observations and experiments are needed to clarify the attraction mechanism in Habenaria obtusata.

Literature Cited


COLONIZATION OF CULISETA MELANURA (COQUILLET) IN THE LABORATORY

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Culiseta melanura is a mosquito involved in the natural history of eastern encephalitis. For over 15 years attempts were made, without success, to rear this mosquito in the laboratory for intensive experimental studies. In 1958, however, Hayes reported the use of induced copulation in attempted colonization of the species, and still more recently Haeger (personal communication, 1968) has been able to establish a colony in Florida. This communication reports success in colonization of the northern form of the species in Connecticut.

Historical. Much of the difficulty encountered in past attempts to colonize C. melanura was associated with the peculiar biology of the species, particularly the long period of suspended growth of the larvae during the fall and winter months.

Smith (1904) and Dyar (1905) published initial accounts of larvae overwintering in New Jersey in water under the ice, and subsequent observations of overwintering larvae of C. melanura were reported by Burbits and Lake (1956), Hayes (1961), Siverly and Schoof (1962), and Stamm et al. (1962).

The cessation of larval development, or diapause, during the winter months did not, however, appear to be an obligatory characteristic. In Connecticut, Wallis (1953) found that larvae hatched from an egg raft deposited in the laboratory by an adult female collected during November, proceeded through larval development to pupation within a 2-week period. In New Jersey, Burbits and Lake (1956) observed that a group of larvae brought into the laboratory during the winter of one year did not pupate until the follow-
ing spring, but that larvae brought in during the next winter continued normal development and progressed to the adult stage within a short time.

In Georgia, Love and Goodwin (1961) collected larvae during November and December and found that up to 2 months was required for development from 4th instar larvae to adults. They showed that the length of time for development was not affected by variations in photoperiod.

But just as the larval diapause did not always occur, so the onset of diapause in the laboratory also proved unpredictable (Wallis, unpublished data from 1961). Workers therefore continued to regard the diapause as a serious obstacle to colonization of *C. melanura*, and a search was begun for ways of experimentally terminating it. Wallis (1962) found that adding liver concentrate to the medium of diapausing larvae consistently stimulated pupation of significant numbers of the larvae. Subsequently, Rutledge and Ward (1965) reported that pupation occurred after natural swamp-water larval medium was enriched with powdered dog food; they obtained two discrete waves of adult emergence, one after 7 weeks and the other after 17 weeks. Still later, Hayes and Maxfield (1967) described a successful method for breaking the larval diapause that involved a similar enrichment of the larval medium plus use of long photoperiods.

The delay in development of *C. melanura* larvae nevertheless remained an undesirable complication in routine rearing in the laboratory, and work on colonization of this species has therefore been continued with the goal of establishing a vigorous colony that could be maintained for experimental purposes on a practical, routine basis.

**Procedure and Results.** Attempts made to colonize *C. melanura* in this laboratory during the summers of 1964–66 ended in failure. Collection of field material was hampered by a decline in the mosquito population associated with unusually dry weather during these years, and only a few larvae were encountered and very few gravid females. Eggs from these females, when put out to hatch on tap water, provided young larvae that developed only slowly and irregularly, and consequently, few laboratory-reared adults of uniform age were obtained for stocking an experimental colony.

During August 1967, however, rainfall returned to normal, and between 27 August and 5 September 55 gravid females were hand-captured in diurnal resting places at Farmington, Connecticut. These were transported alive to the laboratory, where they were identified, placed in cages containing water for oviposition, and then maintained in the insectary at 27°C and 80 percent relative humidity. As egg rafts were deposited, each was put out to hatch in white enameled pans containing starting medium as described by Heal and Pergrin (1947): 50 mg of dry powdered brewer’s yeast and 50 mg of Bacto brain heart infusion powder per liter of distilled water. This procedure was followed because it had proved successful for the rearing of *Culex salinarius* (Wallis and Whitman, 1968).

After the eggs hatched, Gaines dog food pellets were added as nutrient for the larvae. Each pan was covered with a sheet-metal plate and kept covered during the development of the larvae. In contrast to previous experience, larvae developed rapidly and uniformly, and pupation occurred 14–21 days after eclosion. As pupae developed, they were transferred to ½-pint cartons containing water and allowed to emerge as adults in a screened 24 x 24 x 24 inch cage for attempted colonization. The colony was kept under artificial light for daily 16-hour photoperiods and maintained at a temperature of 27°C and 80 percent relative humidity. Between 18 September and 19 October 4814 larvae developed to pupation without diapause. Similar results were obtained with eggs from wild females caught between 16 and 23 October. Fertile egg rafts from the first laboratory-reared adults were obtained on 23 November, 3 Decem-
ber, 12 January, and 22 January. All of these developed without delay in the onset of pupation, which occurred at 12-14 days.

Difficulty was experienced initially in blood-feeding of the adult females in the cage. Although some females died on the human blood that was offered during the daytime, only a small number could be induced to feed on the immobilized young chickens left in the cage overnight for this purpose. It soon became apparent, however, that this low level of feeding lasted only through the first 10 days of adult life; thereafter, feeding increased significantly and by the 3rd week little difficulty was encountered in obtaining blood-feeding on chickens.

Although egg fertility was low and variable in early generations, the percentage of fertile egg rafts subsequently increased, and at present, after 12 months of continuous rearing, the large majority of the egg rafts obtained are fertile. It is now possible to maintain the colony by routine breeding procedures.

Discussion. The success achieved in the present study has several possible explanations. An abundant supply of gravid female C. melanura collected in the field was allowed to oviposit in the laboratory. These eggs hatched on distilled water containing a starter medium composed of brain-heart infusion and provided larvae that developed faster and more uniformly than in previous rearing attempts in which eggs were allowed to hatch in water only. With the rearing method used, growth of larvae proceeded to pupation uninterrupted by diapause, and it was thus possible to stock the experimental colony with large numbers of adults of uniform age, the result being the production of a sufficient total number of egg rafts to offset the low level of fertility encountered in the early generations.

Larval diapause, an obstacle in the past to laboratory rearing of this species, did not occur in larvae hatched from eggs of gravid females that were collected on three different dates in the late summer and early fall. Since larvae obtained from eggs oviposited throughout the winter months by laboratory-reared females also continued to grow without diapause, it seems reasonable to conclude that the onset of diapause is governed by environmental factors rather than by an intrinsic biological-clock mechanism.

Although the factors that trigger the onset of diapause in C. melanura larvae are not yet understood, the present rearing method clearly prevented the occurrence of this phenomenon, possibly by meeting certain environmental requirements. First, newly hatched larvae in the enriched starting medium were not subjected to early nutritional stress. Second, larvae growing in covered pans were isolated from changing photoperiods. Third, temperature was kept uniform, and thus ovipositing females, hatching eggs, and growing larvae were not subjected to the chilling temperatures often encountered in nature in temperate climates late in the mosquito-breeding season.

Summary. Culiseta melanura, in its northern form, was colonized in the laboratory with starting material of 55 gravid females captured in the field. Eggs hatched in water containing brain-heart infusion and yeast provided larvae that developed rapidly and uniformly, and pupation regularly occurred 14-21 days after eclosion. From these, large numbers of adults were obtained to stock an experimental cage that was kept on 16-hour photoperiods and at a uniform temperature of 27°C. The percentage of fertile eggs produced by laboratory-reared females was low initially, but increased during the following months of continuous rearing. At present, a vigorous colony is being maintained in the laboratory on a routine rearing basis.

References


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