The Effect of Artificial Blood Meals on *Culex quinquefasciatus* Fecundity in Colony

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Abstract: Artificial membrane feeding is critical in the maintenance of mosquito colonies. Feeding artificially is said to be as effective as live feeding and has replaced most types of live feeding in laboratories for years, however there is a lack of studies supporting this. *Culex quinquefasciatus* (Diptera:Culicidae) is often used for experiments due to its abundance and ability to vector many medically significant diseases. To study the difference in *Culex quinquefasciatus* fecundity between live feeding and artificial membrane feeding separate cohorts were used. A cohort for live feeding, one for artificial feeding, and one for exsanguinated blood feeding. The fecundity and fertility of each mosquito was observed in each cohort. In this study we found that live feeding resulting in the highest mean egg per raft ratio. This is possibly due to the chemical treatment of blood with the introduction of citrate to prevent clotting. Whether the reduction in egg production is due to the ingestion of chemical or the lack of natural clotting within the mosquito is unclear and would require further research.

Keywords: *Culex quinquefasciatus*, fecundity, fertility, live, feeding

Introduction

Artificial membrane feeding is an economical and ethical method of hematophagous arthropod colony upkeep. Prior to research conducted in the 1970's, artificial membrane feeders were not frequently utilized as they were not abundant. After their development, multiple studies were conducted to demonstrate their effectiveness, and find a method away from the use of live animals (Rutledge et al. 1964, Deng et al. 2012, Luo 2014). During the completion of these studies, there was heavy pressure to switch to the artificial membrane feeding. Since then, they have become a standard practice for the blood feeding of arthropods such as mosquitoes and black flies. While there were multiple studies that demonstrated that artificial feeding is a viable option (Galun 1967, Bailey et al. 1980), there were also studies that demonstrated that live hosts were superior by multiple mechanisms (Pothikasikorn et al. 2010). Phasomkusolsil et al. 2013 shows that feeding rate, survival, fecundity, and hatching rate of mosquitoes were all lower when fed artificial blood meals from animals like sheep (Phasomkusolsil et al. 2013). This study also shows that there is a reduction in larval fitness from parental mosquitoes feeding on artificial vertebrate

blood meals. These show that while artificial feeding is a viable alternative, there is a consequence to mosquito fitness when attempted to keep them in colony. A more recent study suggests that the source of the blood meal does have an effect on the processes that lead to reproduction and can significantly affect fecundity and fertility. (Richards et al. 2010) This study aims to identify any differences between mosquito fecundity in artificial blood meals and live host mosquito feeding, without the bias of evaluating suitable alternatives of blood feeding. From prior studies, it is predicted that mosquito feeding on a live host will produce more eggs, and those eggs will also have a higher hatching rate.

Objectives

1. Identify any differences in mosquito fecundity after feeding on artificial blood meal and live host blood meals.
2. Identify any differences in egg hatch rate between mosquitoes feeding on artificial and live host blood meals.

Materials and Methods

Mosquitoes

A *Culex quinquefasciatus* Sebering colony was utilized for this experiment. Larval conditions were consistent for all mosquitoes at 27° Celsius with a larval food preparation of 12g of liver powder and 8g of yeast per 100ml. All mosquitoes utilized for experiments were not blood fed until experimentation.

Blood Feeding Experiment

Between 200-300 *Culex quinquefasciatus* pupae were isolated and put into a container to emerge. The container was monitored for a week and adults were given a 10% sucrose solution to drink. Once the adults were a week old the container was covered with a trash bag and placed inside of a cooler surrounded by ice for 5 minutes. The container was then taken out and the mosquitoes were separated into males and females. The males were discarded, and the females were further separated into 3 equally sized cohorts. Each cohort was placed in its own soup cup with a mesh lid. Cohorts were labeled EXS, LC, and ART respectively. Each cohort was offered either whole chicken blood freshly extracted from a chicken in an artificial feeder treated with heparin (EXS), blood from a live chicken (LC), or commercially purchased chicken blood on an artificial feeder consistent with the rearing of our other mosquitoes (ART). Blood meals were prepared by placing parafilm held down with an o-ring over two reservoirs. One was filled with ART blood and the other with EXS blood. Medical tape was used to tie down a live chicken by the feet and wings to a notecard. Each container was fed with their respective blood types. The ART and EXS cohorts were fed for an hour and the LC cohorts were fed with two chickens continuously, each for 30 minutes. Cohorts were taken individually after feeding and placed in trash bags and put in a cooler for five minutes. Only fully engorged mosquitos were taken out of the cohort and put in an individual cupboard cups to keep track of individual fecundity. Each cupboard cup contained a smaller cup for water to lay eggs

in. This was repeated with all three cohorts. Each mosquito was given a number to keep track of type of blood fed on. Individual mosquitoes were provided with a cotton ball soaked in 10% sucrose solution replaced daily. Cups of water inside individual cupboard cups were filled daily. Mosquitoes were observed daily and when rafts were laid the cup was taken out of the container. Of these mosquitoes, only those that lay eggs were added to analysis.

