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BIOLOGY OF *CULISETA MELANURA (COQUILLETT)* IN SOUTHEAST GEORGIA [1]

R. E. SIVERLY [2] AND H. F. SCHOOF

Introduction. Interest in the colonization of *Culiseta melanura* has been stimulated by the implication of this mosquito in the transmission of eastern encephalitis. Isolation of EE virus from wild-captured *C. melanura* was first reported by Chamberlain *et al.,* (1951). Since that study, numerous investigations have been made in connection with encephalitis outbreaks in eastern United States (Chamberlain *et al.,* 1958; Wallis, 1959; Bickley and Byrne, 1960; Hayes, 1960).

That *C. melanura* is important in the transmission of EE is based largely upon circumstantial (rather than experimental) evidence. While there is no unanimo agreement in the conclusions drawn various workers, the weight of the e dence suggests that when *C. melanura* involved, the involvement is most like a low-level maintenance cycle which serv to account for endemicity of infecti within an avian population. This mosqui probably is not involved in the type epidemic cycle which accounts for t feed-back of encephalitis virus from avi to human populations.

Experimental evidence is needed to sess better the role of *C. melanura* transmission of encephalitis. Such data c be obtained most readily by laborato experimentation with living material whi can best be provided through coloniz tion of the suspected vector. According studies directed toward colonization *C. melanura* were initiated at the Tec nical Development Laboratories, Sava

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ah, Georgia, in June 1957, and continued ıch summer through 1959. Although the ›lonizing of this species was not accomplished, considerable data on the biology f C. *melanura* were obtained and are ›ported in this paper.

BACKGROUND. According to Carpenter ıd La Casse (1955), *C. melanura* occurs ı some of the states west of the Mississippi, and in all states east of the Mississippi River except Connecticut, Illinois, ıdiana, and West Virginia. It has since ›en reported from Connecticut (Wallis, ›59) and from Indiana (Siverly, 1958). . *melanura* probably occurs in all states ıst of the Mississippi and its range extends from the Atlantic seacoast to the 98th eridian.

The investigations of Burbutis and Lake 956), and Hayes (1958), together with is study, add much to the information ailable on *C. melanura* as reported by arpenter and La Casse (1955). *C. melanura* is a swamp mosquito, probably limed in its flight range and nocturnal or epuscular in habit. Humidity, as well as mperature, apparently is a critical factor determining its range of distribution, ›pulation density, and seasonal prevalence. Developmental rates in nature are ceedingly variable and subject to such ıriations as imposed by weather factors ıd fluctuations in water level. In the uthern states, breeding may be continual, though retarded during the winter onths. In the northern states, development is interrupted during the winter peod; larvae may sustain themselves in otected habitats and complete their delopment the following spring.[3] The ımber of annual generations ranges from w in the northern states to several in e south.

OBSERVATIONS AND EXPERIMENTS: LARVAL DEVELOPMENT. During this investigation, much of eastern Georgia and southern South Carolina near Savannah, Georgia was surveyed for *C. melanura*. The best natural study area was found in Chatham County, Georgia, approximately 15 miles southwest of Savannah (Figure 1). Each small, solid, black circle (35 in all) in this figure represents a rot cavity or similar natural condition in which immature stages were collected. Collection sites at any given location were designated as a station. There were 9 stations. The entire study area is part of the drainage basin of the Ogeechee River.

Figures 2 (Station II) and 3 (Station V) illustrate collection sites in Chatham County. The tree in Figure 2 is a tupelo, the other is cypress. Characteristically, these tree cavities were charred as a result of forest fires and partially filled with water by percolation from the surrounding water. Each site harbored larvae and pupae of *C. melanura*. At times the concentration was as high as 100 specimens per dip.

Immature stages also may be found in pockets in the soil and in root holes. In Alabama breeding was observed in earth cavities created by uprooted trees. A similar micro-habitat was detected in Indiana.

