THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DEPARTMENT OF ENTOMOLOGY

# INVESTIGATING BACULOVIRUS AS A VIRAL VECTOR FOR DELIVERY OF CAS9 TO THE OVARIES OF THE DISEASE VECTOR *AEDES AEGYPTI* FOR USE IN MOSQUITO GENE EDITING TECHNOLOGIES

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## ABSTRACT

Genetic engineering is an emerging tool for reducing the transmission of pathogens such as Zika, dengue, and West Nile viruses from the insect vector *Aedes aegypti* to humans. Recent developments in specific gene modification techniques have led to more widely accessible methods of editing, but with similarly low efficiencies to traditional gene editing protocols. By using the baculovirus *Ac*MNPV as a vector for Cas9, I aim to increase the efficiency of targeted gene modification in *Ae. aegypti*. This manuscript details initial experiments to understand how a baculovirus vector expressing a fluorescent protein gene (mCherry) will enter the ovaries of a female adult mosquito. Based on imaging, it appears that the baculovirus delivers synthetic DNA encoding mCherry into the follicle of the oocyte. This opens possibilities for further experiments including using an ovary specific localization signal to direct Cas9 expressed from the *Ac*MNPV genome for targeted mutagenesis in mosquitoes.

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# Chapter 1

# Introduction

Mosquitoes are responsible for transmitting pathogens that cause disease worldwide. The vector *Ae. aegypti* is known to transmit viruses that infect humans, such as Zika, dengue, and West Nile virus (1). Pathogens such as these account for diseases that cause millions of human deaths per year, and vector control is a crucial part of limiting this number. One of the most recent techniques developed to limit the spread of mosquito-borne disease is genetic editing. By changing the genome of these deadly disease vectors, scientists are able to limit the transmission of a pathogen through a mosquito and into a human host.

## Genetic Engineering in Mosquitoes

CRISPR-Cas9 was discovered in 1987 in *Escherichia coli* (2). In 2012 it was leveraged for the editing of eukaryotic genomes, which has since revolutionized the world of genetic engineering (3). Cas9-mediated genetic engineering arrived first at the insect class via *Drosophila melanogaster* in 2013 (4). Although *Ae. aegypti* has not always been considered a model organism, many advancements in vector biological methods have been established in this organism and there is a vast amount of data concerning the insect. Because of this, and its impact on human health, it is now widely studied as a model for vectors of disease (5). Since the early 2010s, there have been hundreds of papers published concerning genetic editing in mosquitoes, especially on *Ae. aegypti* (6).

There are numerous practical applications for gene editing in mosquitoes, including reducing mosquito populations and replacing populations of disease-transmitting mosquitoes with disease-resistant mosquitoes. Utilizing genetics in a controlled manner has the potential to save millions of lives per year from diseases such as yellow fever, West Nile, Zika, and dengue (1). The CRISPR-Cas9 system has been used to slow the growth of mosquitoes, create super-mendelian gene drives, and even create malaria-resistant mosquitoes (7-11).

The process of making a transgenic mosquito involves editing the germline cells of the embryo, rearing the mosquito to adulthood, mating it, and screening for the desired gene or trait of interest in the next generation to ensure the modification is heritable (12, 13). This can be a tedious and time-consuming task, with varied success. In order to fully utilize the incredible resource that the CRISPR-Cas9 system can be for mosquito biology, it is imperative to develop more widely accessible techniques with which to use it.

## ReMOT Control

The traditional method of generating heritable mutations in a mosquito involves individually injecting each freshly laid embryo with a solution of Cas9 protein and a target guide RNA. This is a high precision technique that involves exact timing, weeks of training and preparation, and large, specialized, and expensive equipment. Many labs around the world do not have these resources, and as such, cannot contribute to the growing pool of knowledge made accessible using CRISPR-Cas9-based techniques.

The Receptor-Mediated Ovary Transduction of Cargo (ReMOT Control) method, developed by the Rasgon Laboratory in 2018, is a more efficient technique to create targeted mutations in mosquitoes (14). Rather than inject each embryo individually, an adult female mosquito is injected intrathoracically and the Cas9-gRNA complex localizes to each egg as it develops (Figure 1). Using this method, hundreds of individual eggs can be targeted for modification, instead of one analogous embryo. The ReMOT Control method is also simple to use, requires inexpensive equipment, and only a few hours of training. Unfortunately, both methods have an efficiency rate of around 1% progeny with a mutation. The technique still has room to be improved upon in order to more efficiently generate mutants.

