OBSERVATIONS OF A LABORATORY COLONY OF THE MOSQUITO *CULEX TRITAENIORHYNCHUS* GILES [1]

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INTRODUCTION. Since the publication of the preliminary report on the laboratory colonization of *Culex tritaeniorhynchus* (Newson, Blakeslee, *et al.* 1956) more detailed observations have been made on the bionomics of the colony and a more complete evaluation has been made of some of the changes noted in the behavior of this species during its adaptation to the laboratory environment. The following account includes information collected subsequent to the preparation of the preliminary report, a description of some of the adaptive changes observed in the early generations of the colony and the procedures followed in establishing a subcolony in a small cage in this laboratory.

COLONY BIONOMICS. The stock colony is now in the estimated twelfth generation and still is maintained in the large room-sized cage under the conditions described in the preliminary report. Egg raft viability has increased slightly above that reported for the sixth generation and approximately 97 percent of the egg rafts currently produced contain viable eggs. The size of the egg rafts may vary considerably. A group of over 200 egg rafts selected at random from those produced over a period of three weeks contained from 97 to 427 eggs each with an average of 214 eggs per raft. An average of 90 percent of the eggs in each of these 200 rafts was viable.

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The average duration of the larval and pupal stages has not changed. Development may be retarded several days by lowering the temperature, but a critical study has not been made of the effect of temperature fluctuations on the time required for larval and pupal development.

Under the conditions maintained in the laboratory colony, males have not been observed to live more than 29 days while the maximum female longevity observed thus far was 107 days. One female produced a normally formed, viable egg raft when 102 days old and took a full blood meal from a rabbit the following night. This female had been isolated from males for 78 days prior to the date the egg raft was produced. The stage in which this species naturally overwinters in Japan is not known. On the basis of this laboratory evidence, it seems quite likely that, from the viewpoint of longevity, some adult female *Culex tritaeniorhynchus* should be able to overwinter successfully and be capable of taking blood meals and producing viable egg rafts in the spring when they again become active.

Some females in the laboratory have become inseminated and have taken their first blood meal as early as 56 hours following emergence. They have produced viable egg rafts 4 days after the blood meal. The majority, however, do not mate or take their first blood meal until 7 to 8 days after they emerge. Some females have been observed to mate repeatedly while there are some which apparently do not become inseminated or take a blood meal during their entire lifetime. Some are known to mate prior to taking a blood meal and others mate afterwards but detailed studies have not yet been made to determine what correlation, if any, exists between these two activities.

A limited study completed by the Virus Department of this laboratory indicated that the mosquitoes in this laboratory colony have retained, to date, their ability to transmit the virus of Japanese B encephalitis in laboratory animals (Gresser, *et al.*).

Growths of protozoa, *Vorticella* sp., occasionally have been observed on pupae and fourth instar larvae in the rearing containers. The infested individuals are sluggish and move slowly when disturbed. While mortality is high in these larvae and pupae, apparently normal adults have developed from individuals which supported very heavy growths of protozoa during their immature stages. The nature and extent of the injury caused by these protozoa has not yet been determined.

BEHAVIOR CHANGES. Green filamentous algae, mainly *Spirogyra* sp., were added to the larval rearing media during the first three generations of the colony, since *C. tritaeniorhynchus* larvae frequently had been observed grazing on algae of this type in natural breeding sites. After the second generation the larvae in the colony no longer ate the algae so its use was discontinued.

Several changes have been noted in adult behavior during the adaptation of the colony to laboratory conditions. The females originally were quite reluctant to feed on humans but now do so readily. Although swarming still occurs both mornings and evenings, the swarming activity has been modified somewhat. In the early generations of the colony, swarming activity in the evening began when a small number of males would begin to swarm near the floor in the dark corners of the cage. Additional males joined and these small swarms slowly increased in size, moved towards the center of the cage and merged into one large swarm as the cage became darker. Copulation occurred as females flew into the swarm. The females apparently were attracted to the swarm by the sound produced by flying males for, when the air conditioning system and aquarium aerators were turned off during the swarming period, females flew from all parts of the cage directly to the swarming males. When this equipment was kept in operation, however, the only females that entered the swarm were those which apparently encountered it during random flight around the cage. The aerators, therefore, are routinely turned off during swarming. At the present time males no longer form small swarms as a prelude to general swarming activity. Swarming occurs throughout the entire cage in the mornings and evenings when light intensity is approximately one foot candle or less and both males and females actively participate in the swarming flights. The flight of both males and females during the morning and evening swarming is much more rapid and erratic than observed during the early stages of colony development and the spatial requirement for the swarming flight is much less.

