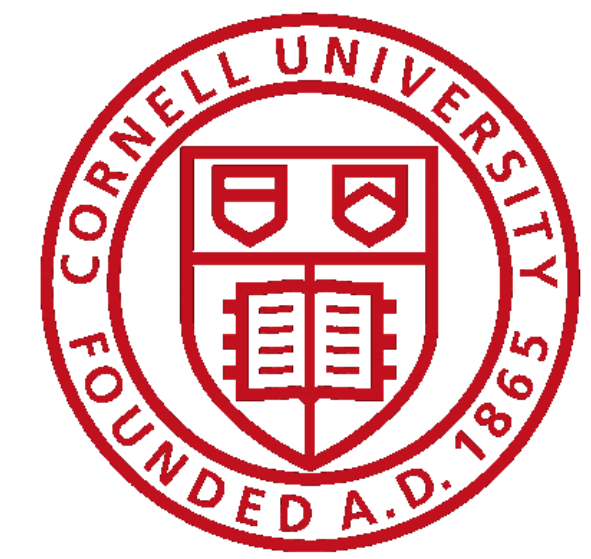# The Genetic and Historical Relationship between Germline Stem Cell Gene *bag of marbles* and the Endosymbiont Bacteria *Wolbachia*

Contact:
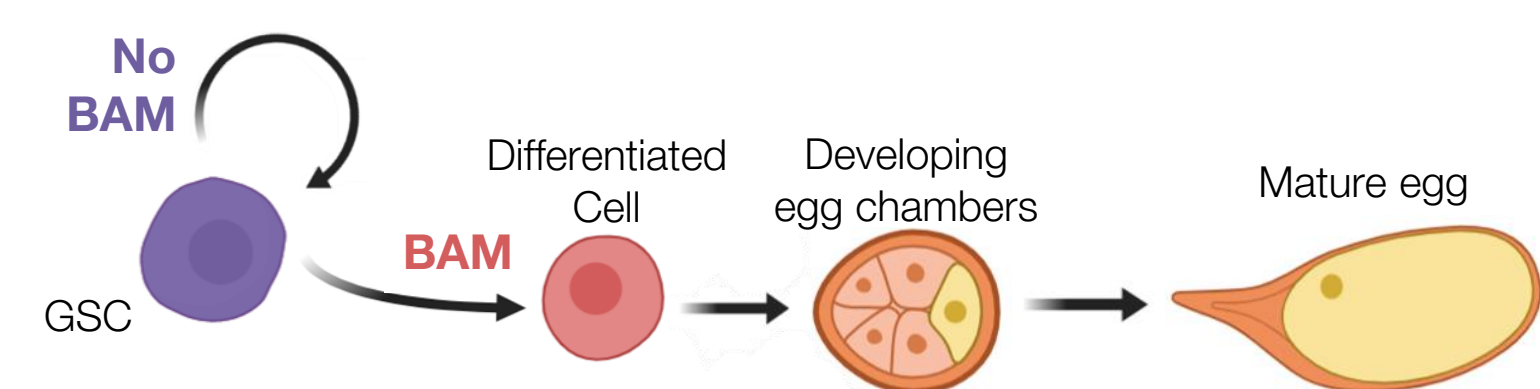**mjw349@cornell.edu**
Would love to discuss!

Miwa Wenzel and Charles F. Aquadro
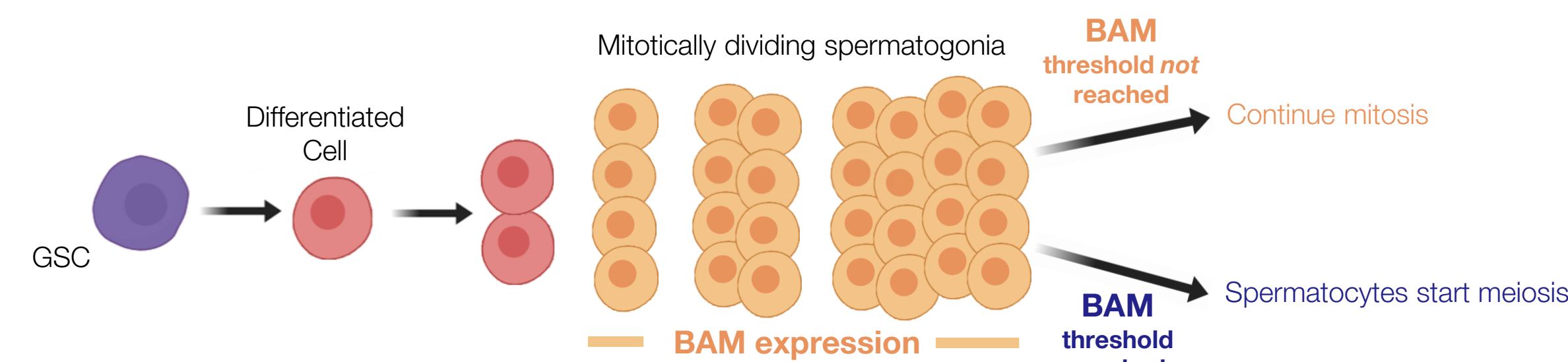**Department of Molecular Biology and Genetics, Cornell University**

Background

The **bag of marbles (bam)** gene plays a key role in *Drosophila* gametogenesis.

