Identification and Characterization of X-linked Hybrid Male Sterility Factors between *Drosophila simulans* and *D. mauritiana*

Rodolfo A. Villegas, Neal Weldon, Gina Mavhezha and Colin D. Meiklejohn

School of Biological Sciences, University of Nebraska-Lincoln

INTRODUCTION

New species are formed through the evolution of reproductive barriers that decrease gene flow between populations. One of these barriers, intrinsic postzygotic isolation, results in sterile or inviable hybrid offspring due to genetic incompatibilities (1).

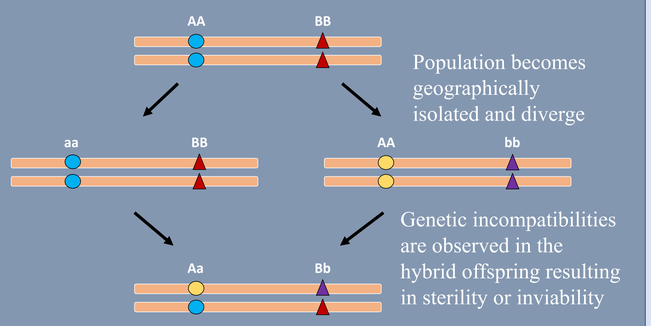

Figure 1: Dobzhansky-Muller Genetic Incompatibilities Model

Genetic incompatibilities preferentially accumulate on sex chromosomes; as a consequence, when hybridization results in the sterility or inviability of one sex, it is the heterogametic sex (Haldane's Rule) (2).

My research aims to identify and describe X-linked factors that drive hybrid male sterility (HMS) in Drosophila to begin to resolve the genetic basis of Haldane's Rule.

BACKGROUND

My research is focused on identifying hybrid male sterility factors within a 4Mb region named 2P6. this region harbors a previously identified HMS factor named *Odysseus* (*OdsH*) (3).

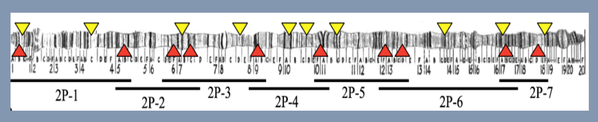

Figure 2: Polytene map of *Drosophila simulans* X chromosome. Each 2P-region denotes introgressed regions of *D. mauritiana* which cause sterility in an otherwise *D. simulans* genetic background.

OdsH is a heterochromatin-binding protein, but the mechanism of HMS is unknown. Protein localization experiments have implicated an interaction with the *D. simulans* Y chromosome as the mechanism of sterility (4).

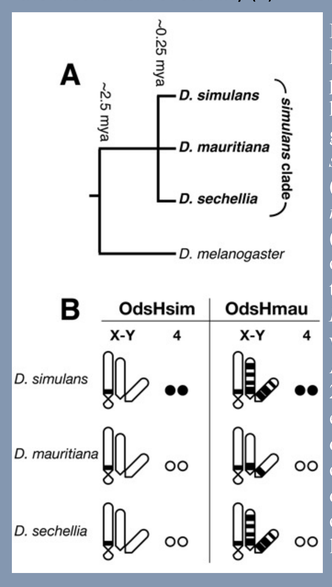

Figure 3: From Bayes and Malik 2009 (4), a summary of protein localization studies highlighting the differences in genomic localization of the *D. simulans* allele of *OdsH* (OdsHsim) and the *D. mauritiana* allele of *OdsH* (OdsHmau) to the Y and 4th chromosomes. A key result is that OdsHmau localizes to the *D. simulans* Y chromosome while OdsHsim does not. Additionally, Bayes and Malik 2009 demonstrate localization of either OdsH protein results in decondensation of that chromosome suggesting the decondensation of the Y chromosome by OdsHmau may lead to the HMS phenotype.

METHODS

- The high resolution genetic map of HMS in 2P6 was produced using visible markers to complete the crossing scheme below:

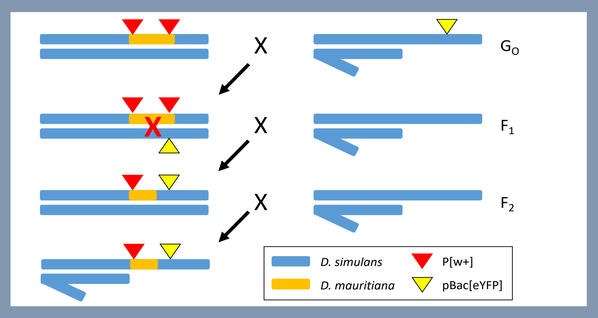

Figure 4: Crossing scheme for results of Figure 5A & 5B

- All fertility tests are conducted in replicates of at least ten using a single focal male and three *D. simulans* (wXD1) virgin females.

