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# GONOTROPHIC CYCLE AND SURVIVORSHIP OF *CULEX QUINQUEFASCIATUS* (DIPTERA: CULICIDAE) USING STICKY OVITRAPS IN MONTERREY, NORTHEASTERN MEXICO

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ABSTRACT: Mark–release–recapture experiments were conducted to determine the length of the gonotrophic cycle and rate of survivorship of *Culex quinquefasciatus* Say in Monterrey, northeastern Mexico. A total of 2,352 field-caught *Cx. quinquefasciatus* females were marked and released at 8–12 h postemergence in 2 field trials. Sticky ovitraps were used to recapture marked gravid females. One hundred and ten (4.6%) marked females were recaptured during a 12-day sampling period. Recapture rates for the 2 individual trials were 6.4% and 3.5%. The length of the gonotrophic cycle, calculated as the average time between the initial blood meal and the time of recapture of gravid females, was 2–3 days. The first blood-fed mosquitoes were recaptured on the 2nd day postrelease. Gravid egg-laying females were most commonly recaptured at 2–3 days postfeeding. Daily survival estimates for the 2 release dates were of 0.871 and 0.883, respectively.

**KEY WORDS** *Culex quinquefasciatus*, gonotrophic cycle, survivorship, Mexico, West Nile virus

## INTRODUCTION

West Nile virus (WNV: Family Flaviviridae) was recently introduced into northern Mexico (Blitvich et al. 2003, 2004; Estrada-Franco et al. 2003; Fernandez-Salas et al. 2003). Although the principal vector of this infection has not been determined, *Culex quinquefasciatus* Say is a prime suspect. This mosquito species is highly abundant in northern Mexico (Elizondo-Quiroga 2002), and nearby populations in the USA were relatively efficient vectors of WNV in laboratory trials (Turell et al. 2001, Goddard et al. 2002). Furthermore, WNV has been frequently isolated from wild-caught *Cx. quinquefasciatus* in the USA (CDC 2002, Reisen et al. 2004).

*Culex quinquefasciatus* larvae are typically found in stream margins and natural and artificial containers in both urban and suburban areas, where they can survive in both clean and polluted water (Vinogradova 2000, Elizondo-Quiroga 2002). At present, the bionomics of *Cx. quinquefasciatus* in Mexico, particularly in respect to disease transmission and vector control, are poorly understood. Government vector-control programs have focused on vector species that transmit diseases, such as dengue and malaria (Mancheno et al. 2001). However, now that WNV has been introduced into northern Mexico, information on *Cx. quinquefasciatus* in this area is urgently required.

The length of the gonotrophic cycle and longevity of female mosquito vectors are critical determinants of vectorial capacity (Garret-Jones and Shidrawa 1969). The gonotrophic cycle is directly related to the biting or feeding frequency, and longevity is related to the time available to acquire and infect humans with viruses. Reisen et al. (1991) reported the daily survivorship of *Cx. quinquefasciatus* in southern California as 0.84. De Meillon et al. (1967a) determined that about 66% of *Cx. quinquefasciatus* that had their blood meal before 24 h oviposited 2 days later (3rd night) and the remaining 34% oviposited after 3 days. Practically all of the mosquitoes that had their blood meal after midnight oviposited by the 4th night. De Meillon et al. (1967b) showed that sugar feeding delayed oviposition; mosquitoes fed on sugar continued to oviposit to the 10th day after the blood meal; by the 5th day, 45% had oviposited; in the sugar-free group, 98% had oviposited.

The mark–release–recapture technique has been successfully used to estimate longevity and the gonotrophic cycle of mosquitoes (Service 1993). Ordoñez-González et al. (2001) employed both mark–release–recapture and ovitraps to estimate the dispersion of *Aedes aegypti* (L.) in northeastern Mexico. We wanted to replicate this approach for *Cx. quinquefasciatus* using sticky ovitraps with grass infusion. This approach was used because *Cx. quinquefasciatus* lay eggs in easily observable rafts on the water surface, and this species has a preference for grass infusion (Vinogradova 2000). Our aims were to determine the duration of the gonotrophic cycle and estimate daily survival rates. This information is necessary to better understand *Cx.*

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*quinquefasciatus* biology and the epidemiology of future WNV outbreaks in northern Mexico.

## MATERIALS AND METHODS

*Study area:* The mark–release–recapture experiments were performed in a single study site in Pesqueria, Nuevo León State, northeastern Mexico (25°47′N and 100°03′W) (11,321 inhabitants). The site is in the Monterrey City metropolitan area and is approximately 20 miles northeast of the city center. It is a suburban area near where cereal crops, such as corn, wheat, barley, and oats, are grown. The elevation range is 330–400 m. The study site is located where human settlements have been established along the Pesqueria River. Vegetation is represented by a brushwood forest of *Prosopis glandulosa* Torrey (Mesquite). Annual average rainfall is 550 mm, but monthly average rainfall during the dry season (June–August) is only 40 mm. Annual mean temperature is 28°C with 40–60% relative humidity. At night, prevailing easterly winds are less than 1.15 km/h.