♀ In females, bam acts as a switch in germline stem cell (GSC) differentiation.[1]

♂ In males, the accumulation of bam acts as a switch to meiotic programming.[17]

- *bam*, along with many other GSC genes, is **rapidly evolving** in some species.[2-4]
- One potential driver of this adaptive evolution is the endosymbiont bacteria **Wolbachia** which inhabits the germline of many arthropods.[5]
- **Wolbachia** rescues female fertility of a *D. melanogaster* **bam** hypomorph.[5]
- Thus, **we are exploring this hypothesis in three ways**.

Wolbachia rescues fertility of bam hypomorph (Flores et al. 2015)
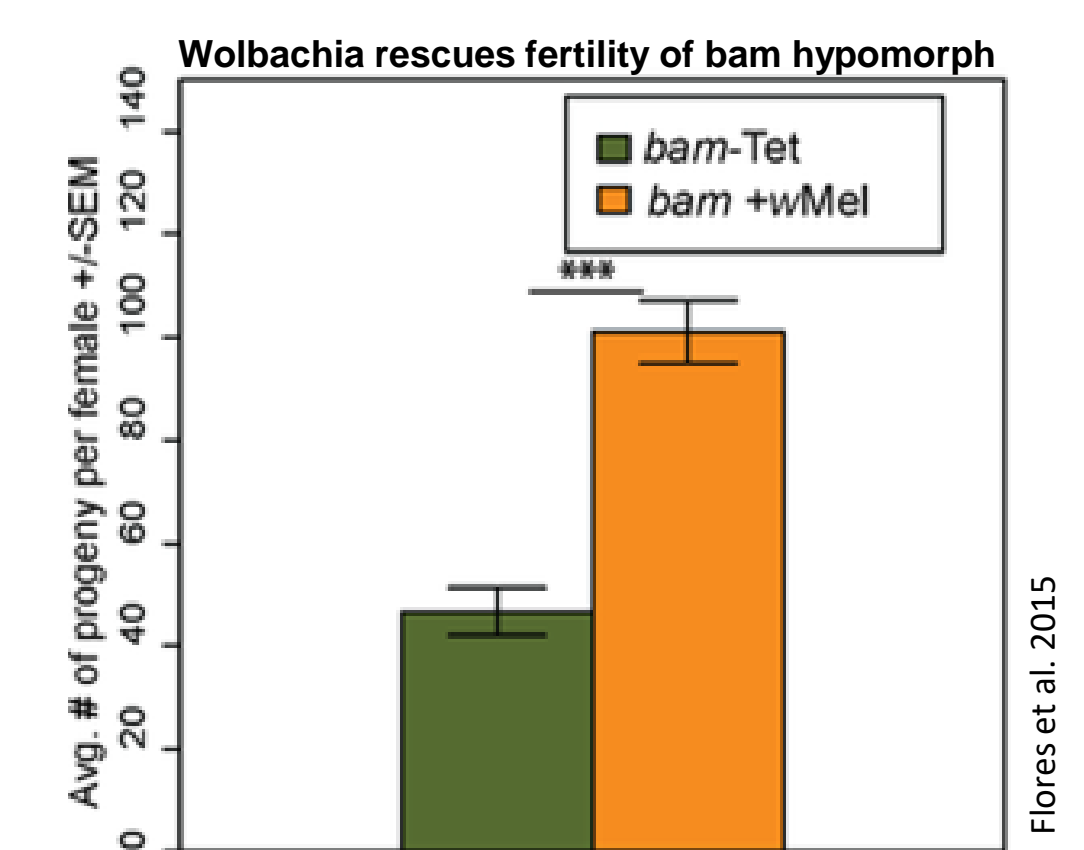
1. Determine if Wolbachia rescues bam directly

