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OBSERVATIONS ON THE LABORATORY BIOLOGY AND MAINTENANCE OF *AEDES TRIVITTATUS*[1]

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ABSTRACT. Data on the biology and laboratory maintenance of *Aedes trivittatus* (Coq.) are reported. Methods used to obtain large numbers of *Ae. trivittatus* for laboratory experimentation with minimum effort involve the collection and storage of eggs from field-collected adults. Each field-collected female produces an average of 62 eggs and nearly 50% hatch, even after storage of up to 1 year. Eighty % survival of larvae and 97% survival of pupae are obtained by using the rearing methods described. Fifty % of adult females survive for more than 26 days, with maximum survival for any mosquito being 60 days. Problems and precautions involved in maintaining *Ae. trivittatus* in the laboratory are discussed.

Aedes (Ochlerotatus) trivittatus (Coquillett) is a medium sized, floodwater mosquito that is widely distributed in North America. It has been reported from 39 of the continental United States (Carpenter and LaCasse 1955; Carpenter 1968, 1970), southern Canada (Carpenter and LaCasse 1955, Trimble 1972), and parts of Mexico and Panama (Howard et al. 1917). *Ae. trivittatus* is found throughout Iowa (Wong et al. 1970, Pinger and Rowley 1972) and can be the most abundant species in certain areas (Christensen and Andrews 1976). It has been reported as the second most abundant mosquito collected in CO_2-baited CDC light traps set primarily near urban areas of Iowa (Wong et al. 1970) and the third most abundant species collected in New Jersey light traps in rural Iowa (Pinger and Rowley 1972).

Ae. trivittatus has been reported to be a vector of several mosquito-borne diseases. Trivittatus virus (TVT) was originally isolated from a pool of *Ae. trivittatus* collected in North Dakota (Hammon et al. 1952), and this species has since been incriminated as the natural vector of this virus (Wong et al. 1970, Rowley et al. 1973, Watts et al. 1976). In addition to TVT virus, arboviruses isolated from field-collected *Ae. trivittatus* include western equine encephalomyelitis, Bunyamwera, Jamestown Canyon, and Flanders viruses (Wong et al. 1970, 1973, Rowley et al. 1973, Anslow et al. 1969, Thompson et al. 1972, Sudia et al. 1971, Rowley, unpublished data). *Ae. trivittatus* also has been reported as a natural vector of dog heartworm, *Dirofilaria immitis*, in central Iowa (Christensen 1977).

Although some data are available on the biology of *Ae. trivittatus* (Abdel-Malek 1948 a, b, Horsfall et al. 1958, Wright and Knight 1966, Pinger and Rowley 1975), most of this information concerns field observations. A paucity of information is available on the laboratory biology and maintenance of this mosquito. *Ae. trivittatus* has been used routinely in our laboratory in studies with TVT virus and *D. im-*

[1] Journal Paper No. J-8935 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa. Project No. 1955.

mitis. These studies have provided numerous data on the laboratory biology and maintenance of this mosquito. Because *Ae. trivittatus* is involved in the natural history and maintenance of several microorganisms, the objective of this paper is to collate data generated in our laboratory that may help other researchers.

METHODS AND RESULTS

Ae. trivittatus colonization is possible with any developmental stage, but as with many other floodwater mosquitoes, adults must be manually copulated. The most convenient and expedient means of obtaining large numbers of *Ae. trivittatus* for use in the laboratory is by field-collecting adult females. Mosquitoes are collected with a mouth aspirator after they have blood-fed on a human host. An alternative is to allow mosquitoes to blood feed in the laboratory on a rabbit after field collection.

After blood feeding, mosquitoes are maintained in lots of 50 in 0.473-liter ice cream cartons with fine-mesh marquisette coverings. Cotton pads, moistened in 0.3 M sucrose solution, are placed on the marquisette. Mosquitoes are maintained in a controlled environment at 26.5±1°C and 80 ± 5% RH under a 16-hr photoperiod with a 90-min crepuscular period at the beginning and end of each light period.

One to 6 field-collected, blood-fed mosquitoes are transferred to individual oviposition cages 3 days after blood feeding. Ovipositional cages are similar to those used by Horsfall et al. (1973) for *Ae. vexans*. Cages measured 2.5 × 2.5 × 15 cm and were constructed of plastic (approximately 3 mm thick) on 2 sides and the ends. One side was covered with metal window screen, and the other with finer mesh plastic screen. A hole in one end accommodated a rubber stopper (No. 00) and allowed for the introduction and removal of mosquitoes.

Cages are placed, wire side down, on an ovipositional substrate consisting of 6 to 8 layers of cheesecloth wrapped around a layer of cotton. Substrate is placed in white enamel trays (25 × 42 × 7 cm) and well moistened with deionized water. Ten to 12 cages can be placed in each tray. Mosquitoes are provided with 0.3 M sucrose, and the trays are covered with glass plates.

Mosquitoes begin ovipositing as soon as 4 days postprandially and continue for up to 23 days. At 3- to 4-day intervals, eggs are collected with a Pasteur pipette, counted, and transferred to moist filter paper in petri dishes. Data on egg production by 1,247 field-collected *Ae. trivittatus* are presented in Table 1.