Regardless of whether the micro-habitat is a rot cavity in a tree, a pocket in the soil lined with roots, an earth cavity, or similar habitat, there appear to be two consistent elements in the nature of breeding places utilized by *C. melanura:* (1) darkness or conditions of low light intensity, and (2) contact with soil. Contact with soil is probably of greatest significance. Many species of mosquitoes require some degree of darkness and the propensity of certain species to utilize cavities enclosed by wood is well known, e.g., tree-hole breeding mosquitoes. With *C. melanura*, however, there is always contact with soil. In no case has breeding been reported in tree holes or in other natural cavities above ground level. The implications of this contact with the soil in artificial rearing will be discussed.

Immature stages of *C. melanura* were

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[3] Larvae of *C. melanura* were taken from an ırth hummock formed by the roots of a fallen e in a bog area near Muncie, Indiana on April 1961. Water temperature was 40° F. Stages larvae ranged from second to late fourth inır. This observation supports the concept that e larval stage constitutes a mode of overwinterg for this mosquito in the northern parts of range.

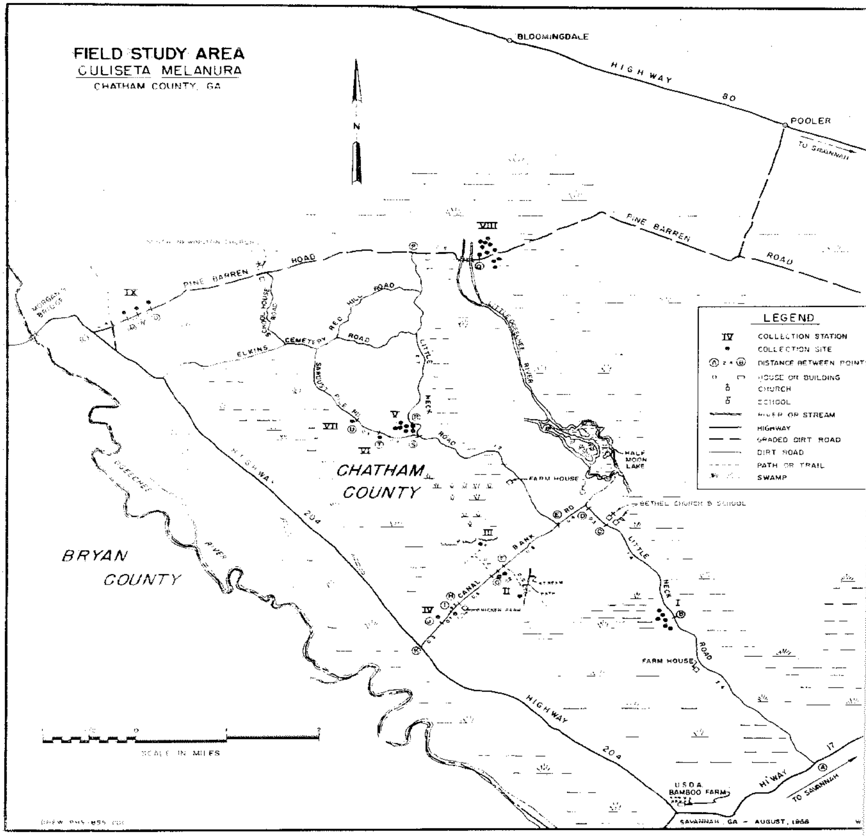

FIG. 1.—Field study area, *Culiseta melanura*, Chatham County, Ga.

collected at natural collection sites and transported to the laboratory. Artificial breeding structures set near natural breeding sites were also used. The artificial structures were assembled as follows: A glass provision jar (two-gallon) was set in the soil with its rim at ground level. It was filled to about half capacity with strained swamp water and some litter from the swamp floor added. A wooden nail keg with a side aperture was then placed over the container and staked in place. The top of the keg was provided with removable cover for convenience in pe odic inspection of the artificial breedi container (Figure 4).

Initially, all stages were collected b later collections were limited to egg raf At the laboratory, rafts were placed tap water and hatching usually occurr within two days at 78° F. to 82° F.

