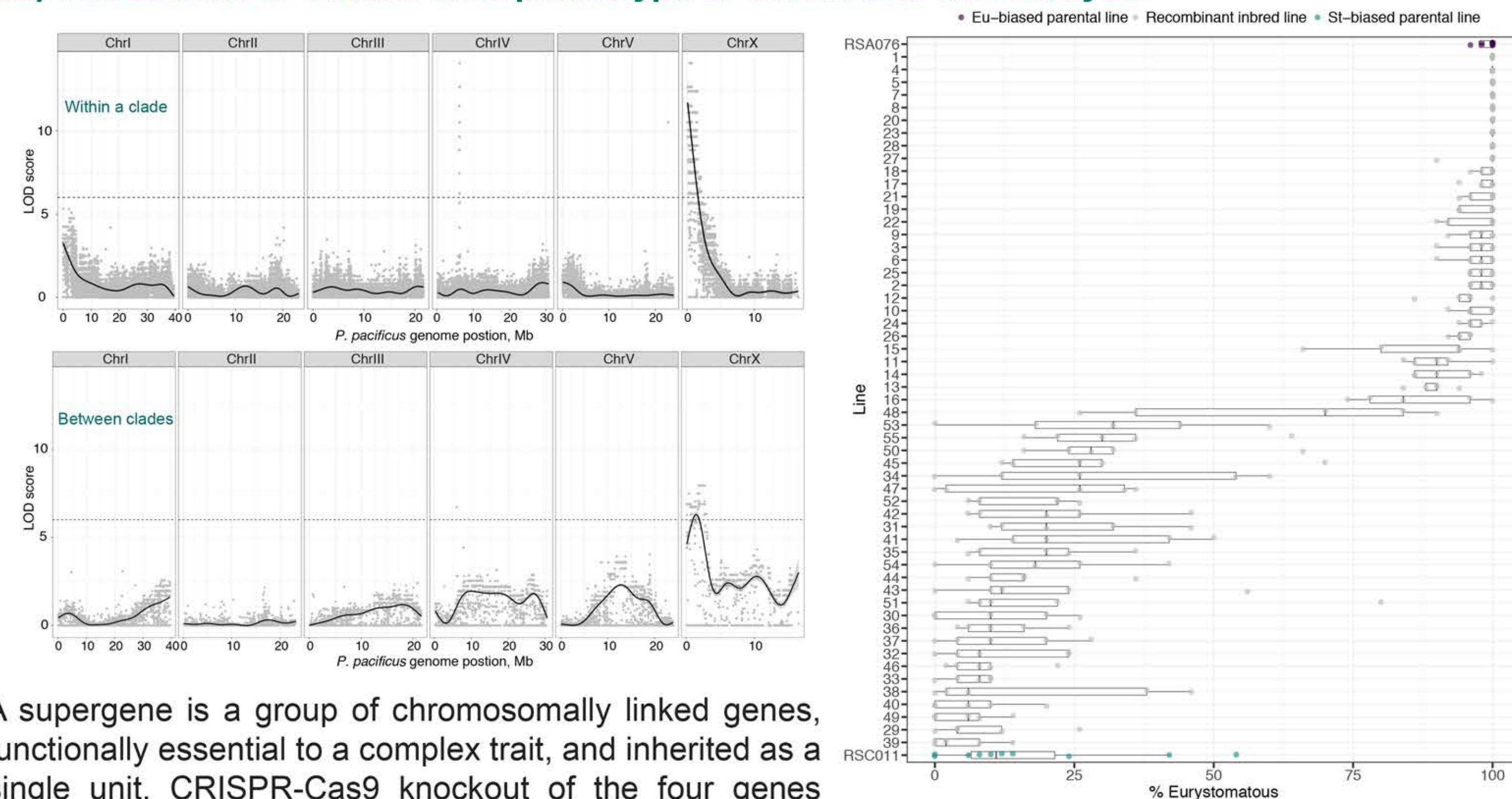
### (2.A) *P. pacificus* phylogeny



### (2.B) Generation of Recombinant inbred lines

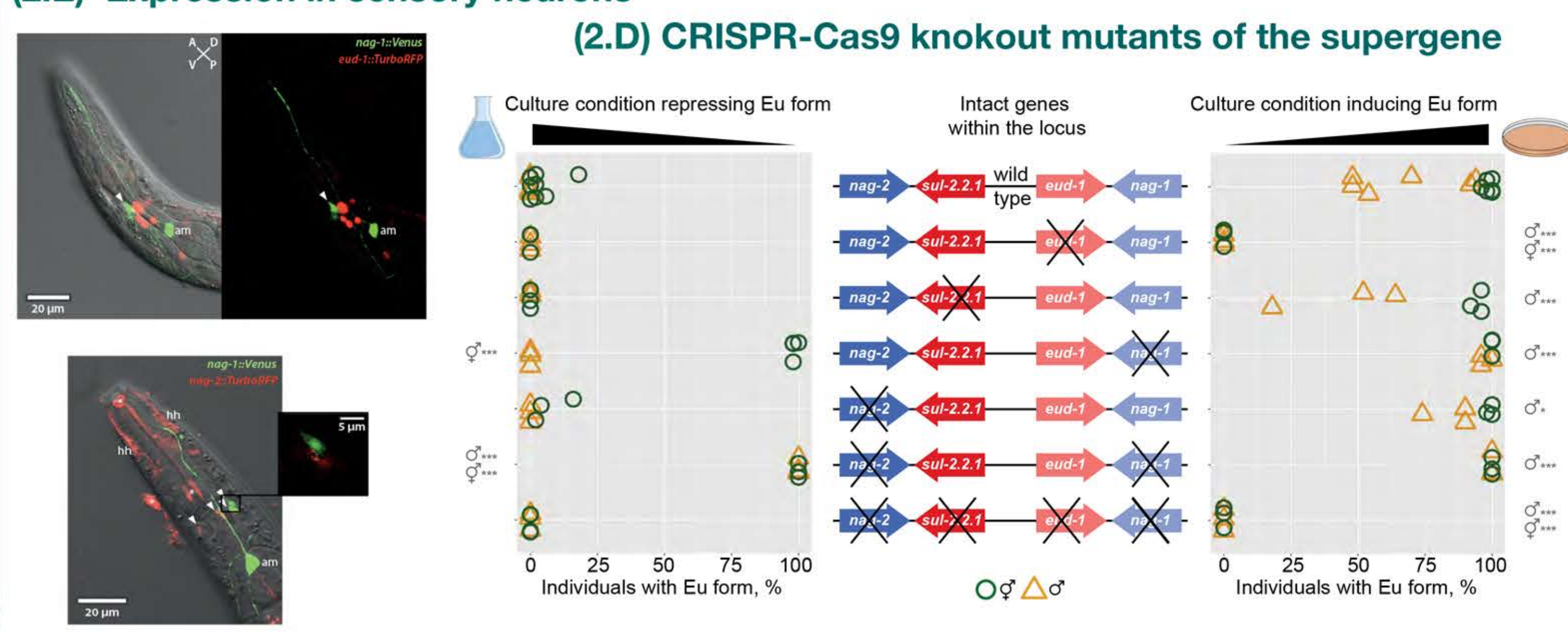


### (2.C) Distribution of mouth form phenotype in the RILs & QTL analysis



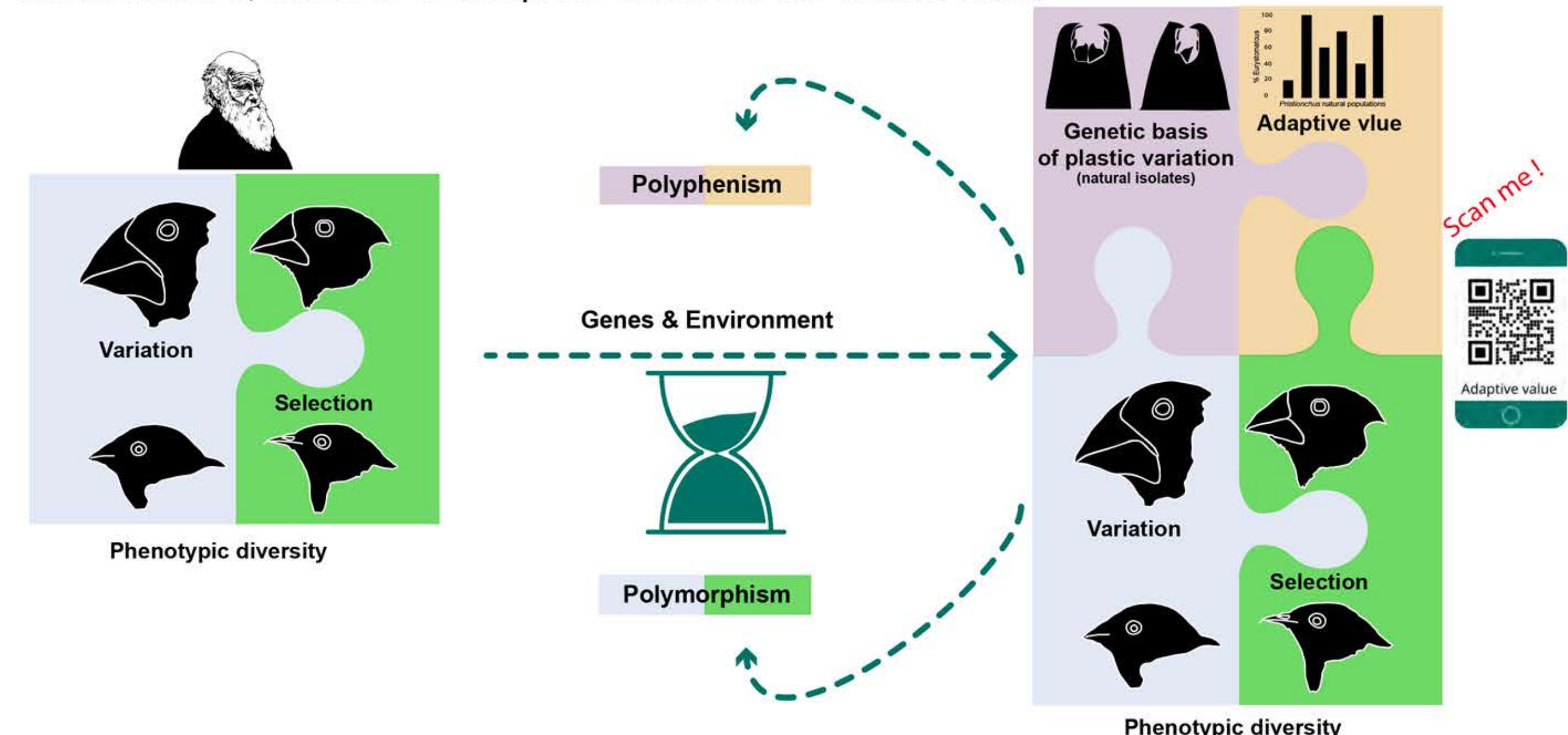
