

## LABORATORY BIONOMICS OF *CULEX* (*MELANOCONION*) *TAENIOPUS*

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**ABSTRACT.** This paper describes the techniques for establishing and maintaining a laboratory strain of *Culex (Melanoconion) taeniopus* from Guatemala. Developmental times, survival rates and other data pertaining to the population dynamics and behavior of this important vector are presented. It was concluded that mating took place during the crepuscular periods 6–10 days after emergence. Females survived for approximately three months and males for two months. Oogenesis was completed as early as 96 hours after the initial bloodmeal. Oviposition did not begin before day 5 and continued beyond 18 days after the bloodmeal. Egg rafts were deposited on the inside of clay flower pots, and hatched in approximately 48 hr. The overall larval survival rate was 82.3%, averaging approximately 13 days for development from eclosion to pupation. The average pupal developmental time was approximately 59 hr with a 92.4% survival. Cross-mating studies between this Guatemalan strain and a laboratory strain from Florida indicate that the two are distinct species.

### INTRODUCTION

Mosquito species of the genus *Culex*, subgenus *Melanoconion*, are important components in the enzootic cycle of Venezuelan encephalitis (VE) viruses (Galindo 1972). Three species in this subgenus have been shown to be natural vectors of these alphaviruses: *Culex (Mel.) aikenii* Dyar and Knab (Galindo and Grayson 1971), since resolved into *Cx. ocosa* Dyar and Knab and *Cx. panocossa* Dyar (Kreutzer and Galindo 1980); *Cx. portesi* Senevet and Abonnec (Aitken 1972); and *Cx. taeniopus* Dyar and Knab, formerly referred to as *Cx. opisthopus* Komp (Cupp et al. 1979).

The importance of these *Cx. (Melanoconion)* species as vectors of enzootic VE virus points to the usefulness of laboratory colonization in determining infection thresholds, extrinsic incubation periods, and subsequent virus transmission rates (Scherer et al. 1981, 1982). Indeed, successful colonization of several species has already been reported, including those reported as vectors of endemic strains of VE virus. Colonization of *Cx. aikenii sensu lato* was achieved by Adams and Galindo (1972); *Cx. portesi* by Takahashi (1968) and Davies and Martinez (1970); and a Florida form of *Cx. taeniopus* (referred to as *Cx. cedecei* Stone and Hair) by Hair (1968). Other *Melanoconion* species colonized include *Cx. peccator* Dyar and Knab by Chapman and Barr (1969) and *Cx. pilosus* (Dyar and Knab) by Hair (1968).

In regard to the colonization of *Cx. taeniopus*, some doubt exists whether or not the Florida and Central America strains of this taxon represent the same species. Until recently, the mosquito referred to as *Culex (Mel.) taeniopus* in this report was called *Cx. opisthopus* (see Sirivanakarn and Belkin 1980). *Culex opisthopus* had been reported as far north as Florida (the

only U.S. report), and it ranged through Grand Cayman, Jamaica, and Puerto Rico, and from Honduras south to Panama (Knight and Stone 1977). Mosquitoes previously thought to be *Cx. opisthopus* in Florida were reclassified as a new species, *Cx. cedecei*, by Stone and Hair (1968). It was under this name that the colonization report was published. However, *Cx. cedecei* was later synonymized with *Cx. opisthopus* Komp by Belkin et al. (1970). More recently, Sirivanakarn and Belkin (1980) synonymized *Cx. opisthopus* with *Cx. taeniopus* Dyar and Knab. All mosquitoes previously referred to as "*taeniopus*," except for the type material, were renamed *Cx. pedroi* Sirivanakarn and Belkin (Sirivanakarn and Belkin 1980).

The main objectives of this study were to establish a laboratory colony of the Guatemalan strain of *Cx. taeniopus*, describe the techniques necessary for maintaining and rearing this mosquito, and report details of the laboratory bionomics of this species. The availability of colonized "*taeniopus*" from Florida (identical to material formerly referred to as "*cedecei*") and colonization of the Guatemalan strain enabled us to make cross-mating experiments to determine genetic affinities between these two geographic strains. Insemination rates, fecundity, fertile egg production, and survival rates of larvae were the criteria measured in the crossing study and are described here. Finally, the life history of these two strains in the laboratory is compared.

### MATERIALS AND METHODS

**INITIATION OF COLONY.** Approximately 100 adult females used as parental stock were collected in La Avellana, Guatemala during late August 1979. These females were offered a bloodmeal from an anesthetized golden hamster on September 5, 1979, and transported to the Schwardt Laboratory at Cornell University,

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Ithaca, N.Y. All subsequent rearings have taken place in the insectary at this facility.

