Journal of the American Mosquito Control Association, 21(4):350–354, 2005

BIOLOGICAL VARIATION IN TWO *ANOPHELES VESTITIPENNIS* POPULATIONS WITH DIFFERENT FEEDING PREFERENCES IN SOUTHERN MEXICO

ARMANDO ULLOA,[1] MARIO H. RODRÍGUEZ,[2] JUAN I. ARREDONDO-JIMENEZ[1] AND ILDEFONSO FERNANDEZ-SALAS[3]

ABSTRACT. The lengths of gonotrophic cycle and egg development and survival rate were studied in *Anopheles vestitipennis* collected in horse and human-baited traps in southern Mexico. The gonotrophic cycle duration was estimated using cross-correlation analysis, whereas the survival rate was assessed using a vertical method. Daily changes of parity rates gave significant correlation indices at 3 and 4 days in the zoophilic and anthropophilic populations, respectively. The minimum time required to develop mature eggs after blood feeding was 54 and 60 h, and the survival rate was 0.93 and 0.88 in zoophilic and anthropophilic female mosquito populations, respectively. These biological differences provide additional support for the existence of subpopulations with distinctive feeding preferences within *An. vestitipennis* in southern Mexico.

KEY WORDS *Anopheles vestitipennis*, anthropophilic, zoophilic, gonotrophic cycle, survival rate

INTRODUCTION

Anopheles vestitipennis Dyar and Knab is distributed from central Mexico, throughout Central America (Vargas 1958, Belkin et al. 1970), to northern South America and the Great and Lesser Antilles (Wilkerson and Strickman 1990). This mosquito has been incriminated as a vector *Plasmodium vivax* in the Lacandon forest of southern Mexico (Loyola et al. 1991, Arredondo-Jimenez 1995), Guatemala (Padilla et al. 1992), and Belize (Achee et al. 2000).

Isozyme analysis and differences in biting behavior of *An. vestitipennis* populations from southern Mexico indicated differences between mosquito populations collected on human and animal baits (Arredondo-Jimenez et al. 1996). These findings were later supported by differences in Random amplification polymorphic DNA (RAPD) markers (Martínez 2001, Murillo-Sánchez 2001) and egg ornamentation (Rodríguez et al. 1999). In addition, mark-recapture experiments also provided support to the possible existence of 2 sympatric populations with different host preferences with specific preferences for human or animals in the region (Ulloa et al. 2002, 2004). To further document the possible distinctiveness between the purported zoophilic and anthropophilic subpopulations of *An. vestitipennis* in southern Mexico, we compared the length of their gonotrophic cycle and oogenesis and their daily survival rates.

[1] Centro de Investigación de Paludismo, Instituto Nacional de Salud Pública. Apartado Postal 537. Tapachula, Chiapas 30700, México.

[2] Centro de Investigación sobre Enfermedades Infecciosas, Instituto Nacional de Salud Pública, Avenida Universidad 655, Cuernavaca, Morelos, 62508 México.

[3] Laboratorio de Entomología Médica, Universidad Autónoma de Nuevo León, Monterrey, N. L.

MATERIALS AND METHODS

Study area: The study was carried out in a village, Nueva Independencia (14°37′30″N, 92°16′14″W, elevation 50 m), with a population of 112 living in 23 households, located on the Pacific Ocean coastal plain of southern Chiapas. Climate in the area is hot subhumid (Garcia 1973), with a wet season extending from May through October. Previously, a high prevalence of *An. vestitipennis* in the village was documented (Ulloa et al. 2002). The vegetation surrounding the village is represented mostly by crops (mango, banana, and corn) and cattle pastures with various forested and flooded tall grass patches, favorable for *An. vestitipennis* breeding (Rejmankova et al. 1998).

