STUDIES ON THE MOSQUITOES IN THE YAEYAMA ISLANDS, JAPAN 6. COLONIZATION AND BIONOMICS OF *AEDES (VERRALLINA) IRIOMOTENSIS* AND *AEDES (VERRALLINA) ATRIISIMILIS*[1]

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ABSTRACT. Two species of mosquitoes, *Aedes (Verrallina) iriomotensis* and *Ae. (Ver.) atriisimilis* have been colonized for the first time in the laboratory. These colonies have been maintained since 1978, during which time they have undergone approximately 30 generations. The eggs of *Ae. iriomotensis* apparently withstood desiccation for a period of up to one month. The eggs of *Ae. atriisimilis* did not withstand complete desiccation but they may be stored under high humidity for 2 months. Larval development of both species was completed within 10 days and 90% of the larvae pupated by the 8th day. Larval mortality was low and appeared to be directly related to amounts of food and rearing density. The pupal stage lasted 1 to 2 days. Males of *Ae. iriomotensis* mated readily 10 hours after emergence with just emerged females on the water surface. *Aedes atriisimilis* males mated most readily 3 to 4 days after emergence. Copulation was usually initiated on the wing and was completed on the ground.

INTRODUCTION

Colonization of mosquitoes is important not only for the study of pathogen-vector relationships but also for a better understanding of their biology and the phylogenetic relationship between closely related species. To meet these needs, we have attempted to colonize a number of mosquito species occurring in the Ryukyu Islands, Japan.

The subgenus *Verrallina* Theobald is primarily restricted in distribution to the Oriental zoogeographical region except with a short extension north to Okinawa, Kyushu, Japan and east into the Carolines, New Guinea, South Pacific islands, and south into northeastern Australia (Reinert 1974). At present, approximately 100 species have been described in the region (Knight and Stone 1977). Three species of the subgenus, *Aedes iriomotensis* Tanaka and Mizusawa, *Ae. atriisimilis* Tanaka and Mizusawa and *Ae. nobukonis* Yamada, have been recognized in the Ryukyu Is., Japan (Tanaka and Mizusawa 1973, Tanaka et al. 1979).

Although adult females of *Ae. iriomotensis* and *Ae. atriisimilis* are abundant biting pests and the former species might be a vector of *Setaria bernardi* Railliet and Henry, a parasite of the wild boar, *Sus riukiuanus* Kuroda (Shoho and Machida 1979, Miyagi and Toma 1979, 1980, 1981), the bionomics of the species are very poorly known.

This paper presents the results of colonization attempts, as well as observations on the bionomics of *Ae. iriomotensis* and *Ae. atriisimilis* in the laboratory.

MATERIALS AND METHODS

In September 1978, 20 engorged female *Ae. iriomotensis* and *Ae. atriisimilis* were collected in a forest on Iriomotejima. All the females collected were transferred to our insectary and maintained for colonization under controlled conditions of temperature (25–27°C), relative humidity (70–80%) and artificial daylight. A photoperiod of 16 hr

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of daylight was provided by two 15-watt fluorescent tubes and a crepuscular period was simulated by gradually dimming 100-watt incandescent bulbs to full darkness in a 30-minute period. The dimming process was reversed in the morning.

Mosquitoes were reared in "connection cages" measuring 25×31×40 cm (Toma and Miyagi 1978). Approximately 1000 larvae were reared in a lower vessel (25×31×10 cm), one-third filled with water. Constant mechanical aeration was provided by means of an airstone attached by a plastic tube to an air pump. Pulverized food for larvae, consisting of equal weights of Ebios (brewer's yeast) and mouse pellets, was sprinkled on the water surface as needed 2 or 3 times daily. Water was not changed during larval and pupal development except to eliminate contamination (indicated by odor or diseased larvae). The upper cage for rearing of adults was connected to the vessel the first day after pupation occurred. Food for adults consisted of 2% sucrose supplied on soaked cotton pads. Pads were changed every 3rd day. Females were allowed to feed on an anesthetized mouse for about one hour. Three to 4 days after taking blood meals, approximately 100 gravid females were removed by an aspirator tube into a small vial (5 cm diam and 10 cm deep) which was provided with a damp paper towel for an oviposition site.