- There is **a single *bam* hypomorph** in females; there are none in males
- We are using **PhiC-31 transgenesis** to create novel hypomorphs in different functional regions of bam
- Differentiated rescue of hypomorphs will inform the scope of Wolbachia's genetic interaction with bam.
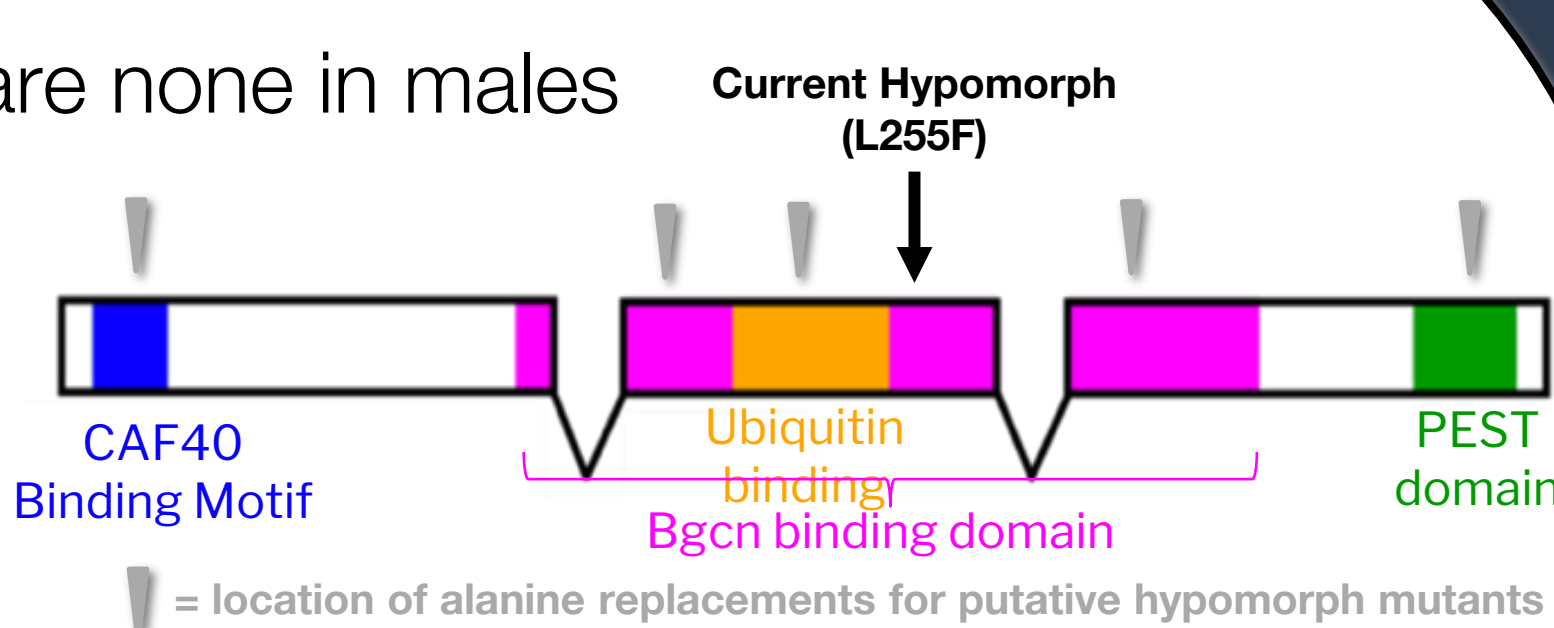

conclusions

- **Five putative hypomorphs are currently being generated and assayed.**
- **We observe the first case of GSC gene rescue by Wolbachia in male Drosophila.**
- **Sustained bam expression in hypomorphs suggests cells are halted in their cell fate.**

methods

- **Control lines were generated** to confirm that the transgenic nature of the allele and a fluorescent Venus tag have a minimal effect on the phenotype.
- Putative hypomorphs mutants are being generated via an **alanine scanning** approach.
- Fertility and cytological defects and Wolbachia (wMel) rescue were assessed in control mutants.
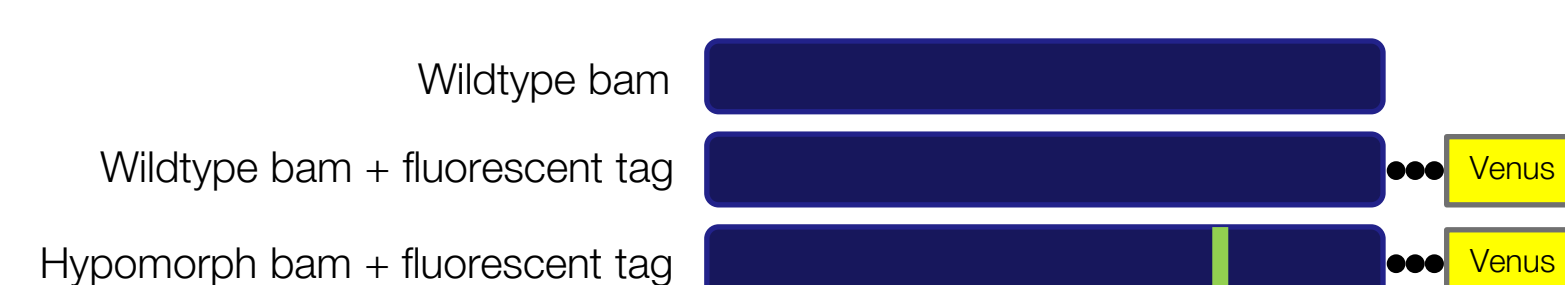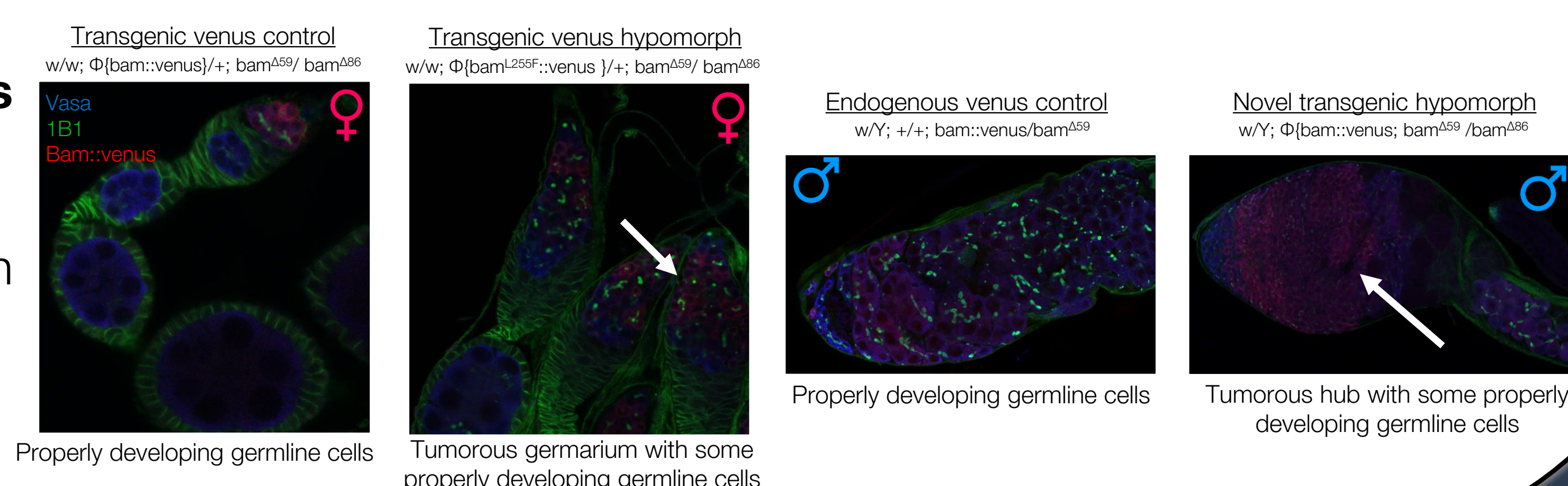

