Detection of *Wolbachia pipientis* infection in tetracycline-treated *Drosophila*

Cynthia K.S. Ulbing, Miwa J. Wenzel, Paula Fernandez Begne, Jaclyn Bubnell, and Charles F. Aquadro
Cornell University Department of Molecular Biology and Genetics

Cornell University

We find evidence of the **endosymbiotic bacteria *Wolbachia*** in *Drosophila* fly lines that have received antibiotic treatment. Multiple tests for the bacterium indicate a **persistent low-titer infection.** We believe this occurrence may be common in *Drosophila-Wolbachia* research labs and therefore seek to document our experience and suggest reliable methods for detecting these infections. We advise against the use of endpoint PCR for detecting *Wolbachia* at very low titers.

- We **treated an isogenic fly line with tetracycline** at a standard[1,2] dose of 0.03 mg/mL food media for 3 generations followed by 10 generations of recovery
- We screened the line using endpoint PCR at multiple timepoints post-treatment and obtained **recurrent evidence of *Wolbachia* infection**
- Our follow-up qPCR analyses confirmed the presence of *Wolbachia* DNA

- We confirmed these results were **not a consequence of amplicon contamination** by exercising clean PCR techniques, running no template controls with our samples, and collecting cytological images of the *Drosophila* ovaries, where *Wolbachia* localizes[3]

- We attempted to increase *Wolbachia* titer to perform whole-genome sequencing of the bacterium from our samples, but were unsuccessful at increasing titer in our lines using techniques found reported in literature[4,5]
- We have begun to **characterize the persistent *Wolbachia strain*** using touchdown PCR and sequencing of five multilocus sequence typing loci[6] and preliminary results indicate the **wMel** strain

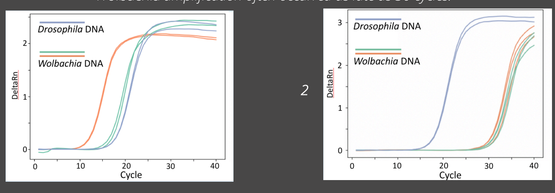
We show that tetracycline-treatment has been inefficient in clearing these flies of *Wolbachia* infection. We believe this result is not unique to our fly lines, as tetracycline resistance has been documented in bacteria[7] and incomplete removal of the bacteria has been documented in other arthropods[8]. We believe the incomplete removal of *Wolbachia* is an important consideration when designing and interpreting research on *Wolbachia-Drosophila* interactions.

Methods for detecting low-titer *Wolbachia* infections

-PCR methods-

We found **qPCR** to be among the most reliable methods for diagnosing a low-titer *Wolbachia* infection in our flies. Frequently, qPCR showed amplification of *Wolbachia* loci from the same genetic samples which endpoint PCR did not. *Wolbachia* loci amplified relatively late in low-titer samples, compared to positive controls (below).

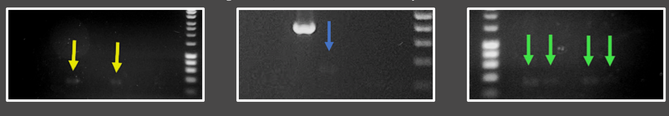
Amplification plots of 1) a Wolbachia *positive control and 2) a low-titer sample from our tetracycline-treated isogenic strain. Amplification of* Wolbachia *loci in high-titer strains occurs as early as 10 cycles whereas in low-titer strains* Wolbachia *amplification often occurred as late as 30 cycles.*



We determined **touchdown PCR** to be reliable at amplifying *Wolbachia* in low abundances, however we only tested this method on a small subset of samples.

Endpoint PCR was the least reliable method for detecting *Wolbachia* in low-titer samples. Although this method is effective when *Wolbachia* titer is high, our evidence suggests this method produces only lowly detectible positives and can produce false negatives when titer is low.

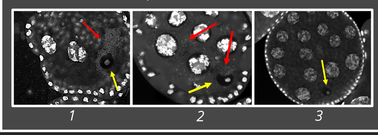
Although endpoint PCR was largely unreliable, efficiency was best when targeting the repeat "ARM" region of the Wolbachia *genome, consistent with results from Schneider et al.*[9]



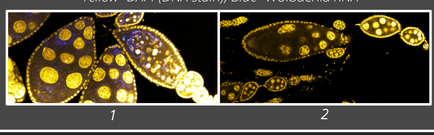
-Cytological imaging-

We used imaging to confirm the presence of *Wolbachia* in the ovarian cells of tetracycline-treated *Drosophila*. We used **Syto11 nucleic acid staining**[10] to visualize bacteria in the egg chambers. We identified a small fraction of infected cells in tetracycline-treated flies. To more precisely visualize *Wolbachia* localization in the low-titer samples we began employing **RNA-FISH**, however it appears that titer is not high enough for detection with this method (below).

Syto11 staining of egg chambers in 1) Wolbachia *positive line, 2) tetracycline-treated line, 3) true* Wolbachia *negative line. Yellow arrow=pole cell, Red arrow=*Wolbachia



RNA-FISH of Drosophila *egg chambers in 1) a positive control infected with* Wolbachia *and 2) a tetracycline-treated line Yellow=DAPI (DNA stain), Blue=*Wolbachia *RNA*



We find that the most suitable method for detecting a low-titer *Wolbachia* infection is combining qPCR and cytological imaging.

Imaging methods are safe from the risk of amplicon contamination while qPCR can be more specific to *Wolbachia* and less costly if the infection occurs at a very low abundance. Either of these methods appears suitable, however combining both provides the highest confidence.

Questions?

While there is no virtual Q&A session scheduled for this poster, the authors would be happy to respond to questions and hear about shared experiences by other researchers.

Primary contact - Cynthia Ulbing - cku2@cornell.edu
Secondary contact - Miwa Wenzel - mjw349@cornell.edu

Many experiments for this project have been redesigned or delayed by the COVID-19 pandemic. Further analysis with larger sample sixes will resume when possible.

Literature cited: 1 Chrostek et al. 2013. *PLoS Genetics* 9(12), 2 Martinez et al. 2017. *Mol Ecol* 26(15):4072-84, 3 Ferree et al. 2005. *PLoS Pathog.* 1(2):0111-24, 4 Serbus et al. 2015. *PLoS Pathog.* 11(3):e1004777, 5 Grobler et al. 2018. *PLoS Pathog.* 14(11):e1007445, 6 Baldo et al. 2006. *Appl. Environ. Microbiol.* 72(11):7098-1100, 7 Speer et al. 1992. *Clin. Microbiol. Rev.* 5(4):387-99, 8 Wang et al. 2017. *Sci. Rep.* 7:44014, Schneider et al. 2014. *BMC Microbiol.* 14(1):121, 10 Casper-Lindley et al. 2011. *Appl. Environ. Microbiol.* 7(14):4788-4794