Table 1. Egg production by field-collected *Aedes trivittatus* blood fed on a human or rabbit host.

Host	Number of mosquitoes	Number of eggs	Mean number of of eggs/ mosquito
Human	699	43,332	62
Rabbit	548	34,419	62

Eggs are maintained on moist filter paper at 26.5 ± 1° C for 10–14 days to allow for embryonation and then are stored at 4° C for future use. Eggs remain viable for up to 1 year or longer when stored in this manner. Care must be taken to prevent eggs from drying out during storage. Eggs cannot tolerate desiccation at either temperature. Approximately 2 wks before hatching, eggs are removed from refrigeration (4° C) and warm-conditioned at 26.5 ± 1° C. Two steps are involved in the procedure used to hatch *Ae. trivittatus* eggs: (1) eggs are immersed in deoxygenated water (room temperature) for 2 hr, and larvae are removed and counted; and (2) remaining eggs are transferred to a mild nutrient broth (approximately 1 part nutrient broth: 5000 parts deionized water) for 12–14 hr (overnight). Larvae are then removed and counted, and remaining unhatched eggs are discarded.

Records were maintained on the number of larvae hatching during seven different experiments involving 27,895 eggs. Percentage of eggs hatching averaged 49% and ranged from 30% to 57%

(Table 2). The percentage of viable eggs hatching probably was much greater because no attempt was made to remove damaged or unembryonated eggs. Approximately 75% to 80% of the eggs hatched in the 2-hr period in deoxygenated water. Thus, hatching in deoxygenated water affords a simple and quick method of obtaining mosquitoes of uniform age for experimental purposes. The prolonged reduction in oxygen tension that the nutrient broth produces gives a maximum yield of mosquitoes from eggs.

Table 2. Hatchability of *Aedes trivittatus* eggs obtained from field-collected adults.

Experiment number	Number eggs	Number hatching	Percent hatching
1	2,302	1,175	51.0
2	2,050	610	29.8
3	2,349	981	41.8
4	10,108	5,223	51.7
5	4,374	2,489	56.9
6	2,612	1,475	56.5
7	4,100	1,781	43.4
total	27,895	13,734	49.2

Larvae are reared in lots of 200 in deionized water (1.0–1.5 cm deep) in 25 × 42 × 7 cm, white enamel trays. Eight to 12 small pinches of sterile, washed sand are dispersed evenly in each tray. Larvae are fed a slurry of finely ground fish food (Tetramin®) pipetted on top of the sand in amounts that mosquitoes will consume in 24 to 36 hr. Over-feeding to some extent is tolerable, as long as a surface scum does not form.

Mortality rates were determined for 12,676 larvae by using these rearing methods. In six experiments, 80% of all larvae survived to pupation, with a range of 61% to 87% (Table 3).

Pupation begins at 5 days posteclosion, and pupae are harvested on developmental day 6. Lots of 50 pupae are placed in deionized water (3–4 cm deep) in ice cream cartons with marquisette coverings. After mosquito emergence, water is drained, and mosquitoes are lightly anesthetized with gaseous CO_2 and separated according to sex on a refrigerated cold table. Percentage mortality was determined for 5,602 pupae with these rearing methods. In two experiments, a mean of 97% survived through adult emergence (Table 4). Most pupal mortality was attributed to those few mosquitoes in each carton that had not emerged when the water was drained.

Table 3. Larval mortality of laboratory-reared *Aedes trivittatus*.

Experiment number	Number of larvae	Number pupating	Percent larval mortality
1	361	309	14.4
2	522	320	38.7
3	2,766	1,864	32.6
4	5,117	4,472	12.6
5	2,459	2,024	17.7
6	1,451	1,130	22.1
total	12,676	10,119	20.2

Adult *Ae. trivittatus* are maintained in environmental chambers in lots of 50 in ice cream cartons. As before, adult mosquitoes are afforded access to 0.3 M sucrose. Four- to 6-day-old females blood feed well through the marquisette covering on human, rabbit, or canine hosts. A higher percentage feed and imbibe larger amounts of blood when sucrose pads are removed 24 hr before blood feeding.

Longevity of 490 males, 529 females maintained on sucrose only, and 429 females given one blood meal and then maintained on sucrose was determined by using these rearing methods. Mosquitoes were given fresh sucrose pads every other day throughout all experiments. All car-

Table 4. Pupal mortality of laboratory-reared *Aedes trivittatus*.

Experiment number	Number of pupae	Number of adults	Percent pupal mortality
1	4,472	4,376	2.1
2	1,130	1,048	7.3
total	5,602	5,424	3.2

tons were checked daily, and dead mosquitoes were counted and removed. Mosquitoes were checked in this manner until all individuals were dead.

Longevity curves, expressed as proportion surviving, are illustrated in Fig. 1. At time zero, mosquitoes were actually 5 days old. This delay was necessary to obtain blood-fed mosquitoes. Males were much shorter lived than females, showing an LT50 of only 16 days. Only 4% of the males were alive after 27 days, but about 50% of the females in both experimental groups were still alive. The LT 25, 50, and 75 for blood-fed and mosquitoes not blood-fed were 22, 26, 32, and 21, 28, and 36 days, respectively. Maximum survival time for any mosquito was 60 days.

