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LABORATORY STUDIES OF DIEL OVIPOSITION, FECUNDITY, SURVIVAL, AND GONOTROPHIC CYCLES IN *ANOPHELES HOMUNCULUS*

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ABSTRACT. Diel oviposition patterns of *Anopheles homunculus* were determined from field-collected females in Platanal Forest, Cumaca, Trinidad. The timing and number of eggs oviposited were monitored at 2-h intervals for a set of 30 individual females and a group of 130 females. Individual females of *An. homunculus* displayed a strongly nocturnal pattern of oviposition. During the 1st gonotrophic cycle, >65% of ovipositions occurred and >70% of eggs were laid between 2200 and 0200 h. In the 2nd gonotrophic cycle, 79% of eggs were laid during the same time period. The same trend was found for the 130 females caged together, with >80% of eggs laid between 2200 and 2400 h. The length of the gonotrophic cycle ranged from 74 to 102 h (mean 81.8 ± 11.9 h) for the 1st gonotrophic cycle and from 46 to 76 h (mean 56.0 ± 12.6 h) for the 2nd cycle. The fecundity of *An. homunculus* averaged 62 eggs in each gonotrophic cycle. No females survived in the laboratory longer than 10 days. These observations on *An. homunculus* oviposition patterns are the 1st for any species in the subgenus *Kerteszia* and may be useful for future attempts at colonization.

KEY WORDS *Anopheles homunculus*, oviposition, gonotrophic cycle, survival, Trinidad

INTRODUCTION

In the Neotropics the *Anopheles* subgenus *Kerteszia* is represented by at least 10 species (Zavortink 1973), of which 6 are considered primary or secondary vectors of malaria and other disease pathogens (Downs et al. 1943, Zavortink 1973, Wilkerson and Peyton 1991, Chadee 1994). The subgenus *Kerteszia* is poorly known, possibly because of the difficulty associated with its taxonomy (Zavortink 1973, Wilkerson and Peyton 1991) and colonization (Chadee 1994).

In Trinidad, *Anopheles bellator* Dyar and Knab and *Anopheles homunculus* Komp are probably the 2 best studied species of the subgenus *Kerteszia* because of the pioneering work on the bromeliad–*Anopheles*–malaria complex (Downs and Pittendrigh 1946) and studies on the ecoclimatic divergence of the 2 species (Pittendrigh 1950). Chadee (1992a) studied the effects of moonlight on the landing activity of *An. bellator* and Chadee (1994) described the longevity, seasonal abundance, and feeding behavior of *An. homunculus*. Studies on the bloodfeeding and blood digestion kinetics of both *An. bellator* and *An. homunculus* were conducted to develop behavioral and physiological profiles relative to innate vector competence (Chadee and Beier 1995a, 1995b; Chadee et al. 1996). The diel patterns of oviposition and length of the gonotrophic cycle of *An. homunculus* are poorly understood. We present information on the diel oviposition periodicity of *An. homunculus* over 2 gonotrophic cycles. This information has practical applications relative to colonization and timing of vector control efforts.

MATERIALS AND METHODS

Mosquitoes were collected from the Platanal Forest, Cumaca (61°10′N, 10°41′W), a small hamlet located in the northeastern area of the northern mountains approximately 25 km northeast of the Royal Borough of Arima, Trinidad, West Indies (Chadee 1994). Mosquito collections were made between 1600 and 2000 h, the peak landing times of *An. homunculus* (Chadee 1994), from September 20 to 30, 1996. Six men were stationed in the forest at ground level, equipped with a flashlight (turned on only to collect landing mosquitoes), handnet, and aspirator. Chadee (1992b, 1994) and Aitken (1960) have described mosquito collection, storage, and transportation methodologies.

At the Insect Vector Control Division laboratory (Trinidad, West Indies), mosquitoes were lightly anesthetized with chloroform, identified, and counted under a microscope at 40× magnification. *Anopheles homunculus* with any blood in the gut were discarded. *Anopheles homunculus* that survived the light anesthetic were placed into a colony cage (30 × 30 × 30 cm) consisting of wire netting covering a wooden frame. These cages were placed in an indoor insectary using natural lighting conditions. In the cage, adults were provided *ad libitum* with a 10% solution of glucose dispensed through a cotton wick. The indoor insectary, lighting regimen, temperature, and humidity profiles follow Chadee (1992b). Sunset occurred at 1800 h each day. On the 2nd day after collection females were allowed to engorge on blood from R.T.M.'s arm between 1800 and 1830 h in conformity with the

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Table 1. Diel patterns of oviposition and number of oviposition occurrences (*n*) of individual *Anopheles homunculus* during 2 gonotrophic cycles.