Several different rearing techniqu were tested. At first, the use of enan pans for rearing resulted in high mortali

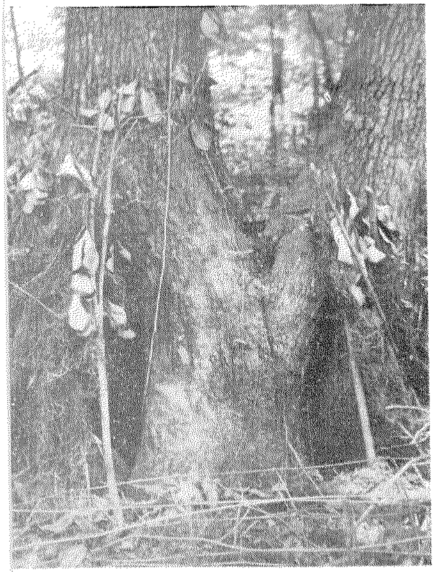

FIG. 2.—Larval and adult habitat of *C. melanura* ı tupelo.

FIG. 3.—Larval and adult habitat of *C. melanura* in cypress.

f larvae in the third and fourth instars. .ater, rearing tanks (28″ by 12″ by 10″) ·ere constructed of marine plywood ¾″). A hinged lid was provided for ıch tank; openings in the lid allowed the ntry of aeration tubing.

Short lengths of plastic tubing were onnected by T-tubes at approximately 4-inch intervals to the main air line. ach short length of tubing terminated t an air stone on the bottom of the tank. ın air compressor driven by a ¼ H.P. lectric motor provided air pressure. 'anks were aerated for two-hour periods ıch morning and afternoon.

In comparison with pan rearing, the igher production in the wooden tanks believed to be attributable to physical ıctors (lower light intensity, increased ıbsurface enclosure area) rather than hemical factors. Chemical analysis showed ıat no nutritive substances or materials could be detected in, or associated with, the wooden enclosures.

Since rearing in tap water was unsuccessful, swamp water from the field study area was substituted. Although superior to tap water for rearing purposes, swamp water produced erratic results that may have been attributable to the variability of the swamp water itself. Its chemical constituents are variable, depending upon its source in nature, the length of time it overlays the swamp, and other factors. Presumably such water contains growth factors essential for larval development. Without natural supplements, however, it may be weakly suppportive or even inert.

In terms of proportionately high production of consistently robust pupae, the best results were obtained when sand was added to the swamp water. Such sand was obtained from the swamp in prox-

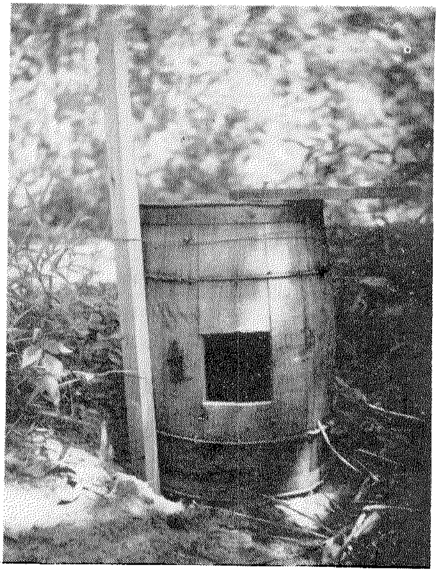

FIG. 4.—Artificial breeding site consisting of nail keg positioned over 2-gallon glass container.

imity to natural collecting sites. Consistently large, vigorous pupae were produced in 12 days after hatching, as compared with 17 to 21 days by conventional pan or wooden tank rearing methods. Under optimum conditions, the time in days to complete the egg, larval and pupal stages was as follows: egg, 2; larva, 10 (first instar 3; second 2; third 2; fourth 3) and pupa, 2.

Larvae were fed 100 mg of pulverized dog food on alternate days. No supplementary food was needed.

THE PUPA AND ADULT. The life span of the adult female *C. melanura* includes: (1) emergence, (2) feeding (survival), (3) resting, (4) mating, and (5) oviposition. In nature these events are accompanied by, or sequential to, periods of short or sustained flight.

Pupae were placed in glass evaporating dishes set inside emergence cartons.[4] Since a high level of humidity is extremely important throughout all phases of the life cycle, the emergence cartons were set i damp sand on the floor of a walk-i cage. Pupae are extremely sensitive t light, and when maintained in half-cov ered dishes under dimly-lighted condi tions, they tend to migrate to the darkes portion of the container. Furthermore darkness appears necessary for successfu emergence. In a darkened room, observa tions with the use of a flashlight indicat that emergence may occur during all hour of the day and is not restricted to evenin or night periods.