results

- **Female data is consistent** with the endogenous phenotype of wildtype and hypomorph lines.

- **Males** show a **novel hypomorph** phenotype and interaction with wMel
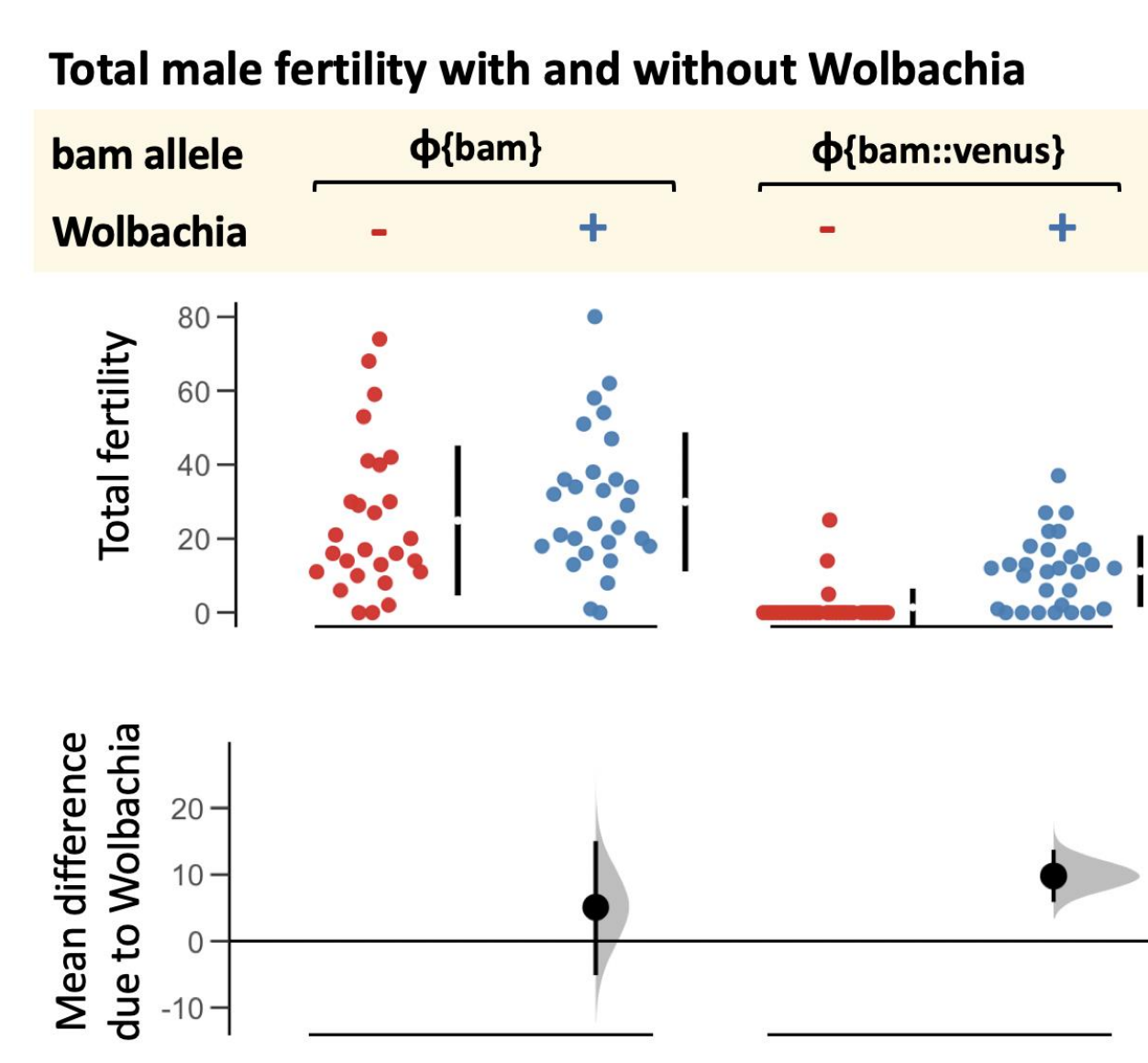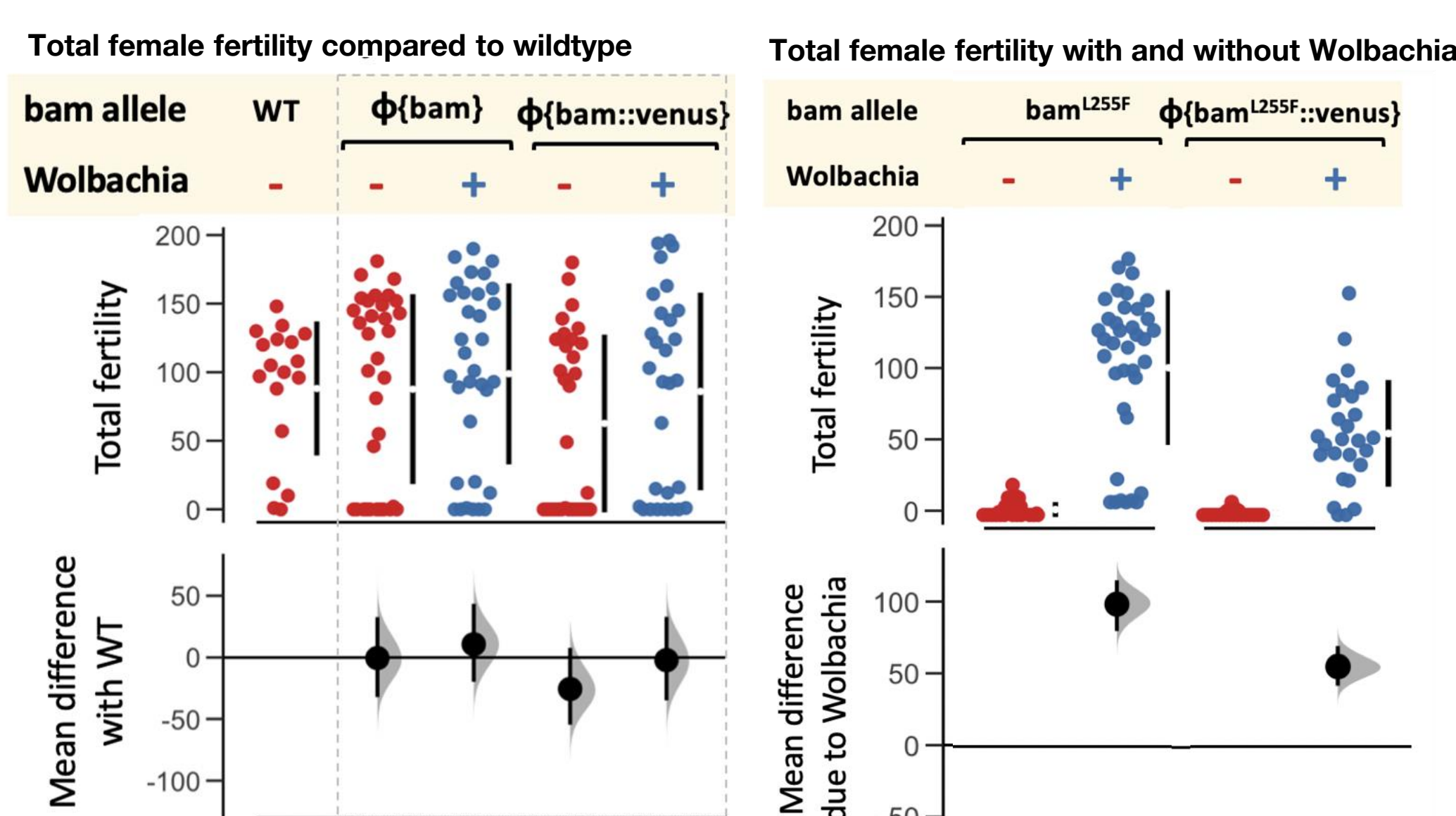
- **Bam expression is sustained** in transgenic hypomorphs of both males and females

## 2. Test for evidence of historical Wolbachia infections

- Current Wolbachia infections are not congruent with selection at *bam*.
- **Horizontal gene transfer** (HGT) by Wolbachia into its host reveals past infections.
- Evidence of past infections in different species will inform us of historical Wolbachia interactions that may have contributed to bam's rapid evolution.

