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EFFECTS OF AGE AND BODY SIZE ON OVARIAN DEVELOPMENT AND FECUNDITY IN *CULEX QUINQUEFASCIATUS* (DIPTERA: CULICIDAE)

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Culex quinquefasciatus (Diptera: Culicidae) is a significant vector of bancroftian filariasis and an expected vector of *Dirofilaria immitis*. These types of mosquitoes are also a possible vector of arboviruses such as West Nile Virus (WNV) and Break Valley fever (Bhattacharya *et al.* 2016). According to the estimation of Ottesen & Ramachandran (1995) about 120 million people worldwide are infected with lymphatic filariae, especially those that cause bancroftian filariasis.

In many areas, mosquito control is getting better, but there are several impediments, such as the rise in pesticide resistance and the dearth of safe, affordable alternatives to pesticides. The need to extend the use of highly effective vector control compounds is necessitated by the rise in insecticide resistance (Kalimuthu *et al.* 2013). Traditional vector control strategies include reducing larval breeding habitats (WHO, 1997) and the use of insecticides, but these methods don't always work to lower the burden of disease (Ooi *et al.* 2006).

As there is presently no approved vaccine or antiviral medication available, vector control is the sole method of preventing these diseases (Swaminathan *et al*, 2009). Genetic control strategies to target disease vectors have received considerable attention over recent years. Therefore, a detailed understanding of mosquito mating biology is necessary to ascertain the minimum requirements that genetically modified mosquitoes must meet to succeed as well as the key performance indicators (Ferguson *et al.* 2005). The relationships between fecundity, age factors, and body size are less well understood. Numerous studies have looked at mosquito reproduction decreases with age, and a number of these have described similar decreases in different mosquito strains (Akoh *et al.* 1992).

To support genetically controlled vector control programs, we sought to ascertain the fecundity and gradual ovarian development in relation to age

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and body size. This work improves our understanding of the complex reproductive biology of mosquitoes and refines estimations of reproductive capacity for genetic control efforts. It also highlights the possible influence of age and body size on *Culex quinquefasciatus* ovary length. Genetic control over *Culex quinquefasciatus* egg-laying ability may be achieved if ovarian development can be stopped at a specific age or size. Further research using cutting-edge technologies is required to elucidate the impact of different insecticides and sperm transfer on ovary development for laboratory-reared and wild mosquitoes.

Larvae of wild *Culex quinquefasciatus* were collected from Savar, Dhaka-1342, Bangladesh, by using a hand-held dipper and kept in a plastic jar. The collected larvae were brought to the Insect Rearing and Environmental Station (IRES), Jahangirnagar University, and reared in a tray containing clean water (as culture medium) at 28 ± 2°C and 80 ± 5 % (RH) relative humidity and a photoperiod of 12:12 (L:D). When the larvae began to pupate, the pupae were transferred into a mosquito-rearing case (30 × 30 × 30 cm) in which the maximum adults emerge from the pupal case the following day. The adult mosquitoes were fed with 10% (Dextrose (D+)-Glucose) solution every day, and after 10 days of eclosion, the mosquitoes were fed with blood.

The adult mosquitoes of different age and body size were isolated, collected, and brought into the entomology laboratory and knocked down by putting them in the refrigerator for 15 minutes. Female reproductive organs (ovaries, Spermathecae and the bursa) were dissected after 3, 5, and 10 days eclosion of small, medium, and large size. For the approximate body sizes, wing lengths were measured. For staining, one drop of eosin was added to the sample for 30 seconds and then the stain was washed with saline water.

Stained organs were placed carefully in a drop of saline water on a microscale slide (1 mm). Then Organs on the slide were examined using a light microscope. Ovary lengths were measured by means of the scale attached to the slide. After measuring the ovary length, the ovarioles were freed carefully by slitting the ovarian sheath with fine pins (0.3 mm). Under a cover slip, the ovarioles were examined without compression and the ovariole diameter was taken. Dissected spermathecae and bursa on the microscopic slide were pressed slightly to tear the spermathecae and analysed for the presence of sperm. After 10 days of eclosion, the mosquitoes were fed blood. The ovaries were then dissected and analyzed after being fed blood for 24, 48, and 72 hours.

Data were recorded in a notebook and then entered and calculated all data in Microsoft Excel 2016. The ovary lengths, mean and standard error were calculated by the same software. The effect of age and body size on ovary length was also analysed by using a line chart in Microsoft Excel 2016.

The ovary length of larger and older female mosquitoes was greater than that of smaller ones (Fig. 1). The average ovary length of small 1-day-old females was 0.75 ± 0.03 mm, medium 0.76 ± 0.02 mm, and large 0.77 ± 0.02 mm; of small 3-days-old 0.86 ± 0.03 mm, medium 0.95 ± 0.02 mm, and large 0.99 ± 0.01mm; of small 5-days-old 1.01 ± 0.06 mm, medium 1.06 ± 0.07mm, and large 1.10 ± 0.06mm; of small 10-days-old 1.08 ± 0.07 mm, medium 1.14 ± 0.03mm; and large 1.15 ± 0.03mm (Figure 1).