	No. of eggs laid by individual females								
	1st cycle			2nd cycle			Cycles combined	Total	
Time (h)	*n*	Mean ± SE	%	*n*	Mean ± SE	%	*n*	Mean ± SE	%
1800–2000	0	0	0.0	0	0	0.0	0	0	0.0
2000–2200	7	31.3 ± 15.4	11.2	2	54.0 ± 8.0	20.7	9	36.3 ± 17.9	13.3
2200–2400	14	55.7 ± 28.1	39.8	4	63.3 ± 15.9	48.6	18	57.1 ± 27.1	1.7
2400–0200	12	42.0 ± 27.9	31.1	2	80.0 ± 7.1	30.7	14	54.6 ± 35.0	31.0
0200–0400	5	43.0 ± 17.3	11.2	0	0	0.0	5	43.0 ± 17.3	8.9
0400–0600	1	27.0	1.4	0	0	0.0	1	27.0	1.1
0600–0800	1	102	5.3	0	0	0.0	1	103	4.0
0800–1800	0	0	0.0	0	0	0.0	0	0	0.0
Total	40	48.6 ± 29.9	100.0	8	65.1 ± 9.0	100.0	48	50.5 ± 29.5	100

approved human use protocol of the National Institutes of Health.

One day later, 30 visibly engorged females were aspirated from the colony cage and each was placed into a separate oviposition cage, identical in structure to the colony cage. Each oviposition cage was furnished *ad libitum* with a cube of sugar in an uncovered petri dish. By monitoring individual females, we were able to record survival, the length of the gonotrophic cycle of each individual, and the diel periodicity of oviposition. All remaining females in the original colony cage were monitored for oviposition periodicity. By monitoring oviposition in individual females and groups of females in the colony cage, we tested for interference among females (Corbet and Chadee 1993, Chadee and Beier 1997).

Oviposition was monitored 36 h after bloodfeeding by placing in each cage 4 small white polyethylene tubs (top diameter 10 cm, bottom diameter 8 cm, height 8 cm, capacity 550 ml) painted black outside, each containing 300 ml of bromeliad water. Bromeliad water was collected 24 h prior to monitoring oviposition. A fresh tub was prepared using temperature-equilibrated bromeliad water about 10 min before being placed into the cage. The diel periodicity of oviposition was monitored by replacing an old tub with a freshly prepared one every 2 h. Monitoring continued in the 30 cages and in the colony cage from 0600 h on the 2nd day after engorgement until oviposition occurred. After oviposition ceased, tubs were removed and all females in the individual cages were immediately bloodfed and monitored for the 2nd cycle oviposition.

Dead females were dissected and relict eggs were counted. Both ovaries of each female mosquito were examined and scored as nulliparous (N) or parous (P) based upon the condition of tracheolar skeins. Both ovaries of all parous females were scored for parity by Polovodov's method based on the number of dilatations and presence of relict eggs. Parous ovarioles with one or more dilatations were scored as P_1 or P_2 (Detinova 1962).

By segregating females in individual cages the length of the gonotrophic cycle and longevity or survival of females were determined. Individual mosquitoes were monitored each day until their death. In addition, the numbers of times individual females laid their full complement or only a small part of their egg complement were recorded. Oviposition data from individuals and the 130 females were analyzed separately. The periodicity data were entered into contingency tables (2 [individual vs. caged] × 8 [time periods]) and subjected to a *G*-test (Flower and Cohen 1995) to determine peak hours and frequency of oviposition.

RESULTS

Individual Oviposition

Table 1 shows the oviposition patterns of *An. homunculus* during 2 gonotrophic cycles. Twenty-five of the 30 individually caged females oviposited during the 1st cycle, and 8 survived to oviposit a 2nd time in the laboratory. During the 1st gonotrophic cycle, oviposition was mainly nocturnal with 94.7% of the eggs being laid between 2000 and 0600 h and 5.3% laid during the dawn period (0600–0800 h), indicating a small crepuscular component. More than 65% of ovipositions occurred in a well-defined peak 5 h after sunset, between 2200 and 0200 h, with 1,378 eggs (>70.1% of total) laid during that time. No eggs were laid between 0800 and 1800 h. During the 2nd gonotrophic cycle, 8 of the remaining 25 females survived to oviposit. Diel oviposition patterns were similar to those observed during the 1st cycle; 78% of ovipositions occurred between 2000 and 0200 h. Peak oviposition started at 2200 h and ended at 0200 h and 413 eggs (79.3% of total) were laid during this time.