Dynamics of parous–nulliparous ratios: Daily changes in the parous–nulliparous ratio in wild caught *An. vestitipennis* were recorded. A set of 2 modified Magoon traps (Service 1993) were baited with 2 men in 1 trap and 1 horse in the other. The 2 traps were placed 5 m apart (30 and 150 m from the nearest house of the village and larval breeding sites, respectively) and were used to collect female *An. vestitipennis* during 6-h periods (1800–2400 h) for 15 consecutive nights during the rainy season. In traps baited with humans, mosquitoes were collected during the 1st 45 min of each hour. Mosquitoes landing on human volunteers were captured with mouth aspirators (World Health Organization 1975, Bown et al. 1987). In traps with animal baits, mosquitoes were collected resting on the inner surface of the traps during the last 15 min of each hour. Female mosquitoes (~50) collected from each trapping method were dissected the same night of collection to determine parity rates (Detinova 1962). Mosquitoes were identified by the key of Wilkerson and Strickman (1990).

Time-series analysis for each 15-day sampling period was conducted. The length of the gonotrophic cycle was estimated using a cross-correlation analysis (Birley and Rajagopalan 1981), with the

following formula: $M_t = P_u T_{(t-u)}$, where M is the number of parous individuals captured on day t; $T_{(t-u)}$ is the total number of females (nulliparous and parous) captured on day $t - u$; u is the length of the gonotrophic cycle, and P the survival rate per gonotrophic cycle, calculated from the slope in a regression model. The r coefficient for day 0 represents the correlation between P_t and T_t data pairs from mosquitoes captured the same day (15 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. The day 2 coefficient 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 cross-correlation coefficient (r) between the time-series expresses a time delay (u) equivalent to the gonotrophic cycle. The highest significant cross-correlation coefficient (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 following formula: $Z_t = X_t - \beta(X_{t-1})$, where Z_t is the time series to be filtered, X_t is the time series to be filtered, and β is 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 the correlation r corresponded to a lag u equivalent to the gonotrophic cycle, with regular peaks at the start of each cycle.

Survival rate: Daily survival rates (p) were calculated using the parity rates of both mosquito populations, with $p = (PR)^{1/CG}$, where PR is the parity rate, and CG is the duration of the gonotrophic cycle (Davidson 1954).

Vitellogenesis: The stages and duration of Christopher stages of ovarian development based on the appearance of follicles (Clements 2000) were determined in a sample of *An. vestitipennis* collected in human or horse baits as described above. Samples of unfed females (without traces of blood) from both collection types were dissected to determine the status of ovarian maturation; the remaining females, held in small cages, were blood fed to repletion through a nylon screen on a tethered horse or a human volunteer; unfed and partially fed mosquitoes were discarded. Blood fed mosquitoes were immediately transported to the Center for Malaria Research (CIP) in Tapachula. Mosquitoes from both populations were supplied with cotton soaked in a 10% sucrose solution and maintained in a temperature controlled room at 70–100% relative humidity with a temperature of 29°C. Beginning at 12 h after blood feeding, and continuing every 6 h up to 78 h, groups of 10 mosquitoes were dissected to determine the Christopher stages. The same methods were used to estimate the duration of Christopher stages in other unfed mosquito samples (human and horse bait-collected) that were fed on the alternative host than they were collected on. Females that did not develop beyond the states of Christopher II or II' 18 h after feeding were reported as pre-gravid (Gillies 1954).

RESULTS

Parity rate: A total of 3,800 *An. vestitipennis* females were collected in both collecting stations: 1,712 from the animal traps and 2,088 from human-baited traps. Parity assessments were carried out dissecting 641 (37.4%) and 663 (31.8%) mosquitoes, respectively, from animal and human bait collections. Of those dissected, parity was observed in 526 females (82 ± 13.52%; range, 47–97% among collecting nights) from the animal traps, whereas 424 mosquitoes (64 ± 19.82; range, 28–89% among collecting nights) from the human-baited trap were parous. Significant difference in parity rates was observed between these *An. vestitipennis* populations ($X^2_{corr} = 7.331$ $P = 0.0068$) (Table 1).