**INSECTARY-ENVIRONMENTAL CONDITIONS.** The insectary was regularly maintained at 27°C. Relative humidity was not controlled, but averaged 40%. Lighting was supplied by ceiling-mounted 40 watt fluorescent tubes. Time switches were set to turn these lights on at 0800 hr and off at 2200 hr. This lighting system was supplemented by a Sunrise-Sunset Simulator (Tatra Electronic Systems), which provided two crepuscular periods, dawn at 0730 to 0800 hr and dusk at 2200 to 2230 hr.

**MAINTENANCE OF ADULT MOSQUITOES.** Adult mosquitoes were housed in 45.7 cm<sup>3</sup> Gerberg Collapsible Insect Cages. Because the humidity within the insectary was not controlled, the cages were covered with a layer of plastic to maintain an average relative humidity of 80% (range 75–85%). Four baby food jars, each containing a folded paper towel "wick" and filled with distilled water, were provided as a source of humidity and daytime adult resting site. Inverted 10.2 cm diam. unglazed clay flower pots standing on a plastic dish with water served as the main oviposition substrate and adult resting site, and provided a secondary source of moisture (Takahashi 1968). Each flower pot had two 13 mm diam. holes (one in the inverted top and one in the side) to allow adult access to the inside where they seemed to prefer to rest during the day and deposit egg rafts. Every other day two fresh cotton balls soaked with a 5% sucrose solution were placed in the plastic feeding hammock of the insect cage as a carbohydrate source, and moistened with distilled water on alternate days. An anesthetized golden hamster was placed within the cage from 2200 to 2230 hr every week or two as a source of blood.

**EGGS.** Egg rafts were collected every 1 to 2 days by rinsing the inside of the flower pots with distilled water. Wicks in the baby food jars were also closely examined. Egg rafts were then individually transferred into baby food jars containing 30 ml of distilled water by a medicine dropper. Hatches were recorded daily and newly hatched larvae were fed immediately. Food for larvae consisted of a slurry made from 1 gm of Fleischmann's Active Dry Yeast and 1 gm pulverized Big Red Rabbit Food dissolved in 50 ml of distilled water. The rabbit food was pulverized in a Waring blender and then sifted with a 60 mesh filter; thus only particles 250  $\mu$  in diam. or smaller were used.

**LARVAE.** On the third day after hatch, larvae were transferred from the baby food jars to 33  $\times$  23  $\times$  5 cm white enameled pans, 25 larvae to a pan. The enameled pans were covered with a 2.5 cm layer of styrofoam insulation to prevent

loss of water. At the outset, each pan contained 400 ml of distilled water and approximately 4 ml of the concentrated food slurry described above (80 mg yeast and 80 mg rabbit chow). On the second day after transfer 3 ml of the food slurry were added. Finally, 2 ml of the food slurry were added every other day beginning the fourth day after transfer and until pupation was completed. Fouling of the water was not a serious problem with this food regimen.

**PUPAE.** Pupae were collected daily with a medicine dropper, rinsed thoroughly with distilled water to remove larval food contaminants, and transferred with fresh distilled water to 120 ml plastic cups 6.5 cm in diam. No more than 125 pupae were put into one cup as crowding diminished the percent of adult emergence. The cups were examined daily. At this time, pupal exuviae were removed and counted, and dead pupae and adults were recorded and removed. The cups were removed from the cage when emergence was complete.

**CROSS-MATING.** Mosquitoes of the Florida strain were provided by D. B. Francy and associates at the USPHS, CDC, Fort Collins, CO laboratory from a colony that is now several years old. Starter material was collected from Mahogany Hammock, Everglades. This strain was maintained at Cornell in the same manner described above. Inseminated females from the 8th generation of the Guatemalan colony and from approximately the 17th generation of the Florida colony were simultaneously bloodfed on an anesthetized hamster. Egg rafts were collected and pooled by strain in a large white enameled pan with 800 ml of distilled water and 8 ml of the food slurry. On the fourth day after hatch, larvae were transferred to the smaller enameled pans and reared as previously described. As larvae pupated, each pupa was placed in a separate labelled vial with a cotton plug. Four groups of adults were then released into the standard adult cages within 48 hr of emergence as follows: Cage 1 (control)—250 Florida males with 250 Florida females; Cage 2 (control)—250 Guatemala males with 250 Guatemala females; Cage 3 (cross)—250 Florida males with 250 Guatemala females; and Cage 4 (cross)—250 Guatemala males with 250 Florida females. Fourteen days later an anesthetized hamster was offered as a bloodmeal source in each cage. At the end of the blood-feeding period, all adults were removed from the cage and engorged females were placed in a separate 30.4 cm<sup>3</sup> cage. The remaining males and unengorged females were returned to the standard cage. Each of these smaller cages was covered with plastic and contained three baby food jar wicks and one inverted clay flower pot. Because initial engorgement rates were varied,

