

# OPERATIONAL AND SCIENTIFIC NOTES

LABORATORY COLONIZATION OF *Culex stigmatosoma*.<sup>\*</sup> The successful establishment of laboratory colonies of *Culex tarsalis* (Brennan & Harwood, 1953; Brennan, Rush & Hubert, 1954; Hubert, Rush & Brennan, 1954) has suggested the possibility that other species of mosquitoes, previously refractory to laboratory cultivation, might be colonized using the same type of technique.

There is apparently no report in the literature of the successful colonization inside a laboratory of *Culex stigmatosoma*. Brookman & Reeves, (1952) according to Trembley (1955), were able to raise *C. stigmatosoma* in outdoor cages, during spring and summer. Brookman, according to a personal communication from R. E. Bellamy (1955), carried a self-perpetuating colony of *C. stigmatosoma* for several months in the summer of 1951 on a screened vestibule adjacent to the laboratory at Bakersfield, but no details have been published. Inasmuch as we have been successful in maintaining *C. tarsalis* in the laboratory for nearly two years under controlled illumination, we decided to apply the same techniques to a colony of *C. stigmatosoma*.

Through the courtesy of Mr. John Shanafelt of the Orange County (California) Mosquito Abatement District, we obtained a number of egg rafts of *C. stigmatosoma* on September 24, 1954. These rafts hatched in the laboratory and the adults produced were placed, on October 10, 1954, in a cage 17½" x 17½" x 36" high and exposed to a lengthened period of daylight of approximately 16 hours, with one hour of twilight, 6½ hours darkness and one-half hour of increasing illumination. After two days of conditioning, 20 female *C. stigmatosoma* along with 10 males were exposed with a canary overnight, but only 2 females engorged. The mosquitoes were again exposed to a canary on the nights of October 13, 16, and 17, but only 2 or 3 more mosquitoes bit, and no egg rafts were obtained.

On November 3, 1954, about 60 *C. stigmatosoma* of both sexes, which had hatched from the rafts collected on September 24, were exposed to 24-hour light conditioning in the same cage as the one used previously. The next night, 20 females bit when exposed with a canary; 2 rafts were found on November 10, 4 on November 12, and 8 on November 14. In all cases, these hatched 2 days after they were laid, and by December 1 had produced a sufficient number of adults for breeding a second generation in captivity.

On this date, about 50 adult mosquitoes were transferred to a smaller cage 12" x 12" x 36"

high and were maintained without controlled illumination in a window-lit laboratory. The adults exposed with a canary on December 1 again produced fertile eggs, with adults emerging on December 19.

As of the present date (April 12, 1955) 3 more generations of *C. stigmatosoma* have been obtained in the laboratory in the smaller of the two cages listed above and without controlled illumination. Laboratory temperature does not drop below 72° C., and a new generation of adults hatches out about 21 days (18-26 days) after the females of the previous generation have taken a blood meal. Fertility is high and there seems to be no reason why the strain should not continue breeding in captivity.

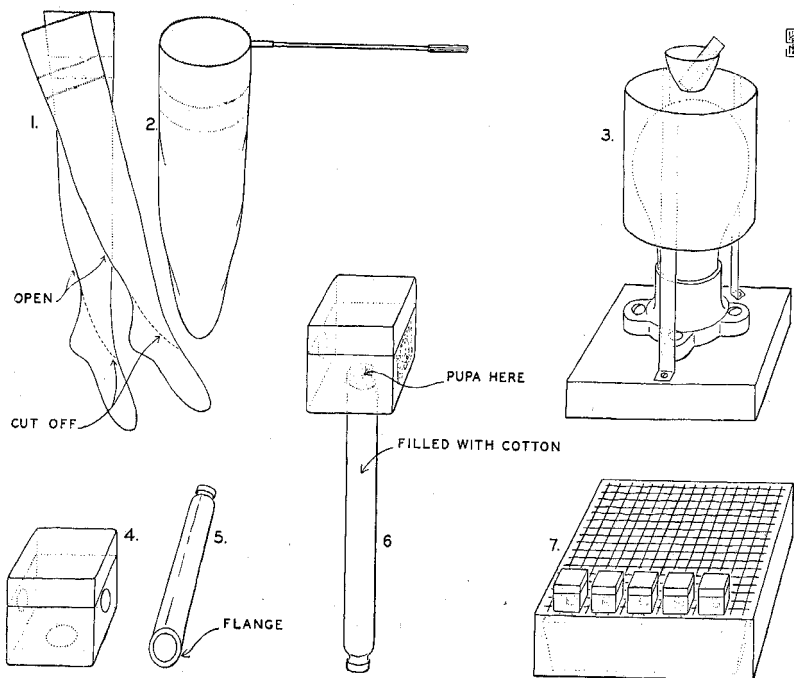
The adaptation of *C. stigmatosoma* to laboratory conditions with successful mating in small cages without controlled illumination is analogous to a similar type of adjustment exhibited in the Rocky Mountain Laboratory colony of *C. tarsalis* (Brennan, Rush & Hubert, 1954; Hubert, Rush & Brennan, 1954). Our own colony of *C. tarsalis*, obtained originally from the Rocky Mountain Laboratory through the courtesy of Dr. Brennan, is being maintained very satisfactorily under these conditions.—Gordon H. Ball & Jowett Chao, Department of Zoology, University of California, Los Angeles.

