watt bulb within a small wooden or metal box will usually provide sufficient heat for drying the mount. It is not recommended that a heating table or hot plate be used, as they tend to cause bubble formation. The heat should come from all directions, rather than just from below.

SUMMARY. Rapid mounting of mosquito larvae has been described. One technique involves 2 hours storage of the larva in a dehydrating-clearing mixture consisting of either 70 cc. of beechwood creosote and 25 cc. of 95 per cent pure ethyl alcohol, or 70 cc. of absolute phenol and 25 cc. of 95 per cent pure ethyl alcohol. Then the larva is cleared fully in pure beechwood creosote for several minutes, and mounted in either euparal or diaphane. The second technique involves the use of lactophenol as a clearing agent or temporary mountant, and polyvinyl alcohol as a clearing agent and permanent mountant. In every case living larvae may be used directly without prior killing. Eggs, egg rafts, and pupae may be mounted also with these procedures. Heating of the finished slide in a drying or cooking oven at 100 degrees F. for 30 minutes assists materially in rapid drying of the mount.

Literature Cited

BURTON, G. J. 1953. Some techniques for mounting mosquito eggs, larvae, pupae and adults on slides. Mosquito News 13(1):7-15.

DOWNS, W. G. 1943. Polyvinyl alcohol: A medium for mounting and clearing biological specimens. Science 97:539.

SIMPLIFIED TECHNIQUES FOR THE CONTINUOUS REARING OF *CULEX TARSALIS* WITH ADDITIONAL NOTES AND OBSERVATIONS [1]

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Since the preliminary report on the colonization of *Culex tarsalis* by Brennan and Harwood (1953), we have been able to refine somewhat the methods earlier used in maintenance of the Rocky Mountain Laboratory's stock colony. A subcolony has been successfully established in a small cage in a window-equipped room of the insectary without controlled lighting, and a considerable amount of miscellaneous biological information has been accumulated. The report cited above is a necessary adjunct to this paper.

MAIN COLONY. The stock colony is still housed in the large walk-in cage. Although there are strong indications that our strain of *C. tarsalis* is now well adapted to conventional rearing techniques, we are still continuing with a simplified conditioning process as a precaution against the loss of the colony. Temperature and humidity are maintained at about 70° F. and 70 per cent, respectively. Light is provided by overhead fluorescent tubes and by a rheostat-controlled 300-watt lamp now directed toward an upper corner of the room. A twilight period is initiated by extinguishing the ceiling lights, giving an exposure meter (Weston Master II) reading in the cage of approximately 6.5. After about 15 minutes, voltage to the rheostat-controlled lamp is rapidly reduced from 110

[1] From the U. S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, National Microbiological Institute, Rocky Mountain Laboratory, Hamilton, Montana.

to about 55. After another 15 minutes the lamp is extinguished and the room is left in darkness for 5 hours. The dawn period is eliminated and all lights are switched on. These time intervals and light intensities are apparently not critical.

Certain behavioral changes have become apparent between the tenth and the present estimated twenty-fourth generations. The mosquitoes feed more readily on humans entering the cage. Twilight swarming now begins at a greater light intensity and also more swarming occurs when the lights are turned on. In addition, the swarms are noticeably larger.

Yeast and dried milk have been eliminated from the diet of the larvae which are now supplied only with the high protein pellets. The initial feeding per pan of 300 to 500 larvae consists of about ¾ grams of pellet, a portion of which is crushed. Thereafter a whole pellet (about ½ gram) is added every 3 or 4 days. Mr. Ian S. Lindsay, Defence Research Board, Suffield Experimental Station, Ralston, Alberta, has reported (personal communication) the successful establishment of a colony from our strain. He states that the larvae are fed ground whole wheat bread supplemented with whole milk powder and brewer's yeast rather than the high protein pellets, that air is bubbled through the larval medium, and that larval mortality has been about 2 per cent.

