OBSERVATIONS ON TWO SPECIES OF *CULEX* OF THE SUBGENUS *MELANOCONION*

JAKIE A. HAIR [1, 2]

U. S. Department of Health, Education, and Welfare
Public Health Service, Bureau of Disease Prevention and Environmental Control, National Communicable Disease Center, Atlanta, Georgia 30333

Recent studies by members of the Arbovirus Ecology Laboratory (AEL), National Communicable Disease Center, have indicated that one or more species of the *Culex (Melanoconion)* group are important in the natural cycle of VEE and other arboviruses in the south Florida everglades (Chamberlain *et al.*, 1964). At the present state of our knowledge, the adult females of this subgenus cannot be identified with certainty; the specific identification being dependent upon characteristics of the larvae and male terminalia. Therefore, precise knowledge of vector relationships is difficult to obtain. To provide specimens of known identity for infection and transmission experiments, attempts were begun in September 1966 to establish laboratory colonies of the various *Melanoconion* species occurring in the south Florida area.

This report presents the procedures used in the laboratory colonization of a new species, *Culex (Melanoconion) cedecei* Stone and Hair (1968) with pertinent laboratory and field biological data. Notes on the biology of *Culex (Melanoconion) pilosus* (Dyar and Knab) are also presented.

COLLECTION AND HANDLING OF PARENT MATERIAL

Female *C. (Melanoconion)* mosquitoes which were used as parent material in the colonization procedures were captured primarily at Everglades National Park in miniature battery-operated light traps (Sudia and Chamberlain, 1962) and rodent-baited lard-can traps (Bellamy and Reeves, 1952). The caged mosquitoes were placed in a styrofoam ice chest cooled by a refreezant can, and transported to the AEL insectary in Atlanta. There they were offered a blood meal and held at 80° F. and 70 percent RH for ovarian maturation. All subsequent work was carried out at the same temperature and with the same humidity conditions.

REARING AND LABORATORY BIOLOGY OF *C. CEDECEI*

SUMMARY OF REARING PROCEDURES. The adult colonies of *C. cedecei* were kept in 9 x 9 x 12 inch plastic cages in the insectary.

Illumination was by fluorescent lights for 14¾ hours each day, preceded and succeeded by 30-minute twilight periods utilizing incandescent lamps (Wood, 1961). Moist cotton towels were placed on the cages daily to increase the humidity in the vicinity of the adults. Absorbent cotton pads wet with 3 percent dextrose in water were provided fresh daily as maintenance food.

Restrained chicks or white mice were placed on the cages twice weekly as a source of blood.

Shortly after the first blood feeding, 4" x 2" culture dishes filled with hay infusion or "used" larval rearing water were introduced as oviposition sites. Egg rafts were removed from the dishes daily and placed in groups of 2 or 3 in 100 ml. beakers of tap water; the beakers were

[1] Present address: Department of Entomology, Oklahoma State University, Stillwater, Oklahoma.

[2] Deep appreciation is extended to Mr. James Haeger, Entomological Research Center, Florida State Board of Health, Vero Beach and Dr. Roy W. Chamberlain, Chief, Arbovirus Infections Unit, for their helpful suggestions, and to Dr. W. D. Sudia, Chief, Arbovirus Ecology Laboratory, for reviewing the manuscript.

then dated and covered with a sheet of glass until the eggs began to hatch.

After the eggs hatched, the young larvae were poured from each beaker into a 7 x 11 x 2-inch enamel pan. Two hundred ml. of tap water, 200 mg. active brewer's yeast, and 10 mg. aureomycin/ml. of water were then added, and the pan was covered with a glass plate to prevent evaporation and to maintain a constant water temperature. At 4-day intervals thereafter, the larvae were fed the yeast-dog food diets as shown in Table 1.

LARVAL REARING. Egg rafts of *C. cedecei* usually hatched within 36–48 hours; however, approximately 5 percent of the rafts had delayed hatching, extending for up to 8 days. In some cases hatching of the entire raft was delayed; in others only a portion of the eggs was involved. This delayed hatching, not previously reported in other *Culex* mosquitoes, may serve as a biological protective mechanism for the species.

A larval diet consisting of yeast and dog food (Table 1) was developed for *C. cedecei* which resulted in pupal production of 90 percent or greater. With this diet the larvae matured within 11–20 days,

TABLE 1.—Diets used for rearing *Culex* (*Melanoconion*) *cedecei* and *C.* (*Mel.*) *pilosus*.

Day	*cedecei*	*pilosus*
1	200 mg. active yeast, 10 μg. aureomycin/ml. of larval water	100 mg. active yeast 10 μg. aureomycin/ml. of larval water
5	50 mg. yeast 50 mg. dog food (non-fat)* 10 μg. aureomycin/ml. of larval water	50 mg. yeast 50 mg. dog food (non-fat) 10 μg. aureomycin/ml. of larval water
9	repeat day 5	repeat day 5
13	repeat day 5	repeat day 5

* General Foods Corporation.