Oviposition Occurrences

During the 1st gonotrophic cycle, peak oviposition occurrences were recorded between 2200 and

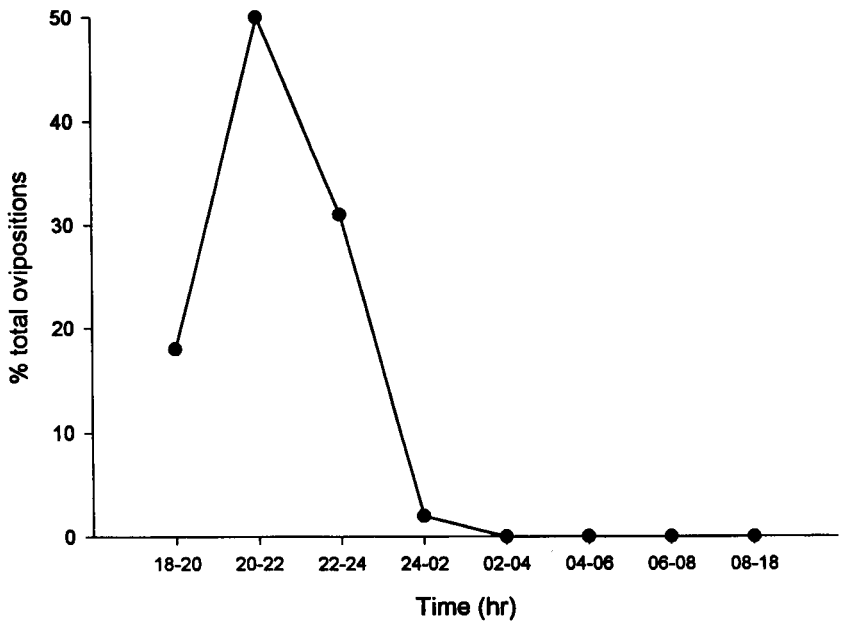

Fig. 1. Diel patterns of oviposition in 130 *Anopheles homunculus* mosquitoes in a cage.

0200 h; although more oviposition occurrences were recorded between 2400 and 0200 (n = 12) than between 0200 and 0400 h (n = 5), the mean number of eggs laid was similar to that observed between 0200 and 0400 h (42.0 vs. 43.0) (Table 1). During the 2nd cycle peak ovipositions were observed between 2000 and 2400 h (n = 4); 2 each were observed between 2000 and 2200 h and between 2400 and 0200 h. Numbers of ovipositions and numbers of eggs laid between 2000 and 2400 h were higher than at other times ($P > 0.01$).

Colony Cage Oviposition

Oviposition patterns among the 130 females in the colony cage were similar to patterns among individual females during the 2nd cycle, with oviposition confined to between 2000 and 0200 h (Fig. 1). Peak oviposition occurred at 2200 to 2400 h (1,210 eggs or >80% of eggs) with significantly more eggs being laid during this period than other periods (G = 39.9; df 6; P = 0.001). Oviposition started at 2000 h but finished earlier (at 2400 h) than that observed during the 1st cycle of the individual females but later than during the 2nd cycle of the individual females (2000–0200 h) (Table 1).

Fecundity

Table 2 shows the fecundity of *An. homunculus* during 2 gonotrophic cycles. The fecundity of *An. homunculus* averaged 62 ± 27.3 (range 46–89) and 62 ± 26.0 (range 36–108) eggs during the 1st and 2nd gonotrophic cycles, respectively. These values were not significantly different. The number of ovipositions by individual females had no significant effect on the total fecundity of mosquitoes, that is 55.0 ± 29.3 and 57.9 ± 27.9 eggs for 1st and 2nd ovipositions, respectively (Table 2). Ranges of 20–39, 40–59, 60–79, 80–99, and >100 eggs were matured by 3 (12%), 3 (12%), 8 (32%), 9 (36%), and 2 (8%) gravid females, respectively. These counts were not absolute because females were refed to monitor the 2nd cycle. During the 2nd gonotrophic cycle, however, 40–59, 60–79, and 80–99 eggs were matured by 3, 3, and 2 gravid females (Tables 2 and 3). These counts of the 8 surviving females included retained eggs and were independent of whether a female had laid any or all of her egg

Table 2. Number of bouts of egg laying (ovipositions) and fecundity of *Anopheles homunculus* during 2 gonotrophic cycles.

