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DIEL OVIPOSITION AND FECUNDITY OF *ANOPHELES OSWALDOI* IN TRINIDAD, WEST INDIES

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ABSTRACT. The diel oviposition periodicity of wild-caught *Anopheles oswaldoi* collected from the forest-ecotone habitat in Valencia, Trinidad, was studied in the laboratory by recording the performance of egg-laying individuals and a colony at 2-h intervals. Oviposition was almost exclusively nocturnal, with 95.3% of eggs and 91.3% of oviposition occurrences being observed during the scotophase. During the rest of the day, only 4.7% of eggs (8.7% occurrences) were recorded after dawn (0600–0800 h). Wild-caught females engorged on human blood under laboratory conditions matured, on average, 61.1 ± 32.3 follicles (range 56–135). Ranges of 50–69, 70–89, 90–109, and >110 follicles were matured by 12, 4, 4, and 3 gravid females, respectively. These findings provide vector operators with a window of time to maximize the impact of insecticides on *An. oswaldoi* populations.

INTRODUCTION

Anopheles oswaldoi (Peryassu) is a suspected vector of malaria in Brazil (Olivera-Ferreira et al. 1990, de Arruda et al. 1986, Deane et al. 1988), Peru (Hayes et al. 1987), and Venezuela (Rubio-Palis and Curtis 1992). In Trinidad, Downs et al. (1943) successfully infected *An. oswaldoi* with falciparum malaria under laboratory conditions. In addition, in western Venezuela *Plasmodium vivax* circumsporozoite protein has been found among field-collected *An. oswaldoi* mosquitoes (Rubio-Palis et al. 1992). Within its geographic range, *An. oswaldoi* breeds primarily in sunlit or partially shaded ponds, river overflows, marshes, ditches, and hoof-prints in forested localities and is both exophilic and zoophilic (Faran 1980). In Trinidad, Rozeboom (1935) reported twice as many female *An. oswaldoi* collected from animal baits as on human bait and found none inside houses. In Brazil, Olivera-Ferreira et al. (1990) reported that peak biting occurred between 1800 and 1900 h. Similar results were subsequently reported by Rubio-Palis and Curtis (1992).

Nothing is known about the diel patterns of oviposition of *An. oswaldoi* either in the laboratory or in the field. In Trinidad, *An. oswaldoi* is usually collected in forest-ecotone habitats, which often lie in close proximity to human habitation. At the Valencia recreational area in Trinidad, considerable environmental changes have occurred, including deforestation, which may have been responsible for the decline in the number of sylvatic animals, the preferred *An oswaldoi* host species. Consequently, this species has become a nuisance to people visiting the area, as well as a potential vector should malaria be imported into Trinidad. This paper presents information on the diel oviposition patterns for *An. oswaldoi,* a component of the species biology that has practical applications relative to the timing of vector control operators.

MATERIALS AND METHODS

Wild *An. oswaldoi* females were collected from Valencia (10°39.52′N, 61°09.94′W), a recreation area located along the Valencia Road, ~35 km east of the Piarco International Airport, Trinidad. The Oropuche River bisects the road from north to south in an area composed of evergreen seasonal forest that has a continuous canopy layer at approximately 36 m. This site has been subjected to deforestation because of development as a recreational area and the representative flora is that of the ecotone with the representative trees being of the *Crappo-Guatecae:* wild debasse type (Beard 1946; Chadee, unpublished data). In addition to the river there are 2 large ponds (100 × 100 m) that were previously used for pisciculture but are now abandoned.

Mosquito collections were made between 1700 and 2000 h, the peak landing times of *An. oswaldoi* (Chadee, unpublished data). Six men were stationed at ground level in the forest-ecotone habitat. Mosquito collections, storage, and transportation methodologies have been described by Chadee (1992a, 1994) and Aitken (1960).

