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GONOTROPHIC CYCLE ESTIMATE FOR *CULEX QUINQUEFASCIATUS* IN MÉRIDA, YUCATÁN, MÉXICO

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ABSTRACT. West Nile virus (WNV) has been present in the Yucatán State, México, since 2002. *Culex quinquefasciatus*, one of the main vectors of WNV transmission in the United States, is also common in Mexico and may be a key vector of WNV transmission to humans in the Yucatán. The aim of this study was to determine the length of the gonotrophic cycle and the survival rates of *Cx. quinquefasciatus* from Mérida, Yucatan, during the rainy versus the dry season. Mosquitoes were collected during 25-day periods in October (rainy season) and in April (dry season), and captured females were classified by abdominal appearance (freshly fed, late-stage fed, half gravid, and subgravid). To determine the age structure as nulliparous and parous females and to calculate the gonotrophic cycle through a time series and the mosquito survival, we used Davidson formulae. Also, vitellogenesis analysis to monitor egg maturity was conducted during both seasons. Cross-correlation data suggested a similar length of the gonotrophic cycle (4 days) in both seasons. Oogenic development required a minimum of 72 h in each season. However, survival of the mosquito population collected in the rainy season was significantly higher (0.91) with a mean temperature of 28 ± 1.57°C than was survival in the dry season (0.78) with a mean temperature of 29 ± 1.10°C. Survival, although higher during the rainy season, did not influence the length of the gonotrophic cycle of *Cx. quinquefasciatus* in Yucatán.

KEY WORDS Gonotrophic cycle, survival rate, *Culex quinquefasciatus*, Yucatán

INTRODUCTION

West Nile virus (WNV) activity was demonstrated in Yucatán State of México in horses and birds in July and December 2002, respectively (Loroño-Pino et al. 2003, Farfán-Ale et al. 2004). *Culex quinquefasciatus* Say in Florida is competent but only a moderately efficient vector of WNV (Sardelis et al. 2001). This species is one of the most abundant *Culex* species in Yucatan. Larval collections from domestic and peridomestic habitats conducted during the rainy season from August to December 2003 (Nájera-Vázquez et al. 2005) and temporal variations in the relative abundance of *Cx. quinquefasciatus* collected in Mérida during 2005 indicated that this species represented 88.7% of the total sample population (García-Rejón, unpublished data).

The vectorial capacity for virus transmission is influenced by the length of the gonotrophic cycle (Mutero and Birley 1987) and the survival rate (Birley and Rajagopalan 1981, Birley 1984). The length of the gonotrophic cycle impacts the frequency of vector–host contact (Rodriguez et al. 1992), and the survival rate determines the total egg production and the stability of mosquito population size (Miller et al. 1973). The latter is the most important factor in estimating the average lifetime infection as vector (MacDonald 1957). To estimate the survival rate during the gonotrophic cycle of a mosquito population of a given age, it is necessary to know the parity rate (mosquitoes with at least 1 event of oviposition during its life) (Davidson, 1954, MacDonald 1957). Meanwhile, the length of the gonotrophic cycle may be stimulated by the physiological longevity of the vector (Detinova 1962, Rodriguez et al. 1992). Climatic conditions influence the length of the gonotrophic cycle as well as vectorial capacity of mosquito populations. The purpose of this study was to determine whether the length of the gonotrophic cycle or survival rate of bloodfed *Cx. quinquefasciatus* differed during the rainy versus the dry season.

MATERIALS AND METHODS

Mosquito collection

The study was conducted in the city of Mérida, Yucatan, México, during 2004–2005. The city of Mérida is located close to sea level and the climate is warm and subhumid, with rains in

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summer and an average annual temperature of 26°C. Mérida has a distinct rainy season (May–October) and a dry season (January–April). The average pluvial precipitation is 1,000 mm during the rainy season and 300 mm during the dry season. Mérida has approximately 700,000 inhabitants (INEGI 2005).

