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probably identify the females by sound (Roth, 1948). The factors that attract the males to the host are unknown, but they could be chemical (Grant, 1969) or visual (Fay, 1968; Sippell & Brown, 1953). The attraction of both sexes to the host is, in the author's opinion, probably the main means whereby the sexes are brought together when they are physiologically ready to mate.

The males fly a horizontal figure-eight pattern toand-fro around the host in groups or singly. In watching this "patrolling" of the males, one gets the impression that they are acting individually and not in concert. The males appear to wait for the females and pounce on them as they appear. The mating occurs with only one male present as well as when several are flying about. Mating definitely seems to be a pair activity and "swarming" is unnecessary. Peyton (1956) observed males of *Aedes varipalpus* swarming over warm-blooded animals and pouncing on the females as they came for a blood meal.

Mating of Ae. aegypti in nature probably occurs most commonly during the peak activity periods. In the present study the observations were made during the morning period of peak activity. McClelland (1959) reported observing mating of Ae. aegypti in Uganda on two occasions between 16.30 and

17.50 hours, during the afternoon peak activity period, while conducting biting catches in Uganda.

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# Laboratory Colonization of Aedes simpsoni (Theobald) and Eretmapodites quinquevittatus Theobald\*

by W. K. Hartberg 1 & E. J. Gerberg 2

Aedes simpsoni is a known laboratory vector of yellow fever (Bauer, 1928; Philip, 1929) and it has played a major part in several epidemics (Mahaffy et al., 1942; Sérié et al., 1968b; Smithburn & Haddow, 1946). Mosquitos of the genus Eretmapo-

dites are good laboratory vectors of yellow fever (Bauer, 1928) and chikungunya (Gilotra & Shah, 1967). Viruses isolated from wild-caught *Eretmapodites* include Rift Valley fever virus (Smithburn, Haddow & Gillett, 1948), Semliki Forest virus (Macnamara, 1953), Spondweni virus (Brottes et al., 1969; McIntosh et al., 1961; Worth, Patterson & deMeillon, 1961), Nyando virus (Ardoin & Simpson, 1965; Sérié et al., 1968a), Okola, Middleburg, and Nkolbisson viruses (Brottes et al., 1969), Bunyamwere virus (Dr B. E. Henderson and Dr D. Metselaar, personal communication), and an unidentified viral agent MTMP 131 (Henderson et al., 1969). It is desirable that laboratory colonies of *Ae. simpsoni* 

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and *Eretmapodites* should be maintained for studies on these arboviruses.

With increasing research on the genetics of mosquito vectors, it has become apparent that strains of known genotypes are of basic importance to the geneticist. The only way to obtain such strains is to colonize field-collected material, genetically analyse the newly colonized material, and then to select strains with appropriate characteristics for study. The uncertainty of field-collected material being available in sufficient quantity for study also makes it highly desirable that laboratory colonies of vector species should be provided for research workers.

Laboratory colonies of several species of the subgenus *Stegomyia* of the genus *Aedes* have been established but the colonization of most of the feral African species, other than *Ae. aegypti*, has been unsuccessful or difficult. This has certainly been true of *Ae. simpsoni* (Mukwaya & Mawejje, 1966) prior to the present study. One species of *Eretmapodites*, *E. chrysogaster*, has already been successfully colonized (Gillett, 1958) and to the best of our knowledge *E. quinquevittatus*, whose colonization is reported herein, is the second species of the genus to be successfully established in the laboratory.

Colonies of both Ae. simpsoni and E. quinquevittatus have been maintained in two laboratories—namely, the WHO East Africa Aedes Research Unit, Dar es Salaam, Tanzania, and Insect Control & Research, Inc., Baltimore, Md., USA. This report includes observations made in both laboratories.

#### Colonization of Ae. simpsoni

Procedures, results, and observations. Ae. simpsoni has been successfully colonized since November 1969. Two cages, one large (52 by 57 by 74 cm) and the other small (30.48 cm³), were set up with several hundred virgin (laboratory-reared) Ae. simpsoni. The adults mated readily in the cages. The mosquitos were reared from eggs laid by Ae. simpsoni adults collected in the Mbagala suburb of Dar es Salaam. The cages were kept in an insectary maintained at a temperature of 26.5°C±1°C and a relative humidity of 80%. No special lighting was used.

Larvae were reared in white plastic containers about 20 by 36 by 7 cm containing approximately 2 cm of water. The larvae were fed on a mixture containing equal parts of liver powder and ground dog food, or on a mixture of 10 parts of liver powder, 8 parts of a proprietary wheat germ product, and 2 parts of brewers' yeast. The adults were fed on

boiled raisins or cotton balls soaked in 10% sucrose solution. The adult females accept the first blood meal 24-28 hours after emergence, and they feed readily on guinea-pigs, rabbits, or man.