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 15 days in females collected in animal traps; however, high correlations on days 3 and 6 suggested a 3-day gonotrophic cycle (Table 2). Similarly, no significant correlation ($P > 0.05$) in daily changes of parity was observed in females collected in human-baited traps, but a significant high correlation ($P < 0.05$) on day 4 was observed using filtered data (Table 2).

Survival rate: The daily survival rate in mosquitoes collected in animal traps was 0.93, whereas that for mosquitoes collected in human-baited traps was 0.88.

Vitellogenesis: All unfed females from both collection types dissected at the beginning of the experiment were at Christopher stage II. Mosquitoes collected in animal traps and fed to repletion on animal blood required a minimum of 54 h after feeding to reach Christopher stage V, whereas in mosquitoes collected in human traps and fed to repletion on human blood, progression to Christopher stage V occurred at a minimum of 60 h after feeding (Table 3). The length of the gonotrophic cycle could be calculated indirectly, by adding 24 h, which is considered the required time to locate an oviposition site, lay eggs, and seek for new host, to the minimum time required to develop eggs to Christopher stage V (Mekuria al. 1991). When this criterion was used in our data, the estimation of the length of the gonotrophic cycle was 3.25 and 3.50 days for the zoophilic and anthropophilic populations, respectively.

In mosquitoes that were maintained up to 78 h to complete vitellogenesis, 47/50 (94%) of those collected on horses and fed with horse blood developed to Christopher stage V, whereas only 24/40 (60%) of those collected on humans and fed with human blood completed vitellogenesis (Table 3). In the latter group, 19/60 (32%) did not devel-

Table 1. Daily parous rate of *Anopheles vestitipennis* populations.

	Zoophilic population				Anthropophilic population			
Day	Collected	Dissected	Parous	Parous rate	Collected	Dissected	Parous	Parous rate
1	80	26	22	84	133	46	28	61
2	75	34	30	88	120	45	28	62
3	85	26	24	92	80	48	29	60
4	150	54	34	62	190	43	26	60
5	140	50	35	70	165	50	43	86
6	130	52	49	94	110	46	39	84
7	190	49	43	87	130	44	37	84
8	90	50	43	86	120	47	42	89
9	140	50	47	94	105	50	31	62
10	84	30	23	77	96	28	22	78
11	82	34	33	97	150	30	19	63
12	120	50	45	90	203	48	37	77
13	140	50	40	80	179	50	18	36
14	120	50	40	80	118	48	15	31
15	86	36	17	47	189	40	11	28
	Σ1,712	Σ641	Σ526	mean = 82 ± 13.5	Σ2,088	Σ663	Σ424	mean = 64 ± 19.8

oped beyond Christopher stage II or II', i.e., remained pre-gravid.

Females collected from animal and human traps that were fed to repletion on the respective opposite host reached Christopher stage V at the same time as females fed on their original host. However, the time to complete ovary maturation (Christopher stage V) extended up to 24 and 12 h for mosquitoes originally collected on animal (48%, 24/50) and human (60%, 24/40) traps, respectively, but pre-gravidity was observed only in the anthropophilic population (33%, 23/70).

Table 2. Correlation indexes in parity rates of *Anopheles vestitipennis* collected in animal- and human-baited traps using cross-correlation analysis of a time-series (data of the first 10 days of collection).

	Zoophilic population		Anthropophilic population	
Day	Raw data[1]	Filtered data	Raw data[2]	Filtered data
0	0.827	0.792	0.383	0.427
1	0.223	0.015	0.087	0.293
2	0.149	0.021	0.011	0.619
3	0.167	0.292*	0.675	0.820
4	0.036	0.019	0.372	0.918**
5	0.023	0.415	0.481	0.478
6	0.564	0.581*	0.115	0.267
7	0.044	0.248	0.497	0.706
8	0.024	0.371	0.681	0.512**
9	0.072	0.439*	0.178	0.193
10	0.365	0.191	0.024	0.77

[1] Cross-correlation analysis using the number of parous individuals captured on day t and the total number of females (nulliparous and parous) captured on day $t - u$ (where u is the length of the gonotrophic).

