

Montclair State University

## Montclair State University Digital Commons

---

Theses, Dissertations and Culminating Projects

---

5-2020

### **Response to Phototaxic Stimulation in Relation to Sex and Reproductive State in the Urban Adapted Mosquito, *Culex pipiens f. molestus***

Karen M. Catalina

Follow this and additional works at: <https://digitalcommons.montclair.edu/etd>

 Part of the [Biology Commons](#)

---

## Abstract

The mosquito, *Culex pipiens f. molestus*, is a scientifically relevant species due to its impact upon both human and veterinary medicine. This city dwelling disease vector lives and reproduces in underground tunnels and sewer systems throughout the world. After reproducing, adults begin searching for food above ground, but little is known about the sensory signals that guide this seeking behavior. Because cues may have a major impact on feeding and reproduction, I compared the phototactic response of adult male and female *Cx. pipiens f. molestus* at different reproductive states. Virgin females, mated females, virgin males, and mated males were monitored to determine how they would respond to a light signal at one end of a tunnel. All groups showed a significant positive phototactic response. However, the response of virgin females was less pronounced than the responses of the other groups. The overall positive phototactic response indicates that *Culex pipiens f. molestus* may be using light signals, at least in part, to initiate seeking behavior. The lesser response of virgin females is potentially due to a biological mechanism that delays host-seeking, even in the presence of initiating cues, until after reproduction has occurred.

**MONTCLAIR STATE UNIVERSITY**

**Response to phototoxic stimulation in relation to sex and reproductive state in the  
urban adapted mosquito, *Culex pipiens f. molestus***

By

Karen M. Catalina

A Master's Thesis Submitted to the Faculty of

Montclair State University

Partial Fulfillment of the Requirements

For the Degree of

Master of Science

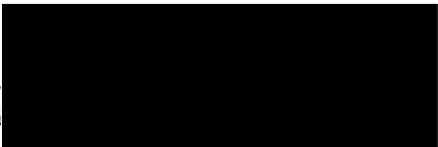
May 2020

College of Science and Mathematics

Thesis Committee:

Department of Biology

\_\_\_\_\_   
Dr. Matthew Aardema, Thesis sponsor

\_\_\_\_\_   
Dr. Scott Kight, Committe

\_\_\_\_\_   
Dr. Lisa Hazard, Committee member

**Response to phototaxic stimulation in relation to sex and reproductive state in the urban adapted mosquito, *Culex pipiens f. molestus***

A THESIS

Submitted in partial fulfillment of the requirements  
for the degree of Master of Science

By  
KAREN M. CATALINA  
Montclair State University  
Montclair, NJ  
2020

Copyright 2020  
By Karen M. Catalina

## **Acknowledgements**

I would like to thank the Department of Biology at Montclair State University for their support and the use of their facilities during this process. I would like to express my gratitude to my research advisor, Dr. Matthew Aardema, for his patience, guidance, and encouragement. His experience was crucial to the completion of this project. Additionally, I would like to thank the rest of my committee, Dr. Lisa Hazard and Dr. Scott Kight, for giving their time and valuable insight. Lastly, I am forever indebted and grateful to my husband, Frank Catalina, my in-laws, Ellen and Frank Catalina, and my parents, Marie and Theodore Wahlstrom. There is no doubt that without them, none of this would have been possible.

## **Table of Contents**

<b>Chapter Title</b>	<b>Page</b>
Abstract	1
Thesis Signature	2
Title Page	3
Copyright Page	4
Acknowledgments	5
Table of Contents	6
List of Figures and Tables	7
Introduction	8
Methods	12
Collection and maintenance	12
Behavioral Trials	13
Analytical Methods	15
Results	16
Control Trial, test for inherent bias	16
Main Trial, test for phototactic response	17
Main Trial, test for behavioral differences between subgroups	18
Discussion	19
References	23
Appendix	26

<b>Figures</b>	<b>Page</b>
Figure 1. Apparatus A and B (Photograph: Karen Catalina)	14
Figure 2. Initial trial mosquito location data	17
Figure 3. Phototaxic response at group level	18
Appendix Table 1. Initial trial data	26
Appendix Table 2. Main trial data	26
Appendix Table 3. Contingency tables for biologically significant pairs	27

## Introduction

*Culex pipiens f. molestus*, the subterranean dwelling form of *Culex pipiens*, is a widespread pest and a capable vector for diseases such as tularemia, Ross River Virus, lymphatic filariasis, Rift Valley fever, and primarily, West Nile virus (WNV) (Byrne & Nichols, 1999, Žiegytė et al., 2014, Kassim et al., 2012, and Vinogradova, 2003). Their geographic distribution includes cities and towns in North America, South America, Europe, Asia, Australia, and Africa (Farajollahi et al., 2011), with a northerly range defined by the presence of suitable buildings and underground refuge rather than climate or the availability of natural habitat. Only the northernmost reaches of the globe are not colonized by these mosquitoes due to the absence of basements and underground systems (Vinogradova, 2000).

