Journal of the American Mosquito Control Association, 17(4):254–259, 2001

ESTIMATION OF SURVIVAL AND GONOTROPHIC CYCLE LENGTH OF *SIMULIUM METALLICUM* SENSU LATO (DIPTERA: SIMULIIDAE) IN SOUTHERN MEXICO

MARIO A. RODRÍGUEZ-PÉREZ,[1] RAFAEL VÁZQUEZ-SÁNCHEZ[2] AND FILIBERTO REYES-VILLANUEVA[3]

ABSTRACT. This study compares the use of a mark–recapture analysis and a time-series analysis to estimate the gonotrophic cycle length and survivorship of *Simulium metallicum* s.l. in southern Mexico. Daily collections were performed with human- and horse-baited traps at 3 sites in a coffee plantation. The mark–recapture and time series experiments on these collections conclusively yielded a gonotrophic cycle length estimate of 3 days. Horizontal estimates of daily survivorship ranged from 0.75 to 0.69 and these values were similar to that estimated vertically of 0.77. The survival to infective stage (9 days) ranged from 0.012 to 0.043, taking into account at least 12 days for development of 3rd-stage larvae of *Onchocerca volvulus.* Mark–recapture and time-series methods were found to be suitable for estimating the gonotrophic cycle length and daily survivorship of *S. metallicum* s.l. under field conditions in southern Mexico.

KEY WORDS *Simulium,* ecology, survivorship, gonotrophic cycle

INTRODUCTION

Simulium metallicum Bellardi s.l. is the primary vector of *Onchocerca volvulus* (Leuckart) in the main endemic area of northern Venezuela (Lewis and Ibanez de Aldecoa 1962, Grillet et al. 1994, Basañez et al. 2000), but it is only a secondary vector in Guatemala and southern Mexico (Dalmat 1955, Garms and Ochoa 1979, Porter and Collins 1988a). *Simulium metallicum* s.l. has been conclusively demonstrated to be a species complex of at least 11 cytospecies (Conn et al. 1989, Procunier 1989, Conn 1990), 3 of which occur in Guatemala and, together with a 4th, in Mexico (Millest 1990, Millest et al. 1999). Experimental infections of *S. metallicum* s.l. with *O. volvulus* in Venezuela and Guatemala (Takaoka et al. 1986a, 1986b; Grillet et al. 1994; Basañez et al. 2000) have shown differences in the vector competence of the *S. metallicum* s.l. population complex. However, the possibility of variation in the vectorial capacity of this species in these 2 different geographic scenarios has not been investigated and no information is available about the estimation of survival and gonotrophic cycle length under field conditions. Variation in the numerous factors influencing vectorial capacity, such as survivorship and gonotrophic cycle, also may account for differences in the ability of the different *S. metallicum* cytospecies to transmit *O. volvulus.*

The time-series analysis technique (Birley and Boorman 1982) has been applied successfully to determine the survivorship and gonotrophic cycle lengths in black flies (Birley et al. 1983, Rodríguez-Pérez et al. 1995), and the results have been similar to those obtained with mark–release–recapture techniques (Milby and Reisen 1989, Collins et al. 1992). Herein, we present an evaluation of time-series and mark–release–recapture techniques in estimating the survivorship and the length of the gonotrophic cycle for animal- and human-biting populations of *S. metallicum* s.l. collected in southern Mexico. Studies to determine the oogenesis stages and development (Reyes-Villanueva and Rodríguez-Pérez 1994) of *S. metallicum* s.l. under field conditions were also carried out in parallel.

MATERIALS AND METHODS

Study area and sampling sites: The studies were conducted during January and March 1998 in Las Golondrinas (15°25′59″; N, 92°39′06″W; altitude 890 m), located in the onchocerciasis-endemic focus of southern Chiapas in Mexico. When the study was done, the village had 339 inhabitants and was hypoendemic (skin microfilaria [mf] prevalence of 13% and a geometric mean community microfilarial load of 0.04 mf/mg; Rodríguez-Pérez et al. 1999). Coffee production constituted the basis of economy of the village. Three sampling sites 300 m apart were randomly chosen within a coffee plantation; site 1 was aproximately 100 m away from the nearest breeding site, whereas sites 2 and 3 were about 400 m away. A population of horse-biting *S. metallicum* s.l. was collected at site 1. *Simulium metallicum* s.l. were also collected at sites 2 and 3 in landing-baits on humans selected from community volunteers, with each working as host and collector.

