

# A PRELIMINARY REPORT ON THE LABORATORY COLONIZATION OF THE MOSQUITO, *CULEX TARSALIS* COQUILLET<sup>1</sup>

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**INTRODUCTION.** In the epidemiology of western equine encephalitis, *Culex tarsalis* appears to be the most important mosquito species concerned. It is the only mosquito from which the virus has been repeatedly isolated during epidemic and non-epidemic years. In some endemic areas this species is probably an important vector in the transmission of the virus to man. At present it seems that the only promising approach to the study of the ecology of the virus is through the bionomics of *C. tarsalis*. Isolations of the viruses of both St. Louis and California encephalitis from this same mosquito suggest its further medical importance. The need, then, of a laboratory colony of *C. tarsalis* is obvious.

Dr. Bernard Brookman working at Bakersfield, California, in 1951, reported (personal communication) the continuous rearing of the species outdoors from May to October when the females, apparently entering a diapause, refused further blood meals and the declining colony was permitted to expire. His attempts to establish a colony indoors were unsuccessful. Also in 1951, the senior author experienced some measure of success in rearing the species in the laboratory through three generations, but efforts to establish a colony were unsuccessful. It thus became apparent that, although *C. tarsalis* is a common and widely distributed mosquito in North America with diversified larval habitats ranging from the thermal springs to liquid manure, the usual rearing tech-

niques were unsuitable for successful laboratory colonization of the species.

A method developed in August 1952 and currently in use at the Rocky Mountain Laboratory, while still somewhat cumbersome, has resulted in the successful colonization of *C. tarsalis*. The colony, now in its tenth generation (estimated, since no effort was made to record generations beyond F<sub>4</sub>), is flourishing. Adult and larval mortality has been minimal. The adults are large and vigorous. There has been no suggestion of a diapause.

The growth of the principal colony is perhaps best reflected in egg raft production. During the period of 15 October to 31 December, 107 rafts (1.4 per day) were deposited; 78 (73%) contained viable eggs. During the period of 1 January to 10 March, 787 rafts (11.4 per day) were deposited; 600 (76%) contained viable eggs.

**MATERIALS AND METHODS.** In earlier experiments, inducing mating in captivity had been the chief obstacle in the continuous rearing of *C. tarsalis* (a eurygamous species). This prompted more critical observations of the correlation between adult sexual behavior and environmental conditions in nature. From these observations it seemed expedient to initiate a rearing technique based on a process of conditioning adults by simulating as accurately as possible in the laboratory the presumed factors which stimulate mating. The conditioning routine was derived from a composite day of a number of summer days in Hamilton, Montana, when prevailing conditions were favorable to swarming, emphasis being placed on the critical periods of dawn and twilight, i.e., the periods of peak activity for *C. tarsalis*.

The technique described below is flexible and should lend itself readily to modification to meet existing needs. Although

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certain steps may not be essential, and others can be eliminated, when feasible, or as evidence of adaptability is obtained from succeeding generations, it appears that a conditioning process, at least for the earlier generations, is fundamental to the success of colonizing *C. tarsalis*.

The Rocky Mountain Laboratory strain is a mixture started with gravid females from Bismarck, North Dakota, and eggs, larvae, and pupae from Hamilton, Mon-

tana, all collected in midsummer of 1952.

Adults of the first laboratory generation were subjected to an artificial day by caging them in a windowless room of the insectary where light, temperature, and humidity are controlled, either automatically or manually, as desired.

Cages. The main colony cage (Fig. 1) of metal screen and wood frame is a large, portable, walk-in model without a floor, and is approximately 7 feet high, 6 feet