[2] Cross-correlation calculated using data filtered using an autoregressive process with a lag of 1 day (see text for complete formulas).

* High correlation index at intervals of 3 days ($P > 0.05$).

** High correlation index at intervals of 4 days ($P < 0.05$).

DISCUSSION

Differences in parity rates, length of the gonotrophic cycles, and survival rates were documented among *An. vestitipennis* collected in human- versus animal-baited traps, adding to the evidence (Arredondo-Jimenez et al. 1996, Rodriguez et al. 1999, Martínez 2001, Murillo-Sánchez 2001, Ulloa et al. 2002, Ulloa et al. 2004) for the existence of 2 cryptic sympatric subspecies with anthropophilic or zoophilic feeding preferences in southern Mexico.

Some of our results require commentary. Data from the anthropophilic sample, adjusted to the criterion of Mutero and Birley (1987), suggested a gonotrophic cycle of 4 days. However, the length of the gonotrophic cycle of the zoophilic sample could not be calculated by differences in correlation coefficient of raw and filtered data. In this case, a length of 3 days was estimated by the presence of a high correlation coefficient value appearing 3 days after feeding, followed by a 2nd high coefficient at the same time interval (Bockarie et al. 1995). This figure is similar to previous estimates of gonotrophic cycle duration in *An. vestitipennis* collected in animal baits, in the same area (Arredondo-Jimenez et al. 1998) and in the Dominican Republic (Mekuria et al. 1991).

Our findings indicate longer survival rates and shorter egg development time in the zoophilic population compared with the anthropophilic one, suggesting differences in nutritious quality of human and animal blood (e.g., Edman 1989) or recent adaptation to human hosts by *An. vestitipennis* mosquitoes (Arredondo-Jimenez 1995).

When mosquitoes were fed with blood sources different than those from where they were collected (data not shown), vitellogenesis time was extended. The extension of the time to progress to Christopher stage V in a group of mosquitoes fed with a

Table 3. Vitellogenesis of *Anopheles vestitipennis* populations collected in animal- and human-baited traps fed on the same host they were collected.

Hours post-feeding	Zoophilic population (Christopher stage)						Anthropophilic population (Christopher 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			100			10		80[2]	20		
30	10			50	50		10		60[2]	40		
36	10				100		10			70	30	
42	10				100		10		10[1]	60	30	
48	10				100		10			20	80	
54[1]	10				30	70	10		10[2]		90	
60[1]	10					100	10				80	20
66	10					100	10		20[2]		50	30
72	10					100	10		10[2]			90
78	10					100	10					100

[1] Minimum time required to develop mature eggs (Christopher stage V): stages I and II, previtellogenic phase; III, initiation phase; IV, trophic phase; V, post-trophic phase (Clements 2000).
[2] Pregravid stage.

different host species to that they were collected on indicates a host effect, probably caused by differences in blood digestion when mosquitoes encounter blood proteins different to those of their preferred host (Clements 2000). This host effect may contribute to the integration of separate subpopulations.

Only anthropophilic females presented pre-gravidity, no matter the blood origin they were fed. This may indicate an inherent characteristics of the anthropophilic population and not an effect of the nutritious quality of the blood. However, previous results of a 6% pre-gravid rate reported with mosquitoes fed on cattle blood (Arredondo-Jimenez et al. 1998) suggest that horse blood could in fact be more nutritious than other blood types.

The pre-gravid stage in anthropophilic females indicates their need for more than 1 feeding to produce their 1st batch of eggs, which increase the contact of this mosquito subpopulation with humans and consequently their chances to become infected and later transmit diseases (Boreham and Garrett-Jones 1973). Additional tests are needed using F1 females from each of the anthropophilic and zoophilic populations to control previous blood meals, age composition, and rearing conditions to confirm this statement.