From May, when the colony was started, until September natural daylight entering the cage through windows provided the only illumination except for the overhead fluorescent lights which were used on cloudy days. Several series of light intensity readings were made in the cage during the dawn, mid-day and dusk periods in June, July and August and detailed notes were made on the behavior of the adults at the various light intensities. The lower light intensities were measured with a Model 200-M Photovolt Exposure Meter and the higher ones with a Model 8DW58Y4 General Electric Exposure Meter. During the last two weeks of September the flying, feeding and mating activity and egg production of the colony were observed to be steadily decreasing. Several females taken from the cage during a routine examination on September 26 were found to have well developed fat bodies. No extensive fat body development had been found in any of the colony females prior to this time. The only conditions changed in the cage between summer, when the adult activity was high, and September, when the described trend towards hibernation was observed, was a decrease of approximately two hours in the time the cage was exposed to light each day. On September 29 the light schedule described in the original report was started. This approximated, both in the intensity and duration, the light conditions recorded in the cage in mid-summer. Within two weeks after the artificial lighting schedule was started, the feeding, flying and mating activities had risen to their former levels. Egg raft production rose sharply and from 94 to 98 percent of all egg rafts produced contained viable eggs. The same light schedule is still being used and the level of activity in the colony remains essentially unchanged.

Major Herbert Barnett, in personal correspondence, stated that in attempts to establish a subcolony of *C. tritaeniorhynchus* at the Walter Reed Army Institute of Research from material shipped to him from this laboratory, it was necessary to maintain a daily photoperiod of 15 hours in order to maintain a satisfactory level of adult activity. It thus appears that one of the critical factors in maintaining adult activity in a laboratory colony of this species is the daily period to which it is exposed to light. A daily exposure period of 15 hours is adequate to maintain adult activity in the colony while a reduction of two hours in the light period may cause a sharp decrease in feeding and mating activity and result in gonotrophic dissociation in the females.

SUBCOLONIZATION IN A SMALL CAGE. A self-sustaining subcolony is now established in this laboratory in a 3 x 3 x 3 feet screened cage. Several groups of pupae were taken from the main colony in October, when it was in the estimated fifth and sixth generation, and placed in the small cage. Adults emerging into the cage were given fresh apple slices, cotton, wads soaked in 2 percent sugar solution and a rabbit as food sources. This small cage was kept at the same temperature and relative humidity and exposed to essentially the same artificial light schedule as the main colony. Larvae were reared outside the cage using the same technique as for the main colony and the pupae were removed from the rearing containers and placed back into the cage where the adults emerged. Seventy-one percent of the egg rafts produced by the first generation and 94 percent of those produced by the second generation contained viable eggs. The subcolony is now in the estimated seventh generation and produces an average of 10 egg rafts per day. Approximately 97 percent of the rafts currently produced contain viable eggs. The rafts compare favorably in size with those produced in the main colony. The adults are large and vigorous and mating and feeding activity remains at a high level.

Summary. Ninety-seven percent of the egg rafts now produced by the stock colony of *Culex tritaeniorhynchus* at the 406th Medical General Laboratory contain viable eggs. The rafts containing viable eggs have an average of 214 eggs per raft of which an average of approximately 90 percent hatch. When maintained at 75° F. and a relative humidity of 80–95 percent no males have been observed to live more than 29 days while females have produced viable eggs at 102 days, taken a blood meal at 103 days and remained alive and active up to 107 days after emergence. Females may take blood meals and mate as early as 56 hours after emergence and can produce viable eggs four days after a blood meal. During adaptation to laboratory conditions changes were noted in the feeding behavior of both larvae and adults and the swarming behavior of adult males and females was modified. Females in the colony have retained, to date, the ability to transmit the virus of Japanese B encephalitis in laboratory animals. It appears that a daily light exposure period of approximately 15 hours is necessary to keep adult feeding and mating activity at a level high enough to establish and maintain a colony of this species in the laboratory. A self-sustaining subcolony has been established in a 3 x 3 x 3 feet cage by using essentially the same rearing technique and lighting schedule as is employed in the stock colony.

Acknowledgment. The authors wish to express their appreciation to S. Toshioka, M. Sakai, T. Shimada, J. Akiyama, Sp/3 R. G. Laraway and Pfc. C. R. Pearce for their assistance.

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