

DOCTORAL THESIS

Identification of loci/genes responsible for hybrid incompatibilities between *Caenorhabditis briggsae* and *C. Nigoni*

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Abstract

Identification of genetic basis of Hybrid Incompatibility (HI) in hybrids between closely related species leads to a comprehensive understanding of speciation. In *Drosophila* species, systematic characterization of HI has been intensively preceded, but similar studies are falling behind in other species, including nematodes. Although model organism *C. elegans* is well-established and utilized in laboratory, it has performed little contribution to this research area, because *C. elegans* failed to mate with other sister species and produce viable progeny. As a sister species of *C. briggsae*, which is close to *C. elegans*, newly discovered *C. nigoni* made it possible to identify the genetic basis of HI in nematode species.

In this study, a new species pair including *C. nigoni* and *C. briggsae* was used to study the genetic and molecular bases of HI between the two. 96 GFP markers were randomly integrated into the genome of *C. briggsae* by biolistic bombardment. Next-Generation Sequencing (NGS) combined with single worm PCR were performed to identify the location of GFP markers. By tracking those markers, the genomic fragments of *C. briggsae* linked to GFP were backcrossed into *C. nigoni*. Such process was repeated for at least 15 generations and total 111 strains carrying independent introgressions were generated. The patterns of HI were dissected by scoring the embryonic lethality, larval arrest, sex ratio and male sterility for each introgression strain. Widespread HI loci were identified on a genome-wide scale for the first time in nematode species, which also supported Haldane's Rule and large X-effect theory between the two species. In this study, *C. nigoni* genome "cn1" was *de novo* assembled by using a hybrid approach, which combined Illumina synthetic long-read technology and massive parallel sequencing of Fosmid mate-pair library. The "cn1" genome will serve as an important resource for comparative analysis in nematode species. Two lines of hybrid sterile males each carrying an independent introgression fragment from *C. briggsae* X chromosome in an otherwise *C. nigoni* background, demonstrate similar defects in spermatogenesis. A similar pattern of downregulated genes that are specific for spermatogenesis between the two hybrids and wild type control was observed. Importantly, the downregulated genes caused by the X chromosome introgressions are significantly enriched on autosomes, suggesting an epistatic interaction between the X chromosome and autosomes. By measuring small RNAs, the results shows that a subset of 22G RNAs specifically targeting the downregulated spermatogenesis genes are significantly upregulated in hybrids, indicating that perturbation of small RNA-mediated regulation may contribute to the X-autosome interaction. Taken together, this thesis provides a comprehensive study of identification of loci/genes responsible for hybrid incompatibilities between *C. briggsae* and *C. nigoni* and comparative genomics analysis between the two species.

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