To feed the adults, a thick layer of ab sorbent cotton soaked in 20 percent suga solution was placed on top of the walk-i cage. The newly emerged mosquitoes o both sexes readily fed through the screen ing.

Difficulties in inducing females to fee on animal hosts have been encountere by other workers (Chamberlain *et al* 1955). Preliminary observations indicat that *C. melanura* females will engorge o blood-soaked cotton pads offered in th same way as sugar pads. The best uptak observed was with one part, by volume of a 20-percent sugar solution in distille water to two parts of blood. Whole cit rated human blood was used. In a sampl of 20 females, immobilized with ether an microscopically examined, better than 5 percent had engorged during a 24-hou period. A few were so replete that fligh was difficult. McLintock (1952) recom mends a blood and sugar mixture fo *Culiseta inornata* in the ratio of 3 part blood to 1 part 10 percent sucrose solutio

C. melanura females do not appear to b attracted to the human host. Hayes an Doane (1958) report an isolated instanc of this mosquito feeding on man in na ture. Wallis (1959) lists man as a bloo source in laboratory tests involving sev eral animals. Two instances of feeding o man were observed in the laboratory i

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[4] A one-gallon ice cream carton in which circular disc was cut from the bottom, leavin a 1-inch rim. This opening was covered b plastic screening, stapled to the rim and th carton was inverted, the top thus becoming removable bottom.

he current study. Both females had been eld-collected from resting boxes (see be- ow). At no time did laboratory-reared nosquitoes attack individuals inside the valk-in cage despite densities of a thou- and or more.

Under natural conditions, adults rest in ark, moist, protected habitats such as hol- ow trees, caves, and cisterns. Artificial esting sites were described by Burbutis nd Jobbins (1958). In this study, resting helters were open boxes (2′ by 1′ by 1′) onstructed from ¾ inch marine plywood. he interior of each box was lined with lack cloth. Over a three-week period, 17 mosquitoes were collected from such helters placed at ground level in prox- nity to natural breeding sites. Of this otal, 56 were *C. melanura* (24 males and 2 females).

Under indoor conditions, *C. melanura* ppears to be a sluggish mosquito and oth sexes spend most of the time in esting. They are extremely sensitive to hanges in humidity and have been ob- erved to retreat inside an artificial "tree- ole"[5] maintained inside a walk-in cage, hen the relative humidity dropped be- ow 80 percent. Although quick to re- pond to a lowered humidity, there is a g in resumption of any form of activity hen favorable conditions of humidity e restored. Undoubtedly, there is a com- lex of environmental conditions involved n incitation to activity. In the laboratory, elative humidity of at least 80 percent, air emperature of 80° F. and dim light are dentifiable factors in this complex.

Under natural conditions, *C. melanura* hould be most active during evening or night, although there may be some iurnal activity in humid, swampy areas ith a dense tree canopy. Daytime ac- vity was not observed in the field, but id occur in an indoor walk-in cage.

According to Hayes' observation (1958), f the swarming of *C. melanura* in nature, mating was occasionally observed in the swarms, and fertile eggs were later obtained from one female collected from a swarm. Whether in nature mating is dependent upon swarming is not definitely established.

Laboratory observations indicate that production of fertile egg rafts from laboratory-reared mosquitoes does not occur in the absence of swarming. This being the case, plus the fact that swarming and mating are associated in nature, suggests induction of swarming as a logical approach to fertile egg production in the laboratory. In this study, considerable effort was directed toward induction of swarming. Failure to obtain fertile egg rafts does not contraindicate the merit of this approach. Time limitations did not permit extended studies needed in determining all conditions necessary for mating. Preliminary observations are as follows:

(1) *C. melanura* requires conditioning by exposure to gradually diminishing light extending through an approximate 40-minute interval.

(2) Swarming activity is triggered at an illumination of about 0.2 foot candle of light.

(3) If the 0.2 foot candle illumination is extended, swarming activity continues for an extended period.