The *molestus* form of *Culex pipiens* is indistinguishable morphologically from *Cx. p. pipiens*, but differs genetically, ecologically, and behaviorally (Byrne and Nichols, 1999). Adapted to urban, subterranean life, they thrive and breed in polluted, confined areas such as sewers, the tunnels of steam and subways systems, and flooded basements (Farajollahi et al., 2011, Vinogradova, 2011). Unlike most other *Culex* species, they are obligately autogenous, meaning they lay one batch of eggs before a bloodmeal. However, after they have laid one batch of eggs, females may make their way to the surface to hunt for a bloodmeal in order to lay subsequent egg batches (Byrne and Nichols, 1999). Once above ground, female mosquitoes use doorways and ventilation systems to gain access to living quarters where they feed, most often overnight, biting the face, neck, and forearms of their human host (Vinogradova, 2000). Females will also obtain nutrients from nectar feeding, but for males nectar is the sole source of nutrition. Without sugar, males are susceptible to rapidly decreasing health, and death can occur within

days after eclosion (Barredo and DeGennero, 2020). The longer a male is without sugar, the less likely it is to have reproductive success (Barredo and DeGennero, 2020). Living underground, where winter temperatures are warmer than above ground temperatures, have made diapause avertable (Vinogradova, 2000), thus, these mosquitoes will breed and feed year-round.

Scientific interest in the *Culex pipiens* complex stems from its effects upon both human and veterinary medicine through the spread of WNV. Up to 80% of infected individuals are asymptomatic (Barrett, 2014 and WHO, 2017), while the other 20% will show signs of West Nile fever. Symptoms include fever, swollen lymph nodes, rash, body aches, fatigue, headaches, and nausea (WHO, 2017). In less than 1% of cases, the disease will develop into West Nile neuroinvasive disease (WNND), manifested as acute flaccid paralysis (AFP), encephalitis, and/or meningitis. Those with encephalitis or AFP often suffer from long-term neurological impairment (Barrett, 2014 and WHO, 2017). The overall fatality rate for patients who have contracted WNND is 10%; however, rates between 15-29% are common in elderly patients or those with compromised immune systems due to organ transplants or autoimmune disease (Spickler, 2013 and WHO, 2017). In 1999, West Nile spread to the United States. The disease emerged in New York and spread throughout the country, where it quickly became established (Vinogradova, 2003 and Barrett, 2014).

WNV is a zoonotic disease transmitted through the bite of an infected mosquito. Mosquitoes harbor the virus in their salivary glands after feeding on infected birds. In nature, the virus is perpetuated through a continuous host-vector-host transmission cycle. Mosquito vectors feed on avian reservoirs where the virus is amplified. The virus is then transmitted when the mosquito takes its next bloodmeal. WNV has been detected in male field collected mosquito

species, which is concerning because it indicates the existence of vertical transmission, the direct passage of a pathogen from female parent to offspring during oogenesis. (Colpitts et al., 2012, Farahollahi et al., 2011). Their ability to interbreed with *Cx. pipiens pipiens* (Byrnes and Nichols, 1999) magnifies the problem, as vertical transmission has the potential to create new generations within two separate species that are carriers of WNV.

Humans are the primary bloodmeal hosts of the *molestus* form, while *Cx. pipiens pipiens* prefer avian hosts (Vinogradova, 2000, Farajollahi et al., 2011). Although *Culex pipiens f. molestus* are highly anthropophilic (Becker et al., 2003), it has been shown that they will feed on birds (Žiegytė et al., 2014). Hybridization between *Cx. pipiens f. molestus* and *Cx. p. pipiens* is evidenced by the discovery of intermediate forms, carrying traits from both parental species (Byrne & Nichols, 1999). Hybridization has implications for the transmission of WNV, as a hybrid of these bird-host and human-host species would be a notably more efficient transmission vector than either parent species alone. This problem is compounded considering female carriers of WNV are capable of vertical transmission (Farajollahi et al., 2011), creating a new generation of disease-carrying vectors.

At some point during their adult lives, *Cx. p. molestus* come above ground to look for food. Males seek out nectar while females look for blood. Searching behavior is the term used to describe behavior exhibited by insects while seeking resources. Three factors converge to comprise the specifics of a species' searching behavior patterns: (i) physiological capabilities and genetic programming, including locomotory capability, sensory perception, and innate responses/behaviors, (ii) environmental factors such as stimuli and resource availability, and (iii) internal factors dictating the insect's immediate needs, such as resource requirements or mating

drive (Bell, 1990). Therefore, searching is a complex behavior controlled by the confluence of natural rhythms, physiological state, and external stimuli. For example, since circadian rhythm can govern behavioral thresholds, an insect may need to feed yet will not search for food if its circadian rhythm dictates an inactive period (Payne et al., 1970).