*Mark–release–recapture experiments:* Dippers were used to capture *Cx. quinquefasciatus* 4th-stage larvae and pupae from larval habitats such as streams and 200-liter drums. Rearing was conducted at collection sites, and larvae were placed in plastic 12" × 12" trays positioned inside screened handling cages. After emergence, adult females were placed in 3.8-liter cardboard containers containing cotton pads soaked with a 10% sugar solution. Mosquitoes were then dusted with dye (Bioquip, Gardena, CA) using Pasteur pipettes. A different color dye was used in each trial. Marked females were released in the evening (1930 h) of June 12 (trial 1) and June 14 (trial 2) at 8–12 h postemergence. Mosquitoes unable to fly were subtracted from the number counted. We recaptured mosquitoes for 12 days following each release. Recapture was by the use of 100 sticky ovitraps baited with a grass infusion (Vinogradova 2000, Ordoñez-Gonzalez et al. 2001) located within a 50-m radius of the release site. Plastic 3.8-liter open black containers were filled with 2 liters of grass infusion with a 7-cm strip of thin black cardboard added in a projection edge as an oviposition substrate. Glue used to catch rodents (Trapper® Bell Laboratories Inc., Madison, WI) was softened by heating and was smeared onto the cardboard strip. Gravid females became stuck when they came to oviposit. Paper clips held the strip inside the container to prevent the water from dissolving the glue. The sticky ovitraps were checked every morning (0800 h).

*Sella's stages:* Sella's stages (WHO 1975), which indicate blood-digestion intervals, were determined for each recaptured female. This enabled us to record blood digestion status for each release, as well as readiness for egg laying. Sella's stages comprise stage I (unfed), the abdomen is collapsed, and the ovaries occupy one third of the abdomen; stage II (freshly fed), stomach with red blood, ovaries occupying 2–3 segments ventrally and 4 dorsally; stage III–IV (half gravid), blood is dark red, ovaries occupying 4–5 segments ventrally and 6 dorsally; stage V (subgravid), blood greatly reduced and dark in color, ovaries occupying most of abdomen; stage VI–VII (gravid), blood only a black trace, a narrow blackish line or completely digested.

The length of the gonotrophic cycle for these females was calculated as the number of days from the time the 1st blood meal was taken (Sella's stage II) to the peak in numbers of recaptured gravid females (Sella's stage VI and VII).

*Survivorship:* The daily survival rate of *Cx. quinquefasciatus* females was calculated as described by Gillies (1961). In this method, the daily recaptures were transformed to $\ln(x + 1)$ and regressed as a function of time. The antilog of the slope of the regression line was then used to estimate daily survivorship. An analysis of variance was used to test the statistical significance of the slope (SPSS 1999). Regression slopes for each mark–release–recapture were compared using the 2-slopes comparison test (Zar 1984). Survivorship estimates for different releases were pooled if slopes were not significantly different at the 5% level (Reisen et al. 1978).

## RESULTS

*Mark–release–recapture studies:* Weather conditions were relatively stable during the study, with a temperature range of 31.5–34°C and relative humidity of 40–56%. Wind speed was lower on June 12 (0.70 m/sec) than June 14 (3.30 m/sec). No rainfall was observed during the study period.

A total of 10,300 *Cx. quinquefasciatus* 4th-stage larvae and pupae were captured and reared for the mark–release–recapture experiment. Of these, 2,668 (25.9%) were females and 7,632 (74.1%) were males. A total of 2,352 adult females were marked with dye and released at 8–12 h postemergence in the 2 trials. One hundred and ten (4.6%) marked mosquitoes were recaptured using sticky ovitraps during the 12-day postrelease period (Table 1). The recapture rates for trials 1 and 2 were 6.4% (59 of 918) and 3.5% (51 of 1,434), respectively. Recaptures peaked at 1 ($n$ = 17), 4 ($n$ = 11), 5 ($n$ = 22), and 8 ($n$ = 15) days postrelease.

