LABORATORY COLONIZATION OF THE MALARIA VECTOR, *ANOPHELES CULICIFACIES*

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ABSTRACT. *Anopheles culicifacies* was successfully colonized twice in wire cages measuring 60 cm on each side. One of the colonies was successfully selected for mating in smaller wire cages measuring 30 cm on each side. Experiments indicate adaptation to the laboratory involved changes in mating behavior of both sexes.

Although *Anopheles culicifacies* Giles, the principal malaria vector for most of the Indian subcontinent, has been extensively investigated ecologically, it has not been studied genetically or physiologically because appropriate laboratory colonies were not available. Russell and Rao (1942) did maintain a colony in a large outdoor cage in the 1940s, but such a colony is not readily amenable to the experimental manipulation required for genetic investigation. During 1967–69 at the Ross Institute of Tropical Hygiene a colony of *A. culicifacies* was maintained with egg batches originating from Maharashtra State, India. Mating occurred in 12-inch cube cages but also had to be supplemented with artificial mating. An artificially mated colony has recently been established at the Ross Institute from egg batches originating in Sri Lanka (Dr. G. Davidson, personal communication, 1976). Successful colonizations of *A. culicifacies* initiated during the summer and fall 1975 are reported below.

Colonies have been successfully established by capturing wild females from cattle shed resting sites in three different villages: Sattoki (40 miles south of Lahore on Multan Road), Kot Baghicha (near Balloki Headworks, about 30 miles south of Lahore). A third colony, Dhariwal (25 miles south of Lahore), was maintained for 4 generations but subsequently lost. In each case, 500 or more wild-caught females were taken to the laboratory and kept in metal wire cages measuring 30 cm x 30 cm x 30 cm and were provided with a mouse at night. Eggs were collected daily in a manner similar to Pal (1945). Specifically eggs were collected in plastic petri dishes lined with filter paper

and flooded with water to a depth of 3 mm. After hatching (36 to 48 hours), the larvae were transferred to enamel pans measuring 45 cm x 22 cm filled to 1 cm depth of water. Jayewickreme (1952, 1953) found that a wide range of larval diets were suitable for *A. culicifacies*. Specifically, larvae were daily fed a finely ground mixture containing equal parts by weight of wheat germ, Kellogg's Concentrate cereal, and brewer's yeast. Egg-to-adult viability was determined and found to vary widely from as low as 10% to as high as 90%. Egg-to-adult viability averaged near 50% when 100–200 eggs were placed in each pan, which appears to be the optimal density. Larvae pupated 8–15 days after hatching, which agrees well with results of Jayewickreme (1953) and Pal (1945) for progeny of wild-caught females. Pupae were isolated and transferred to 50 ml plastic cups with lids. Adults were collected the next day and transferred to metal wire cages measuring 60 cm on all sides which were partially covered with wet towels. Wet towels were important as efficient egg production depended upon their use. Eggs were usually laid beginning on the 6th day of adult life although occasionally as early as the 4th day. Eggs were collected and the process repeated again, except that guinea pigs were sometimes used instead of mice at night as blood hosts.

Insectary conditions were critical to success. The temperature was maintained between 28–30°C. The humidity was kept at 60% RH. Lighting was provided by fluorescent tubes as well as incandescent bulbs. Fifteen hours of "full daylight" was provided during which all the lights were used. At 2130 hours, the fluorescent tubes were turned off, leaving only incandescent bulbs which were dimmed gradually for 80 minutes by the use of a motor-driven power-stat transformer until there was total darkness. The process was reversed in the morning.

Insemination rates were evaluated in an effort to determine how *A. culicifacies* adapted to the laboratory environment. The first laboratory-reared generation of Dhariwal colony (F_1-D) established with approximately 1,000 pairs of first generation virgin adults yielded 500 to 1,500 eggs daily. Twenty-seven percent (n=100) of the females were found to be inseminated. This result differed dramatically from that of the seventh laboratory generation of Sattoki stock (F_7-S), which was also established with approximately 1,000 adult pairs. Forty-eight percent (n=65) of the females were found to be inseminated and 4,000 to 8,000 eggs were laid daily. The fourth laboratory generation of the Kot Baghicha colony, also established with 1,000 adult pairs, had intermediate rates of egg production (1,500 to 2,000 eggs daily) and an intermediate insemination rate (38%, n=50). The insemination rate appeared to increase, (χ^2=6.1, df=2), as the mosquitoes adapted to the laboratory environment. The increase from 27% to 48% inseminated females likely does not account for all of the five-fold or more increase in egg production observed. Therefore, it appears likely that the fraction of blood-fed, inseminated females actually laying eggs increased as the mosquitoes adapted to the laboratory.

Comparisons of the F_1-D and F_7-S male behavior yielded interesting results. When F_7-S males were mated to F_1-D females, 52% of the females were inseminated compared to the 27% mated by the F_1-D males (χ^2=9.3, df=1). However, when F_7-S females were crossed with either male, the resulting insemination rates were not different (χ^2=0.6, df=1). Thus, females of F_7-S appear to mate more readily than F_1-D females given matings to non-adapted F_1-D males. However, lab-adapted F_7-S males mate more aggressively than non-adapted F_1-D males given matings to F_1-D females. Consequently, mating behavior of both sexes was involved in adaptation to the laboratory environment.

Comparing the insemination rates in cages of different sizes indicated that matings occur most readily in 60 cm cubic cages (insemination rate, 48%; n=50) and less readily in cages with a 30 cm base and 60 cm in height (insemination rate, 20%; n=80). However, matings in 30

cm cubic cages were extremely rare (insemination rate, 2%; n=43).

Recently it was discovered that isolating males and females for 48 hours and allowing females a blood meal 24 hours prior to introducing them in small wire cages measuring 30 cm on a side significantly improved mating success. After maintaining 4 generations of small-cage-raised mosquitoes, sub-samples of adults from the large Sattoki colony and of the 4th generation from small cages derived from the large Sattoki colony were dissected after being held 10 days in small wire cages. In the case of progeny from the large Sattoki colony cage, 8 of 82 dissected females were inseminated as opposed to only 1 in 43 when males and females are not isolated prior to introducing new adults in small wire cages. The insemination rate of females in the fourth generation raised in small cages (14 of 62 females were fertilized) was significantly greater than the insemination rate of progeny from the large Sattoki colony cage which were kept in a small cage ($\chi^2=4.5$, 1 df). Thus selection for efficient mating in smaller cages is progressing satisfactorily. However, inbreeding effects were and continue to be important judging from the large number of unusually small larvae and adult progeny from the small cage populations. To overcome this problem, the sixth small-cage-reared-generation adults are being used to establish a circular mating scheme with 16 sub-populations.

The author plans to use small cage adapted mosquitoes to initiate formal genetics of *A. culicifacies*.

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