Intraspecific variation in searching behavior can be attributed to the development of an individual searching strategy, which is “a set of rules stipulating which alternative behavioral pattern will be adopted in any situation through life” (Dominey, 1984). Alternate strategies are employed based on how advantageous a behavior is when considered in conjunction with factors such as physiological state, environmental condition, age, sex, etc. Insects will often change their behavior when faced with a new set of parameters (Bell, 1990). The mosquito species *Aedes aegypti* will cease searching out a blood meal and begin searching for favorable oviposition sites due to hormonal changes (Klowden and Lea, 1979). Physiological status is a significant factor in the type and level of activity shown by *Culex* species. Roberts (2010) has demonstrated that mating status has a causal relationship with flight versus resting patterns. Additionally, a study done in 1991 by Klowden and Chambers indicates substances introduced during insemination of *Cx. pipiens f. molestus* virgins affect oviposition, egg development, and host-seeking in females. Inseminated females react differently to circadian rhythms, decreasing diurnal and increasing nocturnal activity (Chiba et al., 1992).

Across the Culicidae family, the preferred photoperiod of host-seeking is species specific and phototactic cues are thought to initiate the process (Kawada et al., 2006). However, little is understood about the foraging and orientation cues that influence behavioral patterns of subterranean dwelling *Cx. pipiens f. molestus* across the sexes and at different reproductive

stages. This study is intended to test for phototaxis and determine whether there are differences in phototaxis-initiated searching behavior based on sex and reproductive stage. I designed an experiment to determine if phototactic cues are used by *Cx. molestus* to navigate underground systems in search of blood meals and to compare male and female reactions to phototactic stimuli during different reproductive stages (i.e., virgin vs. mated). Post-reproductive females may have a stronger phototactic response due to their need to feed after their initial clutch is laid. Conversely, pre-reproductive females may have a weaker phototactic response since their primary goal, initially, is to reproduce rather than feed. Both females and nectar-feeding males are of interest due to their hybridization potential with *Cx. p. pipiens*. My hypothesis is that there will be a phototactic response within the population, however, the strength of the response will be affected by sex and/or reproductive state.

## **Methods**

### *Collection and Maintenance*

I subjected a total of 280 mosquitoes, descended from a colony collected from New York, NY, to experimental trials between January 2019 and May 2019. I continuously bred mosquitoes in Montclair State University's entomology laboratory throughout the duration of the experiment. I fed larvae a diet of TetraMin<sup>®</sup> Tropical fish flakes. The amount and frequency were dictated by the density of each breeding container's population. Adults were fed an 8% sucrose solution. Subjects were assigned to one of four treatment groups: virgin males (VM), virgin females (VF), mated males (MM) and mated females (MF). I housed MF and MM together in a dome-shaped BugDorm<sup>®</sup> insect rearing tent measuring 60 cm<sup>3</sup>. Once a week I

introduced a shallow, black breeding container (19 cm W x 12 cm L x 5 cm H), filled approximately 3 cm deep with de-chlorinated water and TetraMin<sup>®</sup> fish flakes for the purpose of oviposition. I removed the container after 2-4 days. After hatching, when the larvae reached the second instar (usually within 2-3 days), I transferred them to a 500 ml larval rearing container with a water depth of 8.5 cm. I obtained virgins by isolating individual pupae via pipette and, after eclosion, placing adults in either all male or all female BugDorm<sup>®</sup> Insect Rearing Cages measuring 30 cm<sup>3</sup>.

### *Behavioral Trials*

Experimental trials began March 13, 2019 and ended on May 9, 2019. I began behavioral trials by investigating whether there was potential for inherent room bias within each of the four groups that could ultimately interfere with the legitimacy of the results. These initial trials also served as a control for the light trials (described below), testing whether there was an affinity for either collection containers in the absence of light. I collected and separated mosquitoes based on sex and reproductive stage while they were anesthetized using CO<sub>2</sub> gas. I then placed them in a glass collection jar and, after they recovered from anesthesia, I introduced them into the center of a 2.44 m long clear plastic tube with a 16 cm circumference. At each end of the tube were funnels (7 cm long with one end circumference of 18 cm and the other end circumference of 3 cm) leading into glass collecting jars (L=12.8 cm., C=18 cm.) (Figure 1). Once a mosquito entered the collecting jar it was unlikely to exit due to the shape of the funnel. Black out fabric surrounded both tubes and no light was introduced into the apparatus. The goal of these control trials was to ensure that, after 24 hours, the mosquitoes would behave independently and be

randomly located throughout the length of the tube. Trials were run simultaneously in identical apparatus, A and B. I ran a total of four trials with 80 mosquitoes, 20 from each of the four groups. Each trial consisted of 10 mosquitoes from one group in apparatus A and 10 mosquitoes from another group in apparatus B.



**Figure 1.** Apparatus A and B. Mosquitoes were introduced to the tube in the top, center, jar. A light was placed at one end of each tube, on the outside of blackout material. Holes were cut into the material to let in a limited amount of light. Collection jars at either end of each tube held mosquitoes. Exiting the jar was inhibited by the funnel shape of the entrance.

I conducted the main trials in the same fashion as the control trials with a few exceptions. At one end a 2 cm round hole was cut into the fabric. A Cree® full spectrum, 460 lumen light bulb (CRI rating - 90, Temperature rating - 5,000K) was placed 5 cm from the end of the collection jar, outside of the fabric, directly adjacent to the hole. The light was turned on and the mosquitoes were left for a period of 24 hours. The location of each mosquito was then recorded.