*Gonotrophic cycle:* Of the 17 individuals recaptured at 1 day postrelease, all were unfed (Sella's stage I) (Table 2). Engorged mosquitoes were first recorded at 2 days postrelease ($n$ = 10), and all contained Sella's stage II blood meals. Mosquitoes half gravid (Sella's stage III and IV) were first recorded at 3 days postrelease, and subgravid mosquitoes (Sella's stage V) at 4 days postrelease. On day 5 (72 h postrelease), we recaptured 22 gravid mosquitoes. At day 6 postrelease, only 1 gravid

Table 1. Numbers of marked *Culex quinquefasciatus* females recaptured per day after release in each of 2 mark–release–recapture experiments conducted in Pesqueria, a suburban area of Monterrey City, northeastern Mexico.[1]

| Days after release | Trial 1[2] | Trial 2[3] | Total |
|---|---|---|---|
| 1 | 8 | 9 | 17 |
| 2 | 4 | 6 | 10 |
| 3 | 4 | 5 | 9 |
| 4 | 5 | 6 | 11 |
| 5 | 10 | 12 | 22 |
| 6 | 3 | 4 | 7 |
| 7 | 3 | 3 | 6 |
| 8 | 7 | 8 | 15 |
| 9 | 3 | 2 | 5 |
| 10 | 2 | 2 | 4 |
| 11 | 2 | 1 | 3 |
| 12 | 0 | 1 | 1 |
| Number released | 918 | 1,434 | 2,352 |
| Number recaptured | 59 | 51 | 110 |
| Recapture rate | 6.4% | 3.5% | 4.7% |

[1] Studies were conducted in Pesqueria, a suburban area of Monterrey City, northeastern Mexico, during 2004.
[2] June 12, 2004.
[3] June 14, 2004.

mosquito appeared. A 2nd smaller recapture peak was observed on day 8 in the 2 groups (14 mosquitoes) and in the pooled data (Fig. 1). The length of the gonotrophic cycle, calculated as the average number of days between the 1st blood meal and the recapture of gravid females, is 2–3 days.

*Survivorship:* An overall daily survival rate of 0.877 was computed from the pooled regression coefficient (Table 3). The slope was significantly different from zero ($F = 14.851$, $df = 1,10$, $P < 0.05$). When data from the 2 releases were analyzed separately, they showed a negative linear relationship with time. The slope was significantly different from 0 for the 1st ($F = 17.658$, $df = 1,10$, $P < 0.05$) and the 2nd release ($F = 10.070$, $df = 1,10$, $P < 0.05$). No significant difference ($t = 3.55$, $df = 2,20$, $P < 0.05$) between the 2 slopes was detected, i.e., there are no significant difference between the 2 regression coefficients (Zar 1984).

## DISCUSSION

Our mark–release–recapture study suggests that the typical length of the gonotrophic cycle of *Cx. quinquefasciatus* in Monterrey, Nuevo León State, is 2–3 days and the daily survival rate is 0.871–0.883. The determination of survival rates is important for understanding the epidemiology of WNV transmission by *Cx. quinquefasciatus*. Mosquito female longevity is linked to the WNV extrinsic incubation period (EIP), i.e., the period in a mosquito that a disease-producing organism requires to develop to the point where it can be transmitted. For field mosquitoes, WNV requires a 2-wk EIP, depending on temperature (Valandingham et al. 2004). Our results show that, by day 14, the pooled daily survival rate of *Cx. quinquefasciatus* was 0.159 (about 15.9% mosquitoes survive and are potentially infective), and by day 21, this was 0.056. Moreover, mosquitoes surviving 14 days have bloodfed 3–5 times, which increases the possibility of acquiring and transmitting WNV to birds, horses, or humans.

The overall recapture rate for the experiment was 4.67%, which compares with 7.70% for *Ae. aegypti* with sticky ovitraps (Ordoñez-Gonzalez et al. 2001) and 0.34% for *Cx. quinquefasciatus* with unlit dry-ice-baited miniature surveillance traps (Schreiber et al. 1988).

The recapture rates for trials 1 and 2 were 6.42% and 3.55%, respectively. The lower recapture rate

Table 2. Sella's stage[1] of *Culex quinquefasciatus* recaptured after each day following 2 mark–releases (A = June 12, 2004, B = June 14, 2004) in the municipality of Pesqueria, a suburban area of Monterrey City, northeastern Mexico.

| | Sella I | | Sella II | | Sella III | | Sella IV | | Sella V | | Sella VI | | Sella VII | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Day | A | B | A | B | A | B | A | B | A | B | A | B | A | B | Total |
| 1 | 9 | 8 | — | — | — | — | — | — | — | — | — | — | — | — | 17 |
| 2 | 1 | — | 5 | 4 | — | — | — | — | — | — | — | — | — | — | 10 |
| 3 | — | — | 1 | — | 4 | 3 | — | 1 | — | — | — | — | — | — | 9 |
| 4 | — | — | — | — | — | — | 2 | 2 | 4 | 3 | — | — | — | — | 11 |
| 5 | — | — | — | — | — | — | — | — | — | — | 4 | 4 | 8 | 6 | 22 |
| 6 | — | — | 2 | 1 | 2 | 1 | — | — | — | — | — | — | 1 | — | 7 |
| 7 | — | — | — | — | 1 | 2 | — | 1 | 2 | — | — | — | — | — | 6 |
| 8 | — | — | — | — | — | — | — | — | 1 | — | 2 | 3 | 5 | 4 | 15 |
| 9 | — | — | 2 | — | — | 1 | — | — | — | — | — | — | 1 | 1 | 5 |
| 10 | 1 | 1 | — | — | 1 | — | — | 1 | — | — | — | — | — | — | 4 |
| 11 | — | — | — | — | 1 | — | — | 1 | — | — | 1 | — | — | — | 3 |
| 12 | — | — | — | — | — | — | — | — | — | 1 | — | — | — | — | 1 |