conclusions

- **We have validated our method and are proceeding to evaluate sequences of different Drosophila species for Wolbachia HGTs**
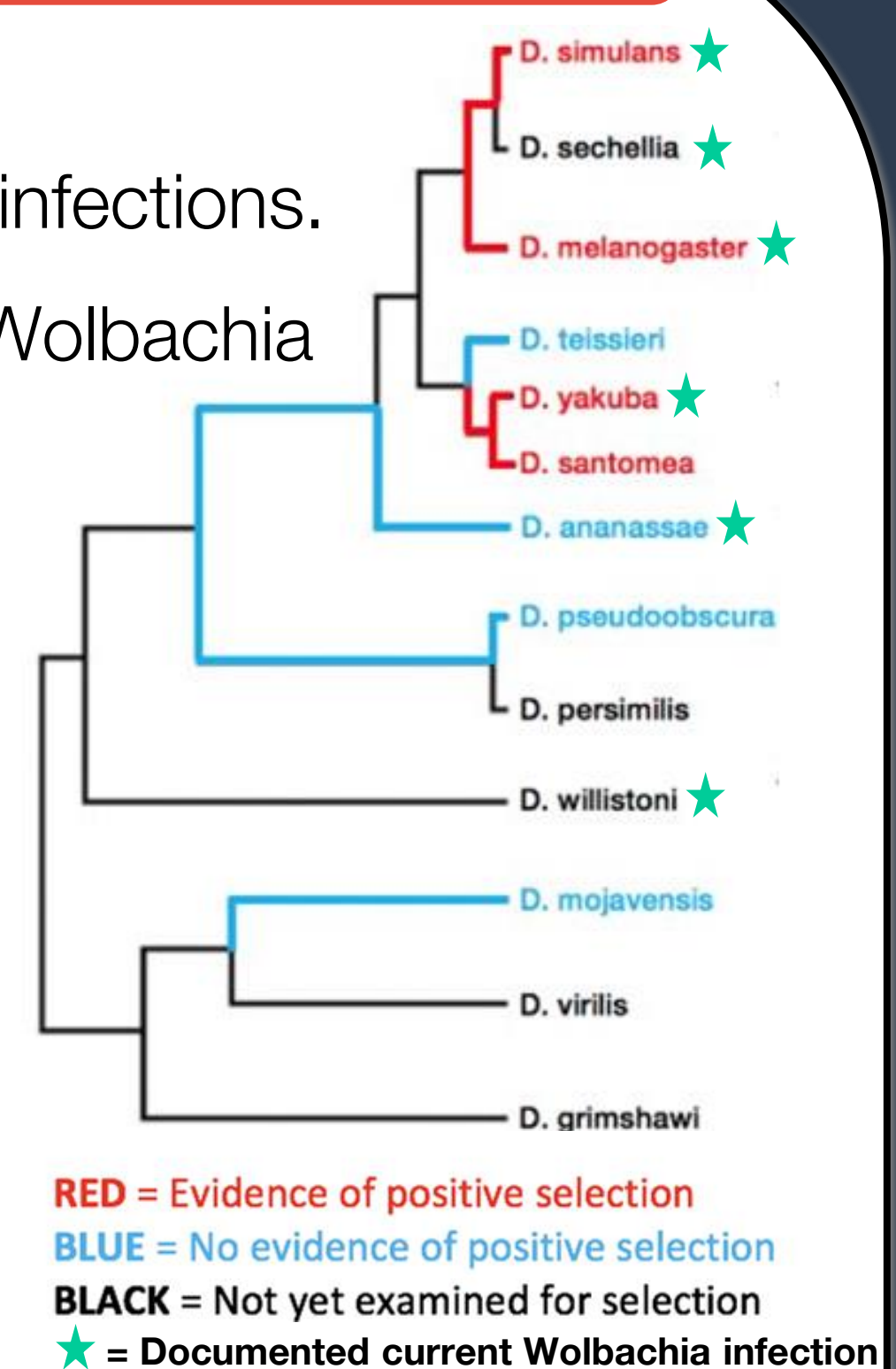
methods

