Wolbachia and Bag of Marbles (*bam*) Interaction in *Drosophila melanogaster*

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Introduction

- **Bag of marbles (*bam*)** regulates Germline Stem Cell (GSC) renewal and differentiation and is known to be under positive selection.
- ***Wolbachia*** *is* a maternally inherited endosymbiont known to **rescue the *bam* mutant phenotype in *D. melanogaster.***
- *Wolbachia pipientis* strain can be divided into two general groups: Wmel-like Wolbachia (characterized by a low titer) and WmelCS-like Wolbachia (characterized by a high titer).

Questions

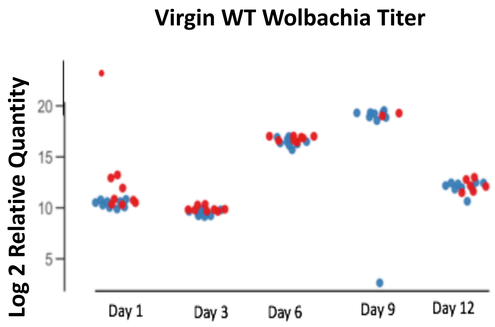
- Does variation in Wolbachia strain type and titer differentially rescue the *bam* hypomorph fertility over time?
- Does *bam* genotype influence *Wolbachia* titer over time?

Methods

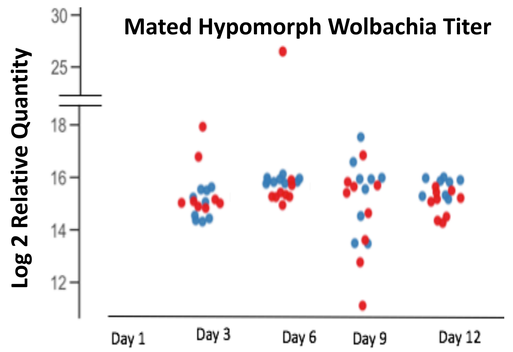
1. Males and females were both of the genotype bam hypomorph/*bam* null.
2. Same age female and male *bam* hypomorphs of 5 different ages (Day 0, 2, 5, 8 and 11) were mated overnight and females are collected the next day.
3. After mating overnight, females were collected, and their ovaries are dissected.
4. Egg producing versus sterile ovaries were counted as a measure of the rescue of the *bam* phenotype.
5. DNA extractions were made of both ovaries and carcass.
6. Wolbachia titer was quantified using qPCR and relative quantification.

Results

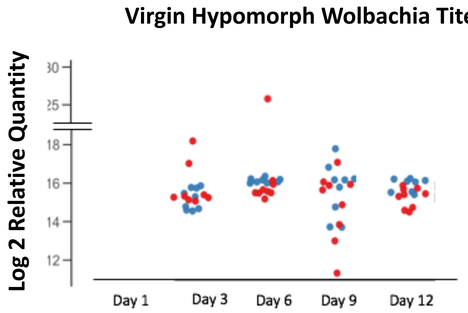
***Wolbachia strain*:** Wmel WmelCS2B



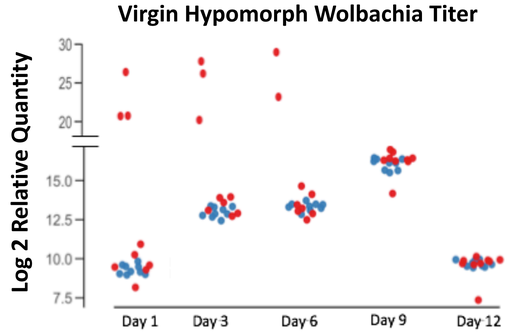
Relative Wolbachia titer in the ovaries of virgin WT flies containing Wmel and WmelCS2B Wolbachia



Relative Wolbachia titer in the ovaries of virgn hypomorph flies containing Wmel and WmelCS2B Wolbachia



Relative Wolbachia titer in the ovaries of virgin hypomorphs containing Wmel and WmelCS2B Wolbachia



Relative Wolbachia titer in the ovaries of mated hypomorph flies containing Wmel and WmelCS2B Wolbachia

Conclusions

- Virgin *bam* hypomorphs have a constant Wolbachia titer over time compared to virgin WT flies
  - **Tight regulation of *Wolbachia* titer in *bam* hypomorphs could be occuring in order to rescue the *bam* mutant phenotype**
- Mated *bam* hypomorphs have an increase in Wolbachia titer over time followed by a decrease in titer at day 12 compared to Virgin hypomorphs
  - ***Wolbachia*** **titer could be increasing over time in mated hypomorphs to ensure that a high titer (or any amount of *Wolbachia*) is passed onto progeny**
  - However, the drop in titer at day 12 could be due sperm reduction in aged males over Time as the initial experiments were done.

Future work

- Revise experiments to use 3-5 day old WT males with aged female hypomorphs to eliminate variation in males.
- Use absolute quantifcation alongside relative quantification to account for any differences in tissue size between wild-type and hypomorph flies
- Repeat experiments with additional *Wolbachia* variants to determine whether the trends remain the same
- RNA-seq at specific time points to determine how *Wolbachia* is impacting *bam* and its interactors

Questions or suggestions?

Contact Catherine Kagemann at chk63@cornell.edu

References:

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Flores, H. et al. (2015). The Drosophila bag of marbles Gene Interacts Genetically with Wolbachia and Shows Female-Specific Effects of Divergence. PLoS Genetics, 11(8).

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