[1] Sella I, unfed, the abdomen is collapsed, and the ovaries occupy one third of the abdomen; sella II, freshly fed, stomach with red blood, ovaries occupying 2–3 segments ventrally and 4 dorsally; sella III–IV, half gravid, blood is dark red, ovaries occupying 4–5 segments ventrally and 6 dorsally; sella V, subgravid, blood greatly reduced, dark in color, ovaries occupying most of abdomen; sella VI–VII, gravid, blood only a black trace as narrow blackish line or completely digested.

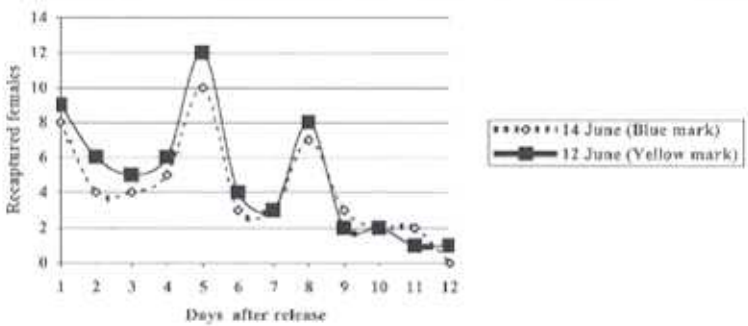


Fig. 1. Numbers of marked *Culex quinquefasciatus* females recaptured per day after release in each of 2 mark–release–recapture studies in the municipality of Pesqueria, a suburban area of Monterrey City, northeastern Mexico.

for trial 2 could be due to the windier conditions on that day. Schreiber et al. (1988) noted that female *Cx. quinquefasciatus* are dispersed on the wind.

We observed the first Sella's stage II of recaptured females on day 2, indicating that mosquitoes fed within 48 h postemergence. A total of 22 females were gravid by day 5 postrelease, or 72 h after having their 1st blood meal, but on day 6, there were 3 females with Sella's stage III, suggesting that they fed the night before. The 2nd oviposition was on day 8 (<60 h), which suggests that the 1st gonotrophic cycle was delayed as a result of bloodfeeding on the morning of the 2nd day postrelease (De Meillon et al. 1967b). We conclude that most marked females bloodfed and oviposited over a 48–72-h period, similar to the results of De Meillon et al. (1967b).

Daily survival probabilities obtained by regression analyses were 0.871 and 0.883 for the 1st and 2nd releases, respectively. These results are similar to daily survivorship of *Cx. quinquefasciatus* in southern California (Reisen et al. 1991), which these authors estimated as 0.84 by Davidson's (1954) method and 0.82 by the Gillies (1961) method. Differences in estimates of survivorship between the Davidson's method and the regression method that we used have been attributed to sampling bias and population fluctuations (Service 1993). Survival rate can be calculated from the proportion of parous females in a population using Davidson's method, but there are many confounders. For example, recruitment of the population must be constant throughout the sampling period but the percentage of parous mosquitoes can be severely reduced by a spike in emergence, thereby lowering estimates of survival. Survival estimates based on the regression method can be strongly influenced by mosquito emigration (Reisen et al. 1982). In our study, the daily decline in numbers of marked specimens was relatively constant, which indicates that population abundance is independent of age ($\beta \neq 0$, $P < 0.05$, slope tests for homogeneity). A stable decline in numbers of recapture specimens excludes possibility of sudden reductions in population abundance due to emigration.

Table 3. Regression estimates of daily survival rates for mark–release–recapture *Culex quinquefasciatus* females in the municipality of Pesqueria, a suburban area of Monterrey City, northeastern Mexico.

| Release date | Regression[1] coefficient (beta) | Coefficient[2] of determination $r^2$ | Daily survival probability |
|---|---|---|---|
| June 12 | −0.138* | 0.638 | 0.871 |
| June 14 | −0.124* | 0.502 | 0.883 |
| Pooled[3] | −0.131* | 0.598 | 0.877 |

[1] ANOVA test for significant linearity; * $P < 0.05$.
[2] Proportion of variance in the dependent variable accounted by the predictor independent variable in the linear model (SPSS 10).
[3] No significant difference among the two slopes, using the two slopes comparison test (Zar 1984).

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