CULTURE TECHNIQUES FOR PERMANENT COLONIZATION OF *CULISETA INCIDENS* (THOMSON) (DIPTERA: CULICIDAE)

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Culiseta incidens (Thomson) occurs in large numbers in many of the isolated still water ponds throughout the Coast Range of California as well as in the Sierra Nevada foothills during late winter and early spring. As summer advances, immatures can be found at progressively higher elevations. It has been widely recognized that *C. incidens* readily attacks man in the field (e.g., Carpenter and LaCasse 1955). This, coupled with its ability to transmit St. Louis and western equine (Hammon and Reeves 1943) and Japanese B encephalitis (Reeves and Hammon 1946) under laboratory conditions, stimulated this attempt to colonize the species, thereby making it available for experimental purposes.

METHODS. Many larvae were collected from creekbed ponds in the Sierra Nevada foothills of eastern Fresno County. They were reared in tapwater under continuous light in 37 x 22 x5 cm enamel pans. The water was aerated to prevent scum formation and kept at 19 to 19.5° C. Food was provided in the form of high protein pellets (Misco High Protein Supplement, Teslow Inc., Bozeman, Montana), and was added to the pans in sufficient quantities to maintain a light infusion. The rearing water had a pH of 7.2 to 7.6. Pupae were harvested and placed in a screen cage 92 cm long, 76 cm high, and 46 cm wide. Adults were provided raisins as a carbohydrate source and were allowed to engorge on a chicken which was secured to the screened top of the cage with a strip of canvas. A chicken was continuously provided. Adults were kept at 20.0 to 22.2° C and 85 to 95 percent relative humidity. Automatic timers provided a light cycle of 19 hours daylight

(about 4 ft-c inside cage) and 3 hours darkness; these were separated by dawn and dusk periods (0.5 ft-c inside cage) of 1 hour each.

RESULTS. After 3 hours of darkness, adults were stimulated to activity (walking or flying) by the weak light of the simulated dawn period. This activity continued until the higher intensity light switched on. The sudden change in light intensity produced an almost instantaneous swarming response. The swarm consisted of males and females flying generally in a circular-like pattern in the center of the cage. Occasionally, copulating pairs could be seen in flight. Swarming continued for about a half hour.

During the early stages of colonization females seemed to have difficulty finding the host. After 16 generations, however, females were attracted to the host immediately after exposure and engorgement occurred quickly.

Females required 8 to 9 days at 22° C for maturation of eggs after each engorgement. Oviposition occurred during or shortly after the simulated twilight. For oviposition, females preferred water with decaying organic material, such as leaves, to tap or distilled water. At 22° C. embryonic development required about 3 days.

Using the stated standard conditions, 250 newly hatched larvae were reared at each of two temperatures, 19.0°±1° C and 24°±1° C. Observations were made every 8 hours until the adults had emerged; the numbers alive and the numbers which had molted were recorded. The data indicate (Table 1) the average time required for development to a given instar.

In the present study a 30.5 cm cubic screened cage was used for maintaining adults of *C. incidens*. About 1000 adults were introduced but only 1 pair was observed *in copula*; they were on the side of the cage. Only 1 out of 87 egg rafts was viable. The resulting F_1 adults, however, failed to produce fertile egg rafts.

In an attempt to increase mating, a larger cage (92x76x46 cm) was constructed and larger numbers of field-collected pupae were introduced. The resultant adults swarmed and produced viable rafts at a ratio of 1 fertile raft to 12 infertile rafts. Owing to adult and larval mortality, relatively few F_1 adults remained. Additional adults from the field were added to the colony during the second through the fourth generations. After 16 generations 85 to 95 percent of the rafts were fertile. In about the 21st generation a subcolony was started in a 30.5 cubic cm cage. There was an immediate decrease in the proportion of egg rafts which were fertile. After several generations of selection, however, fertility increased until it was about that of the larger cage.

DISCUSSION. The chief obstacles to the colonization of this species were the reluctance of the adults to mate and engorge. The feeding difficulty was overcome by providing continuous access to a host and was finally solved by selecting a strain which engorged readily under laboratory conditions. Space was the factor which inhibited mating. Hubert (1953) observed swarming and mating in

TABLE 1.—Time (in days) required for development at two temperatures.

Molt	Median	Range	Survival	Median	Range	Survival
1st	3.8	3.7–6.7	192	3.0	2.7–5.3	187
2nd	6.3	5.7–8.7	175	4.7	4.3–7.3	178
3rd	9.3	8.7–13	162	7.0	6.3–9.7	154
4th	15.3	13.7–21.7	139	10.8	10.3–14.3	136
Emergence ♂	19.2	18.7–24.0	60	14.0	13.3–15.7	61
Emergence ♀	21.2	19.5–25.0	61	15.0	14.3–18.7	65

2.3 x 2.13 x 2.57 meter room; three generations were reared under these conditions. He did not observe mating when the adults were placed in a 30.5 cm. cubic cage. I observed that wild adults rarely mate in small cages. After colonization in a large cage, however, it was easy to select a strain which would swarm and mate in a small cage. The colony, established in 1962, is presently maintained by the Bureau of Vector Control, Fresno, California.

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