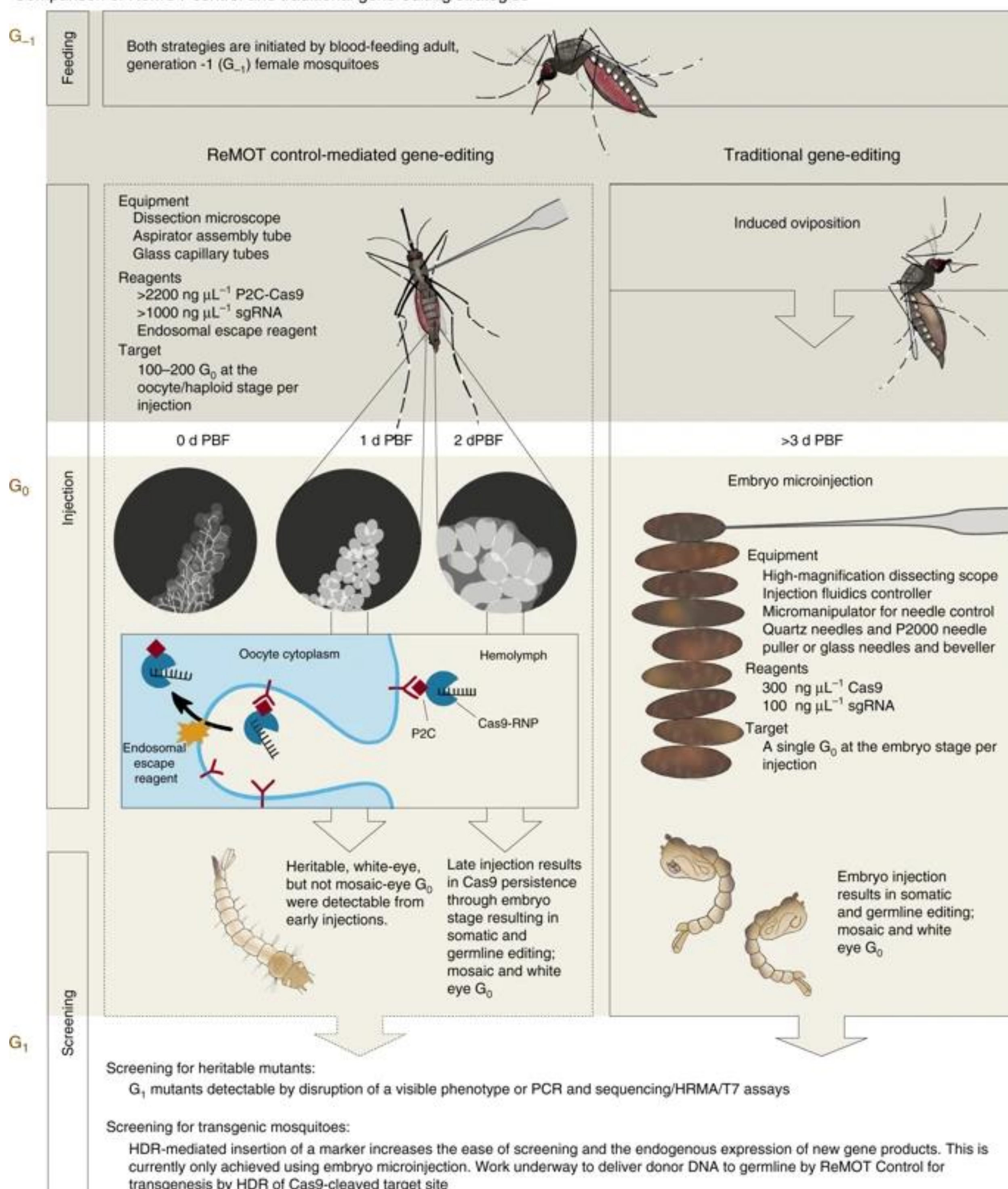


**Figure 1. ReMOT Control vs. Embryo Injection in *Ae. aegypti***

## Viral Vectors

One method of delivery of gene editing agents such as Cas9 to a target genome is by using a viral vector. This method often involves deactivation of a virus, so as not to cause illness, and cloning of editing proteins or sequences into the viral genome (15). Viruses that are commonly used include adenoviruses, adeno-associated viruses, and bacteriophages (16). Not all viruses can be used as a genetic vector- a virus must have low immunogenicity, a large enough capacity to transport the genetic information, and an efficient delivery mechanism for the genetic material. In theory, viral vectors could be encoded to express Cas9 and gRNA, which could be used to alter a mutated disease-causing gene in a patient. Viral vectors are not currently being used as a proven treatment in humans due to the inability to target specific cells in the body for editing (15). This, however, does not exclude the possibility of successful editing in a mosquito using a suitable viral vector.

## Baculovirus

Baculovirus is a family of arthropod specific nucleopolyhedroviruses (NPVs) (17). There are four genera of baculovirus, all of which infect primarily Lepidopteran larvae in nature. One of the more extensively studied baculoviruses is *Autographica californica* multicapsid nucleopolyhedrovirus (*Ac*MNPV), named for the species of moth in which it was discovered- the alfalfa looper (*Autographica californica*). *Ac*MNPV has been thoroughly studied and completely sequenced and is currently being trialed for use in gene therapy treatments for human cancer patients (18,19). Other NPVs have been reported to infect and cause disease in insects outside of the Lepidopteran order, including our species of interest, *Ae. aegypti* (20). *Ac*MNPV, however, is

a virus specific to Lepidopteran species and is not known to replicate in other insects in the wild (17).

The disease caused by baculovirus infection is also known as "wilting disease" because of the way it presents in its host (17). The common caterpillar host is spurred into eating excessively without resting and climbing to the tops of plants. When the caterpillar dies, it "melts" onto the leaves below, spreading virus particles to be eaten by the next unsuspecting caterpillar. In this way, the virus continues its cycle of infection. The reason the host "wilts" or "melts" is because *Ac*MNPV is a lytic virus, meaning as the virus replicates, it bursts the cell in order to release new viral particles.