- Spermatid nuclei were visualized in testes dissections using a *ProtamineB-GFP* transgene. All testes were dissected from ~5 day old males after being placed in a fertility test for at least 3 days.

EXPERIMENT 1: RECOMBINATION-BASED MAPPING OF HMS FACTORS

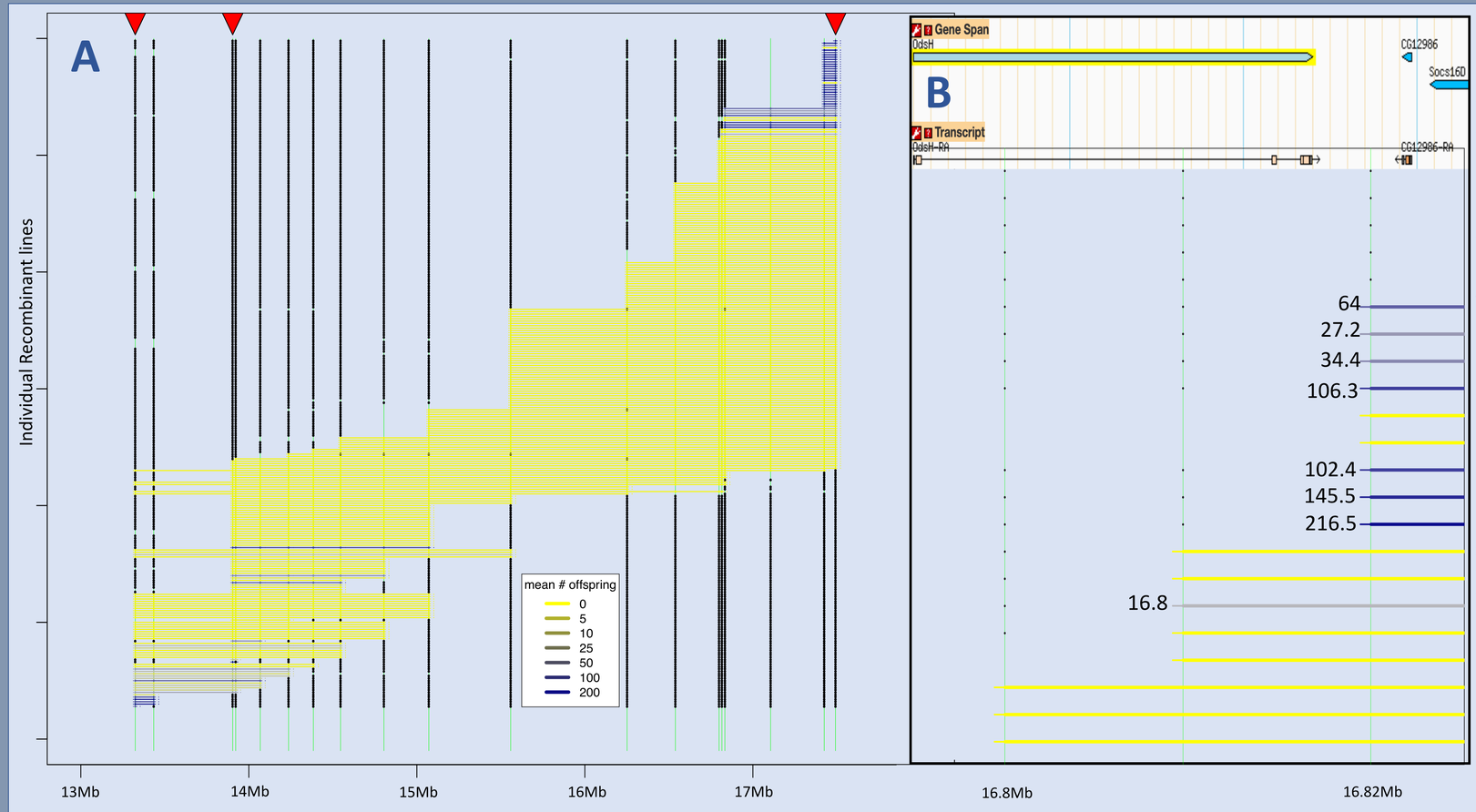

Figure 5: High resolution genetic maps of 2P6
Colored horizontal bars denote the extent of the introgressed *D. mauritiana* genetic material. Vertical lines indicate genetic markers at which each line was genotyped. Red triangles denote P[w+] elements. A black dot at genetic marker denotes the chromosome is *D. simulans* at the marker. The legend denotes the mean fertility of 10 replicate males carrying each chromosome.
A) Genetic map demonstrating all recombinant chromosomes allowing for a single recombination break point (Figure 4).
(B) Zoomed view of 4A with a focus on chromosomes with recombination breakpoints within or near *OdsH*. Above the chromosome map is the *OdsH* gene model from Flybase. The numbers next to each fertile chromosome represents the mean number of offspring produced.