- We developed a pipeline to **identify Wolbachia HGTs**.
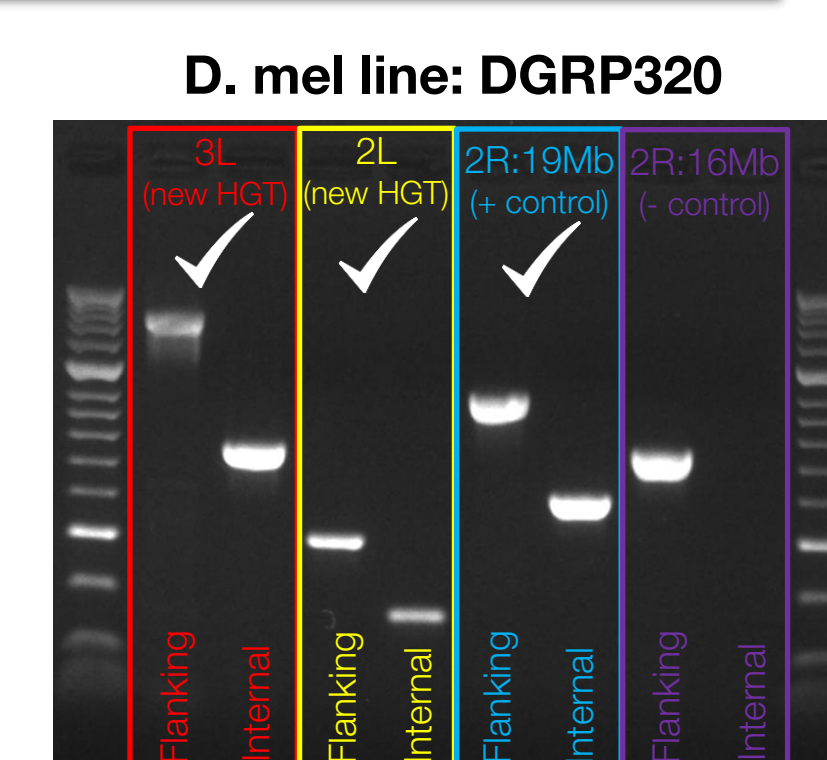
- We tested this pipeline on previously documented HGTs
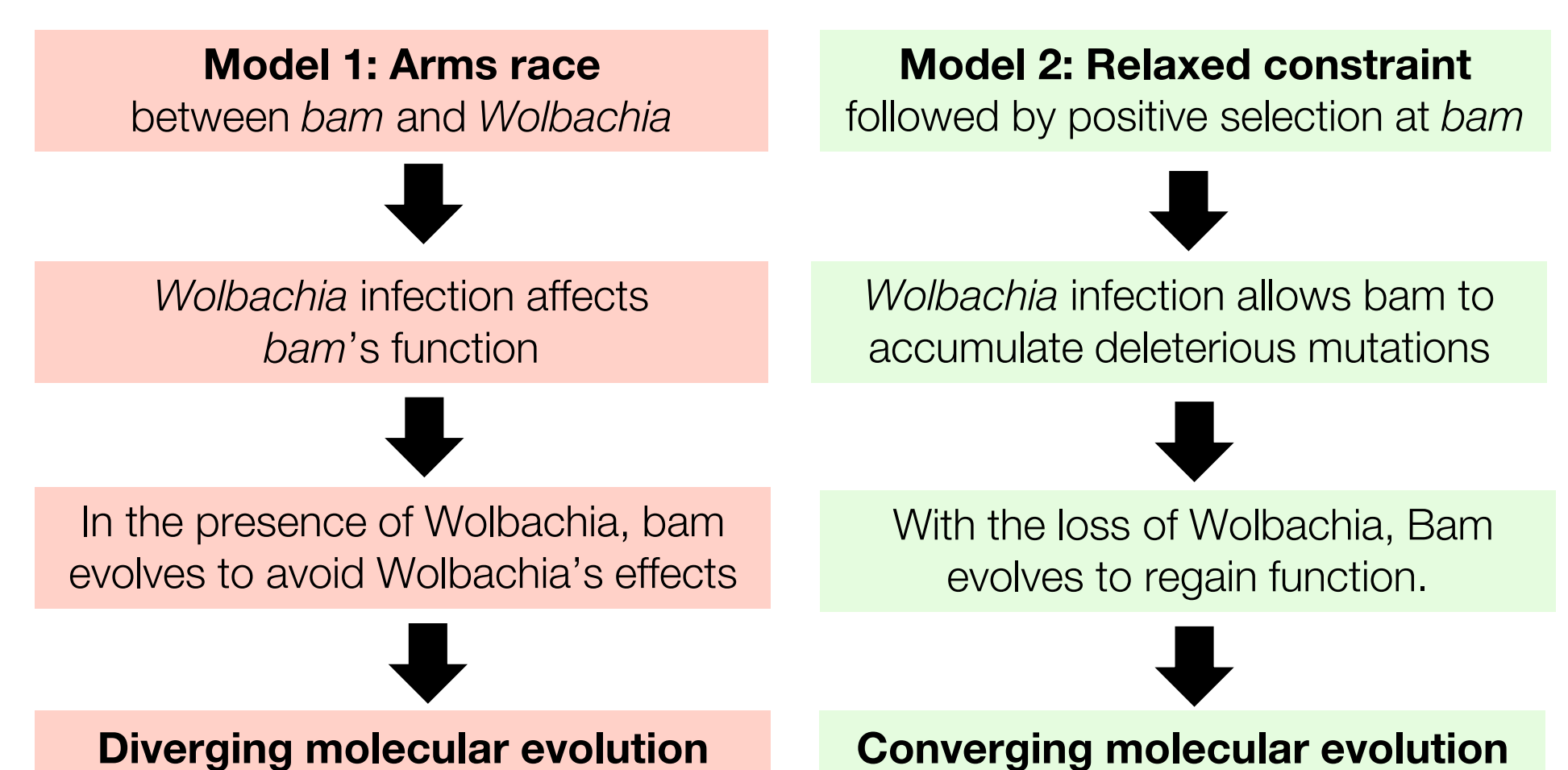- We will run the pipeline on many sequences of various species.

results

- Our method found two previously documented HGTs in D. mel
- We have **found new HGTs** in D. mel and confirmed with PCR

RED = Evidence of positive selection
BLUE = No evidence of positive selection
BLACK = Not yet examined for selection
★ = Documented current Wolbachia infection

3. Test predictions of bam sequence evolution

- *Wolbachia*'s potential as a driver of *bam*'s evolution can be **modeled in two ways**, which may be supported by the different types of substitutions at *bam*.