Pupae were removed daily and placed in beakers of tap water in emergence cages.

Oviposition. After blood-feeding, the pre-oviposition time of *C. cedecei* ranged from 20 to 45 days, with an average time of 30 days. This unusual trait of egg retention has not been reported in any other mosquito species found in the United States. Egg rafts of *C. cedecei* were deposited in a small ovoid mass less than 2 mm. in diameter (Fig. 1) and usually contained from 30–100 eggs. They were smaller and more oval in shape than were most *Culex* rafts, and were seldom boat-shaped or pointed on the ends.

Although hay infusions were used initially as oviposition sites for *C. cedecei,* it was found that beakers of tap water containing pupal exuviae were equally attractive. Rafts were laid almost exclusively on the open water surface.

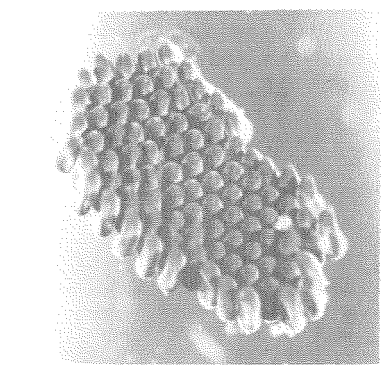

FIG. 1.—Typical ovoid egg mass of *Culex* (*Melanoconion*) *cedecei* Stone and Hair.

with an average time of about 15 days. Aureomycin was added at a rate of 10 mg./ml. of larval rearing water to prevent formation of bacterial surface scums. Without the aureomycin, larval mortality in excess of 20 percent often occurred, and aeration and frequent skimming were necessary. Algal and fungal growth was common even with the aureomycin treatment, but since it was generally subsurface, it was not detrimental to the developing larvae.

The water level of the pans was maintained between 2–5 mm. Although the larvae were subsurface feeders, this shallow water depth permitted many of them to feed on the bottom of the pans where food tended to accumulate. A faster rate of maturation was noted.

Water from a local fish pond was used initially for larval rearing. Good first instar survival was noted, better than with either tap or distilled water. However, in later generations, tap water was used exclusively, with very little mortality.

The duration of the pupal stage of *C. cedecei* in the insectary was approximately 72 hours $\pm$30.

MATING. Adult *C. cedecei* mated reasonably well in the 9 x 9 x 12-inch plastic cages under the insectary light conditions. The fertility rate was 70–80 percent in the F_6 generation, approximately 20 percent higher than observed in the F_1 and F_2 generations. Spermathecal examinations indicated that mating usually occurred within 3 days after emergence.

ADULT HABITS. Adults of *C. cedecei* were negatively phototrophic, resting in the darkest corner of the cage until the twilight and nocturnal periods of the illumination cycle began. They fed in direct light only rarely and seemed to survive better in an area of high humidity (80–100 percent).

Females of *C. cedecei* often refused a blood meal until they were 7–10 days old, or older. Chick, guinea pig, mouse or man were acceptable as hosts, but the latter three were preferred. A second and even a third blood meal was frequently taken prior to the first oviposition.

Adults were relatively long lived, surviving for an average of about 10 weeks under the insectary conditions provided.

LABORATORY BIOLOGY OF *C. PILOSUS*

OVIPOSITION. The pre-oviposition period for *C. pilosus* was somewhat delayed, but generally about 8–10 days shorter than the average of 30 days observed for *C. cedecei*. This trait of egg retention was observed despite the presence of apparently suitable oviposition sites. Perhaps this characteristic is common to all species of the subgenus. The egg rafts of *C. pilosus* were quite different from those of *C. cedecei*. They were extremely long and narrow, about 75 eggs long and only 1–4 eggs wide.

Of numerous media tested as oviposition substrates, hay infusions with hay protruding above the surface of the water were most attractive to wild caught females. Soft, moist mud proved equally attractive to females of F_1 and subsequent generations.

LARVAL REARING. The time required for egg incubation was essentially the same as for *C. cedecei,* and delayed hatching was also observed. King *et al.* (1960) reported that egg rafts of *C. pilosus* could withstand drying, and a random observation made during the present study tends to confirm this report. *Culex pilosus* larvae were seen to hatch upon addition of water to a dried-out oviposition dish. The period of drying was unknown but could have been as long as 2 to 3 days.

The larval diet of *C. pilosus* (Table 1) was essentially the same as that of *C. cedecei* but was administered in smaller quantities and without as great success; pupation of only 50 percent or less was commonly the case. However, it was the best of the many diets tried. Larvae of *C. pilosus* matured faster than those of *C. cedecei;* pupation occurred in 9 to 15 days with an average of 11 days.

Larvae of *C. pilosus* are somewhat more active than those of *C. cedecei* but less active than those of *Culex quinquefasciatus* Say and *Culex nigripalpus* Theobald. They were observed to be subsurface feeders.

