A PRELIMINARY REPORT ON THE LABORATORY COLONIZATION OF THE MOSQUITO CULEX TRITAENIORHYNCHUS GILES

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INTRODUCTION. The mosquito Culex tritaeniorhynchus is considered to be the principal vector of Japanese B encephalitis in Japan. One of the limiting factors in the study of this disease has been the lack of a laboratory colony of this mosquito.

A method developed during the summer of 1956 and now in use at the 406th Medical General Laboratory, has resulted in the successful rearing of this species through an estimated 6 generations. Mortality in all stages is low, the adults are large and vigorous and the colony is flourishing. Over 15,000 egg rafts were produced between June 22, when the first two rafts were collected, and October 22. During the first three weeks of October an average of over 400 egg rafts per day were produced, of which approximately 95 percent contained viable eggs. The 406th Medical General Laboratory Strain is a mixture started with larvae from Okinawa and Kyushu, Japan and eggs from the vicinity of Tokyo, Japan.

CAGE. The rearing cage, which is located in a corner of the insectary, is approximately 11 feet long, 8 feet wide and 8 feet high.

Adults. Cotton wads soaked in a 2 percent sugar solution, and fresh apple slices are provided daily. Two rabbits and a pig are maintained in the cage each night and a rabbit is kept in the cage during the day. The females feed equally well on either the rabbit or the pig, and although some blood meals are taken throughout the day, most females feed at night.

The temperature is maintained at approximately 75°F. and the relative humidity between 80 percent and 95 percent.

Both natural and artificial light are used. Natural light enters through three windows. Artificial light is provided by fluorescent ceiling lights and a rheostat controlled lamp located just outside the cage into which light is reflected by a metal sheet. The light schedule simulates the dawn and dusk light intensities recorded in the center of the cage during July and August. It consists of a dawn period of 1 hour, a day period of 12½ hours, a dusk period of 1½ hours and a night period of 9 hours. Peak mating occurs when the light intensity in the center of the cage is less than one foot candle.

Eggs. Most oviposition occurs during darkness, although a few rafts are deposited during the day. Females usually oviposit in the larval rearing tubes but occasionally oviposit on water puddles on the floor. Hatching occurs within 48 hours.

Larvae. Larvae are reared in tap water containing food pellets of high protein content. The medium, which is contained in tubs 18 inches in diameter and 8 inches deep, is aerated by bubbling compressed air through it. Approximately 2,500 larvae can be successfully reared in a tub of this size. The duration of the larval stage averages 6 days.

Pupae. Pupation, which requires about 2 days, occurs in the larval medium and the adults emerge directly into the cage.

SUMMARY. A colony of Culex tritaeniorhynchus has been successfully reared through an estimated 6 generations in the laboratory. The colony is housed in a cage 11 feet long, 8 feet wide and 8 feet high. Temperature is maintained at

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75° F. and the relative humidity from 80–95 percent. A light schedule is used that simulates the dawn and dusk lighting conditions produced in the cage during July and August and provides a total daylight period of over 12 hours. Sucrose, fresh apple slices, a pig and rabbits are provided as food for adults. Larvae are reared in aerated tap water containing high protein food pellets.

EGG FORMATION AND OVIPPOSITION IN BLOOD-FED Aedes aegypti L.

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Investigation of the blood meal factor which is required by adult female mosquitoes for egg production necessitates the development of a suitable assay to evaluate test diets. Some of the conditions of such an assay have been studied, and it was noted that maximal egg deposition was related to mating. Such a relationship has been discussed by several investigators (MacGregor, 1931; Mellenby and Mellenby, 1937) but the recent studies of Gillett (1955) demonstrated this with a strain of Aedes aegypti. From more recent studies, Lang (1956) suggested that the blood meal is the primary stimulus for egg formation, whereas mating is concerned chiefly with oviposition. The results of further experimentation to elucidate these interrelationships are presented here.

Experimental Procedure. Larvae of Aedes aegypti were reared on a commercial feed medium in enameled pans. Males and females were separated in the pupal stage. Adults were divided into two groups: one contained only virgin females, and the other contained females plus an excess number of males. Both groups of mosquitoes were maintained on a 10 percent sucrose solution for the remainder of the experiment. After 7 to 10 days from emergence, the mosquitoes were deprived of food and water for approximately 20 hours and then offered a blood meal from the forearm of a human host. Following this meal, engorged females from each test group were transferred to individual test cages, and two males were added to the cage of each “mated” female. The control series of both virgin and mated females was maintained on sucrose solution alone. For the next 14 days of the experimental period the cages were kept in a humid condition (greater than 70 percent R. H.) at a temperature of 23–25° C. Cages were examined daily, and the oviposition time of each female was noted. This experiment, which included a total number of 128 virgin and 46 mated females, was replicated 4 times.

In the first three experimental series each female was dissected on the 14th day post-blood meal and examined microscopically after oviposition to ascertain the number of eggs retained in the abdomen. In addition, every mated female was examined to determine the presence of sperm in the spermathecae.

To obtain information on ovarian development and egg formation, a series of mosquitoes from all groups were dissected at daily intervals from the time of emergence through a four-day period following engorgement.

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