an anesthetized hamster was offered as a bloodmeal source to the unengorged females at 7-day intervals for a 3-wk period. Engorged mosquitoes from each bloodmeal offering were added to the appropriate cage.

All egg rafts collected were examined for the number of eggs and then placed into individual baby food jars. Four to six days after collection, each raft was examined for the number of eggs hatched. Eggs that were unhatched but with eye spots were classified as embryonated. Dead and dying females were collected daily, dissected, and their spermathecae examined for the presence of sperm. One week after the final bloodmeal offering, all females were dissected and spermathecae examined for the presence of sperm as an indication of insemination.

## RESULTS

Attempts to colonize the La Avellana strain of *Cx. taeniopus* began in September 1978. However, a crepuscular lighting system had not been installed, and mass mating of adults did not occur. Successful colonization was achieved, with adults collected in La Avellana during late August 1979 and a vigorous strain of this mosquito is currently in the 18th generation.

**EGGS.** Egg rafts are oval and white when first deposited. The eggs darken during the next 2 to 4 hr. The number per raft for 206 rafts counted ranged from 22 to 147, averaging  $86.5 \pm 28.4$  eggs per raft. The mean length and width of 125 egg rafts at their longest and broadest points were  $2.0 \pm 0.5$  mm and  $1.2 \pm 0.2$  mm, respectively.

The period of embryogeny as determined from 32 egg rafts ranged from 39 to 57 hr and averaged 48.8 hr. In none of 27 rafts examined for delayed hatch were larvae collected after the day that hatch was first noted. Hatching was synchronous.

To determine the percent hatch, 99  $F_3$  egg rafts were observed to determine viability and the number of eggs hatched per raft. Larvae hatched from 49 of the 99 egg rafts, with 80.4% hatch from viable rafts.

**LARVAE.** Survival rates are given in Table 1. Using the percentages listed, there was an overall survival rate of 82.3%. The developmental time from egg to pupal stage, determined on a daily basis after transferring larvae to the enameled pans, was 12.7 days.

**PUPAE.** The pupal developmental time as determined from 613 pupae ranged from 26.5 hr to 99.8 hr and averaged 58.8 hr. Data on pupal survival through four generations were also collected throughout the colonization period and averaged 92.4%. The mortalities associated with emergence ranged from situations where

the pupal cuticle split along the thoracic midline and adults were unable to escape, to a situation where adults were almost fully emerged but the hindlegs remained stuck to the pupal exuvia so that the emerging imago was trapped on the surface of the water.

**ADULTS.** The time between egg hatch and adult emergence as well as the sex of the emerging adult were determined for 443 individuals. There were 196 males which averaged 359.7 hr between hatch and emergence, and the remaining 247 females averaged 356.5 hr, indicating that for this particular study, protandry does not occur. The male to female ratio for this group was 1 to 1.3 although this single study may not accurately reflect the true ratio. On three other occasions, 713 males and 701 females were removed from standard cages 1 to 2 wk after emergence, and used for other experimental purposes. Their sex ratio was 1.02 to 1.

After emergence, adults spent most of the daylight hours resting on the inside and outside of the flower pots and the paper towel wicks. Both sexes became very active, flying about the cage during the simulated dusk period. Although mating was never actually observed, it was inferred that copulation took place during the crepuscular periods. In a single study to determine the days after emergence that mating takes place, insemination was not detected until 6–10 days after emergence.

Both sexes of *Cx. taeniopus* are relatively long-lived. Data from four rearings representing 945 males and 771 females indicated that 50% of the males survived for 77 days while 50% of the females survived for 86 days, the last female expiring 133 days after emergence.