At the Insect Vector Control Division laboratory, mosquitoes were lightly anesthetized with chloroform, identified, and counted under a microscope at 40× magnification. *Anopheles oswaldoi* with any trace of blood as well as other species were discarded. All *An. oswaldoi* mosquitoes that survived the light anesthetic after identification were placed in a colony cage (30 × 30 × 30 cm) consisting of wire netting en-

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Table 1. Diel pattern of oviposition of individual and colony *Anopheles oswaldoi* mosquitoes.

		Number of eggs laid			
		Individual		Colony	
Time (h)	*n*	Mean ± SE	%	Total	%
1800–2000	1	126.0	8.6	32	1.6
2000–2200	3	59.0 ± 24.0	14.0	405	21.0
2200–2400	7	85.6 ± 36.3	45.3	1,095	56.7
2400–0200	8	34.3 ± 41.9	14.7	306	15.9
0200–0400	1	86.0	7.0	93	4.8
0400–0600	1	82.0	5.6	0	0
0600–0800	2	33.0 ± 19.8	4.8	0	0
0800–1800	0	0	0	0	0
Total	23	61.1 ± 32.3	100.0	1,931	100.0

closing 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 from a white cotton wick. The indoor insectary, lighting regimen, temperature, and humidity profiles have been previously described by Chadee (1992b). Suntime was used during the monitoring of oviposition and is used throughout the report. During this study sunset fell between 1831 and 1832 h and sunrise between 0551 and 0554 h. On the 2nd day after collection females were allowed to engorge on blood from the experimenter's arm exposed in the evening between 1830 and 1900 h.

One day later, 30 engorged females (condition assessed by eye) were removed by aspirator from the colony cage and each placed into a separate oviposition cage, identical to the colony cage. Each oviposition cage was furnished *ad libitum* with a sugar cube in an uncovered Petri dish. By monitoring individual females, we were able to record the diel periodicity of each individual and assess the contribution each made to the composite periodicity.

From about 36 h after engorgement (i.e., about 1800 h on the day after engorgement) oviposition was monitored by exposing 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, containing 300 ml of pond water. Water samples were collected 24 h prior to monitoring oviposition. A fresh tub was prepared using temperature-equilibrated pond water about 10 min before being placed in a cage. The diel periodicity of oviposition was monitored by replacing an old tub with a freshly prepared one every 2 h. To change the 30 tubs took approximately 15 min, so changing began a few minutes before and finished a few minutes after the scheduled time. Monitoring continued according to this schedule in the 30 cages and concurrently in the colony cage from 0600 h on the 2nd day after engorgement until 0600 h on the 5th day (i.e., continuously for 3 24-h periods). By monitoring individual females in separate cages and in the colony cage, we allowed for possible effects of interference among females (Corbet and Chadee 1993), even though the number of females (about 150) in the colony cage was relatively small. After monitoring ceased, all females in the individual cages were immediately killed and dissected, and relict eggs were counted and recorded. Both ovaries of each female mosquito were examined and scored as nulliparous or parous using Detinova's method, according to the presence of tracheolar skeins. In addition, both ovaries of all parous females were scored for parity by Polovodov's method of counting the number of dilatations on ovarioles and presence of relict eggs (Detinova 1962). In this way the fecundity (the complement of matured follicles) for each female was measured.

Oviposition data from individuals and the colony were analyzed separately. The periodicity data were transformed into contingency tables (2 × 8) and subjected to a *G*-test (Fowler and Cohen 1995) to determine peak hours of oviposition and frequency of oviposition.

RESULTS

The oviposition patterns of *An. oswaldoi* are shown in Table 1. The patterns derived from numbers of eggs laid and occurrences of eggs correspond closely, as shown in Table 1. Of the 30 females observed individually, 23 laid eggs during the 3-day observation period. Among the 23 females that oviposited, 6 retained eggs (2, 3, 5, 27, 41, and 79 eggs). Oviposition by the isolated females occurred between 1800 and

0800 h and showed a well-defined peak between 2200 and 0200 h (>62% of eggs laid; >65% of occurrences) (G = 39.4, df 6, $P < 0.001$), that is, 5 h after sunset. From 1800 to 2200 h, 303 eggs or 21.4% of eggs were laid (>17% occurrence), whereas 11.9% (168 eggs laid; >8% occurrence) were observed prior to dawn (0200 to 0600 h) and 4.7% (>8% occurrence) after dawn (0600–0800 h). No eggs were laid between 0800 and 1800 h (Table 1).

