Dodson, Brittany

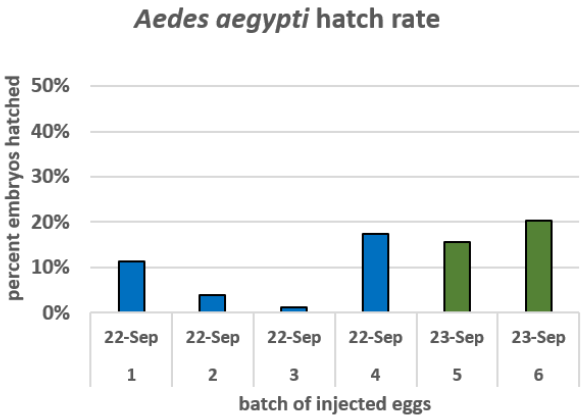
IGTRCN Peer-to-Peer Training Report

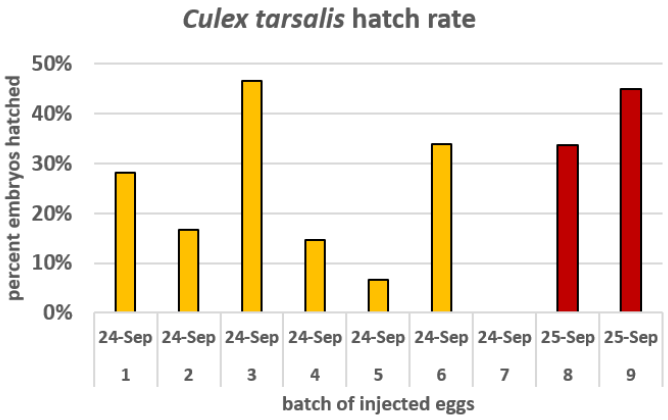
Objective: My PhD thesis involves studying interactions among native bacteria, introduced bacteria and viruses in mosquitoes. The main objective was to learn embryonic microinjection for transgenesis application and *Wolbachia* bacterium transfer.

Accomplishments: With funds from the IGTRCN Peer-to-Peer Training Fellowship, I spent five days at the Insect Transformation Facility (ITF) in Rockville, MD. During that time, I gained knowledge and practical experience in mosquito embryo microinjection needed to perform experiments independently at my institution. Briefly, these skills included:

1. Injection station setup
2. Needle design and fabrication
3. Injection mix preparation
4. Needle filler creation and needle filling
5. Timely embryo preparation:
    a. Collection
    b. Alignment on filter paper
    c. Mounting
6. Microinjection
    a. Proper injection location on embryo
    b. Proper age of embryo for maximum survival and transgenesis success
7. Post injection care of embryos
    a. Aedes: Incubated slide mounted embryos in moist petri dish in insectary for 2 days, then water added and embryos hatched
    b. Culex: Slides mounted with embryos were set in cup with diH20 with posterior pole exposed to air and anterior pole touching the water

Over the course of the week, embryo hatch rate improved and was comparable to rates of experienced staff at the ITF [see figures]. Hatch rate decreases can likely be explained by user fatigue halfway through or at the end of the day.





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Troubleshooting: Most of the week was spent working with *Aedes aegypti* mosquitoes, a system in which embryo collection, setup and injection procedures have been optimized. When I began working with my study species, *Culex tarsalis*, we came across several issues that will need attention:

1. Female mosquitoes laid eggs in a timely manner only once or twice in the morning. This is not amenable to injecting large numbers of embryos of the same cohort.
2. The outer layer of *Cx. tarsalis* embryos have a "leathery" texture, not seen with Aedes mosquitoes. Thus, the needle did not easily pierce the embryo. Troubleshooting during the week suggested that an exceptionally sharp or beveled needle helps alleviate the issue.

In progress:

1. Optimize timely *Cx. tarsalis* egg laying.
    a. May involve using ovipositor stimulant.
    b. May involve selecting for mosquitoes that will lay eggs in 25 minutes.
2. Practice and optimize injections with needles made from our needle puller.

Future experiment: *Wolbachia* is a proposed tool to control mosquito-borne diseases due to its ability to block particular pathogens when transferred to naive mosquito species [1–3]. Contrary to that research, our experiments showed that transfer of *Wolbachia* to *Cx. tarsalis* resulted in enhanced West Nile virus infection [4]. As part of my dissertation, I aim to understand the extent of *Wolbachia*-mediated enhancement. To date, our *Wolbachia* experiments have involved transient infections of the bacterium through adult injection. These infections are considered transient because *Wolbachia* is not stable in the mosquito population. Although natural and transient infections show similar *Wolbachia* tissue tropisms, it is unclear whether adult injection is an acceptable alternative to a natural infection. With new skills obtained with this fellowship, I will establish a laboratory colony of *Culex tarsalis* stably infected with *Wolbachia*. Utilizing the *Wolbachia* infected population, I will then conduct experiments to determine competence for West Nile virus and other viruses.

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References:

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3. Hughes GL, Koga R, Xue P, Fukatsu T, Rasgon JL (2011) *Wolbachia* infections are virulent and inhibit the human malaria parasite *Plasmodium falciparum* in *Anopheles gambiae*. PLoS Pathog 7: e1002043.
4. Dodson BL, Hughes GL, Paul O, Matacchiero AC, Kramer LD, et al. (2014) *Wolbachia* enhances West Nile virus (WNV) infection in the mosquito *Culex tarsalis*. PLoS Negl Trop Dis 8: e2965.