For the main trials, 200 total mosquitoes were tested, 50 from each of the four groups. There were a total of 10 trials. Trials for each mosquito group (VM, VF, MM, MF) were run on both apparatus A and apparatus B (See Table 1). During each trial, 10 mosquitoes from the same group were put in apparatus A and 10 mosquitoes from another group were put in apparatus B. In order to limit confounding variables, the light was switched from one end of the apparatus to the other after the fifth trial. Each group was tested five times. Groups were tested twice with the light on one end and three times with the light on the other end. A sixth test for each group, in order to get an equal number of data points for trials with the light on the left and trials with the light on the right, was not possible due to the limited number of mosquitoes I had to work with. These trials were run to (a) establish the collective population's phototactic response, and (b) to compare each of the four groups to other biologically significant groups in order to identify any differences in behavior based on mating status and/or sex.

### *Analytical Methods*

I analyzed the control trial data, from the experiments using no light, (Appendix Table 1) using Pearson's Chi Square test (Appendix Equation 1). I ran the analysis using all four groups in the data set to determine if there was an overall difference in the observed versus the expected data. My expectation for the purposes of performing the analysis was that there would be no significant difference between the observed location of mosquitoes, and that half of the group would move to the left section of the apparatus and half would move to the right.

For the main trial data (Appendix Table 2) I again used Pearson's Chi Square test, using only light and dark data, to test the hypothesis that there is a phototactic response within the

species. My expectation for the purposes of performing the analysis was that there would be no significant difference in the number of mosquitoes, per group, that were found in the collection jar at the light end of the tube versus the number of mosquitoes that were found in the collection jar at the end of the tube with no light. The p value was calculated for each group based on the chi square value and the degrees of freedom.

Finally, the Chi Square Test for Homogeneity was used to determine if there was a difference in the distribution of the main trial behavior data (Appendix Table 2) when biologically significant groups were compared to one another. Groups were compared pairwise, for a total of four analyses (Appendix Table 3). The Chi-Square statistic was converted to its p-value based on the degrees of freedom and the critical value. The p-value was calculated using GraphPad.com. The overall alpha level was set at 0.05. Because each analyzed pair was tested against two dependent variables (light and dark), Bonferroni's correction was calculated, and the adjusted alpha was set at 0.025.

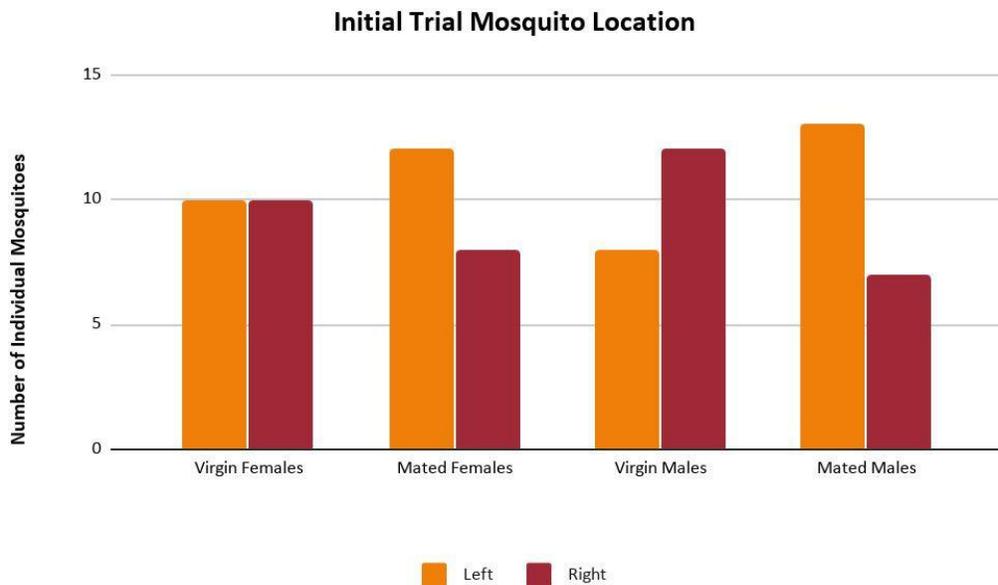
## **Results**

### *Control Trial, test for inherent bias*

The Chi-Square Goodness of Fit test was used to determine whether the actual location of individual mosquitoes at the end of the trials differed from the expected location if mosquitoes were moving at random. Mosquitoes considered to be on either the left or right side included mosquitoes both in the tube and the container on the respective side. In all analyses, no significant difference was shown between the observed and the expected locations of VF ( $p = 1$ ), MF ( $p = 0.3711$ ), VM ( $p = 0.3711$ ), or MM ( $p = 0.1797$ ) (Figure 2) (Appendix Table 1).