Parous–nulliparous ratios and survival dynamics

Mosquito collections were conducted between 0800 h and 1000 h during dry (April) and rainy (October) seasons, using walk-in red boxes (Reisen and Pfuntner 1987). Mosquitoes were identified using the published keys by Darsie and Ward (1981). A time-series analysis of the 25-day sampling period was conducted for each season. Female *Cx. quinquefasciatus* collected were dissected to determine parity rates (Detinova 1962). Daily changes in the parous/nulliparous ratio were recorded. The length of the gonotrophic cycle was estimated using a cross-correlation analysis (Birley and Rajagopalan 1981) with the formula $M_t = P_u T_{(t-u)}$, where M = the number of parous individuals captured on day t; $T_{(t-u)}$ = the total number of females (nulliparous and parous) captured on day $t-u$; u = the length of the gonotrophic cycle; and P = the survival rate per gonotrophic cycle, calculated from the slope in a regression model. The correlation coefficient (r) for day 0 represented the correlation between P and T_t data pairs from mosquitoes captured the same day (15 data pairs). The r for day was obtained by pairing daily P data with the corresponding T data of 1 day before. Likewise, r for each day 1 was obtained by pairing daily P_t data with the corresponding T_t data of 1 day before. The r for day 2 was calculated by pairing daily P_t data with corresponding T_t data of 2 days before, and so on. It was assumed that a significant r between the time series expressed a time delay (u) equivalent to the gonotrophic cycle. The highest significant r obtained after day 0 (u = 0) indicated the number of days per gonotrophic cycle, with descending peaks occurring at multiples of this interval. To eliminate spurious cross correlations, data were filtered using an autoregressive process with a lag of 1 day, with the formula $Z_t = X - ß(X_{t-1})$, where Z_t = the time series to be filtered, X_t = the time series to be filtered, and ß = the estimated auto-regressive parameter (Holmes and Birley 1987). A significant correlation between 2 filtered time series (M_t and $X_{(t-u)}$) was assumed, and r corresponded to a lag u equivalent to the gonotrophic cycle, with regular peaks at the start of each cycle.

Daily survival rates (p) were calculated from the parity rates using the formula $p = (PR)^{1/CG}$, where PR = the parity rate and CG = the duration of the gonotrophic cycle (Davidson 1954).

Vitellogenesis

The duration of Christopher's stages of ovarian development were determined for *Cx. quinquefasciatus* based on the appearance of ovarian follicles (Clements 2000). Mosquitoes were collected using Mosquito Magnets Pro-Liberty (American Biophysics Corp., North Kingstown, RI). These traps were baited with propane and octenol, generating CO_2 as a by-product of propane combustion. These traps contain 2 fans operated by a rechargeable battery: one fan exhausts CO_2 from the trap while the other fan sucks air into the trap (Turell et al. 2003). The traps were operated during the rainy season (temperature 28 ± 1.57°C and relative humidity [RH] 75 ± 5.45%) and the dry season (temperature 29 ± 1.10°C and RH 81 ± 4.73%). Ten unfed females (without traces of blood) were dissected to determine their status of ovarian maturation. The remaining females were allowed to feed on a chicken (unfed mosquitoes without traces of blood were discarded) and immediately transported to the insectary where they were supplied with cotton pads soaked with a 10% sucrose solution and maintained in a temperature-controlled room at 29 ± 1°C and 70–100% RH. Starting at 12 h after bloodfeeding and continuing every 6 h up to 78 h, groups of 10 mosquitoes were dissected to determine their Christopher's stages.

RESULTS

Parity rate

A total of 2,339 *Cx. quinquefasciatus* females were collected and dissected, 1,273 during the rainy season and 1,066 during the dry season. Parity was observed in 69% (883/1,273) of female mosquitoes captured during the rainy season, compared to 37% (408/1,066) collected during the dry season (Table 1). These results indicated a significant difference in parity rates between seasons, $\chi^2 > 3.84$ ($\alpha = 0.05$, df = 1), $P < 0.05$.

Dynamics of parous–nulliparous ratios

Using both raw and filtered data, no significant correlation ($P > 0.05$) was observed in daily changes of parity rates over 25 days in females collected during the rainy and dry seasons; however, a high correlation on days 4 and 8 was found, suggesting a 4-day gonotrophic cycle in both seasons (Table 2).

Survival rate

The daily survival rate was 0.91 for mosquitoes collected in the rainy season and 0.78 in the dry season.

Table 1. Parity rate of female *Cx. quinquefasciatus* from Mérida, México, during the rainy and dry seasons, 2004–2005.

	Rainy season			Dry season		
Day	Dissected	Parous	Parity rate	Dissected	Parous	Parity rate
1	38	19	0.50	39	13	0.33
2	35	24	0.68	50	17	0.34
3	35	25	0.71	29	9	0.31
4	20	10	0.50	43	13	0.30
5	48	20	0.41	43	12	0.27
6	31	8	0.25	40	12	0.3
7	70	36	0.51	30	10	0.33
8	54	35	0.64	34	11	0.32
9	37	28	0.75	20	7	0.35
10	53	40	0.75	45	15	0.33
11	55	36	0.65	50	13	0.26
12	48	29	0.60	45	16	0.35
13	55	43	0.78	37	15	0.40
14	38	22	0.57	39	11	0.28
15	47	28	0.59	53	12	0.22
16	42	27	0.64	41	8	0.19
17	60	48	0.8	36	10	0.27
18	53	43	0.81	35	9	0.25
19	55	41	0.74	61	31	0.50
20	79	51	0.64	46	26	0.56
21	74	63	0.85	53	12	0.22
22	55	42	0.76	36	26	0.72
23	51	41	0.80	47	30	0.63
24	71	64	0.90	68	39	0.57
25	69	60	0.86	46	31	0.67
Total	1,273	883	0.69	1,066	408	0.38