The females oviposit on moist paper towelling lining a cardboard ice-cream carton ( $\frac{1}{2}$  litre) or a disposable plastic cup half filled with water. The eggs are conditioned by slow drying of the paper towelling for 3–6 days under insectary conditions. The conditioned eggs can be stored for some time and still give an excellent hatch rate. The hatching stimulus is either deoxygenated water or 0.01% ascorbic acid solution. After stimulation, the eggs hatch within 6 minutes. The average development times at 26.5°C $\pm$ 1°C and 80% relative humidity are as follows: egg hatch-adult, 10 days; larval stage, 5–8 days; pupal stage, 48 hours.

Ae. simpsoni can be infected with Plasmodium gallinaceum in the laboratory, and sporozoites are developed. Studies are being made to see if this mosquito is capable of transmitting P. gallinaceum. The relative ease with which this strain of Ae. simpsoni was colonized, and the lack of success in the past with strains from other areas, indicate that a wide range of geographical populations should be tested when efforts are being made to colonize difficult species. This approach should increase the chances of success.

### Colonization of E. quinquevittatus

Procedures, results, and observations. E. quinquevittatus has been successfully colonized since July 1969. Colonies of this mosquito do equally well in small (30.48 cm<sup>3</sup>) and large (60.96 cm<sup>3</sup>) cages. The colony was established from larvae collected from a tree-hole in the Kisutu section of Dar es Salaam, and this species does extremely well in the laboratory. The containers, insectary conditions, and feeding mixtures used for larvae are similar to those used for Ae. simpsoni larvae. The larvae tend to crawl up the sides of the trays and even on the underside of the glass plates covering the trays; one larva was observed to travel a distance of over 80 cm out of the rearing medium on the sides of the tray. It is possible that the larvae "browse" on micro-organisms growing on the sides of the rearing containers above the water level.

Bauer (1928) and Haddow (1946) state that larvae of this genus are facultative cannibals, and Gillett (1958) found it necessary to sort the larvae of *E. chrysogaster* according to size in order to reduce

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the loss of larvae by cannibalism. No cannibalism has been noted in the present study with *E. quinque-vittatus* although the larvae will eat cast larval skins.

Gillett (1958) reported that *E. chrysogaster* would not mate in a cage until after it had received a blood meal. However, *E. quinquevittatus* differs in that mating takes place readily before a blood meal, and as soon as the adults are placed in the cage. Within 4 days of adult emergence, females of *E. quinquevittatus* lay eggs without taking a blood meal. In fact, preliminary observations indicate that it is a case of obligatory autogeny. The females refuse to take a blood meal before they have laid their autogenous egg batch. Females lay the first batch of eggs even if they have been given no food as adults. The average size of the autogenous egg batches is 52 eggs per female.

After females have laid their autogenous eggs, they readily accept a blood meal from guinea-pigs, rabbits, or man. When a female is preparing to insert the proboscis, she puts the hind legs down flat and usually spreads the wings, vibrating them rapidly. Once insertion has been effected, the wings are folded back into the normal resting position. All six legs remain in contact with the host during feeding.

The adult mosquitos are fed on boiled raisins, sugar cubes, or cotton balls soaked in 10% sucrose solution.

The females of *E. quinquevittatus* oviposit on moist paper towelling lining ice-cream cartons (½ litre) or plastic cups half-filled with water. They refuse to oviposit on moist cotton when isolated in individual vials. Eggs hatch within 2 days of being laid; the paper towelling must be kept moist (though not submerged in water) since the eggs die if they are allowed to dry. Hylton (1967) reported that *E. chrysogaster* eggs survive in a dry container at a temperature of 26.6°C and 81% relative humidity for as long as 20 days. The moist paper towelling can be placed directly in the larval rearing pans and hatching will take place in the rearing medium.

The average development times at a temperature of  $26.5^{\circ}C \pm 1^{\circ}C$  and 80% relative humidity are as follows: from egg hatch to adult, 10-12 days; larval stage, 8-10 days; pupal stage, 2 days. *E. quinque-*

vittatus has a long life span; 38 days after emergence, only one male had died in the cages under observation. This species is relatively easy to maintain in the laboratory, and could prove to be a very useful mosquito for experimental studies. E. quinquevittatus can be infected with P. gallinaceum in the laboratory and sporozoites develop in the adults. Studies are now being made to determine whether this mosquito is capable of transmitting P. gallinaceum.

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