*Main Trial, test for phototactic response*

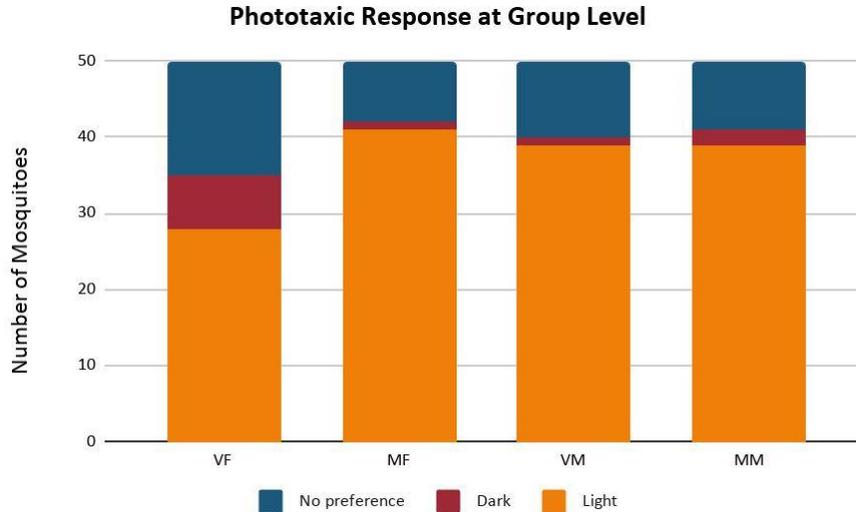
The Chi Square Goodness of Fit test was used to look for a phototactic response within each subgroup of the population. Only data from mosquitoes exhibiting a strong preference for light or dark (within collecting jars) were considered. Mosquitoes exhibiting little or no preference (mosquitoes in tube) were excluded from these data, as they may have been weak or injured (Figure 3). The statistical analysis was calculated with the expectation that there would be no difference between the number of mosquitoes in the collecting jar on the side of the tube with the light when compared with the number of mosquitoes in the collecting jar on the side of the tube with no light. Data for VF ( $p = 0.0004$ ), MF ( $p < 0.0001$ ), VM ( $p = 0.0001$ ), and MM ( $p = 0.0001$ ) showed statistical significance.



**Figure 2.** Initial trial mosquito location data. After a 24 hour period in a dark tube, mosquito location was noted to look for inherent bias or aggregation.

*Main Trial, test for behavioral differences between subgroups*

Using contingency tables, four group combinations were compared to look for differences in the data distribution of light and dark preference (Figure 3). Compared groups included VF to MF, VF to VM, MF to MM, and VM to MM. Groups not compared were VF to MM and VM to MF, since these comparisons are not biologically significant for my purposes. Only data from mosquitoes exhibiting a strong preference for light or dark (within collecting jars) were considered. Mosquitoes exhibiting little or no preference were excluded from these data (mosquitoes in tube) (Figure 3). The Chi Square Test for Homogeneity was used to determine statistical significance. Significant results were found when comparing VF to MF ( $p = 0.0116$ ) and VF to VM ( $p = 0.0143$ ). No significance was found comparing VM to MM ( $p = 0.571$ ), or MM to MF ( $p = 0.5423$ ).



**Figure 3.** Phototactic Response at Group Level. Individual mosquito preference by category during phototactic stimulation.

## Discussion

The results of the initial trials were as expected. Mosquitoes exhibited independent locomotion, evidenced by the random nature of their locations after a period of 24 hours. When compared, there were no groups that displayed significant preferences, indicating the absence of inherent experimental design biases. Additionally, observations suggest the absence of aggregate behavior, as there were no groupings or clusterings.

The main trials showed a strong phototactic response within the population, held across all four of the population subgroups. This result suggests that light cues potentially play an important role in initiating seeking behavior in *Culex pipiens f. molestus*. However, virgin females showed signs of having a relatively inhibited phototactic response when compared to the other subgroups. This is not unexpected since an organism's success is based on their ability to reproduce and leave behind viable offspring (Browne, 1993). There is a constant trade-off between reproduction and survival seen across species. Therefore, behavior responds to the need to obtain resources required for reproduction while limiting risk factors that increase the risk of mortality (Stone et al., 2012, Browne, 1993, Fernandez & Klowden, 1995, Grant & O'Connell, 2007, Bell, 1990). Seeking a blood meal comes with inherent risks. Hosts are generally vigilant against mosquito attacks and defend themselves, resulting in high mortality during feeding (Edman & Scott, 1987). Being autogenous, *Culex pipiens molestus* does not require a blood meal prior to laying their first brood of eggs. Reacting to a host-seeking stimulus would lead to unnecessary and risky behavior while jeopardizing reproductive success (Browne, 1993, Klowden, 1990). A study done by Iwanaga-Sawabe and Kanda (1990) on female, virgin,

blood-starved *Anopheles balabacensis* showed that, when given the choice, mating took precedence over blood feeding. Behavior has evolved to avoid risk until it is required.