To examine the resistance to desiccation of the eggs of both species, wet- and dry-resistance tests were designed as follows. Approximately 8,000 fertilized eggs of *Ae. iriomotensis* were divided into 16 batches of about 400 eggs. Seven of the batches were kept dry in the air for 6 to 150 days and the remaining 9 batches were kept moist on filter paper for 4 to 170 days, and thereafter, each batch was soaked in deoxygenated water and all larvae hatched were counted daily for one month. The unhatched eggs were dissected and checked for embryonic development. In *Ae. atriisimilis,* approximately 4,000 eggs were divided into 12 batches; 4 of the batches were kept dry for 4 to 45 days and the rest were kept moist for 4 to 150 days.

To determine the time required for insemination of the male and the female in both species, 3 mature females and newly-emerged males, and 3 mature males and newly-emerged females of the species were kept in screened cage (20×20×10 cm) for 0 to 100 hours respectively. All females were dissected and their spermathecae were examined for insemination. Five replications were made for each experiment. The same experiments were conducted with the common mosquito, *Ae. (Stegomyia) albopictus* (Skuse), for comparison.

RESULTS AND DISCUSSION

The females preferred to lay eggs singly into the crevices and depressions of the damp filter paper. The mean number of eggs produced per female was variable. Yield of eggs in both species was very low in routinely used oviposition containers placed inside the rearing cage. After several trials, a special method was adopted as follows: approximately 100 gravid females were introduced, using an electric aspirator, into a small glass vial provided with a damp paper towel for oviposition. These gravid females deposited approximately 5,000 eggs (50 eggs/female) in *Ae. iriomotensis* and approximately 1,500 (15 eggs/female) in *Ae. atriisimilis.*

The gravid females of both species in the small glass vials readily laid eggs on crevices and depression of the filter paper. The embryonic period was 2 days in *Ae. iriomotensis* and 3 days in *Ae. atriisimilis.* As shown in Fig. 1, the eggs of *Ae. iriomotensis* apparently withstood desiccation for a period of up to 30 days and after this period the hatching rate was decreased gradually with the prolongation of the period of exposure. While in the moistened eggs, hatching rate was high (90%) up to 80 days and then the rate decreased rapidly. The eggs of *Ae. atriisimilis* did not withstand desiccation. When kept dry in the air for 10 days, the

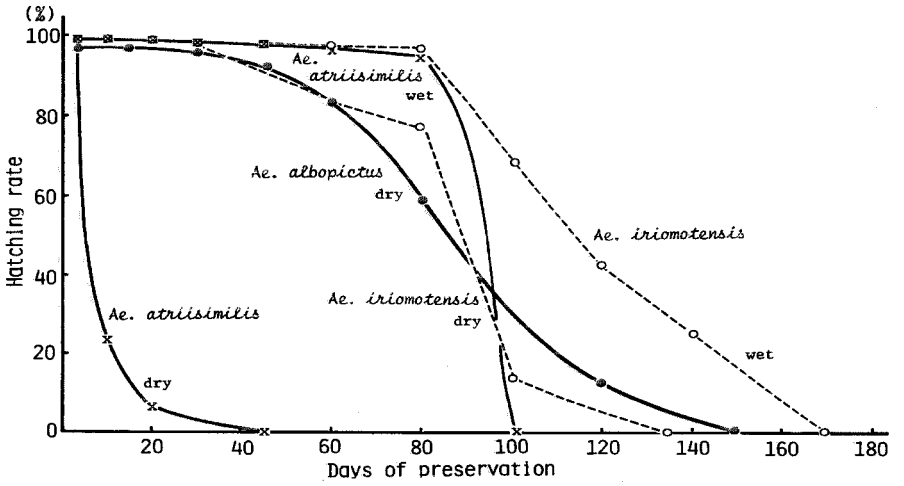


Fig. 1. Relation of hatching rate to period of wet and dry conditions in the eggs of *Aedes iriomotensis*, *Ae. atriisimilis* and *(Ae. albopictus)*. dry–eggs preserved in dry condition; wet–eggs preserved in wet condition.

hatching rate was only 20%. While in the moistened eggs, the hatching rate was high (95%) up to 80 days. Eggs of both species attached on damp filter paper may be stored up to one month, but if the paper is too moist, these stored eggs hatch at once. In contrast, the eggs of *Ae. atriisimilis* when stored in the completely dry condition for one month, may not survive. In comparison with *Ae. albopictus*, the percentage of hatch for desiccated eggs was high (80%) for up to 60 days and then reduced gradually with lengthened desiccation, being 18% at 120 days.