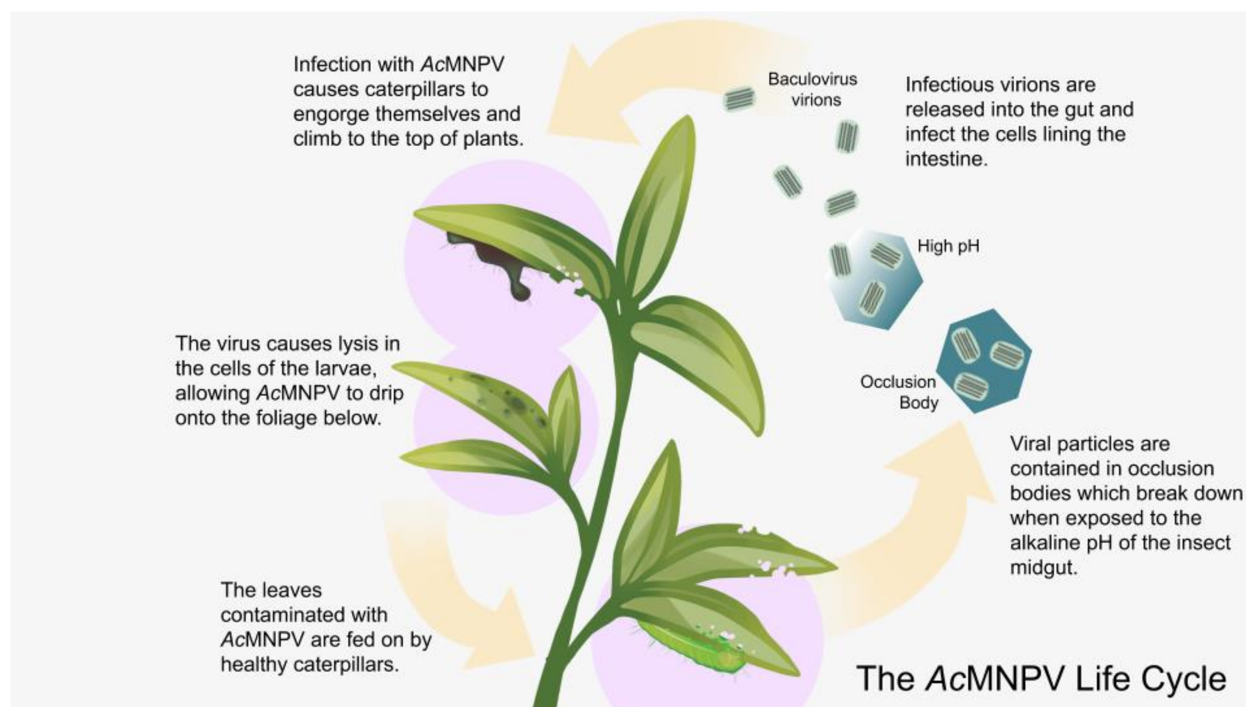


**Figure 2. The Life Cycle of a Baculovirus**

**P2C Peptide**

The mosquito ovary has precautions in place to prohibit entry of large molecules like nucleic acids and proteins, and organisms such as bacteria into the oocyte. In order to bypass these precautions to allow entry of Cas9, a transportation system that is already being used naturally in these cells must be “hijacked”. The ReMOT Control technique was based on the activity of the *Drosophila* yolk protein (YP1) and yolk protein receptors which had previously been shown by immunohistochemistry to translocate protein into the oocytes of *Anopheles gambiae* mosquitoes (21). Recent experiments utilized this finding and confirmed the use of the *Drosophila* yolk protein as a “guide” into the oocytes by attaching enhanced green fluorescent protein (EGFP) to the yolk protein and injecting it into the thorax of live adult mosquitoes (14). The mosquito ovaries were dissected out and imaged. EGFP-YP1 fusion protein injected mosquitoes were compared to EGFP injected mosquitoes, and it was concluded that the yolk protein mediated delivery of the EGFP to the oocytes, where entry of protein without the peptide was blocked. The transport capability of YP1 was localized by deletion mapping to a 41 amino acid fragment dubbed “P2C”. The P2C peptide was shown to be effective in transporting cargo including Cas9 protein into the ovaries of six different species of mosquito, including *Ae. aegypti*.

Because P2C is derived from *Drosophila* YP1, we expect that an insect cell will produce recombinant protein with greater efficiency than other cells such as bacterial *E. coli* (22). This is the motivating theory behind my thesis. When a recombinant baculovirus is encoded to produce a Cas9-P2C fusion protein and is injected into a mosquito, the mosquito cell machinery will

build and fold the protein. If the efficiency of protein folding is higher, this would correlate with higher efficiency of mutation at a specific site.

# Chapter 2

## The Project

Genetic engineering in mosquitoes such as *Ae. aegypti* was achieved in 1998, and greatly improved with Cas9 in 2015- yet leaves much to be desired in the ways of efficiency (12, 13, 23, 24). Development of the ReMOT Control technique improved efficiency from embryo injections by creating a simpler procedure. Making a target mutation using this method, however, still takes significant time for a less than 1% yield. Introducing a viral vector may increase this efficiency, making genetic engineering in mosquitoes a more widespread tool for labs around the world.

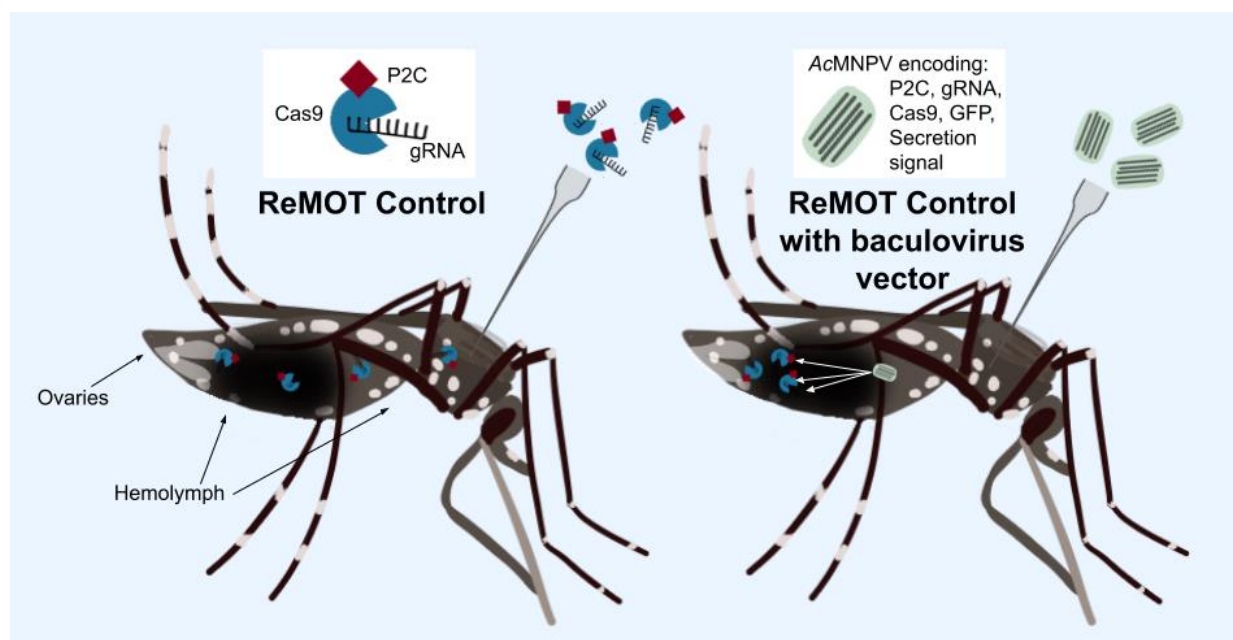


**Figure 3. Comparing Standard ReMOT Control Techniques to Using a Viral Vector**

The long-term project involves three steps, only the first of which is covered in this set of experiments. The first experiment is based on a paper by Naik et al. (25), which documents how GFP/EGFP labelled baculovirus can be injected intrathoracically into four different genera of

mosquito, and the fluorescence is visible throughout the insect body. The researchers working on this paper claim that the baculovirus makes it into the ovaries, and an image of GFP fluorescent ovaries is shown (Figure 4). The image is not clear nor magnified enough, however, to tell if the baculovirus has efficiently made it into the actual oocyte- which would be required for germline editing. There is also a level of green autofluorescence observable in *Ae. aegypti* ovaries, and in this paper no negative control is shown. The first part of the project was replicating this experiment with the focus on the ovaries- and more specifically the oocytes- and how they take up the baculovirus. Because green autofluorescence is prevalent in mosquito tissues, mCherry, which fluoresces red, was chosen as a substitute for EGFP as the fluorescent reporter protein. Baculovirus expressing mCherry was injected into adult female mosquitoes, and their ovaries were dissected with the expectation that if the baculovirus genome successfully infected the ovarian tissues, this could be visualized by mCherry expression.