Oogenesis stages and development: The stages and duration of oogenesis in a sample of 300 wild female *S. metallicum* s.l. were determined. The females were collected on 3 days, between 0700 and 1000 h (when the number of nulliparous females should be high; Rodríguez-Pérez and Reyes-Villanueva 1994). Black flies were bloodfed at repletion

[1] Centro de Investigaciones sobre Enfermedades Infecciosas, Instituto Nacional de Salud Pública, Cuernavaca, Morelos 62508, México.

[2] Centro de Investigación de Paludismo, Instituto Nacional de Salud Pública, Tapachula, Chiapas 30700, México.

[3] Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, San Nicolás de los Garza, Nuevo León 66450, México.

on a horse in a Malaiselike trap located at collection site 1; each bloodfed fly was maintained in a polystyrene tube (1.0 × 7.5 cm), and immediately placed in shade provided by a coffee tree to approximate the conditions of a natural resting site. Every day, a fresh cotton pad soaked in 10% sucrose solution was provided, and the temperature and humidity in the shelter were recorded concurrently. The temperature ranged between 18 and 30°C with a mean of 24°C, relative humidity was 55–80%, and no rainfall or wind occurred. Beginning at 2 h after bloodfeeding, and continuing every 2 h up to 60 h, groups of 10 black flies were dissected to determine the Christophers' stages of oogenesis development (Cupp and Collins 1979).

Black fly capturing, marking, and releasing: Black flies for this study were captured at site 1. Temperature and humidity were the same as indicated above. A total of 2,420 females was captured in plastic cylinders on 4 consecutive days, between 0700 and 1000 h. Black flies were bloodfed to repletion on a horse in 2 Malaiselike traps. Engorged black flies were marked with fine fluorescent dusts (green, red, yellow, and orange color applied on consecutive days) blown through the plastic cylinders with a simple insufflator. At the capture site, marked black flies were allowed to disperse immediately; individuals unable to fly were discarded. Recaptures were attempted for 9 days after release from 0700 to 1730 h beginning at 24 h after release. Collected black flies were examined with the aid of a portable black-light lamp in a 1-m^3 dark wooden box to detect previously marked individuals, which were removed. Marked females were dissected to determine Christophers' stages of oogenesis development and the presence of large sacculate dilatations in the follicular tube, which can be observed 2–4 h after oviposition (Cupp and Collins 1979). Daily survival rates (S) were estimated with the parity rate (PR) of the average figure from dissections of black flies collected at sites 1 and 3, with $S = (PR)^{1/GC}$, where GC is the gonotrophic cycle length estimated in this study (Davidson 1954).

Dynamics of parous–nulliparous ratios: Daily changes in the parous–nulliparous ratio in wild-caught *S. metallicum* s.l. were recorded for 11 consecutive days at 3 collecting sites between 0800 and 1430 h. All female *S. metallicum* s.l. were dissected in 0.9% saline solution and classified as nulliparous or parous by the presence of sacculate dilatations and follicular relics in the ovarian tunica (Cupp and Collins 1979). During the collecting process, the temperature at the 3 sites ranged between 20 and 32°C (mean 26°C), humidity was 60–80%, and no rainfall or wind occurred. Time-series analysis for each 11-day sampling period at each collection site was constructed. The length of the gonotrophic cycle was estimated with a cross-correlation analysis (Birley and Rajagopalan 1981). The number of parous (P_t) was used as the dependent variable (Y) and total females (T_t) as the independent variable (X) in the correlation analysis of each series. The r-coefficient for day 0 represents the correlation between P_t and T_t data pairs from black flies captured on the same day (11 data pairs). The r-coefficient for day 1 was obtained by pairing daily P_t data with the corresponding T_t data of 1 day before (10 data pairs), and so on. A significant r-coefficient between the time-series expresses a time delay (u) equivalent to the oviposition cycle. The highest significant ($P < 0.05$) r-coefficient obtained after day 0 ($u = 0$) indicated the number of days per gonotrophic cycle. Data were also transformed (filtration) to eliminate spuriously significant r-coefficients by using an autoregressive process with a time-delay of 1 day of $Z_t = X_t = \beta(X_{t-1})$, where X_t is the time series to be filtered, and β is the estimated autoregressive parameter (Holmes and Birley 1987). The equation $P_t = S(T_{t-u})$ was fixed through the origin (by regression analysis) and the value of β (slope) was the estimated survival rate (S). Best estimates of daily survivorship were calculated by taking the uth (= gonotrophic cycle length) root of S.