The survival rate obtained in zoophilic *An. vestitipennis* was only slightly higher than that of the anthropophilic population, probably reflecting limitations of the vertical method (Davidson 1954) for its calculation. The basic assumption of this method is that the population has a distribution of stationary age and that mortality is constant with age (McHugh 1989), and it is based on the parity rate and the duration of gonotrophic cycle. In the calculation of the survival rates, the combination of a higher parity rate and a shorter gonotrophic cycle length in the zoophilic population produced similar results to that of the combination of a lower parity rate and a longer gonotrophic cycle in the anthropophilic one. Nevertheless, taken as a whole, these differences confirm the existence of 2 different subpopulations. The identification of a subpopulation of *An. vestitipennis* with anthropophilic preferences and its purported need for more than 1 feeding during its 1st gonotrophic cycle reflects the importance of this mosquito species in malaria transmission in the area.

ACKNOWLEDGEMTS

We thank field technicians Pedro García, Eufronio Garcia, and Rafael Robledo for participation. This project was partially funded by Grant SIBEJ/Chis 02-005.

REFERENCES CITED

Achee NL, Korves CT, Bangs MJ, Rejmankova E, Lege M, Curtin D, Lenares H, Alonzo Y, Andre RG, Roberts DR. 2000. *Plasmodium vivax* polymorphs and *Plasmodium falciparum* circumsporozoite proteins in Anopheles (Diptera: Culicidae) from Belize, Central America. *J Vector Ecol* 25:203–211.

Arredondo-Jimenez JI. 1995. Comparative ecology of allopatric populations of *Anopheles vestitipennis* (Diptera:Culicidae). Ph.D. dissertation. University of California, Davis, CA.

Arredondo-Jiménez JI, Gimnig J, Rodríguez MH, Washino RK. 1996. Genetic differences among *Anopheles vestitipennis* subpopulations collected using different methods in Chiapas State, southern México. *J Am Mosq Control Assoc* 12:396–401.

Arredondo-Jiménez JI, Rodríguez MH, Washino RK. 1998. Gonotrophic cycle and survivorship of *Anopheles vestitipennis* (Diptera:Culicidae) in two different eco-

logical areas of Southern Mexico. *J Med Entomol* 35: 938–942.

Belkin JN, Heinemann J, Page WA. 1970. The culicidae of Jamaica. *Contrib Am Entomol Inst (Ann Arbor)* 6:1–458.

Birley MH, Rajagopalan PK. 1981. Estimation of the survival and biting rates of *Culex quinquefasciatus* (Diptera:Culicidae). *J Med Entomol* 18:181–186.

Bockarie MJ, Service MW, Barnish G, Toure YT. 1995. Vectorial capacity and entomological inoculation rates of Anopheles gambiae in a high rainfall forested area of southern Sierra Leone. *Trop Med Parasitol* 46:164–171.

Boreham PFL, Garrett-Jones C. 1973. Prevalence of mixed bloodmeal and double feeding in a malaria vector (*Anopheles sacharovi* Fabre). *Bull WHO* 54:155–158.

Bown DN, Frederickson CE, Angel-Cabañas G, Méndez J. 1987. An evaluation of bendicarb and deltamethrin and their impact on populations of *Anopheles albimanus*. *PAHO Bull* 22:121–135.

Clements AN. 2000. *The biology of mosquitoes. Development, nutrition and reproduction.* London: Chapman & Hall.

Davidson G. 1954. Estimation of the survival rate of anopheline mosquitoes in nature. *Nature* 174:792–793.

Detinova TS. 1962. *Age-grouping methods in Diptera of medical importance.* Geneva, Switzerland: World Health Organization.

Edman JD. 1989. Are mosquitoes gourmet or gourmand? *J Am Mosq Control Assoc* 5:487–499.