EXPERIMENT 2: *D. MAURITIANA* Y CHROMOSOME RESCUES FERTILITY OF *ODSH*-MEDIATED STERILITY

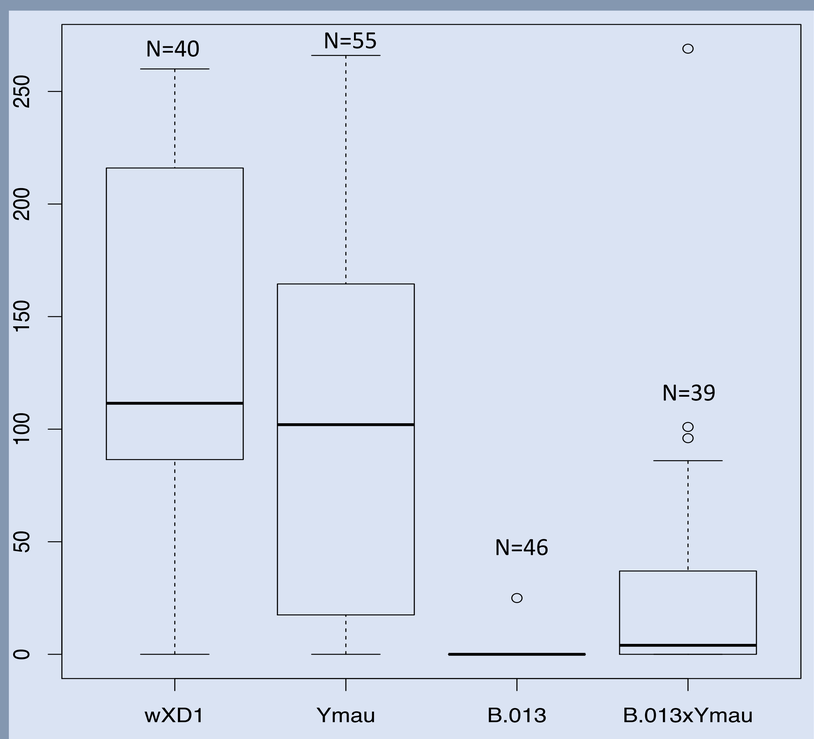

Figure 6: The *D. mauritiana* Y chromosome was introgressed into a short *OdsH*-containing sterile recombinant (B-013) and fertility was assayed. Our *D. simulans* control is labeled wXD1 while Ymau is the *D. mauritiana* Y chromosome in an otherwise *D. simulans* background. The introduction of the *D. mauritiana* Y chromosome rescues fertility of B-013 males, but does not reach levels of fertility of either of our controls.

CONCLUSIONS

- **We demonstrate evidence of at least 4 hybrid male sterility regions within this 4Mb region, including a previously identified HMS factor, *OdsH (3)*.**
- **The *D. mauritiana* Y chromosome rescues fertility of an *OdsH*-containing recombinant chromosome reinforcing the hypothesis that *OdsH* is interacting with a *D. simulans* Y-linked loci to cause sterility.**
- **As the amount of *D. mauritiana* introgression increases the developmental defects occur earlier in spermatogenesis with B-013 (*OdsH*-containing sterile recombinant) producing individual mature sperm while 2P6b fails to individualize and even reshape spermatid nuclei.**