No. of ovipositions	No. of ovipositing mosquitoes	Mean No. of eggs ± SE	Range
First cycle			
1	12	55.9 ± 29.3	36–108
2	12	57.9 ± 27.9	39–99
3	1	94	94
Total	25	62.3 ± 27.3	36–108
Second cycle			
1	8[1]	62.5 ± 26.6	46–89
2	0	0	0
3	0	0	0
Total	8	62.5 ± 26.6	46–89

[1] All females died postoviposition.

Table 3. Time intervals from bloodfeeding to oviposition among individually caged *Anopheles homunculus* that survived 2 gonotrophic cycles (n = 8).

Mosquito number	First cycle		Second cycle	
	Time interval (h)	No. of eggs laid	Time interval (h)	No. of eggs laid
1	76	73	42	62
2	76	45	46	63
3	76	36	62	46
4	102	60	46	47
5	74	86	62	73
6	76	39	76	87
7	100	99	46	54
8	74	94	68	89
Mean	81.8	66.5	56	65.1

complement, but none of the P_1 individuals survived beyond day 8. All 8 females were parous (P_1 and P_2), indicating that these females were initially nulliparous and parous (P_1) when collected and monitored during the 1st gonotrophic cycle in the laboratory, but some had oviposited 1st in the field. Three P_2s laid between 46 and 63 eggs each, whereas the 5 P_1s each laid more than 70 eggs.

Gonotrophic Cycles

The length of the gonotrophic cycle, the time from bloodfeeding to oviposition, varied among the individual females (Table 3). Of the 25 females that oviposited in the 1st gonotrophic cycle, 16 oviposited 74–76 h postbloodfeeding. During the 2nd gonotrophic cycle, the 8 surviving females oviposited earlier at 42 h postbloodfeeding.

Survival

Figure 2 shows the survival rates of mosquitoes individually monitored in the laboratory. None survived more than 10 days under laboratory conditions.

DISCUSSION

The pattern of diel oviposition periodicity in *An. homunculus* shows a well-defined single nocturnal peak and is similar to that observed for 3 other Neotropical anopheline species, *Anopheles albitarsis* Lynch-Arribalbazaga (Chadee 1995), *Anopheles aquasalis* Curry (Chadee and Mohammed 1996), and *Anopheles oswaldoi* Peryassu (Chadee and Beier 1996), but is different from that of *Anopheles albimanus* Wiedemann (Chadee et al. 1993). Our methodology allowed us to determine whether *An. homunculus* females lay their whole egg complement during one bout of oviposition or whether oviposition was spread over more than 1 day. *Anopheles homunculus* adopted 2 different oviposition strategies: 13 females laid their whole egg complement within a single 2-h period, similar to that observed among 4 Neotropical anopheline species (*An. albitarsis* [Chadee 1995], *An. oswaldoi* [Chadee and Beier 1996], *Anopheles freeborni* Aitken [Chadee et al. 1993], and *An. aquasalis* [Chadee and Mohammed 1996]); and 12 females laid their egg complement over more than 1 day, similar to that observed among *An. albimanus* (Chadee et al. 1993).

This is the 1st study on the fecundity of *An. homunculus*. The fecundity of the 25 individual *An. homunculus* females averaged 62 eggs during the 1st and 2nd gonotrophic cycles. The number of