Fecundity & Fertility

Eggs were removed immediately after being laid. They were then taken to a dissecting microscope for a digital image to be taken. Eggs were counted from these images and recorded. Then, each egg raft was added to a cup of water with larval food. Three days was determined to be the cutoff of egg viability. Larvae were counted three days after egg raft collection using a petri dish and dissecting microscope in order to determine overall hatch rates.

Analysis

A one-way analysis of variance (ANOVA) was used to compare the mean egg masses laid by each mosquito in each treatment. Any significant outliers were removed from the study. An ANOVA of larval hatch rate was also conducted on the percent hatch means of each mosquito in each treatment

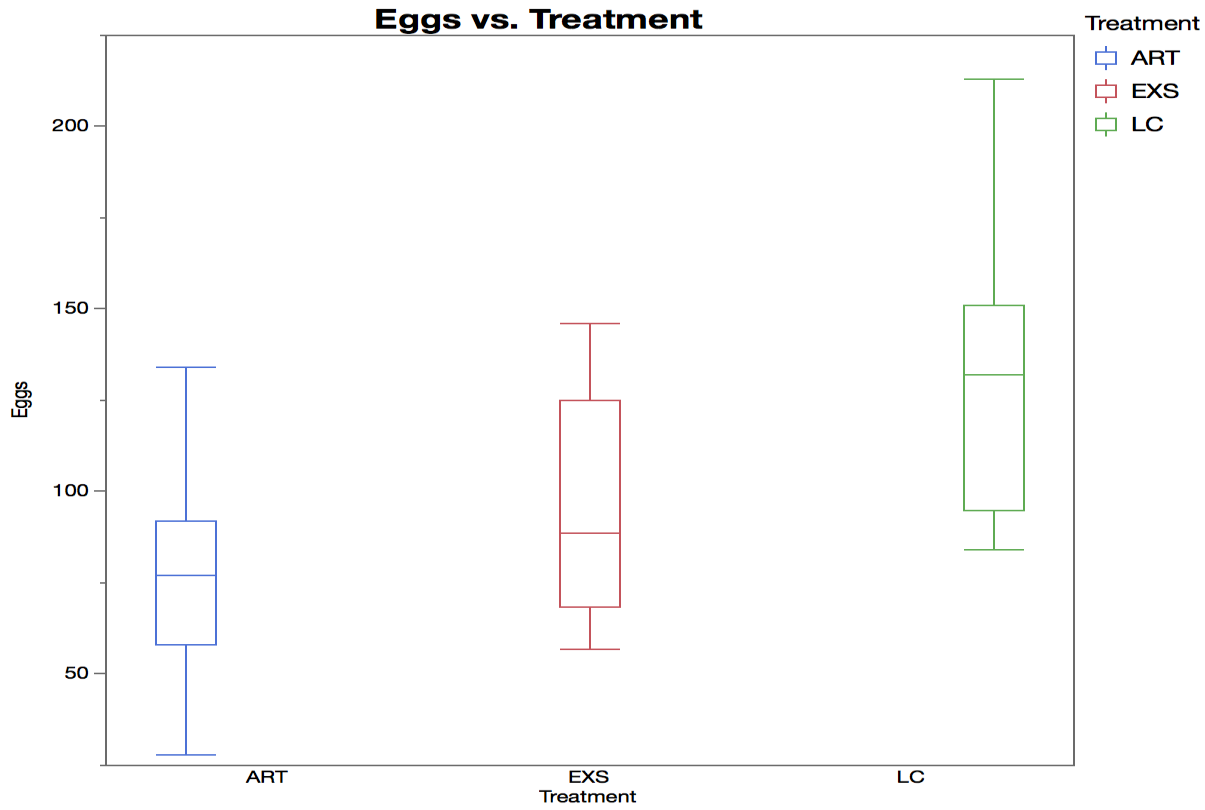

Fig. 1. Comparison of data gathered from amount of eggs per raft in each treatment. The cohort are listed in this order, artificial membrane (ART), exsanguinated (EXS), and Live Chicken (LC)

Results

Fecundity

Overall, there was a significant difference between the means of the egg masses laid by the treatments. Live chicken (LC) cohorts laid significantly more eggs than both the artificial membrane (ART) feeding (P<.0001) and the exsanguinated (EXS) chicken (P=.0003). There was not a significant difference between the exsanguinated chicken treatment and the artificial membrane feeding treatment (P=.1674). (Figure 1)