A supergene is a group of chromosomally linked genes, functionally essential to a complex trait, and inherited as a single unit. CRISPR-Cas9 knockout of the four genes influenced mouth form phenotype to various degrees (Fig 2.D). And transcriptional reporters showed non overlapping expression of these genes in different sensory neurons (Fig 2.E).

### (2.E) Expression in sensory neurons



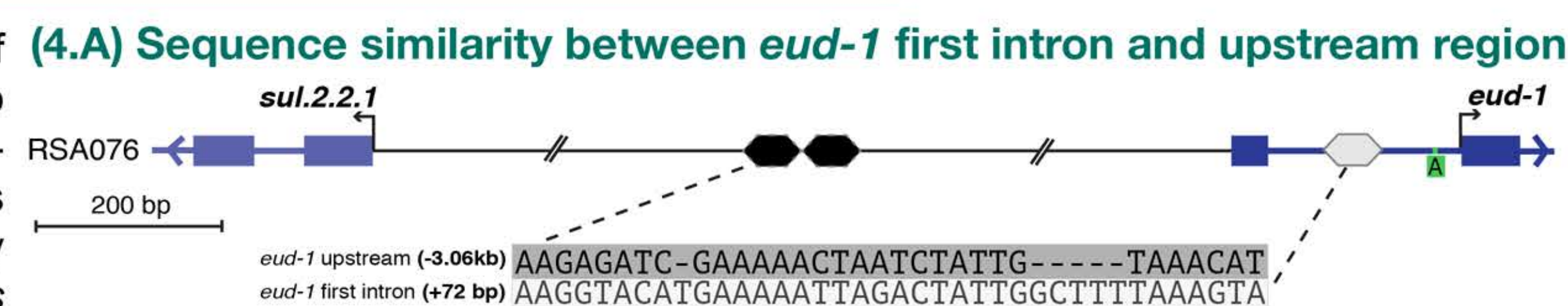
## Highlights (Polymorphism behind Polyphenism)

- Pristionchus pacificus* has a developmentally plastic mouth, which can either develop into a **predatory** or **bacterivorous** 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.