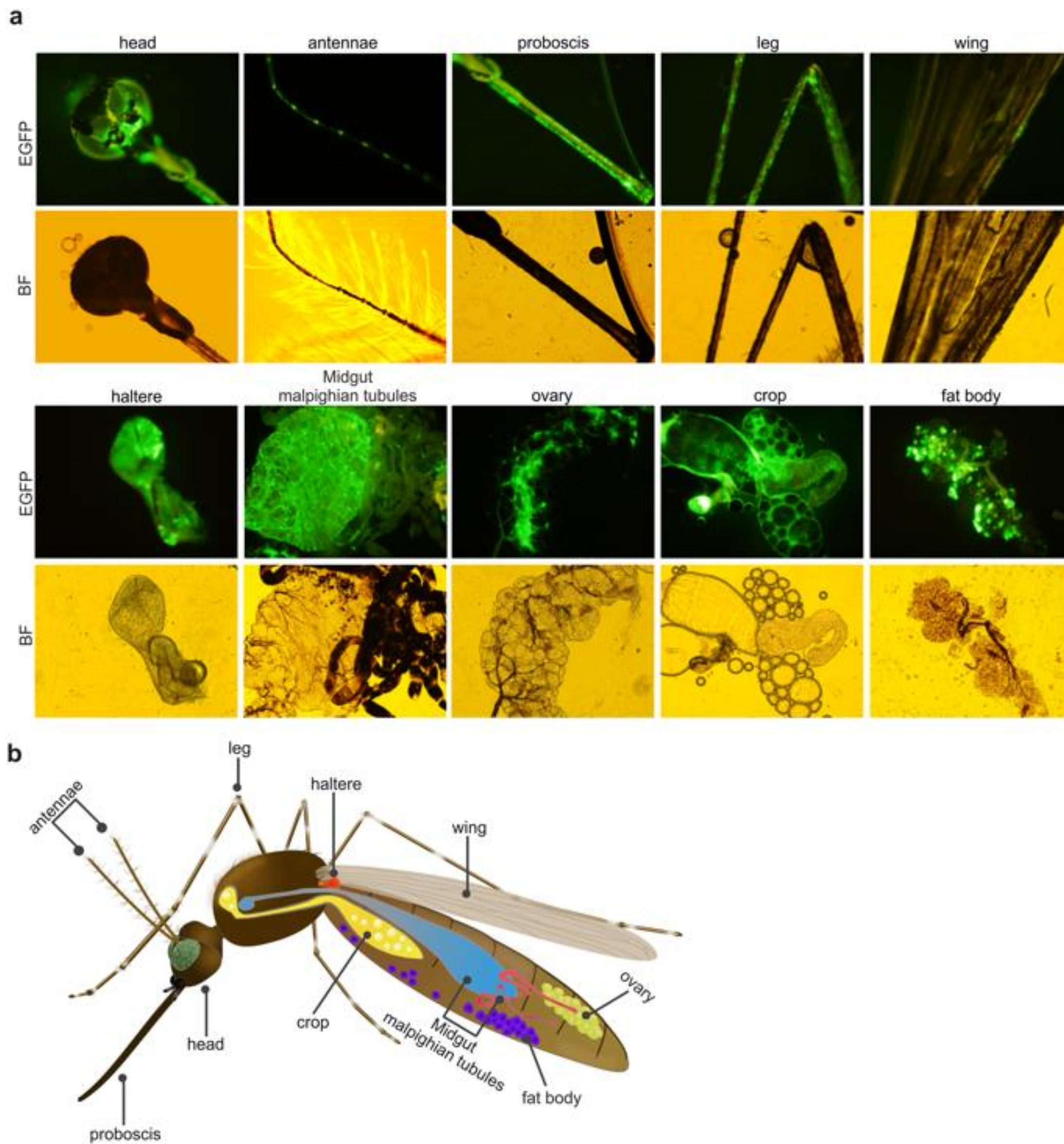


**Figure 4. EGFP delivery in *Ae. aegypti* via baculovirus, from Naik et. Al (2018)**

"Tissue tropism of baculovirus in adult A. aegypti. (a) Dissection of baculovirus transduced adult mosquito. A. aegypti adult mosquitoes were microinjected intra-thoracically with 1 × 105 PFU of vBacb1EG-irEG baculovirus and observed at 15 days post-transduction. EGFP fluorescence was observed in head, antennae, proboscis, leg, wing, haltere, midgut Malpighian tubules, ovary, crop, and fat body tissues of adult mosquitoes. EGFP: green fluorescence, and BF: bright field images. (b) Schematic representation of adult mosquito tissues. For simplicity, cartoon of adult mosquito showing various tissues representing respective images of (a) is shown" (Naik et. al 2018)

This visual assessment of the specific tissue tropism of baculovirus in the mosquito ovaries allows identification of which ovarian tissues cargo can be delivered to using recombinant baculovirus. If the baculovirus is localized to the oocyte, it can be used directly for delivering Cas9-guideRNA complexes. However, considering that the oocyte is highly protected, it is possible that we will not see fluorescence in the oocyte even if fluorescence is present in the maternal follicle cells or the nurse cells. In this case, the next step is adding a localization signal such as P2C onto the construct so that it encodes a fusion protein P2C-mCherry. The P2C would bring the molecule into the oocyte, allowing for a specific pattern of fluorescence upon dissection of the ovaries. Dissected ovaries from infected mosquitoes with baculovirus expressing P2C-mCherry are expected to have increased fluorescence in the oocyte compared to those visualized in the first step. This is based on evidence from previous experiments demonstrating that P2C fusion protein localizes to the oocyte following intrathoracic injection of the protein (26). The last step involves coding the P2C-mCherry-Cas9 fusion protein and gRNA into the viral genome. Following infection of adult females (generation -1, $G_{-1}$) with this recombinant virus, screening for knockouts of a visible marker in the $G_0$ and $G_1$ can determine if heritable mutations have been made, and the efficiency can be calculated (Figure 1).

In this project, I test the hypothesis that *Ac*MNPV can infect ovarian tissue of *Ae. aegypti* and deliver a transcript for expression, and I examine the tissue specificity for this delivery. I demonstrate that the expressed protein complex localizes to the maternal follicle cells of the ovariole, located in the ovary (Figure 5). This data suggests that the cargo could be moved into the oocyte, which would allow for germline editing.