The percentage of rafts containing viable eggs has remained high and during the period of 11 March to 13 July showed an increase when 1392 rafts of 1607 deposited (87 per cent) contained viable eggs. A consideration of single day's production of viable rafts reveals the number of eggs per raft to be similar to the value previously reported, but a marked increase is noted in the number of eggs hatched per raft. A review of the data obtained from the rafts of May 22, 1953, to cite a specific example, shows that of 4418 eggs in 19 rafts, 4110 eggs hatched, a viability of 93 per cent. The average number of eggs per raft was 232.5. The minimum number and maximum number per raft were 73 and 352 respectively. It is interesting to note that there was a 100 per cent hatch from each of these extremes.

The following experimental rearings suggest that the large amount of space provided by a walk-in cage may no longer be necessary.

SUBCOLONIES WITH CONTROLLED LIGHT. A secondary colony was started in a sleeve equipped cage, 16 x 16 x 24 inches, with F_5 pupae from the principal colony. This subcolony, as well as the others mentioned in this section, is in the same room as the main colony, and is therefore subject to controlled light. Both guinea pigs and chickens have been used as sources of blood meals. In the first 9 months after its establishment this colony produced 76 egg rafts, of which 396 (52 per cent) contained viable eggs. This low viability level has been more than adequate for maintenance of the colony which is now in its approximate twelfth generation.

In another small cage of mosquitoes guinea pigs only were used as a source of blood. This colony was started with eggs from the approximate F_7 generation of the stock colony, and is now in about its ninth generation.

Humans have been the only source of blood for mosquitoes in a third cage. This colony originated from a single egg raft from a female which had fed on human blood. During the first 4 months of the colony's existence 27 (84 per cent) of the 32 egg rafts produced contained viable eggs, a viability comparable with that of the main colony. The mosquitoes feed on the blood donor's arm which is held against the screen of the cage. This colony is now in approximately the ninth generation.

Another colony of mosquitoes was maintained through 3 generations in a cage of less than ½ cubic foot volume. The only source of blood was a local garter snake (*Thamnophis* sp.) which was kept in the cage at all times. The experiment was discontinued upon death of the snake. The few egg rafts produced were very small.

SUBCOLONY WITHOUT CONTROLLED LIGHT. When the main colony was in ap

proximately the seventh generation, several hundred first instar larvae were removed to a room having windows. It was desirable to determine whether a subcolony could be established and maintained with a minimum of attention and effort. In all respects, except for the manner of obtaining eggs, the method followed duplicates that now used at this Laboratory in rearing *Aedes aegypti*.

In this room the temperature and relative humidity are about 77° F. and 70 per cent, respectively. No attention is given to the illumination, except that on dark winter days a point is made of leaving the artificial lights on during most of the working hours. The cage is the 16 x 16 x 24-inch size mentioned above. Guinea pigs are used as the only source of blood and are usually provided during the hours of daylight as often as necessary for maintenance of the colony. Occasionally it has been necessary to supplement the daylight feeding with a blood meal at night. Although feeding has been observed at all daylight hours, maximum feeding occurs with the onset of twilight.

The immature stages are handled essentially like those of the main colony. High protein pellets are the only food for the larvae. When pupation begins, the entire contents of the pan are poured into a smaller container which is placed inside the cage for emergence.

No accurate count was kept of the number of egg rafts or the number which hatched. However, in most generations 70 to 80 per cent of the rafts contained viable eggs. The critical period occurred in the F_3 generation, when only about 40 per cent of the rafts produced any larvae. This colony is now in the F_{16} generation and is thriving. In a representative sample of eight F_8, well-formed, viable egg rafts produced over a period of several days, there was an average of 194 eggs per raft, with 97 per cent viability, or a hatch of 1511 from a total of 1550 eggs.

MISCELLANEOUS EXPERIMENTS AND OBSERVATIONS. The approximate length of the egg stage at room temperature was determined for 22 rafts that were either seen during the act of oviposition, or were collected while they were still white. All hatched sometime during the third day. Of 8 white rafts collected at the same time, one hatched 54 hours later; the remainder hatched within the next 2 hours.