RESULTS

Oogenesis stages and development

The duration of complete oogenesis in wild female *S. metallicum* s.l. was determined. After feeding, 10 females were dissected every 2 h up to 60 h ($n = 300$ females). The minimum time required to develop mature eggs (Christophers' stage V) was 46 h. At 16 h, 68 females had reached Christophers' stage I–II ovaries. Christophers' stage III and IV were reached at 18–26 h (39 females) and 28–44 h (69 females), respectively. Ovaries of 69 females reached the last Christophers' stage between 46 and 60 h.

Black fly capturing, marking, and releasing

Of 2,420 female *S. metallicum* s.l. released, only 28 were recaptured (1.2%). In general, host-seeking activity of marked flies resumed 72–96 h (or between 3 and 4 days) after bloodfeeding. Five out of 7 (72.0%) green-colored females recaptured 72 h (or 3 days) after release had sacculate dilatations, which are indicative of recent oviposition; 1 (14%) had follicular relics indicating that she had oviposited within 12–20 h (or 3.5 days) of the time of her recapture (Reyes-Villanueva and Rodríguez-Pérez 1994). The other 1 (14%) was at Christophers' stage IV, and she would need to wait at least 12 h before ovipositing, totaling a maximum of 96 h (or 4 days). Similar numbers were found for the other color-marked and recaptured bloodfed female *S. metallicum* s.l, indicating that the gonotrophic cycle is quite uniform in length (Table 1).

Table 1. Marked bloodfed female *Simulium metallicum* s.l. recaptured after oviposition and the stages of follicular dilatations in their ovaries.

Hours after release	No. females released	No. females examined	No. females recaptured	Stage of follicular dilatations		Christophers' stage
				Sac[1]	Relics[2]	
0	600 green	0	0	—	—	—
24	620 red	630	0	—	—	—
48	600 yellow	622	0	—	—	—
72	600 orange	625	7 green	5	1	1 (IV)
96	0	575	6 red	5	1	—
120	0	454	9 yellow	5	2	2 (IV)
144	0	491	6 orange	5	0	1 (IV)
168	0	460	0	—	—	—
192	0	406	0	—	—	—
216	0	418	0	—	—	—

[1] Large sacculate dilatation in the follicular tube.
[2] Follicular relics in the ovarian tunica.

Dynamics of parous–nulliparous ratios

The total number of unfed females collected at site 1 (horse trap) was more than one half of the total number collected at sites 2 and 3 (human traps; Table 2), whereas mean parity rates for each site were similar and ranged from 0.45 to 0.53. Raw data of individual collection sites 2 and 3 showed significant peaks on day 6 ($r = -0.90$; $P = 0.03$) and 5 ($r = 0.91$; $P = 0.01$), respectively, but this was not supported by filtered data. In addition, the raw and filtered data of parity rates over 11 days did not give significant correlation indices at any time intervals at collection site 1. Birley and Boorman (1982) suggested pooling traps that had a high initial cross-correlation between parous and nulliparous individuals, but a basis for pooling trap collections should also be to observe a similarity between the traps and the mean parity rates (Work et al. 1991). To evaluate these conditions, data on parity rates were pooled and further analyzed. Time-series analysis included all possible combinations of the same sites as follows: 1, 2, and 3; 1 and 2; 1 and 3; and 2 and 3. Only the combination 1–3 showed significant peaks ($P < 0.05$), with both the raw and filtered data, and similarity between parity rates; therefore, only this combination was considered for further analysis. A total of 5,240 *S. metallicum* was collected at sites 1 and 3 with an average parity rate of 0.46 (2,417 parous females). The lowest capture occurred on day 3 (381 black flies), and the highest capture (539 black flies) occurred on day 10 (Table 3). Raw data produced a significant correlation index ($r = 0.745$; $P = 0.03$) at 3-day time intervals, suggesting a 3-day gonotrophic cycle. After data filtration, this time series showed a definite pattern in the cross-autocorrelation analyses between total and parous females. The cross-correlation coefficient was $r = 0.76$ ($P = 0.04$) for this time delay ($u = 3$; Table 4), conclusively indicating a 3-day gonotrophic cycle. This finding is in accordance with the oogenesis and mark–release studies undertaken in the same area. The slopes obtained from the linear regression analysis with raw and filtered data were $Y = 32.33 + 0.43X$, and $Y = -21.06 + 0.33X$, respectively. These values were considered an adequate estimation of the survival

Table 2. Number of total and parous female *Simulium metallicum* s.l. captured in 11 days of sampling in 3 collection sites in a coffee plantation in southern Mexico.