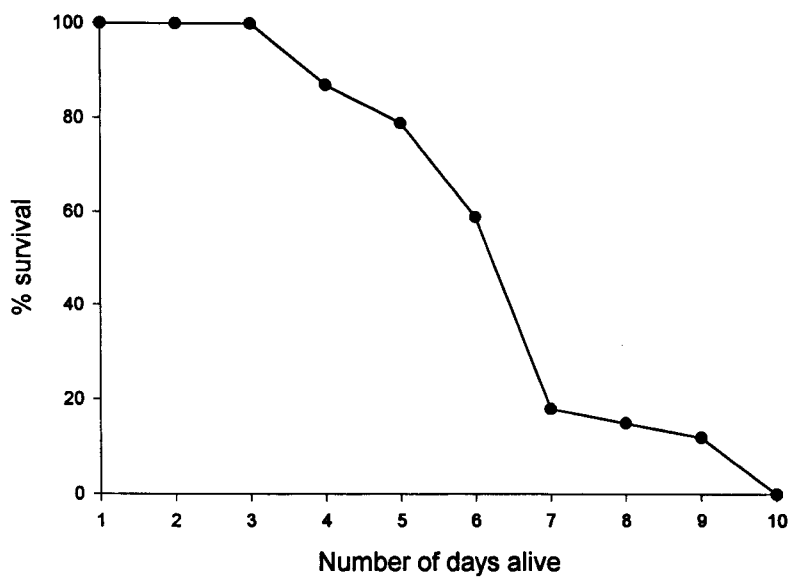

Fig. 2. Survival patterns of *Anopheles homunculus* under laboratory conditions.

eggs laid during any single time interval was moderate, exceeding 50 eggs on only 19 occasions. The gonotrophic status of the field-collected *An. homunculus* that completed 2 gonotrophic cycles in the laboratory showed that 3 P_3 laid between 46 and 63 eggs, whereas the 5 P_2 laid more than 70 eggs. Clements (1992) reported that the fecundity of anopheline mosquitoes tends to decline after 3 or 4 gonotrophic cycles. The present data are consistent with these findings because the mean numbers of eggs matured by *An. homunculus* during 2 gonotrophic cycles were almost identical (Table 2). Overall the fecundity of *An. homunculus* (50.5 ± 29.5 eggs) was similar to that observed for *An. oswaldoi* (61.1 ± 32.3 eggs) but lower than that reported for 3 other Caribbean anopheline species belonging to different subgenera (Chadee and Beier 1996).

During the present study oviposition by *An. homunculus* occurred consistently between 2000 and 0200 h, despite the time of day that follicles reached Christophers' stage V. Chadee and Beier (1995b) reported *An. homunculus* ovarian follicles matured to Christophers' stage V after 48 h but during the present study oviposition did not occur until 74–76 h after bloodfeeding, a difference of more than 24 h during the 1st gonotrophic cycle. During the 2nd gonotrophic cycle oviposition occurred at 42–46 h after bloodfeeding, 2–6 h earlier than the time observed for egg development (Chadee and Beier 1995b) and 32 h earlier than that observed during the 1st cycle of oviposition (mean 81.7 h 1st cycle vs. 56.0 h 2nd cycle) (Table 3). These results show the strength of the circadian rhythm and suggest an earlier initiation and completion of the 2nd gonotrophic cycle. Similar results were observed in *Aedes aegypti* Linn., suggesting that although stage V follicles were present, oviposition was delayed to coincide with peak oviposition periodicity (Gillett 1962).

Table 1 illustrates 2 points. First, the peak landing times of *An. homunculus* in Trinidad (Chadee 1994) preceded the periodicity of oviposition. Second, host seeking and possibly malaria transmission occurred between 1600 and 2000 h, declining at midnight (Chadee 1994) when peak oviposition was observed. In Brazil and Trinidad, *An. homunculus* is considered an important vector of malaria (Pittendrigh 1950, Zavortink 1973). However, Deane (1988) suggested that this species may be of secondary importance. Pittendrigh (1950) reported that "there can be no doubt of its vector status," with Chadee (1994) providing parity rates of 58% during both wet and dry seasons, supporting the possibility of malaria transmission during both seasons. The 10-day survival duration of the field-collected *An. homunculus* was sufficient to possibly complete 2 or 3 gonotrophic cycles and the P_1 mosquitoes initially collected from the field may survive long enough (at least 12 days) for sporogonic development within the mosquito species.

It is interesting to note that *An. homunculus* oviposited at night after the main biting cycle. This suggests that they do not feed until the next night. Consequently, a gonotrophic cycle of 2 days would translate to a 3-day feeding cycle and a 3-day gonotrophic cycle would translate to a 4-day feeding cycle. So for *An. homunculus* (and probably many other species as well), the diel periodicity of oviposition, together with the "fixed" feeding cycle may lower vector competence. The extrinsic incubation period for most *Plasmodium* spp. is at least 10 days. *Plasmodium vivax* may only require 8–9 days. However, further field studies on the survival of *An. homunculus* are required before its vector potential can be determined.

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