

SCIENTIFIC NOTE

LABORATORY COLONIZATION OF *Aedes japonicus japonicus*

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ABSTRACT. We describe methodology used for the laboratory colonization of *Aedes japonicus japonicus*, an exotic mosquito species native to eastern Asia and first collected in New Jersey as larvae in 1999. We created a free mating colony in 2000 that readily bloodfed on restrained bobwhite quail (*Colinus virginianus*). A larval diet of finely ground Purina Lab Diet[®] dissolved in dechlorinated water has proven acceptable. This is the first report of *Ae. j. japonicus* colonization from mosquitoes collected in the United States.

KEY WORDS *Aedes japonicus japonicus*, colony, egg collapse, egg desiccation, New Jersey

Aedes japonicus japonicus (Theobald), the Asian bush mosquito, is an exotic East Asian mosquito first detected outside its native range in the northeast United States in 1998 (Peyton et al. 1999). Since that discovery the mosquito has spread across the United States and is now found in 22 different states and parts of Canada (Saenz et al. 2006). The introduction of West Nile virus (WNV) into the United States in 1999 coupled with the discovery of *Ae. j. japonicus* as a competent laboratory vector of this virus (Turell et al. 2005), as well as St. Louis encephalitis (Sardelis et al. 2003), Eastern equine encephalomyelitis (Sardelis et al. 2002a), and La Crosse encephalitis (Sardelis et al. 2002b), has greatly increased interest in this species. The Centers for Disease Control and Prevention (CDC) has reported the detection of WNV from *Ae. j. japonicus* pools in each year since 2000 (CDC 2007). The vectorial capacity of this species for multiple established and emerging arboviruses, its rapid spread since the initial introduction into the United States, and the lack of information on basic ecology and biology of this species provided the impetus for establishment of a laboratory colony. This study describes the specific conditions under which a free-mating colony of *Ae. j. japonicus* has been established.

Initial populations for establishment of the colony were collected from Kentuckiana Horse Farms, located in Ocean County, New Jersey (40°3'N, 74°30'W). Larvae were collected from automatic watering troughs in outdoor pens where horses were held. These founder mosquitoes were later supplemented with additional

larval samples collected from artificial containers in Hutcheson Memorial Forest, located in Somerset County, New Jersey (40°30'N, 74°33'W), to minimize inbreeding or founder effects on the colony.

All life stages in the colony were kept at room temperature (25 ± 1°C) with a 16L:8D photoperiod without crepuscular periods. Larvae were reared in 30 × 18 × 5 cm white enameled trays that contained ~1.5 liter of dechlorinated tap water. They were fed a diet of finely ground Purina Lab Diet[®] daily suspended in 50 ml of dechlorinated water and then added to the trays. The amount of Lab Diet per 50 ml of water was variable depending on the average size of the larva. Larval trays containing pre-hatch eggs supplemented with 0.1 g Lab Diet in 50 ml dechlorinated water. Trays containing first instars were given 0.2 g in 50 ml, second instars up to 0.2 g in 50 ml, third instars up to 0.3 g in 50 ml, and fourth instars a max of 0.5 g in 50 ml dechlorinated water. Water surface of larval trays was skimmed daily to remove excess bacterial scum and larval exuviae. Water was completely changed in the pans every 48 h, with additional cleaning conducted as needed. When kept at a water temperature of 28°C, larvae spent 2.2 ± 0.5 days in the first instar, 1.6 ± 0.8 days in the second instar, 2.2 ± 1.2 days in the third instar, and 5.6 ± 2.6 days in the fourth instar (Scott 2003). Pupae were individually picked from larval trays using a 1.5 ml pipette and transferred to emergent dishes put inside adult colony cages. Males spent 2.0 ± 2.8 days before emerging as adults, and females spent 5.5 days before adult emergence (Scott 2003).

Adults were housed in 0.61 × 0.61 × 0.61 m cages (BioQuip, Rancho Dominguez, CA), and a 125 ml Erlenmeyer flask containing 10% sucrose solution with 3 cotton wicks protruding from the top was kept in the cages as a carbohydrate source. Adults were fed 3 times weekly on a bobwhite quail, *Colinus virginianus* (L.), re-

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strained inside a pantyhose stocking. The quail was left in the colony cage for 1 h before being removed. Time of day varied, but feeding always took place under lighted conditions.