(4) Swarming activity is enhanced by the use of dark blue filters and screening attached at the light source. Evidently, the screening serves to disperse the light.

(5) Swarms consist entirely of males. Resting females near the site of the swarm are often approached by males, but appear to resist these advances.

In the study, swarming activity was induced by directing four flood lights (with dark blue filters and wire screening attached) toward a piece of projection screen bent in an inverted dome shape and mounted on the ceiling of a walk-in cage. The intensity of lights was gradually diminished over a 40-minute interval to simulate the dusk period in nature. One instance of coupling was observed under these conditions. The female had evidently dropped into the swarm. The pair became

[5] A rectangular cypress chimney, three feet in eight and nine inches across, immersed in a ooden tank of water.

attached in flight, alighted on the side of the cage and remained together for approximately 15 seconds.

These and other observations suggest that natural swarming occurs at dusk, and that the swarming site may be found where indirect light from the setting sun is reflected in broken pattern under latticework of leaves or branches. Hayes' observation (1958) supports this hypothesis.

Conditions favorable for oviposition include darkness and high humidity. In the study area there were several breeding sites where the tree canopy was very dense. At no time during the summer season was there a great deal of light penetration at these sites. Just prior to, during, and immediately after summer storms, the breeding sites were quite dark, regardless of the time of day.

Egg raft collection data indicate that deposition was more frequent immediately after or during a rain. Afternoon

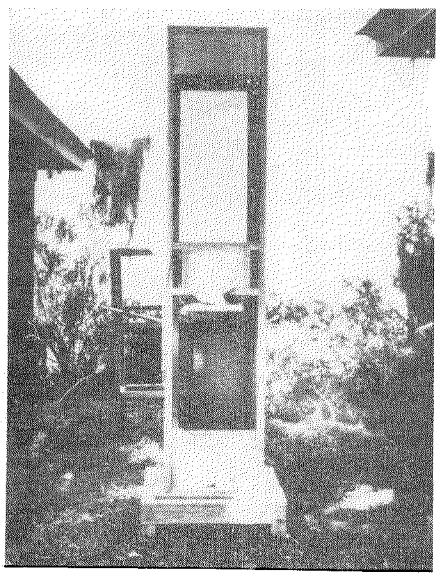

FIG. 5.—Tower cage used to induce swarming and blood feeding.

collections of rafts still light in color indicate that oviposition may occur wheneve suitable temperature, darkness, and a high humidity prevail. In southern Georgia egg rafts may be collected during al months of the year. As many as eigh rafts have been collected from a single tree hole. The egg raft of *C. melanur* has been described by Chamberlain *et al* (1955).

In the laboratory, egg rafts were produced by field-collected females. However from 34 females held in a cage 18" by 16" by 14", only two egg rafts wer produced. Temperature was at 80° F. relative humidity from 70 to 95 percent Presumably most of these females wer inseminated. The poor ovipositional response may be attributable to a low proportion of blood-fed adults, or to failur in maintaining a cage environment favorable for oviposition. Whether inductio of oviposition will present a problem i colonization can best be determined whe the prior conditions for survival and mat ing are satisfied. However, oviposition appears to be a less formidable barrier to colonization than does mating.[6]

To induce swarming, mating and blood feeding, a tower-type cage 18" square and 12' in height was constructed on the laboratory grounds (Figure 5). Within thi cage, a cypress chimney was partiall immersed in a container of water, an pupae placed in a recess at its bottom Adult emergence occurred inside the cage and both sexes survived for as long a three weeks. A caged pigeon was suspended each night from the ceiling c the cage by a pulley arrangement. Ther was no evidence of blood feeding, nor c swarming.

Mosquitoes remained inside the cypres chimney during daylight hours, but at th approach of nightfall, they migrate downward to the aperture at the base c the chimney and then upward on th screened enclosure. An exit hole at th top of the chimney was never used fc

[6] Chamberlain, Roy W. Personal communica tion.

this movement. The pattern of migration always was the same. In the early morning hours the path of migration was reversed. These observations suggest that under natural conditions a similar pattern of vertical migration occurs each day.