An attempt to determine the preoviposition period was made in a separate experiment with 29 females given their first bloodmeal 20 days after emergence. Subsequently, an anesthetized hamster was offered as a bloodmeal source at 2-week intervals. In a separate study of ovarian development, it was noted that eggs are not fully developed before 4 days after the bloodmeal. Therefore, egg rafts collected before day 4 after a blood meal must have come

Table 1. Survival of *Culex taeniopus* larvae. Percent survival to 2nd instar based on 3729 1st instar larvae. Percent survival to 3rd and subsequent stages based on an initial number of 2581 2nd instar larvae transferred to enameled pans.

% survival 1st to 2nd instar	% survival 2nd to 3rd instar	% survival 3rd to 4th instar	% survival 4th instar to pupa
95.0	92.9	97.2	95.6

from a previous bloodmeal. We observed that oviposition begins on day 5 after a bloodmeal and continues for at least 18 days after engorgement. Of the 29 bloodfed females that started in the cage, 97 bloodmeals were taken from which 83 egg rafts were collected. This represents approximately 2.9 egg rafts per female.

A summary of the results of this laboratory study of *Cx. taeniopus* is given in Table 2. These data are compared with those obtained by Hair (1968) for *Cx. "cedecei"* from Florida.

**CROSS-MATING.** Table 3 gives a summary of the data collected for this set of experiments. Insemination rates were relatively high for both intrastain controls and the Guatemala female  $\times$  Florida male cross, and very low for the Florida female  $\times$  Guatemala male cross. The percent hatch was significantly higher in the intrastain controls than the interstrain crosses.

Furthermore, unhatched embryos in the interstrain crosses were generally poorly developed in that they lacked clear-cut segmentation. Larvae from the intrastain controls survived at

a normal rate. Of the interstrain crosses, only 16 of 11,926 eggs from the Guatemala female  $\times$  Florida male cross hatched. Of these, only three survived beyond the second instar, producing one female and two male adults. Dissection and examination of the reproductive organs revealed normal development.

## DISCUSSION

The results of the cross-mating study indicate that the two colonized strains of *Cx. taeniopus* indeed appear to be different species. It is worth noting that there was a very low rate of insemination for the Florida female  $\times$  Guatemala male cross, suggesting that pre-copulatory mechanisms were important. This was not the case for the Guatemala female  $\times$  Florida male cross where a high rate of insemination coupled with a low rate of embryonation indicated that a post-copulatory, pre-zygotic isolating mechanism was involved. Based on external morphological evidence, Stone and Hair (1968) concluded that these two popula-

Table 2. Summary of the biology of *Culex (Melanoconion) taeniopus* and *Cx. (Mel.) "cedecei"* under laboratory conditions.

	<i>Cx. taeniopus</i>	<i>Cx. "cedecei"</i> **
Preoviposition period	5-18+ days	20-45 days
Oviposition site	Inner wall of flower pot (raft)	$\bar{x}$ = 30 Water surface (raft)
Number of eggs per raft	22-147 $\bar{x}$ = 87	30-300 $\bar{x}$ = 85
Egg incubation	39-57 hr $\bar{x}$ = 87	36-48 hr
Delayed egg hatch	Not observed	Observed in 5% of eggs
Larval development	10-20 days $\bar{x}$ = 13	11-20 days $\bar{x}$ = 15
Pupal duration	$\bar{x}$ = 59 hr	$\bar{x}$ = 72 $\pm$ 30 hr
Mating after emergence	Within 6 days	Within 3 days
Multiple bloodmeals	Possible	Observed before 1st oviposition
Adult longevity	Females: $\bar{x}$ = 86 days Males $\bar{x}$ = 71	$\bar{x}$ = 70 days**

\* After Hair (1968).

\*\* Adult longevity is cited as 90 days in Table 2 of Hair (1968).

Table 3. Fecundity, fertility, and insemination rates of crosses of geographic strains of *Culex taeniopus*.

Cross	Total no. rafts collected	Total no. eggs	Average no. egg per raft	Percent hatch	Percent embryonation	Total no. females dissected	Percent insemination
Florida females $\times$ Florida males	90	5672	63.0	69.6	29.5	229	84.3
Guatemala females $\times$ Guatemala males	120	9656	80.5	76.8	12.1	222	89.2
Guatemala females $\times$ Florida males	128	11,926	93.2	0.1	25.4	219	94.1
Florida females $\times$ Guatemala males	15	762	50.8	0	8.0	222	3.2

tions were distinct species. The tarsi of the Florida population were described as entirely dark or with only a trace of pale bands, and male terminalia exhibited constant differences. In our laboratory studies, it was seen that banding patterns of the hind tarsi were much more distinct in the Guatemala strain, making separation possible at the level of the naked eye. Furthermore, Kreutzer (1983, personal communication) has demonstrated species differences in isoenzyme patterns of these two strains.