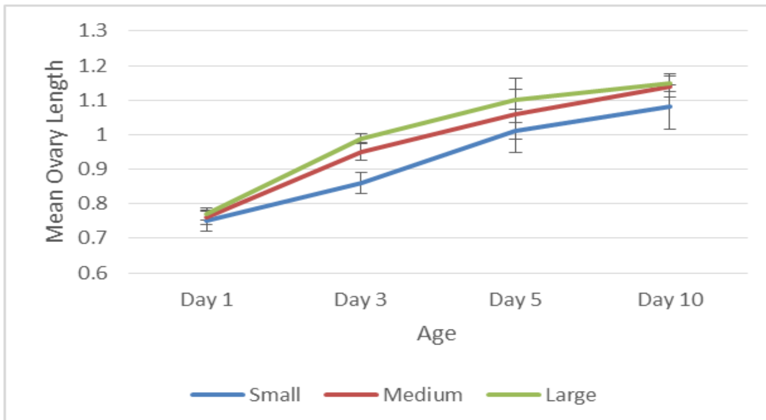

Fig. 1. Mean Ovary Length of Female *Culex quinquefasciatus* of Different Age (1, 3, 5, and 10 Days Old) and Body Size (Small, Medium, and Large)

Dissected spermathecae show numerous sperm stored since the female has already been copulated. The presence of sperm in the spermathecae indicates that the insemination status is positive (Figure 2). The ovary began to grow quickly after 10 days of eclosion when blood fed. Each ovariole grew into a fully formed egg that was ready to lay in approximately 72 hours (Figure 3).

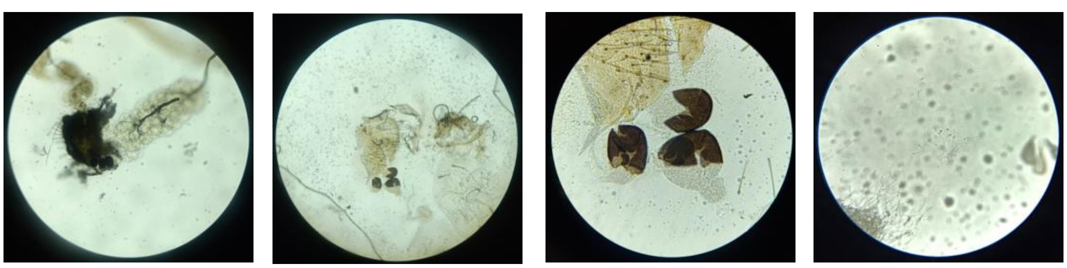

Fig. 2. Sperm and Spermathecae Analysis under Microscope

Our study indicates that age and body size effect the ovary length of *Culex quinquefasciatus*. The mosquitoes of larger body size have relatively larger ovary than the medium and smaller ones. The size of the uninfected female *Cx. quinquefasciatus* mosquitoes and their ability to produce eggs were found to be significantly correlated in a recent study(Lima *et al.* 2003). Afterward,

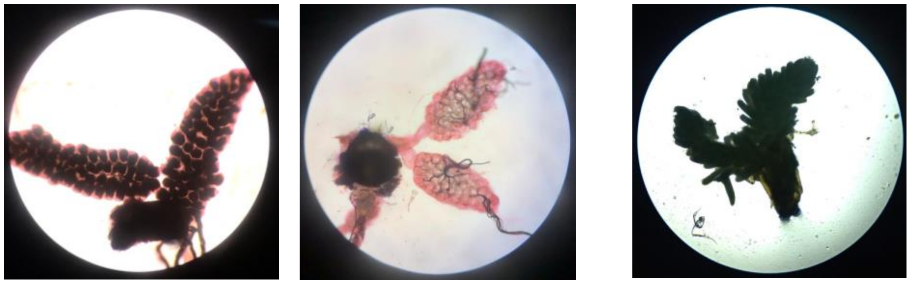

Fig. 3. Ovary Development After 24, 48, and 72 Hours of Blood Fed

the blood feeding of *Cx. quinquefasciatus*, microscopic analysis shows the ovaries rapid development and mature just after 72 hours. Among the factors influencing female fecundity, age and body size were the factors that showed the greatest effect in our results. Ponlawat and Harrington's (2007) study also shows the possible impact of age and body size on the mating capacity of *Aedes aegypti.* Based on our laboratory results, large 10-day-old females show larger ovaries. A similar study shows a positive correlation between fecundity and body size up to 13 days old (McCann *et al.* 2009).

For the better control of the diseases associated with *Culex quinquefasciatu*s, we defined the development of ovaries in relation to age and body size in this study. These findings provide insightful knowledge for optimizing genetic control methods by understanding mosquito ovary development based on age and size. To support genetically controlled vector control programs, we find out the optimal age and body size for female reproductive success.

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