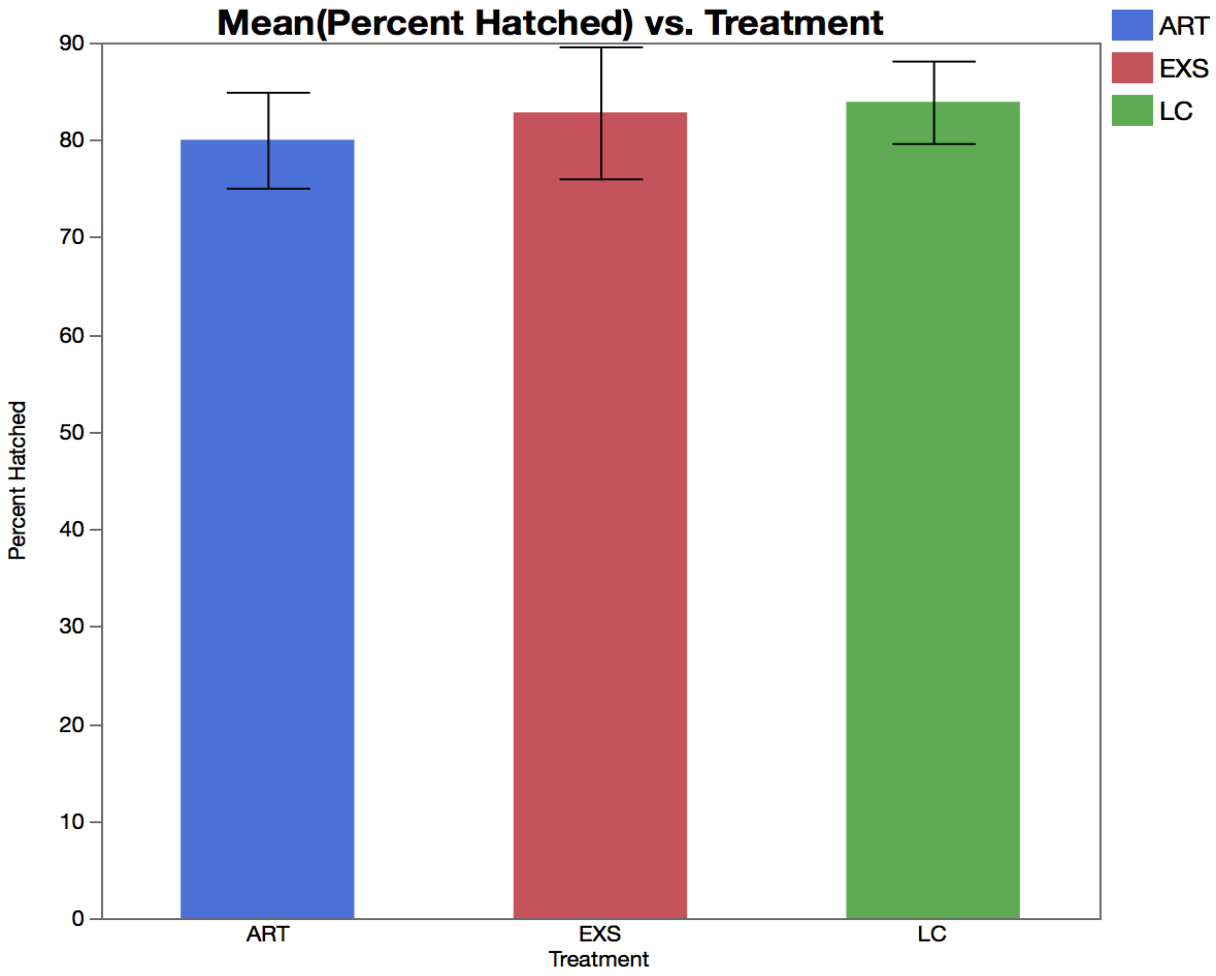

Fig. 2. Comparison of data of the mean (percent of eggs hatched) of each treatment.

Larval Hatching

There was not a significant difference in the number of larvae hatched between egg raft between treatments. The treatment hatch rates between the ART and EXS had a p-value of .7217, the hatch rates between ART and LC had a p-value of .5638, and the p-value between the hatch rates of LC and EXS were .8925. All treatments had between an 80-84% hatch rate (Figure 2).

Discussion

With these results, it is clear that blood meal source affects the fecundity of *Culex quinquefasciatus* mosquitoes. Those mosquitoes that fed on a live chick had a higher mean number of eggs per raft. Mosquitoes fed exsanguinated chicken blood through an artificial feeder also maintained an overall better number of eggs per raft and hatch rate than that of the commercially purchased chicken blood in artificial membrane. When comparing the number of eggs per raft our data shows that those that fed on live chicks had close to double the amount of those fed on the control (LC ~133 eggs/raft ; ART ~75 eggs/raft). This is possibly due to the chemical treatment of blood with the introduction of citrate to prevent clotting. Whether the reduction in egg production is due to the ingestion of chemical or the lack of natural clotting within the mosquito is unclear. Having a higher number of eggs per raft is vital in an attempt to artificially replicate the numbers that occur through natural reproduction in a controlled environment. Current artificial mechanisms used have been developed in order to best replicate the conditions in which mosquitoes feed naturally on live animals, but at a cost of mosquito fecundity as we have demonstrated. Further experiments and observations are necessary in order to comprehend what parameters affect these physiological processes and how we can improve those in order to get a similar result when compared to live-host feeding, which is not always readily available.

These results suggest that blood meal source does not have an impact on the viability of offspring once the eggs have been laid, since there was over an 80% hatch rate for all treatments. Further studies are needed to understand what processes impact larval viability.

Future studies should be done on other common species that are routinely reared in labs for experiments. This includes *Aedes aegypti*, *Aedes albopictus*, and other significant species.

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