Virgin female behavior, in this study, is an example of intraspecies behavioral variation, which is the result of how advantageous a behavior is (Klowden & Lea, 1979). Environmental cues and endogenous factors, such as state of development, insemination, sex, circadian rhythm, and need (i.e. hunger, homeostasis, mating, etc.), are integrated within the central nervous system. Sensory receptors are then activated in a way designed to mediate risk assessment associated with fixed action patterns (Browne, 1993, Fernandez & Klowden, 1995, Klowden, 1990, Bell, 1990). Therefore, behavior can change with priorities (Browne, 1993).

The physiological processes that control these behavioral variations are complex and varied. There is some evidence that the development of sensory systems may be delayed in order to prioritize reproduction and defer host-seeking (Grant & O'Connell, 2007). Additionally, proteins introduced by the male accessory gland (MAG) during copulation are known to modulate a number of behaviors in different dipteran species (Dattorini et al., 2007). Analysis of the mosquito (*Anopheles gambiae*) genome uncovered 46 MAG proteins, many of which are orthologous to *Drosophila melanogaster* MAG proteins that are known to be regulators of female behavior (Dattorini et al., 2007, Klowden, 1999). Studies have shown that these proteins stimulate ovulation and oviposition (Leahy & Craig, 1965, Fuchs & Kang, 1978) and modify host-seeking behavior (Fernandez & Klowden, 1995). They alter circadian rhythm in *Anopheles gambiae* (Jones and Gubbins, 1977), *Aedes aegypti* (Jones, 1981), *Anopheles stephensi* (Rowland, 1989), *Culex quinquefasciatus* (Jones and Gubbins, 1979), and *Culex pipiens molestus* (Chiba et al., 1990 & 1992). MAG proteins also inhibit female receptivity to mating. A

study done by Craig (1967) suggested *Aedes aegypti* female proclivity toward mating decreased after being implanted with MAG substances. Indeed, this effect was not species specific. When female *Aedes aegypti* were introduced to MAG proteins from a number of other mosquito species the effect was the same. Therefore, this mechanism is prevalent throughout the Culicidae family.

For anautogenous mosquitoes that do require a bloodmeal before oviposition, host-seeking is the default behavior (Klowden, 1999). MAG proteins and hormones produced during oogenesis inhibit host seeking and cause females to prioritize oviposition, which suggests adaptive significance for these factors (Klowden and Chambers, 1991, Fernandez & Klowden, 1995). It follows that, in autogenous mosquitoes like *Culex pipiens molestus*, where oviposition occurs prior to taking a bloodmeal, reproduction is the default behavior. MAG proteins of *Culex pipiens molestus* potentially play a role in lowering the action potential threshold of neuron membranes in the central nervous system that are responsible for initiating host-seeking behavior following a phototaxic cue.

The physiological explanation of these data is likely multifactorial. The natural delay in the development of the sensory system in order to minimize risk and maximize benefit indicates age as a primary behavioral factor. A study done by Grant and O'Connell (2007) showed older *Aedes aegypti* females were more responsive to host-seeking stimuli. However, if age were significant in this study, similar results would have been shown across all four categories, as age was random. Age was also not recorded so I am unable to completely dismiss this as a contributing factor. Additionally, I assumed that all mated females had oviposited since they were given ample time and had access to oviposition trays. Due to the size of the colony and the

nature of their living situation, individual oviposition was also not recorded. These data would have been useful in ensuring all mated female behavior was not influenced by individuals within the population carrying fertilized eggs.

A follow-up study should follow similar procedures, comparing virgin females to females of the same age that have been artificially introduced to MAG proteins. Virgin females should also be directly compared to mated females that have oviposited and are of the same age. This study would help clarify whether MAG proteins or the post-oviposition stage of reproduction is more influential in the female *Culex p. molestus* response to phototactic cues.

Understanding both the male and female roles in the host-seeking behavior of this medically important species is helpful in understanding and controlling the spread of disease. The feeding pattern of *Cx. p. molestus* and *Cx. p. pipiens* results in highly competent bridge vectors that increase the frequency of incidental transmission of WNV to human hosts (Pinho et al, 2013, Kilpatrick et al., 2007). In the United States, as bird populations dwindle in northern regions during late summer and early fall, most notably, the American Robin, there is a shift in the feeding behavior of *Cx. p. pipiens* from bird host to human host (Pinho et al, 2013, Kilpatrick et al., 2007). Opportunistic patterns of feeding are likely influenced by genetics, as *Cx. p. molestus* ancestry increases the frequency of feeding on human hosts (Kilpatrick et al. 2007). Understanding searching cues and behavior is crucial for the development and implementation of novel strategies that look to limit the spread of mosquito borne disease.