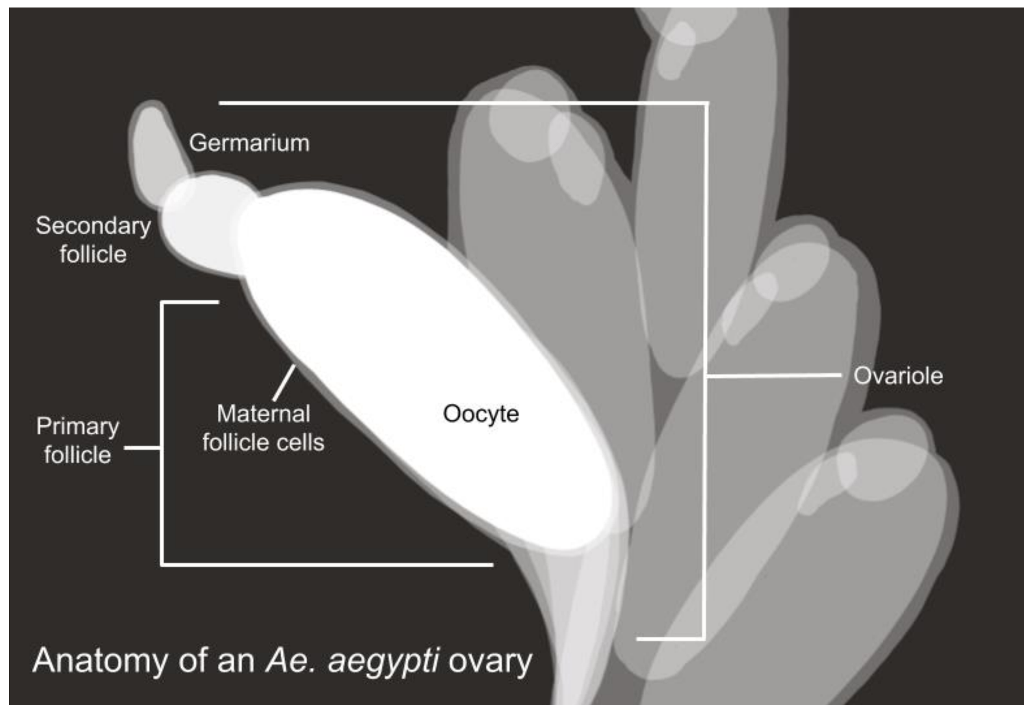


**Figure 5. Anatomy of an *Ae. aegypti* ovary**

# Chapter 3

# Methods

## Cell Culture and Virus Production

ExpiSF9 strain of *Spodoptera frugiperda* cells were cultured in SPF9 media, counted, and passaged regularly to maintain a colony of approx. 0.4 x$10^6$. Frozen *Ac*MNPV was thawed at 37°C and added to flasks of ExpiSF9 cells. Infected cells were incubated for 5 days, then centrifuged at 4500x g for 15 minutes. Supernatant was pipetted into 500µL aliquots and frozen at -80°C until further use.

## Mosquito Rearing

*Ae. aegypti* (Liverpool strain) were reared in a contained insectary with regulated temperature of 27°C and relative humidity of 75 ± 10%. The insects were kept on a 12 hour light 12 hour dark schedule. Larvae were reared in pans with reverse osmosis (RO) water and fed with a 1:2 mixture of ground Tetramin fish flakes to baker's yeast, measured by volume. Adults were fed 10% sucrose via soaked cotton balls on the top of their cages *ad libitum*. When bloodfeeding was required for experiments, adult mosquitoes were placed under a water-jacket membrane feeding system and were provided with anonymous human blood (Biospecialty Corp.).

## Injection Mixture Preparation

Phosphate buffered saline (PBS) was diluted from 10x stocks to 1x before injection and was kept on ice. Aliquots of 8.5 $x10^5$ IP/mL virus stock were thawed in a 37°C water bath and kept at room temperature during injections. For earlier experiments, virus stock was diluted 1:10 and 1:100 in 1x PBS and kept at room temperature throughout injections.

## Injections

Adults were blood fed at 5 to 14 days post emergence. Within 2 days of blood feeding, adult females were chilled at 4°C to be immobilized and kept on ice. Each female that showed visible evidence of a blood meal was injected with approximately 1.5 microliters of solution via a glass needle. Needles were pulled via Sutter P2000 from glass capillary tubes (World Precision Instruments). An aspirator (A5177, Sigma) was attached to a plastic transfer pipet to perform the injections so as to maintain proper infection control procedures with a BSL1 pathogen.

The adult female mosquitoes were injected in two different trials. These trials used different concentrations of virus and a different number of experimental groups (Table 1). In addition, mosquitoes from trial 1 were injected 48 hours after taking a blood meal and dissected at 24 and 48 hours after injection. Mosquitoes from trial 2 were injected 24 hours after taking a blood meal and dissected 24 hours after injection.

**Table 1. A comparison of trials 1 and 2**

| | Trial 1 | Trial 2 |
|---|---|---|
| Groups | Non-inject control | Non-inject control |
| | 1x PBS | 1x PBS |
| | 8.5 x$10^5$ IP/mL | 8.5 x$10^5$ IP/mL |
| | 8.5 x$10^4$ IP/mL | |
| | 8.5 x$10^3$ IP/mL | |
| Injected ____ after blood feeding | 48 hrs | 24 hrs |
| Dissected ____ after injection | 24 hrs | 24 hrs |
| | 48 hrs | |
| Mounting | 1x PBS (temporary) | Mounting solution (permanent) |
| Imaging | Olympus BX41 microscope, iPhone XR camera | Zeiss LSM 880 confocal microscope, Zen Blue Imaging Software |

### Dissections

1 to 2 days after injection, adult females were chilled at 4°C and kept on ice for immobilization. Pairs of ovaries were dissected from each female in 1x PBS using forceps and placed in a tube of 1x PBS on ice. Ovaries from trial 1 were split into two groups, one group was dissected at 24 hours post injection, and the other group at 48 hours post injection. The ovaries from trial two were all dissected out 24 hours post injection (see Table 1). If ovaries were underdeveloped or if they burst during dissection, ovaries were not included in results.

## Mounting and Imaging

Ovaries from trial 1 were transferred onto glass microscope slides into 10-20µL 1x PBS. Double stick tape was used to line the slide and attach the coverslip in place. Ovaries were viewed through an Olympus BX41 microscope and images were taken through the eyepiece with an iPhone XR. To view the ovaries from trial 2, I used mounting liquid (S36942, Thermofisher) to fix the specimens to the slides, following protocol and adding immersion oil beneath the coverslip. Ovaries were viewed through a Zeiss LSM 880 confocal scope and images were taken using Zen Blue Imaging Software.

# Chapter 4

# Results

## Trial 1

Adult female *Ae. aegypti* were dissected at 24 and 48 hours post injection. Ovaries from non-inject and PBS injections had slight pinkish autofluorescence in the oocytes and tracheal tissue. Ovaries from 8.5 $x10^4$ IP/mL and 8.5 $x10^3$ IP/mL baculovirus injections had little visually detectable increase in red fluorescence, and those images are not shown. In Figure 5, images of ovaries from the 8.5 $x10^5$ IP/mL virus injections after 24 hours are compared to the PBS injected ovaries. The baculovirus-mCherry injected ovaries are brighter red than the control ovaries. Figure 6 is an image of an ovary from the 8.5 $x10^5$ IP/mL virus injected group at 48 hours post injection. This ovary is brighter red than those dissected at 24 hours. In this image, the mCherry molecule is detected in the ovaries, although it does not appear that it is getting into the oocyte itself. The fluorescence appears localized to the maternal follicle cells that surround the oocyte.