## Literature Cited

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DO-IT-YOURSELF ENTOMOLOGICAL EQUIPMENT: Net for capturing *Culicoides*, midges, black flies and mosquitoes in flight: Carefully open the back seams of two or three nylon stockings from the heel to the top; then cut the feet off at the desired arc, Fig. 1. Two stockings give a net

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diameter of approximately 9 inches, Fig. 2, and three stockings approximately  $13\frac{1}{2}$  inches. Outsize stockings offer a still larger net diameter. Carefully sew the sections together with nylon thread, using overcast stitching for the rolled seams. The material will not "run" at the seams because it is not taut. The tops of the stockings already provide a deep hem for the insertion of the frame loop. Specimens can be quickly removed from the inverted net with an aspirator while the collector is looking *through* the net. If the net becomes wet while capturing surface-skimming insects a few swings will suffice to dry it. A plastic cover, similar to those used for tennis rackets, will prevent the net from becoming snagged when not in use and the cover also serves as a handy seat when the ground is damp.

**HEATER FOR CLEARING INSECTS IN KOH OR LACTIC ACID SOLUTIONS, FIG. 3:** The necessary materials for this heater are: a block of wood about  $3 \times 3 \times \frac{3}{4}$  inches thick; three strips of aluminum about  $\frac{1}{4}$  inch wide and  $\frac{3}{4}$  of an inch longer than the combined length of an electric light socket plus a 40 or 60 watt bulb screwed in place; a tin can slightly bigger than the diameter of the light bulb; an electric cord and switch; a small crucible about an inch in diameter, containing water and a little shell vial containing the solution and specimen for clearing. Trace the outline of the can on the block of wood and

mount the bulb socket in the center of that circle. Bore a little hole in the center of each strip of aluminum about  $\frac{1}{4}$  inch from one end. Bend each strip at a right angle about  $\frac{1}{2}$  inch from the end containing the hole. Screw the metal "L's" to the block just inside the circle and equidistant from each other. Insert the switch in the cord. Screw the bulb in place and invert the tin can over the aluminum supports. Place the vial containing the solution and the specimen in the crucible of water and heat them on the can. My heater has been in use periodically ten years with only one change of bulb.

**INDIVIDUAL PUPAL-ADULT EMERGENCE CAGES FOR BLACKFLIES:** The following rearing equipment has proved very satisfactory for association of pupal and adult blackflies, yielding almost 100 percent perfect adult specimens and requiring no daily care other than a glance to see which ones have emerged.

The necessary materials for the cages consist of transparent plastic boxes  $22 \times 22 \times 20$  mm, high; glass tubing  $63 \times 8$  mm. (dental "procaine" tubes with the lip were used); nylon "tape" or ribbon; absorbent cotton; and household cement. The racks are made of  $\frac{1}{2}$  inch hardware cloth stapled to a square wooden frame about  $10 \times 10 \times 2\frac{1}{4}$  inches high, which fits over pans  $8 \times 8 \times 2$  inches high containing about an inch of water.

Drill a hole through the bottom of the plastic boxes just big enough to accommodate the large

end of the dental tubes, Figs. 4 and 5. Drill a smaller hole through each of two sides of the cages and glue patches of nylon mesh ribbon over these vents. In the absence of equipment for heating glass to put a flange on the broad end of the tubes, a ring of household cement will serve the purpose. The tubes may be cemented to the cages intruding a few mm. or they may be just dropped through the hole, resting on their flange, Fig. 6. The latter takes less storage space when the equipment is not in use, but requires more careful handling, to avoid jamming the tube against the lid and squashing the pupa when moving the cages about on the racks. (Some cages were fitted with thin sheet-cork bottoms, containing a hole for the tube, and a strip of adhesive tape on the two sides not containing the vents, while others were roughed-up on the inside with steel wool. These provisions for providing the flies with better footing are apparently not necessary.)