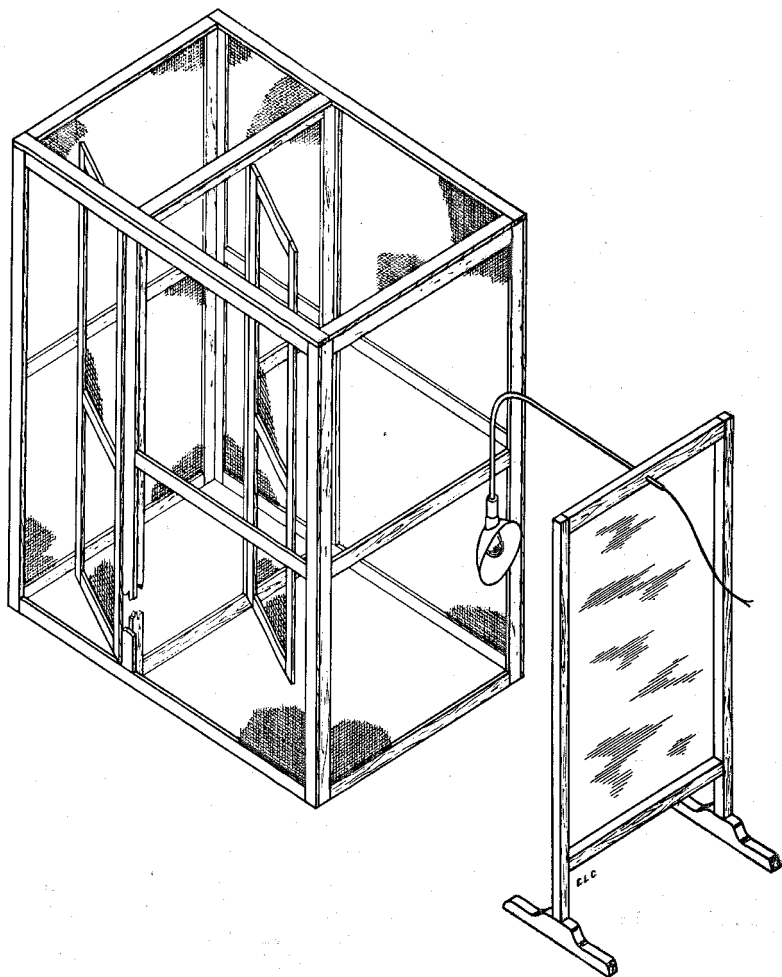


FIG. 1. Isometric projection of main colony cage, rheostat-controlled lamp and screen as arranged for the conditioning and continuous rearing of *Culex tarsalis*.

long, and 4 feet wide. The cage is divided into two compartments. An outer screen door opens into an entrance lock, or trap, (2 x 4 feet) from which an inner screen door opens into the mosquito chamber (4 x 4 feet). The doors are so placed that the inner can not be opened until the outer is closed. A small sleeve in the wall between the mosquito chamber and the trap is convenient.

Small cages (16 x 16 x 24 inches) of the conventional sleeve-equipped type are also used, but to date, not with the degree of success achieved in the walk-in cage since a large space is more conducive to swarming.

**ADULTS: CARE AND CONDITIONING.** The conditioning routine is begun immediately with the  $F_1$  adults in the walk-in cage. The daily cycle is divided into four parts: A day period of about 16 hours, a twilight period of about 1 hour, a night period of about 6 hours, and a dawn period of about 1 hour.

During the day period the temperature may range from  $75^\circ$  to  $79^\circ$  F with a relative humidity of about 40%. The source of light is entirely artificial and is produced by overhead fluorescent tubes of the daylight type and a rheostat-controlled 300-watt lamp located just outside the mosquito compartment into which it is reflected by a white screen (Fig 1). Under conditions of full daylight, with all ceiling lights operating and the rheostat set at 110 volts, the intensity is such that a reading, taken in the center of the cage, on a Weston Master II Exposure Meter directed toward the reflecting screen is 13.

Throughout this lengthy day period the mosquitoes are resting in the darker portions of the cage, for the most part concentrated on the higher parts of the framework. They exhibit little activity except when disturbed, or when feeding on a 10% sugar solution which is constantly available in soaked cotton pads distributed on top of the cage.

A short period of twilight provides a gradual transition from day to night. This critical period is begun by setting thermostat and humidistat at  $60^\circ$  and 70% re-

spectively and switching off ceiling lights. In the center of the cage the exposure meter reading is 6.5. For the next half hour or longer the light intensity from the rheostat-controlled lamp is slowly decreased by reducing the voltage, in about 10 steps, from 110 to 0. This operation is performed manually since it is desirable to make frequent observations of mosquito responses.

Mosquito activity begins early in the twilight period and is continuous throughout. Swarming is performed by the males with an occasional female participating. This behavior in the laboratory parallels closely the phenomenon as observed in the field. Swarming is accompanied by few to numerous matings on the wing as females fly through the swarm, and usually reaches a peak when voltage settings on the rheostat are between 45 and 30. At this interval the readings on the exposure meter in the center of the cage are from 0.3 to 0.1 (estimated). When it is desired to prolong the swarm period, the stepping-down procedure with the rheostat is discontinued for nearly an hour, then continued to darkness.

Two or three times weekly, during the night period, blood meals are provided for the females by suspending a half-grown chicken in a restraining cage of chicken wire from the top of the mosquito cage. Guinea pig blood is acceptable, but chicken blood appears to be preferred and definitely contributes to the production of larger egg rafts.

The dawn period is merely a reversal of the twilight period. The thermostat and humidistat are set at  $75^\circ$  and 40% respectively. The intensity of the rheostat-controlled lamp is gradually increased from 0 to 110 volts and the overhead lights are switched on. Swarming and mating occur at this time, but not to the extent observed at twilight.

This conditioning process was continued as a daily routine for about 6 generations when it was demonstrated that certain steps could be eliminated without jeopardizing the colony. With the next generation temperature and humidity were main-

tained at 70° and 70% respectively throughout the 24-hour cycle, and with the succeeding generation the dawn period was eliminated entirely. The decreasing light intensity during the twilight period seemed essential and was continued. Occasionally the colony was purposely neglected and it remained over week ends under constant daylight conditions with no adverse effects.