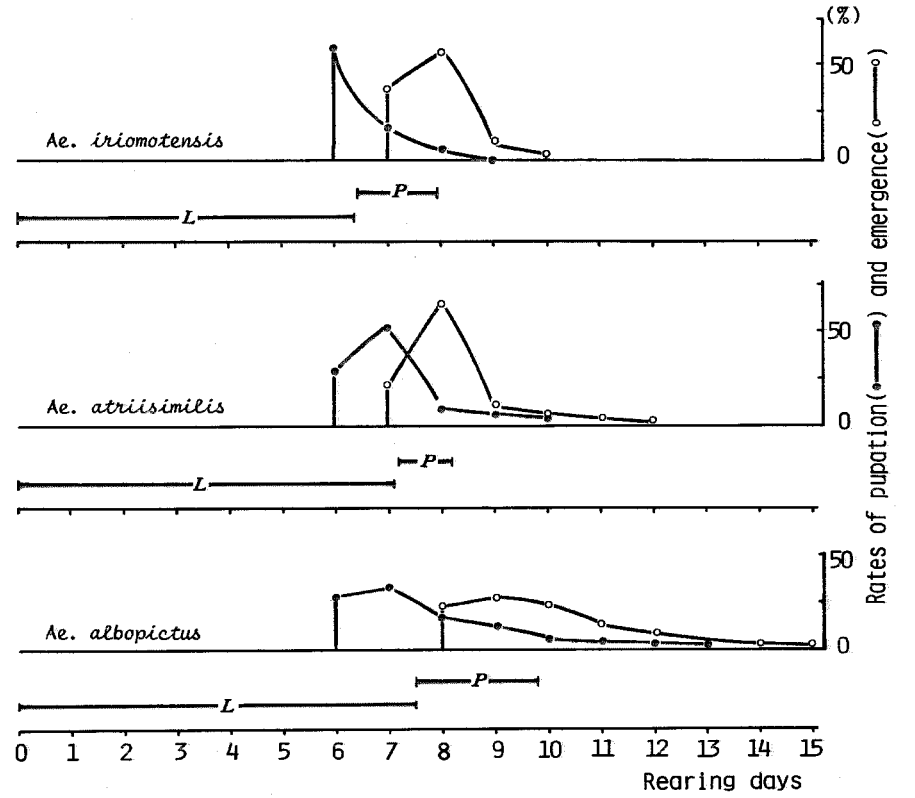
As shown in Fig. 2, the duration of larval stages varied from 5 to 8 days (mean = 6.4 days) in *Ae. iriomotensis* and 5 to 9 days (mean = 7.2 days) in *Ae. atriisimilis*. Approximately 90% of the larvae of both species pupated by the 8th day, but some lasted to the 9th day in *Ae. iriomotensis* and to the 10th day in *Ae. atriisimilis*. The mean durations of the pupal stages lasted 1 day in *Ae. atriisimilis* and 1.5 days in *Ae. iriomotensis*. The larval duration of *Ae. albopictus* varied from 5 to 12 days (mean = 7.5 days) and mean pupal duration lasted 2.3 days. When 1000 larvae per vessel (25×31×10 cm) were fed the following amounts per day of the mouse pellet-yeast diet (0.2–0.3 mg. for 1st and 2nd stage larvae and 0.4–0.5 mg for 3rd and 4th stage larvae), 80 to 90% of them survived to the pupal stage. Mortality during the pupal stage was less than 10%.

The females of *Ae. iriomotensis* took a blood meal 2 to 3 days after emergence. They fed vigorously on man and an anesthetized mouse. In *Ae. atriisimilis*, the females took a blood meal 4 to 5 days after emergence. It was often observed that in the female of *Ae. atriisimilis*, subsequent blood meals were taken at intervals of 2 to 3 days with at least 2 blood meals necessary before oviposition. In the first to several generations, the females fed on humans, as they did not feed well on anesthetized mice and guinea pigs in the cage. After several generations, the females fed readily on an anesthetized

mouse placing on the bottom of the cage. Egg laying began 4 days after engorgement in both species.

