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Rearing *Aedes aegypti* Mosquitoes in a Laboratory Setting

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Many species of insects have been used in a plethora of scientific studies.[3] Laboratory reared mosquitoes are particularly integral to arthropod-borne disease experiments, especially with the recent emergence in North America of Zika virus in the human population. *Aedes aegypti* (Figure 1) is the preferred model for researchers because of their capacity to spread viruses such as, Zika, dengue, chikungunya, and yellow fever[4] as well as their relatively short rearing times in a laboratory insectary and the ability to store their eggs for up to 6 mo.

At any given time, the insectary at the Centers for Disease Control (CDC) National Center for Emerging and Zoonotic Infectious Diseases, Division of Vector-Borne Diseases in Fort Collins, CO, has 7 different *Aedes aegypti* strains that are used in a variety of experiments, including vaccine and vector competence research.[2] The maturation process from egg to adult takes approximately 8 d. The 4 stages of development in the mosquito life cycle includes egg, larval, pupal, and adult stages (Figure 2). Eggs of *Aedes aegypti* can be stored in an airtight container on seed germination paper (The Bangalore Paper & Pulp Mills) (Figure 3) for up to 6 mo. After the eggs hatch, the immature larval and pupal stages follow (Figure 4). During the distinct larval growth there are four molting stages. The development and growth between the molts are known as instar stages 1–4 and each instar takes between 24–30 h to complete.

Ultimately the non-feeding pupal stage follows the fourth instar larval stage and lasts from 24 to 48 h. After pupae emerge to adult mosquitoes, they are provided a 10% sucrose and deionized water solution throughout their life using a repurposed baby food jar wrapped with a 4 × 4 in gauze, secured with a rubber band (Figure 5). The sucrose jar is then placed upside down on top of the holding cage and is replaced weekly. With each egg batch, females can lay upwards of 100–200 eggs and can generate up to 5 batches during a lifetime. Unlike some other mosquito species that lay a cluster of several hundred eggs in a raft on the water surface, the *Aedes aegypti* lay their eggs individually on a moist substrate. The number of eggs produced is dependent on the volume of the bloodmeal ingested. Blood meals are provided using a Hemotek Blood Feeding System (Discovery Workshops United Kingdom) (Figure 6).

Arthropod Rearing; From Egg to Adult

When a request for mosquitoes is received from a research scientist, eggs (on seed germination paper) are pulled from a sealed storage container. The egg papers (Figure 7) are submerged in deionized water in a 16 oz MicroGourmet cup (Reliable Paper Company) and placed in a desiccator attached to a vacuum line (Figure 8) for 10 min. Placing eggs in a desiccator stimulates the eggs to hatch by slowly decreasing the available oxygen and simulating bacterial growth[1] which significantly decreases the time it takes for eggs to hatch. First instar larvae begin to hatch from eggs almost immediately and may continue this process for up to 1.5 h.

The first instar larvae are poured into a 12 × 24 × 2 in larval pan (~300 larvae per pan) filled with 16 oz deionized and 16oz tap water then placed in an incubator maintained at 28° C with >80% humidity. Larvae are provided with the appropriate diet daily, which consists of ground fish flakes (Tetramin), rabbit pellets (TekLad), and liver powder (MP Biomedicals). This diet is sprinkled on the water surface starting at 0.5 grams in a volume of 32 oz on the first day and then gradually increased daily for 4 to 7 d depending on the number of larvae in the pan and any left-over food from the previous day. Once pupae develop, they are sieved or handpicked from the larval pan and transferred to a MicroGourmet cup filled halfway with deionized water. The cup is covered with the plastic lid and vinyl funnel of a Mosquito Breeder (BioQuip) then placed in a 12 × 12-in mesh cage with a lab mat liner (Figure 9). Pupae emerge into adults and are physically released into the cage daily. The holding cage is accessed through a fabric tube sock that is used to minimize the risk of mosquitoes escaping from the cage (Figure 10). Both female and male adults are maintained in this cage for their entire life cycle where mating takes place. At 5 d of age the female receives her first citrated sheep’s blood meal using a Hemotek Blood Feeding System heated to 37° C (Figure 6).

The day following a blood meal, seed germination paper is placed in a MicroGourmet cup with 1 in of deionized water and the cup is situated in the cage, the germination paper serves as an egg laying substrate. This seed germination paper is unique because of its excellent wet strength, moisture content, as well as its structure making it an ideal surface for the eggs to attach to in the egg collection stage. Prior to use, the paper should be free of toxins, evenly finished and free from bacteria and fungi by being stored in a dry and sealed container. The egg paper and cup are removed from the cage 3 d later. The paper is air dried for 30 min, and then stored in a sealed plastic bag and kept in an airtight storage container for up to 6 months. These storage containers are kept at ambient temperatures in an environment with >80 % humidity and are visually checked weekly for humidity and potential mold growth. Humidity is achieved by placing a 2 × 2 in sponge in each container and soaking with tap water as needed.