## References

- Barredo, E., & DeGennaro, M. (2020). Not Just from Blood: Mosquito Nutrient Acquisition from Nectar Sources. *Trends in Parasitology*.
- Bell, W. J. (1990). Searching behavior patterns in insects. *Annual review of entomology*, 35(1), 447-46
- Browne, L. B. (1993). Physiologically induced changes in resource-oriented behavior. *Annual Review of Entomology*, 38(1), 1-23.
- Byrne, Katharine and Nichols, Richard A. (1999). *Culex pipiens* in the London Underground tunnels: Differentiation between surface and subterranean populations. *Heredity*, 82, 7-15.
- Chiba, Y., Shinkawa, Y., Yoshii, M., Matsumoto, A., Tomioka, K., & Takahashi, S.Y. (1992). A comparative study on insemination dependency of circadian activity pattern in mosquitoes. *Physiological entomology*, 17(3), 213-218.
- Chiba, Y., Yamamoto, Y., Shimizu, C., Zaitu, M., Uki, M., Yoshii, M., & Tomioka, K. (1990). Insemination-dependent modification of circadian activity of the mosquito, *Culex pipiens pallens*. *Zoological science*, 7(5), 895-906.
- Colpitts, T. M., Conway, M. J., Montgomery, R. R., & Fikrig, E. (2012). West Nile Virus: biology, transmission, and human infection. *Clinical microbiology reviews*, 25(4), 635-648.
- Craig, G. B. (1967). Mosquitoes: female monogamy induced by male accessory gland substance. *Science*, 156(3781), 1499-1501.
- Barrett, A. D. (2014). Economic burden of West Nile virus in the United States. *The American journal of tropical medicine and hygiene*, 90(3), 389.
- Dominey, W. J. (1984). Alternative mating tactics and evolutionarily stable strategies. *American Zoologist*, 24(2), 385-396.
- Dottorini, T., Nicolaides, L., Ranson, H., Rogers, D. W., Crisanti, A., & Catteruccia, F. (2007). A genome-wide analysis in *Anopheles gambiae* mosquitoes reveals 46 male accessory gland genes, possible modulators of female behavior. *Proceedings of the National Academy of Sciences*, 104(41), 16215-16220.
- Farajollahi, A., Fonseca, D. M., Kramer, L. D., & Kilpatrick, A. M. (2011). “Bird biting” mosquitoes and human disease: A review of the role of *Culex Pipiens* complex mosquitoes in epidemiology. *Infection, Genetics and Evolution: Journal of Molecular Epidemiology and Evolutionary Genetics in Infectious Diseases*, 11(7), 1577–1585.
- Fernandez, N. M., & Klowden, M. J. (1995). Male accessory gland substances modify the host-seeking behavior of gravid *Aedes aegypti* mosquitoes. *Journal of Insect Physiology*, 41(11), 965-970.

- Fuchs, M. S., & Kang, S. H. (1978). Evidence for a naturally occurring inhibitor of oviposition in *Aedes aegypti*. *Annals of the Entomological Society of America*, 71(4), 473-475.
- Gomes, B., Sousa, C.A., Vicente, J., Pinho, L., Calderon, I., Arez, E., Almeida, A., Donnelly, M., & Pinto, J. (2013). Feeding patterns of molestus and pipiens forms of *Culex pipiens* (Diptera: Culicidae) in a region of high hybridization. *Parasites & Vectors* 93(6), <https://doi.org/10.1186/1756-3305-6-93>
- Grant, A. J., & O'Connell, R. J. (2007). Age-related changes in female mosquito carbon dioxide detection. *Journal of medical entomology*, 44(4), 617-623.
- Iwanaga-Sawabe, K., & Kanda, T. (1990). Studies on the Effective Attractants of Anopheline Mosquitoes: I. Blood-Feeding, Mating and Flight Activity of Female *Anopheles balabacensis* (Diptera: Culicidae). *Applied Entomology and Zoology*, 25(2), 231-238.
- Jones, M. D. R. 1981. The programming of circadian flight-activity in relation to mating and the gonotrophic cycle in the mosquito, *Aedes aegypti*. *Physiol. Entomol.* 6:307-313.
- Jones, M. D. R., & Gubbins, S. J. (1977). Modification of circadian flight activity in the mosquito *Anopheles gambiae* after insemination. *Nature*, 268(5622), 731-732.
- Jones, M. D. R., & Gubbins, S. J. (1979). Modification of female circadian flight-activity by a male accessory gland pheromone in the mosquito, *Culex pipiens quinquefasciatus*. *Physiological Entomology*, 4(4), 345-351.
- Kassim, N. F. A., Webb, C. E., & Russell, R. C. (2012). Is the expression of autogeny by *Culex molestus* Forskal (Diptera: Culicidae) influenced by larval nutrition or by adult mating, sugar feeding, or blood feeding?. *Journal of Vector Ecology*, 37(1), 162-171.
- Kawada, H., Tatsuta, H., Arikawa, K., & Takagi, M. (2006). Comparative study on the relationship between photoperiodic host-seeking behavioral patterns and the eye parameters of mosquitoes. *Journal of insect physiology*, 52(1), 67-75.
- Kilpatrick, A. M., Kramer, L. D., Jones, M. J., Marra, P. P., Daszak, P., & Fonseca, D. M. (2007). Genetic influences on mosquito feeding behavior and the emergence of zoonotic pathogens. *The American journal of tropical medicine and hygiene*, 77(4), 667-671.
- Klowden, M. J. (1990). The endogenous regulation of mosquito reproductive behavior. *Experientia*, 46(7), 660-670.
- Klowden, M. J. (1999). The check is in the male: male mosquitoes affect female physiology and behavior. *Journal of the American Mosquito Control Association-Mosquito News*, 15(2), 213-220.
- Klowden, M. J., & Chambers, G. M. (1991). Male accessory gland substances activate egg development in nutritionally stressed *Aedes aegypti* mosquitoes. *Journal of Insect Physiology*, 37(10), 721-726.
- Klowden, M. J., & Lea, A. O. (1979). Humoral inhibition of host-seeking in *Aedes aegypti* during oöcyte maturation. *Journal of Insect Physiology*, 25(3), 231-235.