Garcia E. 1973. *Modificaciones al sistema de clasificación climática de Koppen.* Instituto de Geografía, Universidad Nacional Autónoma de Mexico (UNAM). Mexico D.F.

Gillies MT. 1954. The recognition of age-groups within populations of *Anopheles gambiae* by the pre-gravid rate and sporozoite rate. *Ann Trop Med Parasitol* 48: 58–74.

Holmes PR, Birley MH. 1987. An improved methods for survival rate analysis for time series of haematophagous dipteran population. *J Anim Ecol* 56:427–440.

Loyola EG, Arredondo JI, Rodriguez MH, Bown ND, Vaca MA. 1991. *Anopheles vestitipennis*, the probable vector of *Plasmodium vivax* in the Lacandon Forest of Chiapas, Mexico. *Trans R Soc Trop Med Hyg* 85:171–174.

Martínez ME. 2001. Análisis de poblaciones zoofílicas y antropofílicas de Anopheles (Anopheles) vestitipennis (Diptera: Culicidae). B.S. thesis. Universidad Autónoma de Chiapas, Tapachula, Chiapas.

McHugh CP. 1989. Ecology of a semiisolated population of adult *Anopheles freeborni*: abundance, trophic status, parity survivorship, gonotrophic cycle length and host selection. *Am J Trop Med Hyg* 41:169–179.

Mekuria Y, Granados R, Tidwell MA, Williams DC, Wirtz RA, Robert DR. 1991. Malaria transmission potential by Anopheles mosquitoes of Dajabon Dominican Republic. *J Am Mosq Control Assoc* 7:456–461.

Murillo-Sánchez MH. 2001. Variabilidad genética de subpoblaciones de Anopheles vestitipennis (Diptera: Culicidae) mediante el uso de RAPD-PCR. M.S. thesis. Universidad Autónoma de Nuevo León, Monterrey, México.

Mutero CM, Birley HM. 1987. Estimation of the survival rate and oviposition cycle of field populations of malaria vectors in Kenya. *J Appl Ecol* 24:853–863.

Padilla N, Molina P, Juárez J, Bown D, Cordón-Rosales C. 1992. Potential malaria vectors in northern Guatemala. *J Am Mosq Control Assoc* 8:307–308.

Rejmankova E, Pope KO, Roberts DR, Lege MG, Andre R, Greico J, Alonzo Y. 1998. Characterization and detection of *Anopheles vestitipennis* and *Anopheles punctimacula* (Diptera:Culicidae) larval habitats in Belize with field survey and SPOT satellite imagery. *J Vector Ecol* 23:74–87.

Rodríguez MH, Chavez B, Hernández-Avila JE, Orozco A, Arredondo-Jiménez JI. 1999. Description and morphometric analysis of the eggs of *Anopheles (Anopheles) vestitipennis* (Diptera:Culicidae) from southern Mexico. *J Med Entomol* 36:78–87.

Service MW. 1993. *Mosquito ecology: field sampling methods.* 2nd ed. London: Elsevier Applied Science.

Ulloa A, Arredondo-Jiménez JI, Rodríguez MH, Fernández-Salas I. 2002. Mark-recapture studies of host selection by *Anopheles vestitipennis*. *J Am Mos Control Assoc.* 18:32–35.

Ulloa A, Arredondo-Jiménez JI, Rodríguez MH, Fernández-Salas I, González-Cerón L. 2004. Innate host selection in *Anopheles vestitipennis*. *J Am Mosq Control Assoc* 20:337–341

Vargas L. 1958. Nuevos datos sobre la distribución de anofelinos mexicanos. *Bol Epidemiol* 32:33–56.

Wilkerson RC, Strickman D. 1990. Illustrated key to the female anopheline mosquitoes of Central America and Mexico. *J Am Mosq Control Assoc* 6:7–34.

World Health Organization. 1975. *Manual on practical entomology. Parts I and II.* Geneva, Switzerland: World Health Organization.