We therefore believe that the data presented on the laboratory bionomics of the Guatemala strain are unique. While certain aspects are similar to those reported by Hair (1968), two important exceptions were found. A "delayed egg hatch" as reported for the Florida strain was not observed in our laboratory colony and constitutes a significant difference. A second important difference was observed in the preoviposition period. We observed oviposition 5 to 18 days after the initial bloodmeal, whereas Hair (1968) reported a range of 20 to 45 days, averaging 30 days for the preoviposition period of the Florida strain. This discrepancy could be due to several factors. The number of bloodmeals needed in the laboratory for complete egg development could vary. Hair reported that multiple bloodmeals were necessary for the completion of the first gonotrophic cycle. This was not the case for the Guatemala strain. The oviposition sites also differed. We used inverted clay flower pots (Takahashi 1968) while Hair collected egg rafts from an open water surface. When females of the Florida strain were maintained in our laboratory in the same manner as described above, the preoviposition period did not differ from the Guatemala strain.

Despite the difference noted in the length of the preoviposition period, it is still long compared with many other mosquito species (Gerberg 1970). It should also be noted that a lengthy preoviposition period (21 days) was observed for *Cx. pilosus* (Hair 1968) and *Cx. portesi*, where it was termed a "deferred oviposition phenomenon" (Davies and Martinez 1970). *Culex portesi* did not commence oviposition until 6 days after the bloodmeal and continued to oviposit through 18 days after blood engorgement. The exact reason for the lengthy preoviposition period exhibited by these *Melanoconion* species is not clear but may be a fixed genetic characteristic within the group. However, the following points should be considered. First, the oviposition site offered in the laboratory may not duplicate the natural situation. Second, evidence supporting the hypothe-

sis that deferred oviposition is a laboratory-induced phenomenon is given by Shroyer and Sanders (1977). These authors demonstrated that *Aedes vexans* (Meigen) began oviposition 1 to 2 days later than inseminated females. The delayed effect of virginity on oviposition has also been demonstrated by Das et al. (1967) for *Culex quinquefasciatus* Say (as *fatigans* Wied.) and Lang (1956) for *Aedes aegypti* (Linn.). In our laboratory colony at the time of data collection, insemination rates ranged from 60–70%; thus this factor may have contributed to delayed oviposition by as many as 40% of the females. However, many egg rafts collected up to day 18 after the bloodmeal were viable, indicating that insemination is not the sole explanation for this phenomenon. Third, carbohydrate feeding has also been shown to inhibit oviposition (de Meillon et al. 1967, Hudson 1970, Shroyer and Sanders 1977). During our study a source of sucrose was constantly available and thus could have been an important factor.

Under laboratory conditions described here, the adults of both strains are long-lived, as has been observed for other species of *Culex* (*Melanoconion*). During the portion of our study in which females were offered bloodmeals every 2 weeks, it was possible that some females took as many as 8 bloodmeals and completed 8 gonotrophic cycles. On average, however, by comparing the number of egg rafts collected to the number of females that started the experiment, each female completed 3 gonotrophic cycles with a longevity of approximately 90 days. This observation is of considerable importance when one considers vector efficiency for arboviruses. A long-lived adult female allows for (1) increased opportunities to encounter a viremic host, (2) the virus to reach and multiply in the salivary glands after an infective bloodmeal, (3) transmission of virus in later bloodmeals to susceptible hosts, and (4) the infected female to serve as a reservoir for the virus.

Both strains of *Cx. taeniopus* also displayed a relatively long period of larval development compared to colonized *Culex* (*Culex*) species (Gerberg 1970). This finding is similar to that observed for other species of *Culex* (*Melanoconion*), i.e. *Cx. portesi* (Davies and Martinez 1970); *Cx. aikenii* s.l. (Adames and Galindo 1972); and *Cx. pilosus* (Hair 1968). Inadequate nutrition or suboptimum temperatures might have prolonged larval development if the period noted is indeed longer than normal for this species. However, the combination of rabbit chow and yeast used as a larval diet in this study produced sufficient numbers of viable adults without high larval or pupal mortality rates to exclude experimenting with other diets. Furthermore, the

temperature at which the immatures were reared (27°C) was within the range of the ambient air temperature in La Avellana (23–33°C) during the season in which these mosquitoes are found in high numbers.

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