Summary

Mosquitoes play an important role in vector-borne disease research; their successful development and growth is pivotal to the efficacy and strength of the studies they support. While most strains of mosquitoes are hardy in their natural environment, they are very delicate in lab settings making rearing challenging, thus consistency is key. Through trial

and error, we have determined that *Aedes aegypti* mosquitoes thrive better when fed citrated sheep blood, rather than avian or bovine blood, ultimately increasing the number of females ingesting a blood meal and producing larger, healthier eggs. The better fed the mosquito, the less they bite their handlers too!

If at any stage of development overfeeding occurs, this can lead to “repugnant” water which causes bacterial growth and could potentially kill the larvae. If there are high temperature or humidity fluctuations in the incubators this too can be deleterious to the rearing of mosquitoes and their egg production efficacy. In our facility, the 10 % sucrose solution was found to be a better alternative to the previously determined 5 % because it increased the survival rate of the mosquitoes. Mosquitoes do not take holidays or weekends so dedicated and committed staff is crucial for the maintenance and survival of the colony. *Aedes aegypti* have significant contributions to research, especially when studying disease transmission. In the laboratory they are relatively easy to maintain, lay eggs that can be stored up to 6 mo and can be grown from egg to adult in 8 d, making them an accessible and reliable tool for experiments.

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Figure 1.
Image of male (center left) and female (center right) Aedes aegypti mosquitoes.

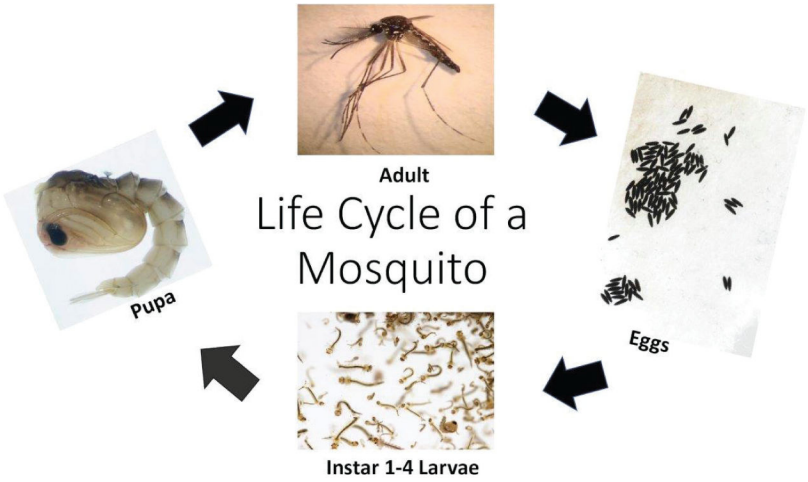

Figure 2.
The life cycles of the mosquito.

Figure 3.
Egg papers in a sealed plastic bag stored in an airtight container.

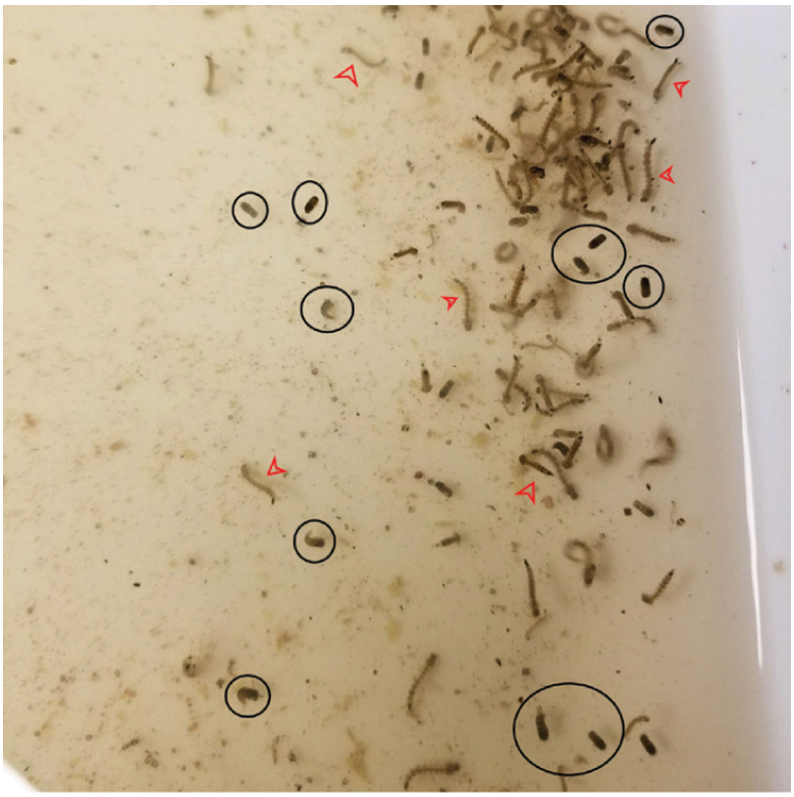

Figure 4.
Larval pan with immature aquatic stages, both larvae (red arrows) and pupae (circled).