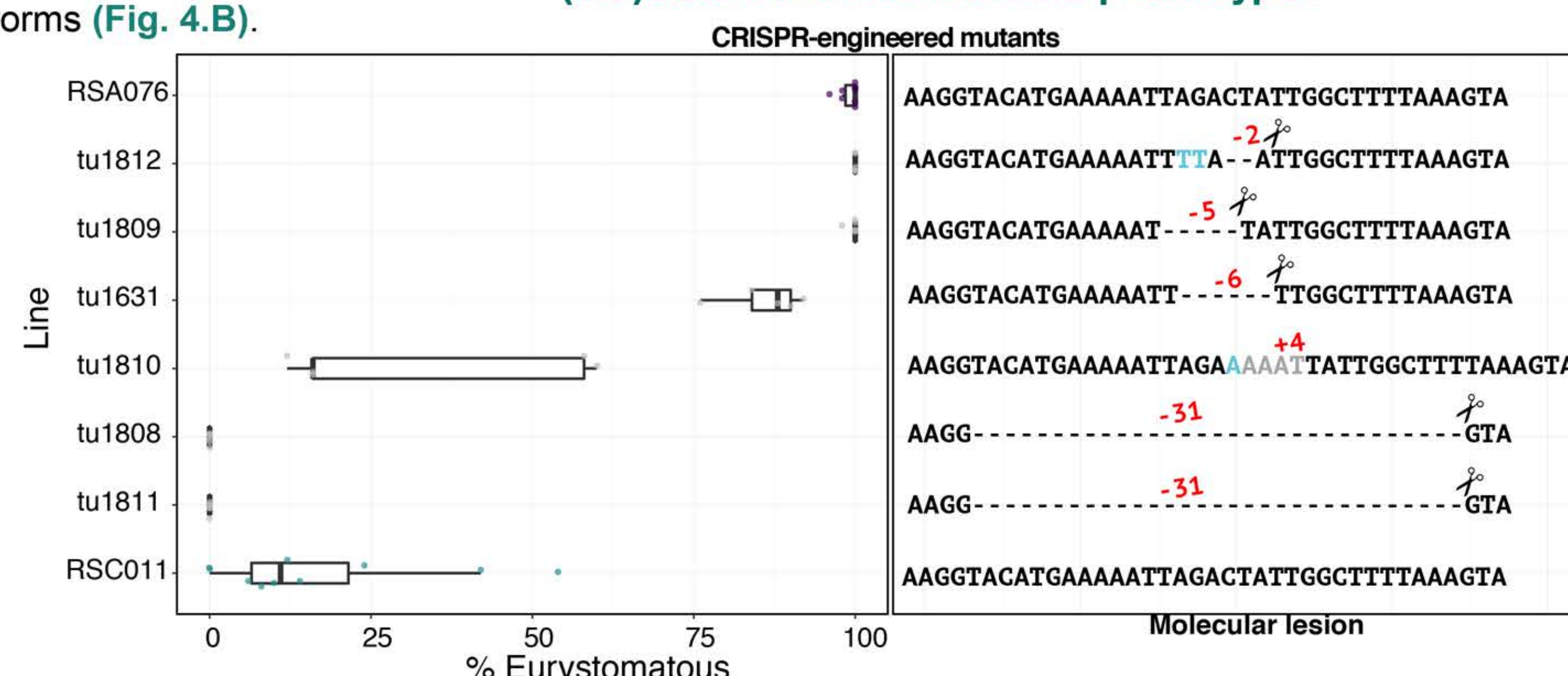


## 4. Additional Intronic regulatory elements

In *eud-1* first intron, a region of 38 bp displayed high similarity to the potential binding site upstream *eud-1* (Fig. 4.A). This region has shown to be highly conserved within *P. pacificus* natural isolates, therefore, not involved in natural variation. However, the deletion of 31bp results in all-St worms (Fig. 4.B).



### (4.B) *eud-1* intronic mutants phenotypes



## References

- Sieriebriennikov, B., Dardiry, M.,\* Prabh, N.,\* Witte, H., Rödelberger, C., Röseler, W., Kieninger, M. R. & Sommer, R. J. (2018): A developmental switch generating phenotypic plasticity is part of a conserved multi-gene locus. Cell Reports, 23, 2835-2843.  
Rödelberger, C., Meyer, J. M., Prabh, N., Lanz, C., Bemm, F. & Sommer, R. J. (2017): Single-molecule sequencing reveals the chromosome-scale genomic architecture of the nematode model organism *Pristionchus pacificus*. Cell Reports, 21, 834-844.  
Ragsdale, E. J., Müller, M. R., Roedelberger, C. & Sommer, R. J. (2013): A developmental switch coupled to the evolution of plasticity acts through a sulfatase. Cell, 155, 922-933.