Oviposition was facilitated by placing round black plastic rice dishes (13 cm diam, 5 cm depth) into the adult cages and filling them approximately halfway with dechlorinated tap water. Seed germination paper (38 lb weight) was placed around the edge of the dish as an oviposition substrate (Anchor Paper, St. Paul, MN). Dishes with egg-laden strips were removed from the cages 2–3 days after oviposition and replaced with fresh dishes and seed paper. Removed papers were placed directly into loosely closed plastic bags and stored at $25 \pm 1^\circ\text{C}$ for a period of 10–14 days. After this time, papers with deposited eggs were submerged into larval pans filled with 1.5 liter dechlorinated tap water with a small amount of larval food added. Egg papers were removed from larval pans after 48 h.

While the above outlined methods are currently used, we arrived at this protocol after trying many different approaches and experiencing several problems. A primary issue was the establishment of a suitable oviposition substrate. When presented with standard filter paper used in other established colonies to facilitate oviposition, it was observed that *Ae. j. japonicus* would not readily oviposit viable eggs. Through further experimentation, we found that the 38 lb seed germination paper described above was very productive and led to much better oviposition rates.

Egg mortality became a second important issue, when we observed that most eggs were prone to desiccation and egg collapse (Fig. 1). Unlike other *Aedine* mosquito eggs that are highly resistant to desiccation, *Ae. j. japonicus* eggs desiccate more frequently. To alleviate this problem, egg-laden papers were placed into loosely closed plastic bag and stored at $25 \pm 1^\circ\text{C}$. This storage system maintained the relative humidity in storage bags at a high level and prevented desiccation of the eggs. This simple modification may be a major factor in the success of *Ae. j. japonicus* colonization.

Another issue in colony establishment related to mating habits. Our colony of *Ae. j. japonicus* requires dense populations for effective free-mating to occur. For the first few generations of the colony, natural mating was supplemented by force-mating pairs of mosquitoes following the techniques outlined in Gerberg et al. (1994). However, after 2–3 months of forced-mating efforts, adult populations attained higher densities of approximately 200–300 individuals per cage, and free mating was observed.

An additional issue we observed concerned the host preference of this species, because the first colony adults were unenthusiastic feeders. Indi-

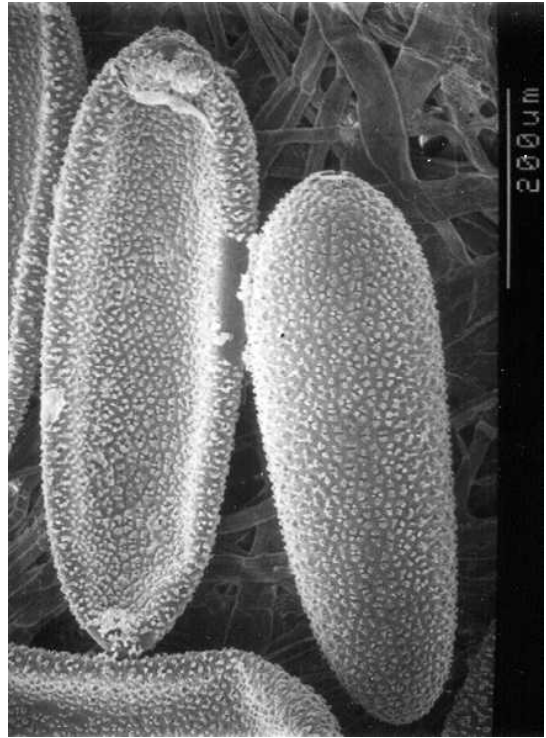


Fig. 1. SEM micrograph of *Ae. j. japonicus* eggs, showing a collapsed specimen on the left as compared to a viable egg on the right.

viduals took partial blood meals from restrained guinea pigs, *Cavia porcellus* (Erxleben), and bobwhite quail, with only a few individuals taking full blood meal. After 4–5 generations, however, adult populations fed more readily on offered hosts, and at present feed on restrained quail.

Using the methods outlined in this paper, we have maintained *Ae. j. japonicus* in colony since early 2000. Since that time we have completed approximately 28 full generations. The colony now feeds on restrained bobwhite quail and mates freely, and egg desiccation issues have been minimized, allowing stable population numbers to build. Mosquitoes from this colony have been used by mosquito control districts around the country to help train local inspectors in identification of this species. Many researchers have also used individuals from this colony for studies ranging from basic biology and life history of the species to genetic analysis of introduced populations (Widdel et al. 2005) as well as other attempts at colonization of *Ae. j. japonicus* (C. Apperson, personal communication). The information obtained from the work presented in this study will contribute to a better understanding of this exotic species and will be invaluable in

applied studies that require the use of *Ae. j. japonicus*.

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