Catch day	Site 1 (horse trap)		Site 2 (human trap)		Site 3 (human trap)	
	Total no. females	No. parous females	Total no. females	No. parous females	Total no. females	No. parous females
1	466	181	99	43	24	09
2	368	172	74	38	19	05
3	370	202	78	42	11	05
4	380	183	27	11	21	10
5	488	180	37	12	44	23
6	365	152	73	32	41	18
7	488	186	84	39	50	27
8	511	265	47	19	16	11
9	460	218	77	42	48	27
10	456	224	299	134	83	54
11	460	222	193	107	71	39
Total	4,812	2,185	1,088	519	428	228

Table 3. Number of total and parous female *Simulium metallicum* s.l. captured in 11 days of sampling at 2 collection sites (sites 1 and 3) in a coffee plantation in southern Mexico.

Catch day	Total no. females	No. parous females	Σ total no. females	Σ no. parous females	Σ no. parous females/Σ total no. females
1	490	190			0.39
2	387	177	877	367	0.42
3	381	207	1,258	574	0.46
4	401	193	1,659	767	0.46
5	532	203	2,191	974	0.44
6	406	170	2,597	1,144	0.44
7	538	213	3,135	1,357	0.43
8	527	276	3,662	1,633	0.44
9	508	245	4,170	1,878	0.45
10	539	278	4,709	2,156	0.46
11	531	261	5,240	2,417	0.46

rate per gonotrophic cycle because intercept values were statistically (t for H_0: intercept = 0) different from zero ($P = 0.01$; and $P = 0.05$, respectively). The estimated daily survivorship based on a 3-day gonotrophic cycle ($0.43^{1/3}$) was 0.75 with raw data, and 0.69 ($0.33^{1/3}$) with filtered data.

In addition, the daily survival rate was estimated vertically to be 0.77 (daily parity rate = 0.46, gonotrophic cycle = 3) according to the Davidson's formula (Davidson 1954). These values were similar to those obtained by using time-series analysis. The same value of 0.77 raised to the power of 3 was 0.46, the survival rate for the gonotrophic cycle. Considering at least 11–12 days for *O. volvulus* 3rd-stage larvae to develop (Porter and Collins 1985), the survival to infective age was calculated to be $0.77^{12} = 4.3\%$. In the time-series analysis, these values ranged from 3.2% (0.75^{12}) to 1.2% (0.69^{12}) for raw and filtered data, respectively.

DISCUSSION

The gonotrophic cycle of hematophagous insects consists of host-seeking activity, bloodfeeding, egg development (oogenesis), and the search for an appropriate breeding site for oviposition (Beklemishev 1940). In this study, female *S. metallicum* s.l. produced mature eggs (Christophers' stage V) as early as 46 h and up to 50 h after engorgement, which is in accordance to 48 h described in another study (Ramírez-Pérez 1977). In the mark–release and time-series experiments, a 3-day gonotrophic cyle was documented, indicating that the eggs of marked females became fully mature during the morning of day 2 after release. The females probably attempted to oviposit during the morning of that day because the time of oviposition has been documented to be between 0800 and 1100 h (Ramírez-Pérez 1977). Females presumably resumed host-seeking activity in the afternoon shortly after oviposition (Garms and Ochoa 1979, Porter and Collins 1988b) because the vast majority (20 of 28 = 71%) of recaptured females had sacculate dilatations. Those females that were not able to find a host probably attempted to refeed on the morning of day 3 because approximately 14% (4 of 28) had follicular relics, indicating that they had oviposited within 12–20 h earlier. These results are in accordance with an estimated period of 2–3 days for the gonotrophic cycle reported in Guatemala for *S. metallicum*; here the eggs of *S. metallicum* s.l. became fully mature when maintained at 20–27°C (Garms and Ochoa 1979). During the experiments, the weather was uniform (mean ± SD temperature of

Table 4. Cross-correlation values (lower and upper 5% levels of significance given in parentheses) between total and parous female *Simulium metallicum* s.l. for a time series of 11 days of sampling at 2 collection sites (sites 1 and 3) in a coffee plantation in southern Mexico.