Pack the tubes firmly, but not too tightly, with absorbent cotton, being careful to fill the last quarter inch with a separate wad of cotton. Drop the tubes into the cages and place the cages on the racks with the tubes extending through the hardware cloth so that the bottoms of the tubes are immersed in water, Fig. 7. After the cotton becomes wet at the top the loose strands can be tamped into place. Then put an intact dark pupa, ventral side down, on the moist cotton and replace the lid of the box. When the adult emerges the date can be written on the lid with a wax pencil and the following day the specimen killed and labeled along with the associated pupal exuviae. Specimens are easily killed by inverting the cage and carefully removing the lid, then tapping the cage while it is held about  $\frac{1}{4}$  inch above a Syracuse dish of alcohol. The writing can be wiped off the lids and the cages used again. After a few weeks the top of the cotton sometimes becomes corroded or mouldy so the uppermost wad should be removed and a clean one substituted.—Kathryn M. Sommerman, Arctic Health Research Center, Public Health Service, Department of Health, Education, and Welfare, Anchorage, Alaska.

**THE EGG AND IDENTITY OF ALASKAN *Anopheles*.** About a hundred anopheline eggs were skimmed from a pond at Eklutna, Alaska on June 6, 1956 and examined on the spot under the binocular microscope. Twenty of 25 selected perfect, unhatched ova were plainly banded dorsally, fitting reasonably well the description given by Rozeboom (1952, *Am. J. Trop. Med. & Hyg.* 1:477-83) of barred eggs of *Anopheles earlei* Vargas in Montana. Departures from Dr. Rozeboom's photograph and description included: (1) one egg grey, unicolor; (2) four eggs mottled, unbanded; (3) nearly half the eggs with two pigmented lines on the dorsum, along the floats, which is evident also in Marshall's fig. 84, egg photograph 3 of *A. maculipennis* var. *typicus* of Europe. A large majority of the seventy-odd remaining

imperfect ova, mostly hatched, also showed transverse bars under the lowest power of the microscope, 9x.

Since acceptance (about 1948) of *Anopheles earlei* Vargas as a northern species distinct from *A. occidentalis* D. & K., culicidologists have often assumed on larval morphological grounds (*earlei*—branched antepalpal hair 2 on abdominal segments IV and V; *occidentalis*—those hairs single) that the Alaskan anopheline is *A. earlei*. The egg of the Alaskan *Anopheles* has not been described. I have lately used *Anopheles* sp. even though aware the antepalpal hairs are almost invariably multiple in Alaskan large larvae. I avoided use of "*earlei*" chiefly because the ova I collected from a pond near Chitina, Alaska and preserved, some in alcohol some in formalin, appeared entirely grey-brown, unbanded. It now seems likely that the Chitina eggs should have been examined fresh to reveal the color pattern. The purpose of this note is to acknowledge the morphological identity of Alaskan anophelines, at least from the coast of central Alaska, with *A. earlei* Vargas and to call attention for heuristic reasons to biological differences from Montana *earlei*.

Rozeboom (l.c.) suggests "that fundamental relationships between closely related species or subspecies are revealed more clearly through genetic and biological studies than through morphological comparisons." He showed that the multivoltine Montana form of *A. earlei* can be bred in the laboratory uninterruptedly generation after generation, that it will swarm and mate readily even in small cages simply upon insertion of the observer's hand, and that the female takes blood without protracted delay for an obligatory diapause. He considers the stenogamous behavior a specific character separating *earlei* from the morphologically similar *maculipennis* (type species) of Europe which requires a very large cage for swarming and mating.

Now, just such fundamental biological idiosyncrasies appear to separate Alaskan *earlei* from Montana *earlei*, including: (1) life cycle of the *Culiseta impatiens* type, i.e. single-broodedness with the female mating the season she emerges, engorging and ovipositing, however, only after hibernation nearly a year later; (2) courtship and mating eurygamous, requiring specific stimuli, still conjectural after two years' unsuccessful attempts at colonization. No mating took place in small cages or even in a large walk-in insectary under the stimulation of various arrangements of colored and white lights.

Hence, further biological studies like Dr. Rozeboom's work with Montana *earlei*, permitting comparison of Alaskan, Canadian, and United States populations of *A. earlei* with each other and with Siberian *A. maculipennis* might lead to more correct and significant names than the present ones.—William C. Frohne, Arctic Health Research Center, Public Health Service, Department of Health, Education, and Welfare, Anchorage, Alaska.