**EGGS.** Oviposition takes place, either during darkness or light, directly in the larval pans which, for convenience and to save space, are kept in a cross-stacked pile on the floor in the walk-in cage. The egg rafts are collected daily in beakers of distilled water.<sup>4</sup> Upon hatching, which takes place 2 to 3 days after oviposition, the young larvae are poured into freshly prepared pans.

From Table 1, based on one day's egg

TABLE 1. Egg production and viability  
(23 January 1953)

Raft No.	No. Eggs	No. Eggs Hatched
1	272	106
2	302	302
3	138	138
4	221	221
5	271	271
6	117	112
7	333	333
8	291	212
9	295	295
10	239	135
11	294	294
12	187	129
13	272	266
14	143	121
15	139	139
16	162	106
17	193	180
18	208	162
19	227	224
20	279	279
21	265	12
Total	4848	4037

Average eggs per raft: 230. Percent viability: 83.3.

<sup>4</sup> Distilled water is used throughout our rearing technique. The tap water available is undesirable because of the formation of a pellicle after standing.

production, it will be noted that the average number of eggs per raft was 230 with a viability of over 83%, and that on this particular day 100% hatching occurred in 9 of 21 egg rafts produced.

**LARVAE.** In preparing a fresh larval culture, a white enameled pan, 2½ x 12 x 18 inches in size, is filled with distilled water to a depth of about ½ inch. About 0.5 gm. of a crushed pellet<sup>5</sup> of high protein content (32%) and about 1.0 gm. of a mixture of equal parts brewer's yeast and dried milk are diffused through the water. A pan thus prepared readily accommodates 300 to 500 larvae without crowding. A whole high protein pellet is added every third day until pupation.

The larvae are for the most part surface feeders and their activities appear sufficient to keep the water fairly clean. When an occasional surface film forms, it is easily removed by sweeping the water surface with a sheet of paper. Dead larvae are not removed. The duration of the larval stages varies from 8 to 13 days, and averages 10 days.

**PUPAE.** Pupae may be handled as described. Pupation, which requires about 3 days, occurs in the larval pans and the adults emerge directly into the cage.

From the above it will be seen that in the continuous maintenance of *C. tarsalis*, the entire life cycle and the concomitant overlapping of generations occur within the main colony cage. Under the conditions described, the time required to complete the life cycle is approximately 25 days.

As suggested earlier, continuous rearing in smaller cages is not so productive as in the walk-in cage. When about 350 F<sub>5</sub> and F<sub>6</sub> pupae from the main colony were transferred to small cages in the conditioning room, the emerging females and those of two succeeding generations deposited 375 egg rafts at the rate of 4.6 per day during the period of 13 January to 3 April. Of these, only 167 (44%) contained viable eggs. However, except for this lower via-

<sup>5</sup> *Hi-Protein Supplement*, Misco Mills, Missoula, Montana.

bility index, no other significant change has been observed. The adults continue to be robust, feed readily, and swarm. Larval and adult mortality appears to be no greater than normally expected.

The erratic results from attempted rearings (again starting with F<sub>5</sub> and F<sub>6</sub> pupae from the stock colony) in small cages in a window-equipped room at a constant temperature and humidity of about 70° and 70% do not warrant further reporting at this time.

Up to the present time no serious effort has been made to determine longevity of captive adults. However, some males have been observed to live 2½ months and some females more than 4 months.

#### SUMMARY

A method is described for the continuous rearing of *Culex tarsalis* Coq. in the laboratory.

The adults are eurygamous; therefore it appears that successful colonization is contingent upon their induced adaptation to captivity by a conditioning process

which has as its basis a day simulated from nature when conditions are favorable to sexual responses. A relatively large space and a variable light intensity are the principal factors required to stimulate swarming and mating.

A satisfactory diet for adults is chicken blood and sucrose; and for larvae a proprietary pellet of high protein content supplemented with brewer's yeast and dried milk is suitable.

Approximately 75% of all egg rafts from the principal colony contain viable eggs. In one day's production of 21 rafts, the average number of eggs per raft was 230 and the viability was somewhat greater than 83%.

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The isometric drawing of the mosquito cage was prepared by Mr. E. L. Cole, biological engineer of the Rocky Mountain Laboratory.

## AN ADULT MOSQUITO SAMPLER

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It has long been realized that light traps (Mulhern, 1942)\* used for sampling mosquito populations are subject to several undesirable limitations. One of these is the inclusion of large numbers of unwanted insects with each trap collection, thereby adding to the task of mosquito identification. Another handicap is that conventional light traps must be located near sources of electricity for power and light,

which greatly restricts the area in which they can operate. An attempt to overcome some of these difficulties was made by Lindquist *et al.* (1945), who used mosquito biting or landing rates as an index to their population densities. Other workers have used various modifications of this technique to meet special situations. The umbrella-trap described here is a further attempt to improve upon these methods. Its use enables one person to collect all of the insects that would normally congregate around him in a definite volume of air during a given length of time. It can be operated during the night or day in almost any area accessible to an individual, and

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\* See also Mulhern, T. D., this issue of *Mosquito News*, page 130.—Ed. Note.