Day	Raw data	Filtered data
0	0.698 (0.168, 0.915)*	0.617 (−0.021, 0.898)**
1	0.464 (−0.234, 0.846)	0.018 (−0.654, 0.674)
2	0.471 (−0.281, 0.865)	0.086 (−0.659, 0.746)
3	0.745 (0.086, 0.951)*	0.761 (0.018, 0.962)*
4	0.207 (−0.647, 0.831)	−0.238 (−0.880, 0.711)
5	0.120 (−0.849, 0.766)	0.262 (−0.807, 0.929)
6	−0.405 (−0.948, 0.743)	−0.456 (−0.985, 0.899)

* Statistically significant at $P < 0.05$.
** Statistically significant at $P = 0.05$.

22 ± 4°C; mean ± SD relative humidity of 68 ± 8%; and no rainfall or wind) and 2 streams supporting the development of large numbers of immature *S. metallicum* s.l. were at a distance of approximately 100–400 m from the collection points. This indicated that the feeding of most females, their oviposition, and return for another blood meal in 3-day cycles was completed within nearby breeding sites. However, because the recapture rate was extremely low (1.2%), it may reflect either a high mortality rate (Service 1993) or movement of a high proportion of flies into other areas and where they could not be recaptured.

On the basis of cytologic criteria, species complexes of the familiy Simuliidae have shown variations in their susceptibility to be infected with *O. volvulus* (Grillet et al. 1994, Charalambous et al. 1997). Four cytospecies of *S. metallicum* (A, B, H, and I) are found in Mexico and Guatemala, whereas 2 cytospecies are found in Venezuela (D and E; Conn et al. 1989, Procunier 1989, Conn 1990). This diversity raises the possibility that the vector competence and vectorial capacity of *S. metallicum* cytospecies may differ within these 2 geographic scenarios. To the best of our knowledge, no studies have been performed on parameters related to the vectorial capacity of the different cytospecies of Simuliidae, such as the duration of the gonotrophic cycle and survivorship rates.

In this study, *S. metallicum* s.l. was very abundant, but the bloodfeeding rate was significantly higher on animals than on humans. A total of 4,812 female *S. metallicum* s.l. was collected at 1 site with 1 horse trap, whereas only 1,516 females were collected at 2 sites with 2 human traps (Table 2), in agreement with the zoophilic preference reported for this species (Dalmat 1955, Porter and Collins 1988b). *Simulium metallicum* s.l. of Mexico and Guatemala has been reported to have a very low degree of vector competence (Collins 1979, Ito et al. 1980) and to be mainly zoophilic, and, therefore, is considered to be only a minor vector. The survival rate of *S. metallicum* to infective age of 1–4% was lower than the 6% survival rate of the main vector, *Simulium ochraceum* Walker s.l. in the same area (Takaoka et al. 1981; Porter and Collins 1985, 1988b; Rodríguez-Pérez et al. 1995). Because interspecific variations exist, it would be interesting to estimate the gonotrophic cycle length and survivorship of cytotypes D and E from Venezuela where, in spite of their relatively low vector competence, their high human-biting rates and anthropophilic behavior (Lewis and Ibanez de Aldecoa 1962, Ramírez-Pérez 1977, Basañez et al. 2000) perpetuate the transmission of *O. volvulus*. Although in this study the cytospecies of *S. metallicum* were not determined, comparison of these parameters among the different cytospecies in Central America would be interesting.

Mark–release and time-series methods approximated similar estimates of the duration of the gonotrophic cycle and survivorship rate of *S. metallicum* s.l. in southern Mexico. However, limitation of these methods cannot be ignored. Only 1.2% of female *S. metallicum* s.l. were recaptured in the mark–release technique. As a consequence, the results may indicate a bias in the estimation of the gonotrophic cycle. The sampling method used in the time-series analysis was not always consistent, suggesting differences in the age structure of populations in individual collecting sites, that is, 11 days of sampling were probably not enough for a time-series analysis, and use of different hosts may have introduced a bias in the proportion of parous individuals caught (Work et al. 1991; Lord, personal communication).

ACKNOWLEDGMENTS

We are much obliged to the Consejo Nacional de Ciencia y Tecnologia (CONACyT), reference number 34486-M, which partially funded this study. R.V.-S. was the recipient of a bachelor scholarship at the Centro de Investigacion de Paludismo with support from CONACyT. We also thank Olga Real-Najarro (Complutense University, Spain) for contributing by critical scientific reading of the manuscript.

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