

Review of: "The Assembly of the Y Chromosome Reveals Amplification of Genes Regulating Male Fertility in *Bactrocera dorsalis*"

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Review of the Manuscript: "The Assembly of the Y Chromosome Reveals Amplification of Genes Regulating Male Fertility in *Bactrocera dorsalis*"

The manuscript by Wu et al. represents a significant contribution to our understanding of the genetic basis of male fertility in *Bactrocera dorsalis*. This study is particularly noteworthy as it delivers the first successful assembly of the Y chromosome for this species, applying a robust and integrative methodological framework that includes PacBio HiFi sequencing, Hi-C analysis, and male/female resequencing data. This high-quality assembly facilitated the identification of essential genes, notably the multi-copy male-determining gene MoY and the amplified gyfY gene, both of which play crucial roles in male fertility.

Strengths

1. **Comprehensive Methodology:** The authors employ advanced sequencing technologies, including ddPCR and RNAi validation, which lend substantial support to their findings and underscore the assembly's quality and reliability.
2. **Substantive Findings:** The amplification of MoY and gyfY genes is a critical discovery, providing novel insights into the genetic mechanisms underlying male fertility in *B. dorsalis*. The study's findings hold substantial promise for genetic pest control strategies, including potential CRISPR/Cas9-based applications targeting the Y chromosome.
3. **Experimental Validation:** The RNAi experiments focused on gyfY provide robust functional evidence of this gene's role in male fertility, underscoring the practical relevance of the genetic insights presented.

Areas for Improvement

1. **Y Chromosome Assembly Clarity:** The authors report a Y chromosome size of 7.6 Mb, which requires further discussion regarding its expected size. In *Drosophila melanogaster*, for example, the most recent release of the reference genome (Release 6) includes only 3.7 Mb of the ~40 Mb Y chromosome, with approximately 10 Mb recently added by Chang & Larracuenta (2019). Clarifying whether this 7.6 Mb represents a near-complete or a small part of the Y chromosome in *B. dorsalis* would add valuable context to the findings.
2. **Expanded Gene Analysis:** While the study successfully identifies key genes, additional functional analysis of non-

gyfY and MoY genes on the Y chromosome would enhance the manuscript by providing a more comprehensive understanding of their roles in fertility and potential utility in pest control strategies.

3. **Language and Clarity:** Minor language revisions are recommended to enhance readability and ensure clarity for an international audience. As an example, all figure descriptions are repeated in the text main body.

Overall Evaluation

This manuscript delivers a well-executed investigation into the Y chromosome of *Bactrocera dorsalis*, with implications that extend into genetics, pest management, and evolutionary biology. The study's methodological rigor, compelling experimental evidence, and potential applications emphasize the significance of this work. Although minor enhancements could strengthen the manuscript, its scientific rigor and the novelty of its contributions justify its acceptance.