Vitellogenesis

All unfed females collected in both seasons at Christopher's stage II and completed their oogenic development in a minimum of 72 h (3 days) after feeding to reach Christopher's stage V. The length of the gonotrophic stage could be calculated indirectly by adding 24 h (which is considered the required time to locate an oviposition site, lay eggs, and locate a new host) to the minimum time required to develop eggs to Christopher's stage V (Mekuria et al. 1991). When this criterion was applied to our data, the estimation of the length of the gonotrophic cycles was 4 days.

Table 2. Correlation indices of the parity rates of *Cx. quinquefasciatus* collected in Mérida, México, during the rainy and dry seasons of 2004–2005, by cross-correlation analysis of a time series (9 days of collection).

Day	Filtered dates of the rainy season	Filtered dates of the dry season
0	0.889	0.583
1	0.034	0.037
2	0.141	0.115
3	0.155	0.237
4	0.175	0.325
5	0.06	0.162
6	0.184	0.185
7	0.123	0.174
8	0.257	0.426
9	0.216	0.317

The percentage of females failing to develop beyond Christopher's stage II was 23% (14/60) in mosquitoes collected in rainy season, whereas 25% (15/60) of those collected in dry season had remained pregravid (Table 3).

DISCUSSION

The length of the gonotrophic cycle and the longevity of female mosquito vectors are critical determinants of vectorial capacity (Garret-Jones and Shidrawi 1969). The gonotrophic cycle is directly related to the biting or feeding frequency, and longevity is related to the time available to acquire virus and then infect humans during a subsequent blood meal. Our data were analyzed first by several criteria based on mathematical formulae (Birley and Rajagopalan 1981, Mutero and Birley 1987), and the correlation index obtained each day of collection was not adjusted to those criteria. However, the correlation index was adjusted according to the Bockarie criteria (Bockarie et al. 1995) and gave a gonotrophic cycle length of 4 days in both seasons. We concluded that the effect of the rain did not modify the gonotrophic length. This result was confirmed by the oogenic development as evident

Table 3. Vitellogenesis of *Cx. quiquefasciatus* populations collected in Mérida, México, during the rainy and dry seasons, 2004–2005.

Hours postfeeding	Rainy season (Christopher's stage)						Dry season (Christopher's stage)					
	No. dissected	I	II	III	IV	V	No. dissected	I	II	III	IV	V
0	10		100				10		100			
12	10		100				10		100			
18	10		100				10		100			
24	10		90[1]	10			10		90[1]	10		
30	10		30[1]	70			10		40[1]	60		
36	10		10[1]	90			10		20[1]	80		
42	10			100			10			100		
48	10			30	20	50	10			40	20	40
54	10				40	60	10				30	70
60	10				40	60	10				30	70
66	10				10	90	10				10	90
72	10					100	10					100
78	10					100	10					100

[1] Pregravid stage.

from vitellogenesis analysis, where it was observed that the females had egg maturation to Christopher's stage V on day 4. The results were the same for both seasons.

The time of gonotrophic cycle of *Cx. quinquefasciatus* from Mérida was in concordance with time obtained by Birley and Rajagopalan (1981) in India and with the study conducted by Milby and Reisen (1989) in California through the recapture–release technique. However, in a study conducted in northern México, the gonotrophic cycle of *Cx. quinquefasciatus* captured in Monterey was calculated between 2 and 3 days (Elizondo-Quiroga et al. 2006).

At the same time, we calculated the survival rate through Davidson's method, in which the survival rate of mosquitoes is considered an important factor in the determination of the transmission of disease (Mutero and Birley 1987), because it determines the stability of the population and their total production of eggs (Miller et al. 1973). We obtained a daily survival rate higher in the rainy season compared with the dry season. The average temperature was lower during the rainy season, contributing to the higher time of survival. Similar data were obtained in Japan in studies conducted with *Cx. quinquefasciatus* and *Cx. pipiens* (Oda et al. 2002). The vertical method described by Davidson (1954) was based on the mosquito parity rate and the length of the gonotrophic cycle, indicating a distribution of age stationary and the mortality constant regarding the age (McHugh 1989).

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