Other workers (Carpenter and La Casse, 1955; Favorite and Davis, 1958) report *C. melanura* as being attracted to light traps. Current findings support these observations to a limited extent only. Four light traps were run for five nights within 50 yards of a breeding focus and in a second instance within 300 yards of an active breeding site over a period of three weeks. At either location the yield was fewer than 10 specimens.

DISCUSSION. In the laboratory, larvae frequently fail to survive although provided with abundant microfauna supplemented by foods of high protein content. Fresh swamp water often is a prerequisite to the completion of development. Obviously, in nature the essential materials are present; consequently, a study of the microflora and microfauna would seem to be a worthwhile approach (Hinman, 1933; Rozeboom, 1935). The use of growth-promoting agents also merits consideration (Williams, 1953).

The successful rearing of larvae in the laboratory is a forerunner to the intensive studies needed on adult survival, mating, and activity. Much of the present work on activity has been handicapped because of limited numbers of specimens available for that purpose.

The mating of adults under caged conditions is a critical barrier to colonization. Presumably, more intensive experiments with light control may induce mating as has been true for other species (Levin *et al.*, 1958).

Application of the induced copulation technique (McDaniel & Horsfall, 1957) [7] suggests another possibility for perpetuation of a colony, provided conditions favorable for oviposition are established.

Oviposition in nature is associated with high levels of humidity, and a relative humidity of 80 percent or higher appears necessary for adult survival in the laboratory. In nature, humidity may be a critical factor in limiting the range of dispersal from the swamp habitat.

Failure to complete colonization of *C. melanura* may be largely due to reliance on procedures successful with other species. In meeting the challenge of colonization of this mosquito, the entomologist must consider carefully each phase of the life cycle in terms of its special limitations. This mosquito manifests a narrow range of adaptation in each stage of development. Successful colonization is a corollary of completion of required conditions within each successive developmental stage.

SUMMARY. 1. Attempts were made to establish a colony of *Culiseta melanura* from adults, larvae, or eggs collected in swamps in Chatham County, Georgia.

2. Principal breeding sites were water-filled, ground-level, tree holes (cypress, tupelo), soil pockets, and root holes. The chief characteristics of these sites were darkness and soil contact.

3. Under favorable conditions the time in days for the various stages were egg (2), larva (10) and pupa (2). Addition of swamp sand to the rearing medium enhanced larval development.

4. Observations indicate that swarming is induced at an illumination of approximately 0.2 foot candle. Swarms consist entirely of males.

ACKNOWLEDGMENT. Thanks are expressed to Dr. Harry D. Pratt, Chief, Insect and Rodent Control Training Section, Training Branch, Communicable Disease Center, Atlanta, Georgia, for his advice and assistance in field work in the initial stages of the project, and to Dr. Roy W. Chamberlain, Chief, Virus-Vector Research Unit, Laboratory Branch, Communicable Disease Center, Atlanta, Georgia, for suggestions on colonization procedures, and for providing larvae.

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[7] As a consultant, Dr. W. R. Horsfall was successful in effecting sperm transfer in laboratory-reared *C. melanura*, but mechanical failure of temperature controls resulted in the death of the inseminated females.

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CARLYLE NIBLEY, JR.

"It is with a heavy heart that we report the death of Maj. Carlyle Nibley, Jr., in an auto accident on 18 May. Nibs was returning home on Highway 1's "killer strip" late at night from College Park, where he has been taking graduate work at the University of Maryland, as was reported in the December issue.

"From the moment that he arrived in Korea as part of the Preventive Medicine Company, at the commencement of the Korean War, Nibs began making an impact on all who worked with him, by his energy, his quick mind and his warm personality. This impact increased with the years and it will be with a real sense that something is gone from their lives that those who knew him will realize that they will not again feel that warmth and energy. The loss of his entomological ability and experience will be greatly felt in the program of military entomology.

"Nibs is survived by his wife and their three teen age children, by two brothers Dr. Reed Nibley of Vienna, Virginia, and Capt. Owen Nibley of the Air Force Academy, by a sister, Miss Jeremy Hardy and by his mother, Mrs. Karl J. Hardy both of Washington, D.C."

(Sent in by Dr. Bickley, through Mr. Morrill.)