Ae. trivittatus does not mate naturally in captivity; therefore, to obtain fertile eggs from laboratory-reared mosquitoes, each female must be manually copulated. The easiest and most successful method we have found is a modification of that reported by Ow Yang et al. (1963).

Adult *Ae. trivittatus* are maintained on sucrose until they are 5 to 6 days old. At this time, males are collected with an aspirator and gently blown between layers of cotton. A minuten pin attached to a wooden applicator stick is inserted through the thorax, and the male's legs, wings, and head are removed. Three to 5 males are prepared at one time in this manner. Females are aspirated into clear plastic tubes (12 × 75 mm), lightly anesthetized with nitrogen gas, and placed ventral side up under a dissecting microscope. A pinned male mosquito is presented, ventral side down, at about a 45° angle to the female. After copulation is initiated, the pair is lifted off the substrate and held until the male releases the female. A minimum of 3 to 4 sec mating time seems sufficient to insure insemination of the female. We generally use individual males to mate a maximum of 2 to 3 females. Mated females are transferred to clean cartons and blood fed the next day. It is possible for one person to mate 250 to 300 mosquitoes in 1 day by using this method.

Forty-eight eggs per manually copulated female (10,406 eggs/217♀♀) were ob-

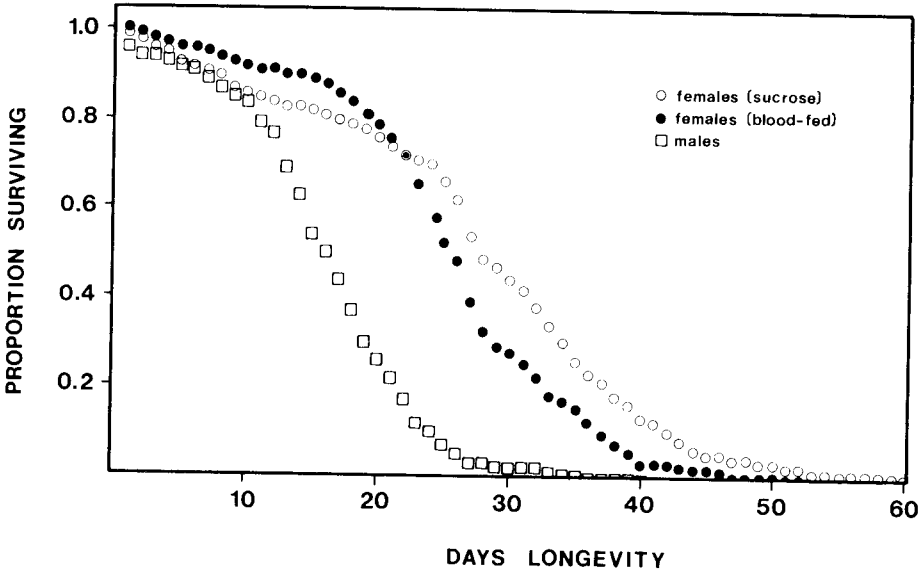

Fig. 1. Survival curves for adult *Aedes trivittatus* maintained at 26.5 ± 1° C and 80% RH.

tained in the 1st oviposition with use of the oviposition cages and substrate described. Number of eggs per female is approximately the same after a 2nd blood meal (47 eggs/0; 2,099 eggs/45 ♀♀). Few mosquitoes survive to oviposit more than twice, but of those that have, we recorded means of 61 (668 eggs/11 ♀♀ and 40 (158 eggs/4♀♀) eggs per female in the 3rd and 4th ovipositions, respectively.

There are 2 basic problems with manual couplations of this mosquito. First, in studies in which data were recorded, only 217 of 365 (60%) manually mated females oviposited. An explanation for this is being investigated. Second, the time required for rearing and mating is pronounced in relation to the number of eggs obtained. The method is satisfactory, however, when mated mosquitoes are required for an experiment.

DISCUSSION AND CONCLUSIONS

The methodology described has allowed us to routinely maintain and use *Ae. trivittatus* in large numbers for experimental purposes. These methods are perhaps not optimal for maximum egg hatching, larval rearing, etc., but they are convenient and therefore provide sufficient quantitites of mosquitoes with minimum effort.

The most efficient method available for obtaining *Ae. trivittatus* for use through the winter is that of field collecting adult females to obtain eggs. We annually collect 60,000 to 100,000 eggs to store at 4°C for future use. This also provides mosquitoes that have not passed several generations in the laboratory; thereby adding more validity to vector studies performed in the laboratory.

A major concern, however, when using eggs from field-collected adults is that TVT virus is transovarially transmitted by *Ae. trivittatus* (Andrews et al. 1977, Christensen et al. 1978). Experiments involving TVT virus require additional precautions to ascertain that eggs came from uninfected mosquitoes. We generally assay field-collected females in groups of 5 to 10 following oviposition to insure that the eggs collected came from uninfected mosquitoes.

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