EXPERIMENT 3: EARLIER DEVELOPMENTAL DEFECTS WITH INCREASING AMOUNTS OF D. MAURITIANA INTROGRESSIONS

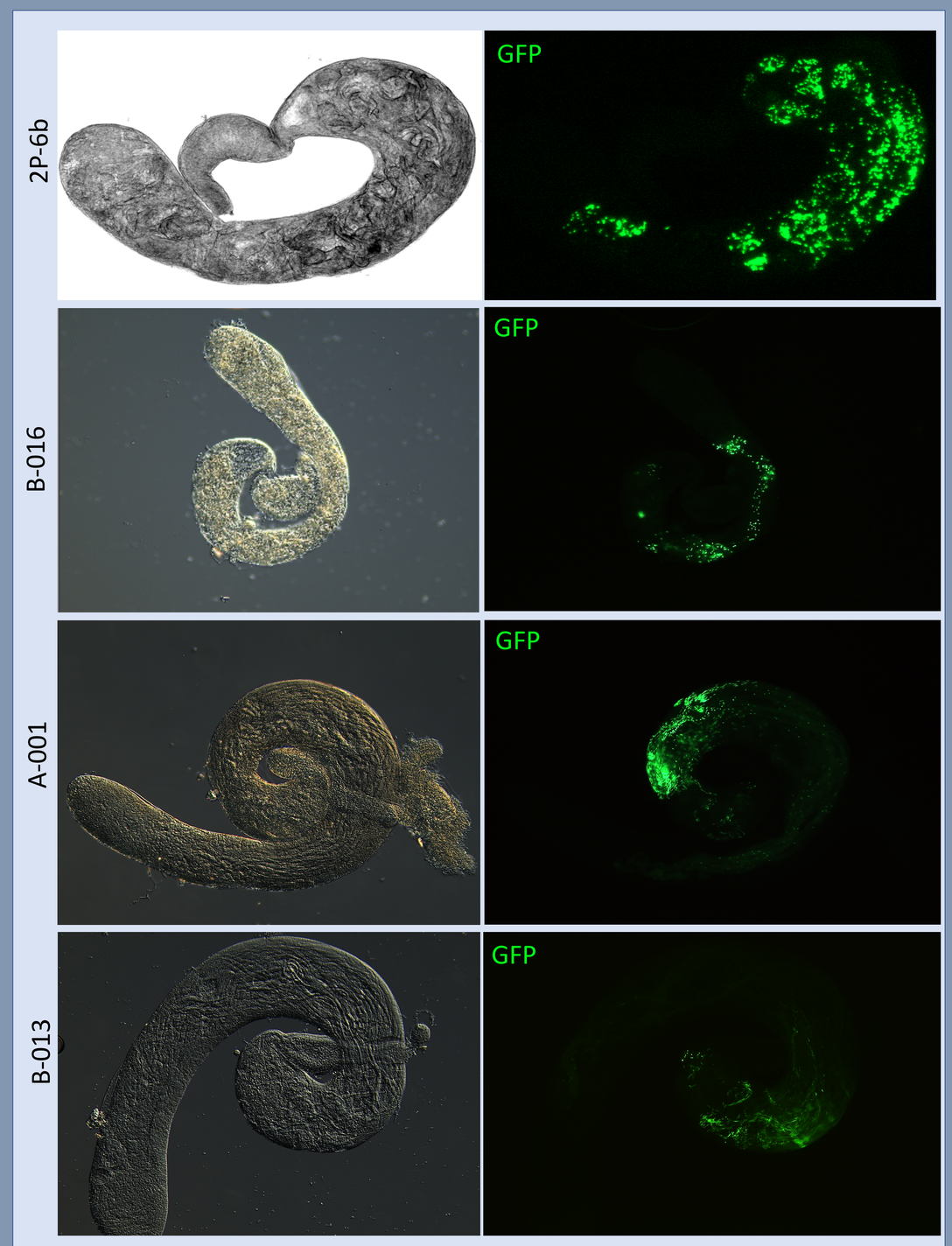

Figure 7: In 2P6b testes, sperm bundles are disorganized, and sperm bundles fail to remodel; instead round ProtB-GFP-positive nuclei appear to remain clustered in cysts throughout much of the testis(5). B-016 appears to have a very similar phenotype while A-001 appears to undergo some reshaping of spermatid nuclei but failure to individualize. B-013 testes appear to make mature and elongated sperm, but are found throughout the testis instead of sequestered in the seminal vesicles.

Funding was provided by the University of Nebraska-Lincoln, specifically through the Blair Baxton Udale Fund and the GAANN Fellowship. Funding also provided by the NIH: R01GM123194.

[1] Coyne and Orr. 2004 Speciation [2] Coyne 1992 *Nature* 355: 511-15 [3] Perez et al. 1993 *Genetics* 261-75 [4] Bayes and Malik 2009 Science 1538-41 [5]Meiklejohn et al. 2019 eLife 2018;7:e35468

NATIONAL INSTITUTES OF HEALTH NSF UNVEIL