Model 1: Arms race between *bam* and *Wolbachia*
- *Wolbachia* infection affects *bam*'s function
- In the presence of Wolbachia, bam evolves to avoid Wolbachia's effects
- **Diverging molecular evolution**

Model 2: Relaxed constraint followed by positive selection at *bam*
- *Wolbachia* infection allows bam to accumulate deleterious mutations
- With the loss of Wolbachia, Bam evolves to regain function.
- **Converging molecular evolution**

conclusions

- **There is preliminary support for Model 2 based on the sliding window analysis.**
- **We are simulating these two models with SLiM with the Messer lab (Cornell).**

methods

- We compared a **sliding window McDonald-Kreitman (MK) test** with an assessment of **physiochemical differences** in amino acid divergences between *D. mel* and *D. sim*
- We are **creating simulations** to determine sequence evolution patterns of the two models

results

- Regions of **high alpha values** from the MK test often coincide with **low physiochemical differences** in amino acid divergences.

Miyata scores & MK test
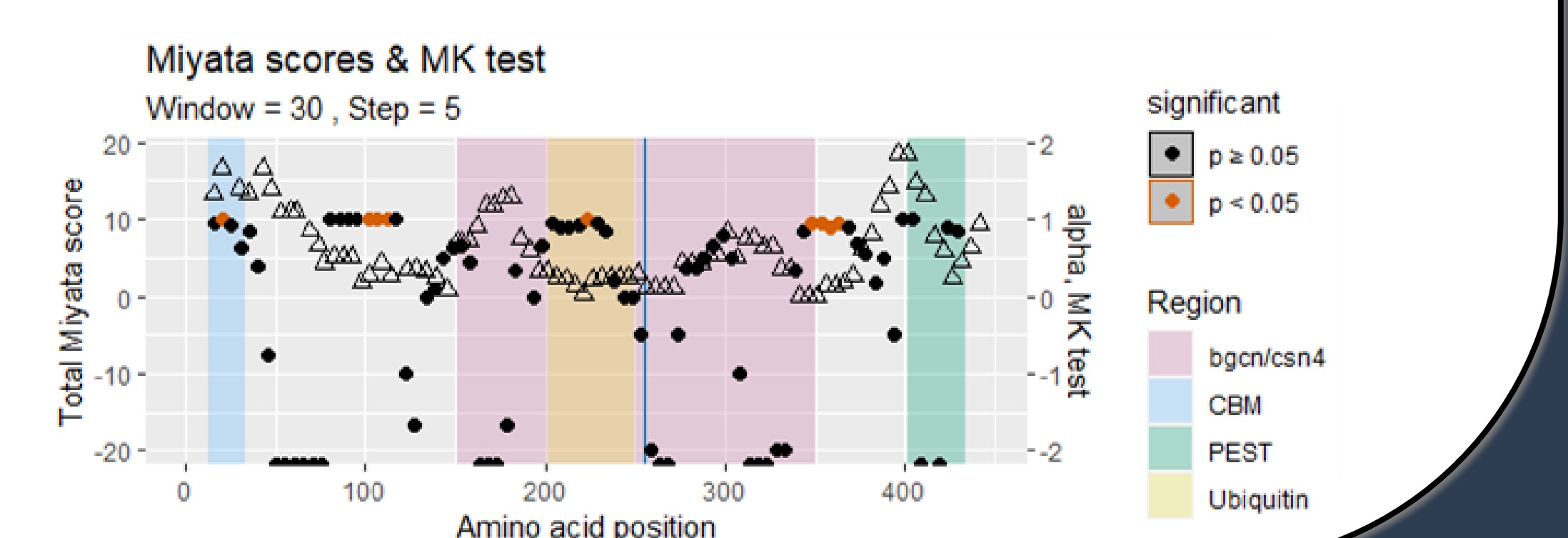
References

1, McKearin, D. and B. Ohlstein, Development, 1995. 121(9): 2937-29471. **2.** Civetta A., et al., Mol Biol Evol, 2006. 23(3): 655-662. **3.** Bauer DuMont, V.L., et al., Mol Biol Evol, 2007. 24(1): 182-192. **4.** Flores, H.A., et al., G3 (Bethesda), 2015. 5(4): 583-592. 5. Flores, H.A., et al., PLoS Genet, 2015. 11(8): e1005453. **6.** Mateos, M., et al., Genetics, 2006. 174(1): 363-376. **7.** Cooper, B.S., et al., Genetics, 2017. 205(1): 333-351. **8.** Muller, M.J., et al., Infect Genet Evol, 2013. 19: 232-239. **9.** Lack, J.B., et al., Genetics, 2015. 199(4): 1229-1241. **10.** Yachdav, G., et al., Nucleic Acids Res, 2014. 42(Web Server issue): W337-43. **11.** Choi, Y. and A.P. Chan, Bioinformatics, 2015. 31(16): 2745-2747. **12.** Hotopp, J.C.D., et al., Science, 2007. 317(5845): 1754-1756. **13.** Huang, W., et al. Genome Res, 2014. 24(7): 11930-1208. **14.** Salzberg, S.L., et al. Genome Biol, 2005. 6(3): R23. **15.** Choi, J.Y. and Aquadro, C.F. Mol Biol Evol, 2014. 31(9): 2457-2471. **16.** Grantham, R. Science, 1974. 185(4154): 862-4. **17.** Miyata, T, et al. J Mol Evol, 1979. 12(3):219-36. **17.** Insco et al. Proc. Natl. Acad. Sci. USA. 2009. 106(52): 22311-22316.
This research is funded by NIH grant R01 GM095793