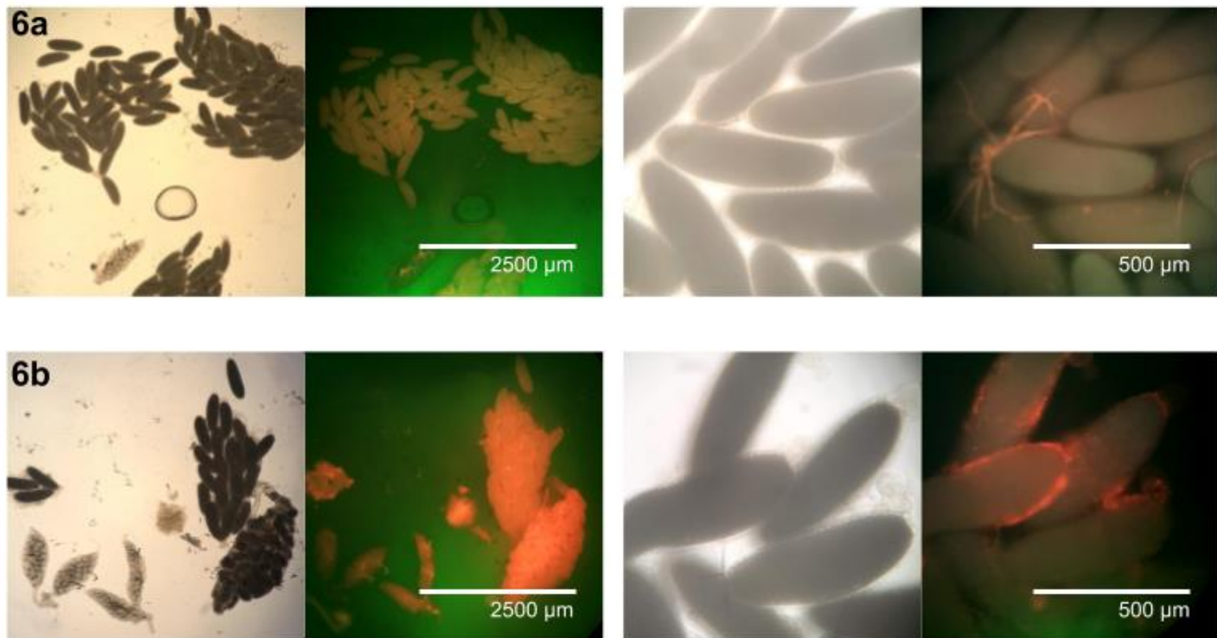


**Figure 6. Visualization of baculovirus expression of mCherry in *Ae. aegypti* ovaries**

**Ovaries dissected from (A) PBS injected and (B) *Ac*MNPV-mCherry injected females at 72 hrs post blood meal and 24 hours post injection**