The males lived for at least one week in *Ae. iriomotensis* and 10 to 15 days in *Ae. atriisimilis*. The females of both species survived for 20 to 25 days.

As shown in Fig. 3, in *Ae. atriisimilis*, the males did not usually mate until they were 36 to 40 hours old. The time required for 180 degree rotation of the male terminalia was approximately 40 hours. The youngest male attempted copulation at an age of 31 hours. The females began to copulate at more than 30 hours after emergence and approximately 100% of the females in the cage had copulated at 100 hours after emergence (Fig. 3). Copulation was usually initiated on the wing. When a female flew, a male dashed out from his resting place and clasped her in flight. The hindtarsal claw of the fe-



L: Mean duration of larval stage. P: Mean duration of pupal stage.

Fig. 2. Duration of larval and pupal stages, and rates of pupation and emergence in 3 *Aedes* species. *L*–mean duration of larval stage; *P*–mean duration of pupal stage.

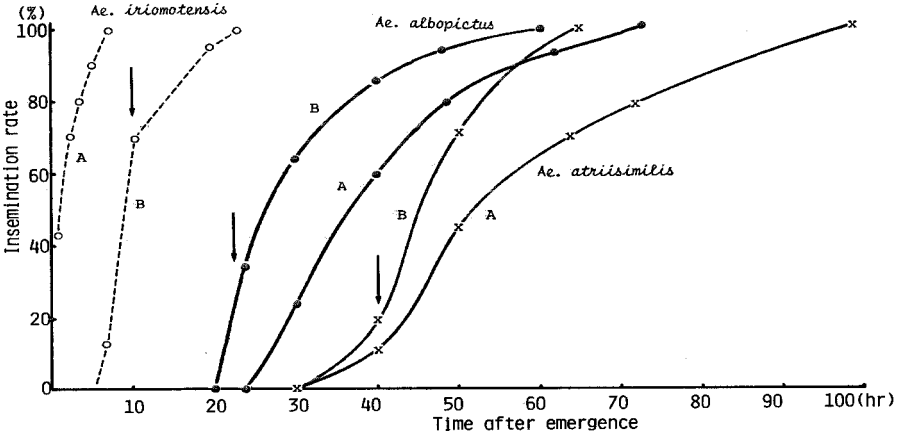


Fig. 3. Time required for insemination of emerged females (A) and males (B) of 3 *Aedes* species. A–3 mature males and newly-emerged females were reared in a cage; B–3 mature females and newly emerged males were reared in a cage. Five replicates of each test were conducted. The arrow shows the time of 180° genital rotation in male.

male was tapped by that of the male and the couple tumbled down the wall of the cage. Copulation took place while both individuals were resting on the wall. The female settled on the wall after being seized and the male hung with its head downwards during coitus. Copulation lasted for about 30 minutes.

In *Ae. iriomotensis*, the time required for 180 degree rotation of the male terminalia was 10 to 12 hours (Fig. 3). The rotation through the initial 90 degrees occurred more rapidly than the final 90 degrees. It was found that a complete rotation of the male terminalia was not always necessary for copulation. The minimum time required for copulation by the male after emergence was 7 hours. Approximately 100% of the females in the cage copulated during the initial 10 hours of emergence (Fig. 3). As soon as the female emerged from the pupal skin, it was ready to accept the male. The male faced the female and then rapidly positioned himself upside-down beneath the female abdomen. Copulation occurred end-to-end with the male and female quietly in copula for 4 minutes to 3 hours.

The mating behavior of both species is species specific. They are both stenogamous and copulation takes place easily in small test tubes (5 cm deep and 1 cm diam). A detailed description of the mating behavior of *Ae. iriomotensis* will be published in a separate paper (Miyagi and Toma 1981).

The rearing method given here is based on experience with 30 generations of the species. Both species of *Verrallina* are adapted to mass colonization and can be used in studies on comparative sexual behavior and pathogenvector relationships.

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