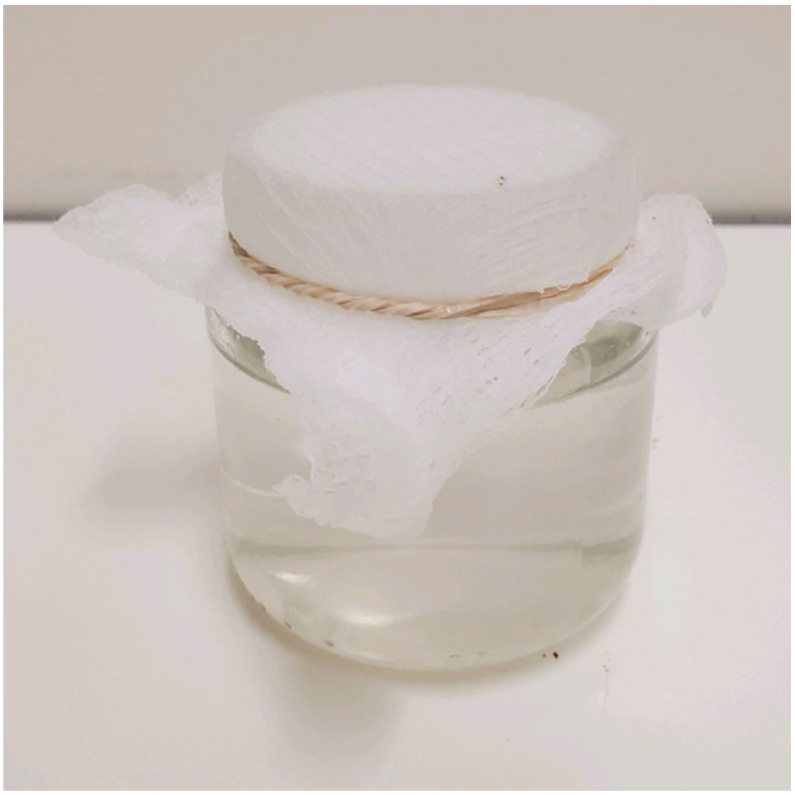

Figure 5.
Repurposed baby food jar wrapped with a 4×4 in gauze and secured with a rubber band.

Figure 6.
Mesh cage holding adult mosquitoes that are being offered a blood meal.

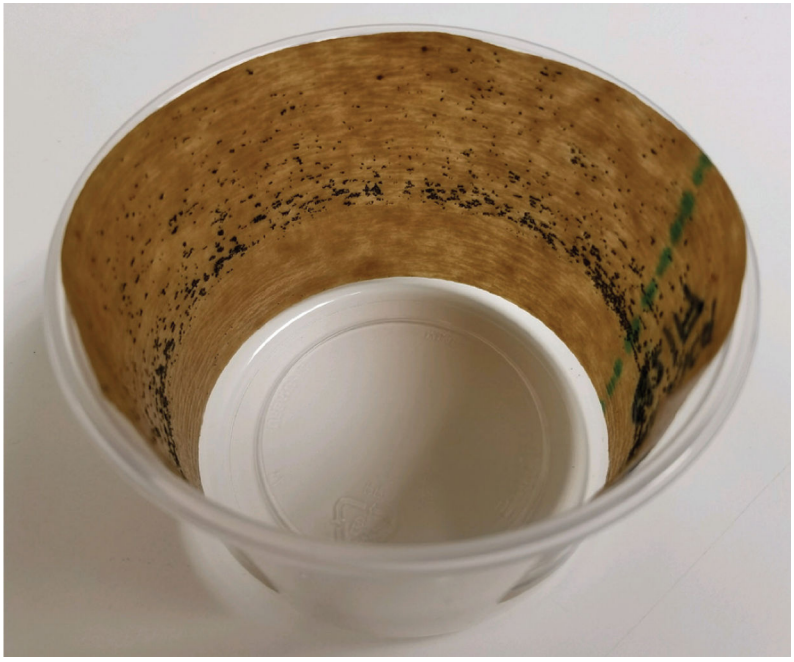

Figure 7.
Egg papers submerged in deionized water in a 16oz MicroGourmet cup.

Figure 8.
Egg papers hatching in a desiccator.

Figure 9.
Mesh holding cage containing pupae and adult stages of development.

Figure 10.
Accessing the holding cage through the fabric tube sock.