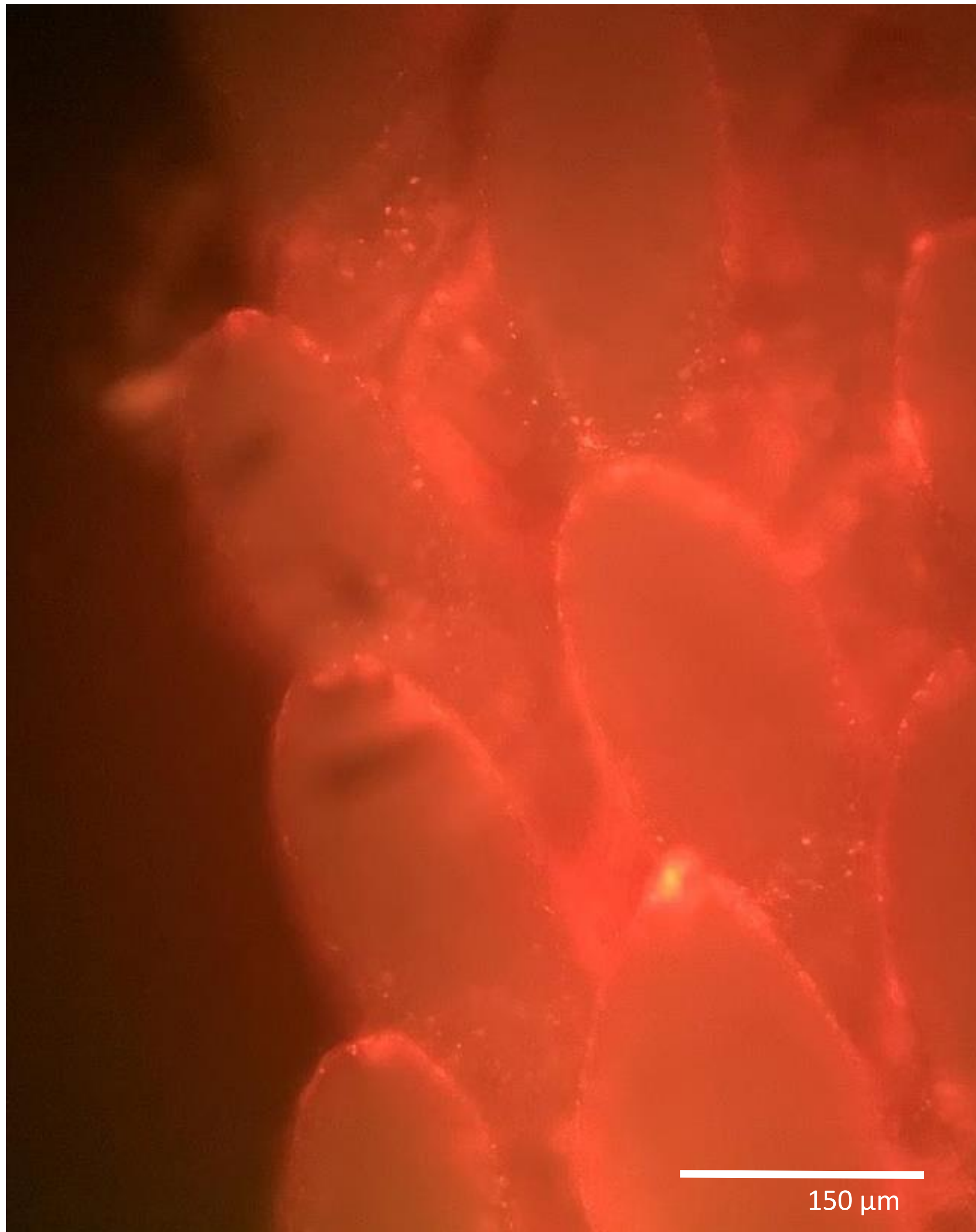


**Figure 7. mCherry fluorescence in a baculovirus infected ovary 96 hrs post blood meal and 48 hrs post injection**

## Trial 2

Because no fluorescence was detected in ovaries dissected from females injected with 8.5 $x10^4$ IP/mL and 8.5 $x10^3$ IP/mL virus in trial 1, trial 2 used only non-injected controls, PBS controls, and 8.5 $x10^5$ IP/mL virus injections. In this trial, I observed a high mortality in the virus injected mosquitoes, though survival was not quantified. The ovaries dissected from virus injected females were also less developed than the control mosquitos' ovaries. The ovaries that were developed enough to be viewed were mounted and viewed through a confocal microscope for better imaging. A construction of Z-stack images of fluorescent ovaries show a clear pattern of red fluorescence in the maternal follicle and not in the oocytes (Figure 8). On cross section of the image, it can be observed that the red mCherry protein is not being expressed within the oocyte.

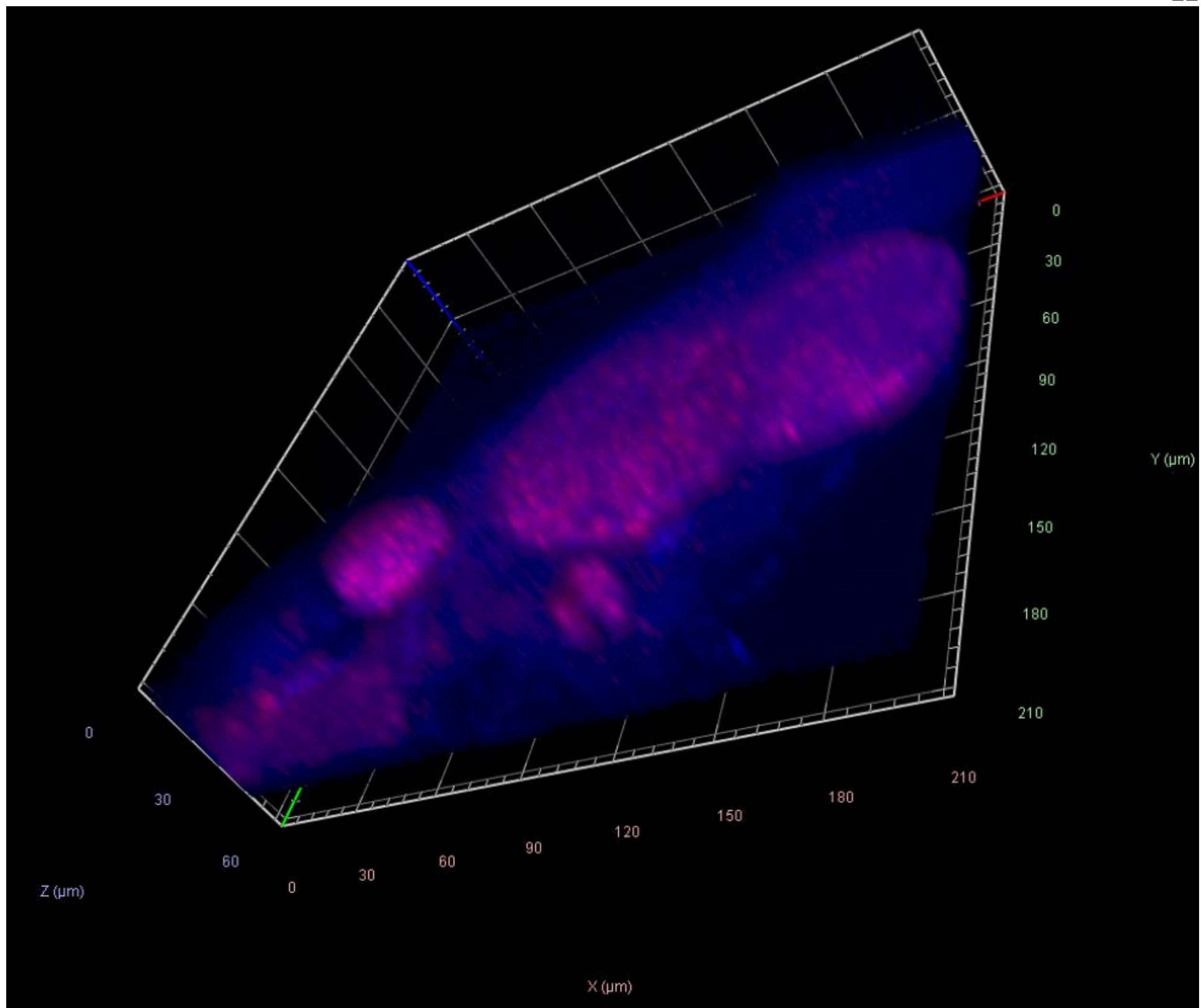


**Figure 8. 3D model of mCherry expression in the ovariole**

# Chapter 5

## Discussion

The fluorescence seen in the baculovirus-mCherry injected ovaries suggests that baculovirus is capable of infecting the maternal follicle cells of the ovary, but not the oocyte. Transmission into the oocyte is critical for germline editing, so finding a way to bring the cargo from the virus through the follicle is a necessity.

The next step in this experiment involves adding an ovary localization signal into the viral genome. Originally, we were expecting to use P2C, but since the protein already seems to locate to the ovaries on its own, introduction of a 3' UTR that encodes localization of transcript from the follicle cells to the ovaries, such as that of the *nanos* gene, may be enough to bring the cargo out of the follicle and into the oocyte (27, 28). An additional experimental group using a baculovirus strain cloned with the localization signals would allow us to confirm whether this system could be used for Cas9 delivery to the germline.

It appeared from the second trial that there was an observed decrease in survival of the mosquitos that were injected with baculovirus-mCherry. Other experiments showed that *Ac*MNPV does not cause lytic disease in mosquito cells, which is how a baculovirus usually kills its host. A decline in a laboratory strain of mosquitoes could alternately be due to crowded larval rearing or a problem with a blood meal. A replication of the experiments presented including an additional quantification of infection efficiency and survival would allow us to create an

optimization of virus injection parameters. If *Ac*MNPV causes decreased survival in *Ae. aegypti,* this contrasts with current published works in this species (16).