The duration of the pupal stage was approximately 60 hours±15.

MATING. Under insectary conditions the fertility rate of *C. pilosus* was lower than that of *C. cedecei,* being only about 50 percent in the third generation. Mating usually occurred within several days after emergence.

ADULT HABITS. Unlike *C. cedecei,* the adults of *C. pilosus* were not strongly negatively phototrophic. They were very active and rested at any position within the cage during the light cycle, and fed readily on sugar or blood during this period. They appeared to require less humidity than *C. cedecei* and survived well at 70 percent RH.

Some of the *C. pilosus* observed took a blood meal within 48 hours after emergence. Although they fed on chicks more readily than did *C. cedecei, C. pilosus* also seemed to prefer the mammalian hosts.

Multiple feedings prior to oviposition were common, as with *C. cedecei.* Also, the *C. pilosus* were relatively long lived, surviving for an average of about 10 weeks. A summary of the laboratory biology of *C. pilosus* and *C. cedecei* is presented in Table 2.

FIELD NOTES

Larvae of *C. cedecei* were collected on several occasions from solution holes on Big Ficus Hammock in Everglades National Park. Apparently the species prefers permanent water for breeding. Breeding areas observed for *C. pilosus,* on the other hand, were temporary pools similar to those described by King *et al.* (1960).

Field observations of adult *Melanoconion* on Big Ficus Hammock have indicated that large numbers of one or more undetermined species, probably *pilosus* or *cedecei,* frequent the sides of solution holes in the limestone rock where the light is subdued, the temperature moderate, and the humidity high.

Terminalia of males which were light-trapped in the Everglades area by members of the Arbovirus Ecology Laboratory between 1963–1966 were examined to determine species composition. *Culex pilosus* comprised 20–90 percent of the catch, depending on the time of year and locality, while *C. cedecei* made up 0–80 percent. During 1964 *C. cedecei* populations, as judged by these male samples, reached a peak in October and November, whereas *C. pilosus* populations were higher in

TABLE 2.—Summary of laboratory biology of *Culex cedecei* and *C. pilosus* at 80° F. and 70% RH.

	cedecei	*pilosus*
Age before first blood meal	5–10 days	1 day
Age before mating	2–5 days	2–5 days
Preoviposition incubation	20+, $\bar{x}$=30 days	15+, $\bar{x}$=21 days
Egg incubation [1]	1½–2 days	1½–2 days
Larval development [2]	11–20, $\bar{x}$=15 days	9–15, $\bar{x}$=11 days
Pupal duration	3±1 day	2½±½ day
Adult longevity	$\bar{x}$=90 days	$\bar{x}$=75 days
Egg raft shape	ovoid	long-narrow
No. eggs/raft	30–100, $\bar{x}$=85	100–290, $\bar{x}$=160
Oviposition substrate	hay infusions, old rearing water	damp mud
Oviposition site	open water	attached to mud surface

[1] Delayed hatching of egg rafts, partially and wholly, has been observed.

[2] Insectary temperature, 80° F.; insectary relative humidity, 70%. Pans covered with glass plate.

May and June. Since *C. pilosus* apparently breeds best in temporary pools, its increase correlates with spring rains following the dry season.

Largest numbers of *C. cedecei* males have been light-trapped along the transition zone in the Everglades area between brackish water and fresh water, with indications of decreased populations as the water became either more saline or wholly fresh. Breeding of *C. pilosus* in the brackish areas, on the other hand, has been found to be quite limited.

Fourteen egg rafts laid by *Melanoconion* caught at Mahogany Hammock in lard-can traps baited with cotton rats *(Sigmodon hispidus)* and cotton mice *(Peromyscus gossypinus)* were hatched to produce larvae of *C. cedecei* only.

The distribution of *C. cedecei* beyond the confines of the Everglades National Park is still uncertain. From their study of various museum specimens, Stone and Hair (1968) believe this species has been confused with *Culex opisthopus* Komp, and that it may entirely supplant *opisthopus* in Florida. The confusion is easily understood, since the two species have only small microscopic differences in larval character. The male terminalia of both species also resemble each other so closely that a detailed comparison must be made for accurate differentiation.

SUMMARY. *Culex (Melanoconion) cedecei,* a newly recognized species previously confused with *C. opisthopus,* has been colonized and successfully maintained in the laboratory for over nine months and has been carried through at least six generations. *Culex pilosus* was maintained by similar methods for three generations prior to loss of the colony. Failure to establish *C. pilosus* was attributed to the lack of an adequate larval diet.

Limited information on host preferences, feeding habits, longevity, and general behavior was obtained. Both species apparently preferred to feed upon rodents. *Culex cedecei* was the more negatively phototrophic.

The laboratory establishment of *C. cedecei* and other *Melanoconion* species from the south Florida everglades regions will permit detailed taxonomic observations and provide material for experimental arbovirus infection studies.

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