The results of limited experimentation to determine the optimum number of larvae per unit area and volume of water and optimum diet cannot yet be accurately interpreted because of variables not now understood. However, it was learned that high protein pellets alone are an adequate diet. A disturbing factor in such experimentation is the fact that in an occasional larval pan of a presumably identical series, the pellet material becomes scummy and a pellicle forms on the water surface with subsequent high larval mortality.

Repeated observations have indicated, as might be expected, that a blood meal is necessary for oviposition. It has been noted further that at 70° F. and 70 per cent humidity blood meals will be accepted as early as the third day after emergence, and that oviposition can occur as early as the fourth day after a blood meal.

We have observed that although up to 90 per cent of the females readily accept an initial blood meal when offered, usually no more than 3 per cent can be induced to refeed at a particular offering, nor more than 15 per cent throughout a course of repeated offerings. In virus studies with *C. tarsalis* in this Laboratory, average refeeding rates for caged lots have been less than 3 per cent, the greatest having been about 20 per cent, and the average refeeding rate for isolated individuals has been less than 10 per cent. The implications are highly significant in the epidemiology of an arthropod-transmitted disease, and of such consequence in experimental transmission that a special study is being made of the blood-feeding tropism with emphasis on the factors which stimulate refeeding.

In certain laboratory experimentation it is sometimes desirable to retard the development of stages in the mosquito life cycle. Although precise information is

not available at this time, preliminary studies have indicated that eggs and pupae can be held at about 32° F. for 10 and 4 days, respectively, and remain viable. Critical studies of this nature are currently in progress.

SUMMARY. Simplified methods of maintaining *Culex tarsalis* at the Rocky Mountain Laboratory are described. The percentage of viable egg rafts produced by the main colony remains high and at times has risen to 87 per cent.

Colonies can be maintained in small cages with or without controlled lighting conditions. Chickens, guinea pigs, humans, or snakes can be utilized as sole sources of blood, and high protein pellets alone are satisfactory larval food. These colonies, with the exception of the snake-fed colony, have all survived for at least 9 generations.

It was determined that at room temperature females will accept blood as early as 3 days after emergence, that the initial feeding rate is high but the refeeding rate is disproportionately low, that a blood meal is required for oviposition, that oviposition can occur as early as 4 days after a blood meal, that eggs hatch on the third day after oviposition, and that the development of eggs and pupae may be retarded by refrigeration yet remain viable.

From the rearing techniques described it would appear that *Culex tarsalis* has, after several generations, become well adapted to insectary conditions. Methods which were inadequate for colonization from wild stock are now used successfully for maintenance of laboratory colonies. Thus, it is seen that the species can now be reared continuously in the absence of controlled light. It is also noteworthy, both from the biological standpoint and from that of laboratory convenience, that our strain of this naturally eurygamous species will mate successfully in confined spaces. It is of great interest to us that the more conventional rearing techniques can be applied so soon to a mosquito initially refractory to all attempts at colonization.

Literature Cited

BRENNAN, JAMES M. and HARWOOD, ROBERT F. 1953. A preliminary report on the laboratory colonization of the mosquito, *Culex tarsalis* Coquillett. Mosquito News 13(2):153–157.

"It is our conviction that the pest mosquitoes should receive more attention from health authorities than they have in the past. Public health has become something more than the absence of disease. PHYSICAL EFFICIENCY AND COMFORT, ON WHICH MENTAL EQUANIMITY DEPENDS TO A SUBSTANTIAL DEGREE, MAY BE SERIOUSLY DISTURBED BY THE CONTINUED ANNOYANCE OF PESTIFEROUS MOSQUITOES WHICH MAY OR MAY NOT HAVE DISEASE-TRANSMITTING POTENTIALITIES." (George H. Bradley, U. S. Public Health Service, Communicable Disease Center, Atlanta, Georgia.)