If genes encoding fluorescent protein can be successfully directed to the oocytes, the last step toward heritable targeted gene editing using baculovirus delivery of Cas9 involves encoding Cas9 into the viral genome, rearing and injecting recombinant virus into adult females ($G_{-1}$), and screening the $G_0$ and $G_1$ progeny for knockouts. Because the vector in question here is *Ae. aegypti,* we will target the kynurenine monooxygenase gene (KMO), which has been widely used to validate mutagenesis methods (14, 24). At this point we can detect mutations in the eyes of the progeny of the injected mosquitoes by visual screening, and quantify the efficiency compared to traditional gene editing through embryo injection and compared to standard ReMOT Control procedures.

Using a viral vector does introduce some issues, such as the requirement for BSL1 precautions when handling the virus and infected mosquitoes. This includes secondary containment for mosquito cages and use of gloves and lab coat when handling viral specimens. This also means that a mouth aspirator, which is used for non-toxic injection material, is not a viable means of injecting mosquitoes, so I used a plastic transfer pipette, which was more difficult to maneuver.

The predicted benefits, however, may outweigh the extra procedural steps needed when using a viral vector. The P2C protein is a *Drosophila* protein, but when synthesized in the lab, is assembled, folded, and extracted from lab-strain *E. coli* bacteria. If P2C was encoded into a virus

which hijacked a mosquito's own cells to replicate, an insect cell would be synthesizing the P2C peptide, possibly leading to higher accuracy in folding and function. The theory behind this is that an insect cell will more accurately fold an insect protein than a bacterial cell will.

Genetic modification of *Ae. aegypti* is essential to vector control measures across the world, however, current gene editing techniques for this insect remain inefficient and unattainable for many laboratories. By aiming to increase the efficiency of creating heritable mutations by using baculovirus as a viral vector, this technique has the potential to increase the number of laboratories that can experiment with gene modification in such an important disease vector.

Using baculovirus as an expression vector to target specific mutations in *Ae. aegypti* carries the possibility of improving efficiency for gene modification tools. It may allow more widespread use of genetic editing in laboratories around the world, contributing to the development of vector control methods regarding *Ae. aegypti* as well as other mosquito and insect species. Herein I have demonstrated that the infection properties of the baculovirus *Ac*MNPV in *Ae. aegypti* are appropriate for nucleic acid delivery to the ovaries for future gene-editing techniques, but that additional design will be necessary to direct moieties to the germline tissue to make heritable edits to the genome.

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**ACADEMIC VITA**


# Sage G. McKeand

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## Education

**The Pennsylvania State University**
**Schreyer Honors College | Spring 2020**
Immunology and Infectious Disease
Minor: Entomology

## Publications

***Cas9-mediated gene-editing in the malaria mosquito Anopheles stephensi by ReMOT Control*** — April 2020
**G3: Genes, Genomes, Genetics**
Vanessa M Macias*, **Sage McKeand***, Duverney Chaverra-Rodriguez*, Grant L. Hughes, Aniko Fazekas, Sujit Pujari, Nijole Jasinskiene, Anthony A. James, Jason L. Rasgon
**These authors contributed equally to the manuscript*
Worked primarily on injections of adult mosquitoes, mosquito rearing, and screening.

***Targeted delivery of CRISPR-associated endonuclease 9 (Cas9) into arthropod ovaries for heritable germline gene editing*** — August 2018
**Nature Communications**
Duverney Chaverra-Rodriguez*, Vanessa M. Macias*, Grant L. Hughes*, Sujit Pujhari, Yasutsugu Suzuki, David R. Peterson, Donghun Kim, **Sage McKeand**, Jason L. Rasgon
**These authors contributed equally to the manuscript*
Contributions to this project include manuscript preparation and figure illustration.

## Presentations

***Cas9-mediated gene-editing in the malaria mosquito Anopheles Stephensi by ReMOT Control*** — November 2019
Vanessa M Macias, **Sage McKeand**, Duverney Chaverra-Rodriguez, Grant L Hughes, Aniko Fazekas, Sujit Pujari, Nijole Jasinskiene, Anthony A. James, Jason L. Rasgon.

Presented as a poster at the 68th Annual American Society of Tropical Medicine and Hygiene (ASTMH) conference. Significant contributions to the experiments include injections and screening, as well as data recording and presentation. Contributions were also made in figure design and creation.

## Research Positions

**Jason Rasgon Lab** Fall 2017 to present

***The Pennsylvania State University***

Undergraduate Research assistant under postdoctoral scholar Dr. Vanessa Macias, working directly with Cas9 genetic modification of *Aedes aegypti* and *Anopheles stephensi* mosquitoes. Worked on a number of additional projects with graduate students or postdoctoral scholars and taught various students and scholars in the lab how to take care of the mosquitoes in the insectary. Coordinated with others to complete projects in a timely manner.

## Skills

**Mosquitoes**

Mosquito rearing and husbandry for various *Anopheles, Aedes,* and *Culex* species
Adult injections in *An. stephensi and Ae. aegypti* via mouth injectors and Nanoject III
Embryo microinjection
Ovary dissection
Species level identification

**Stink Bugs**

Brown marmorated stink bug rearing and husbandry
Brown marmorated stink bug adult injections

**General Laboratory Procedures**

Fluorescent and basic microscopy
Cell culture in non-mammalian cell lines
Polymerase chain reaction
Gel electrophoresis
Plasmid minipreps
Documentation and keeping a laboratory notebook

**Other Skills**

Scientific illustration/figure drawing
General insect identification

## Relevant Coursework

**VBSC 520 Pathobiology** Dr. Jeanne Lumadue
**VBSC 432 Advanced Immunology** Dr. Pamela Giblin
**VBSC 435 Viral Pathogenesis** Dr. Tony Schmitt

**VBSC 451 Immunotoxicology** Dr. Sandeep Prabhu and Dr. Pamela Giblin
**BMB 480 Cancer Progression and Development** Dr. Emily Bell and Dr. Lorraine Santy
**BMB 464 Molecular Medicine** Dr. James Howell
**VBSC 418H Bacterial Pathogenesis** Dr. Bhuvana Katkere
**MICRB 410 Principles of Immunology** Dr. Troy Sutton
**BMB 401H General Biochemistry** Dr. Ying Gu
**ENT 432 Insect Diversity and Evolution** Dr. Andy Deans
**BIOL 222H Genetics** Dr. Paul Babitzke
**BMB 251H Molecular and Cellular Biology** Dr. Yanming Wang and Dr. Lorraine Santy
**CHEM 212H/213H Organic Chemistry II/Lab** Dr. Katherine Masters
**CHEM 210H Organic Chemistry I** Dr. Raymond Funk

## Other Interests/Activities

**Penn State Triathlon Club** Spring 2018 to May 2020
Vice President, Training Coordinator, Member
**Volunteer Emergency Medical Technician** Dec. 2018 to present
**Camp Takodah** ***Richmond, NH*** Summers 2016 to 2018
Cabin Leader, Kitchen Crew Chief