- Leahy, M. G., & Craig Jr, G. B. (1965). Accessory Gland Substance as a Stimulant for Oviposition in *Aedes aegypti* and *A. albopictus*. *Mosquito News*, 25(4), 448-52.
- Payne, T. L., Shorey, H. H., & Gaston, L. K. (1970). Sex pheromones of noctuid moths: factors influencing antennal responsiveness in males of *Trichoplusia ni*. *Journal of insect physiology*, 16(6), 1043-1055.
- Roberts, D. M. (2010). Comparison of the Physiological age of Adult Resting Versus Flying *Culex quinquefasciatus* (Diptera: Culicidae). *Sultan Qaboos University Journal for Science [SQUJS]*, 15, 9-17.
- Rowland, M. (1989). Changes in the circadian flight activity of the mosquito *Anopheles stephensi* associated with insemination, blood-feeding, oviposition and nocturnal light intensity. *Physiological entomology*, 14(1), 77-84.
- Spickler, A.R. (2013). West Nile Virus Infection. Retrieved from [http://www.cfsph.iastate.edu/Factsheets/pdfs/west\\_nile\\_fever.pdf](http://www.cfsph.iastate.edu/Factsheets/pdfs/west_nile_fever.pdf)
- Vinogradova, E. B. (2000). *Culex pipiens pipiens mosquitoes: taxonomy, distribution, ecology, physiology, genetics, applied importance and control* (No. 2). Pensoft Publishers.
- Vinogradova, E. B. (2003). Ecophysiological and morphological variations in mosquitoes of the *Culex pipiens* complex (Diptera: Culicidae). *Acta Soc Zool Bohem*, 67(March), 41-50.
- World Health Organization: WHO. (2017, October 3). West Nile virus. Retrieved from <https://www.who.int/news-room/fact-sheets/detail/west-nile-virus>
- Žiegytė, R., Bernotienė, R., Bukauskaitė, D., Palinauskas, V., Iezhova, T., & Valkiūnas, G. (2014). Complete sporogony of *Plasmodium relictum* (lineages pSGS1 and pGRW11) in mosquito *Culex pipiens pipiens* form *molestus*, with implications to avian malaria epidemiology. *The Journal of Parasitology*, 100(6), 878-882.

## Appendix

Appendix Table 1. Initial trial data.

	Virgin Females		Mated Females		Virgin Males		Mated Males	
	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.
Left Side	10	10	12	10	8	10	13	10
Right Side	10	10	8	10	12	10	7	10
P-value	1		0.3711		0.3711		0.1797	

Appendix Table 2. Main trial data.

Trial *	Virgin Males			Virgin Females			Mated Males			Mated Females			Apparatus	
	Light	Dark	Tube	Light	Dark	Tube	Light	Dark	Tube	Light	Dark	Tube	A	B
1				6	0	4				10	0	0	VF	MF
2				6	3	1				7	0	3	MF	VF
3	6	0	4				7	1	2				VM	MM
4	8	0	2				8	0	2				MM	VM
5							9	0	1	9	0	1	MM	MF
6							5	1	4	8	0	2	MF	MM
7	9	0	1	3	0	7							VF	VM
8	9	0	1	6	2	2							VM	VF
9							10	0	0	7	1	2	MF	MM
10	7	1	2	7	2	1							VM	VF

Appendix Table 3. Contingency tables for biologically significant pairs

<b>Chi<sup>2</sup> Contingency Table for VF vs. MF</b>				<b>Chi<sup>2</sup> Contingency Table for VM vs. MM</b>			
Vcrit (for p-value 0.05)=3.841, df=1				Vcrit (for p-value 0.05)=3.841, df=1			
$\chi^2 = 6.365519324$				$\chi^2 = 0.3210365854$			
	VF	MF	Total		VM	MM	Total
Light preference	28	41	69	Light preference	39	39	78
Dark preference	7	1	8	Dark preference	1	2	3
Total	35	42	77	Total	40	41	81

<b>Chi<sup>2</sup> Contingency Table for VF vs. VM</b>				<b>Chi<sup>2</sup> Contingency Table for MM vs. MF</b>			
Vcrit (for p-value 0.05)=3.841, df=1				Vcrit (for p-value 0.05)=3.841, df=1			
$\chi^2 = 5.999300373$				$\chi^2 = 0.3713390437$			
	VF	VM	Total		MM	MF	Total
Light preference	28	39	67	Light preference	39	41	80
Dark preference	7	1	8	Dark preference	2	1	3
Total	35	40	75	Total	41	42	83