Oviposition in the colony cage was similar to individual periodicity of females except that oviposition was confined to between 1800 and 0400 h. Peak oviposition started earlier, at 2000 h, and ended at 2400 h (78% of eggs laid) with significantly more eggs being laid during this period (2000–2400 h; G = 43.6, df 6; $P > 0.001$) than at any other time. Oviposition started earlier and finished later among individuals (1800–0800 h) than among colony cage mosquitoes (1800–0400 h) (Table 1).

Peak oviposition occurrences were recorded between 2200 and 0200 h and showed that although more oviposition occurrences were observed during 2400–0200 h (8) than at 2200–2400 h (7), the mean and total number of eggs laid was significantly higher ($P < 0.001$) than that observed at 2400–0200 h (85.6 vs. 34.3 eggs) (Table 1).

The fecundity of 23 individual *An. oswaldoi* females averaged 61 ± 32.3 follicles (range 56–135). Ranges of 50–69, 70–89, 90–109, and >110 follicles were matured by 12 (52.2%), 4 (17.4%), 4 (17.4%), and 3 (13.0%) gravid females, respectively. The counts included retained eggs and were independent of whether a female has laid any or all of her egg complement. Most females dissected for parity determination were parous (P_2s and P_3s), indicating that they were old females. It was not possible to determine the parity status of some females with relict eggs, especially those bearing >20 eggs in each ovary. Thirteen P_2 and P_3 females laid between 56 and 69 eggs each, whereas the 8 P_1 females laid >70 eggs each.

DISCUSSION

The diel oviposition periodicity for *An. oswaldoi* in the laboratory was well defined with a single, nocturnal peak. This pattern is very similar to that observed for 2 other Neotropical anopheline species, *Anopheles albitarsis* Lynch-Arribalzaga (Chadee 1995) and *Anopheles aquasalis* Curry (Chadee and Mohammed 1996), but different from that of *Anopheles albimanus* Wied (Chadee et al. 1993).

By recording the behavior of individual females, we have determined that *An. oswaldoi* females usually lay the whole egg complement during one bout of oviposition, seldom if ever spreading oviposition over more than one day. Eighteen females laid their whole egg complement within a 4-h period (i.e., 2 consecutive monitoring intervals), whereas 5 females laid their whole egg complement within a single 2-h period. This pattern has been observed in two other Neotropical species, *An. albitarsis* (Chadee 1995) and *An. aquasalis* (Chadee and Mohammed 1996). The large number of retained eggs observed during this study may be an artifact associated with experiments conducted in small cages under laboratory conditions. In the field, it would be difficult to monitor oviposition because of the location of breeding sites, time of oviposition, and the difficulty associated with observation in aquatic habitats. Moreover, under field conditions it would be impossible to determine the fecundity of wild females and whether females spread oviposition over a period of time, because it would be impossible to determine if the eggs collected represented part or the full complement of eggs.

The fecundity of the 23 individual females averaged 61.1 ± 32.3 (range 56–135). Counts included retained eggs and therefore were independent of the number of eggs a female had laid. The number of eggs laid during any single time interval (i.e., an oviposition occurrence) was fairly large, exceeding 56 eggs on all 23 occasions. The gonotrophic status of the field-collected *An. oswaldoi* showed that the 13 P_2 and P_3 females had laid between 56 and 69 eggs each, whereas the 8 P_1 females laid >70 eggs each. The present data are the only fecundity information available on this aspect of the biology of this species. Clements (1992) reported that the fecundity of anopheline mosquitoes declined only after 3 or 4 gonotrophic cycles. The present data show that the fecundity of old parous (P_2 and P_3) and relatively young parous (P_1) females were different and did show a decline in the number of follicles matured with age. In fact, the results suggest a rather similar pattern to that observed for *Aedes aegypti* (Linn.), with 15% fewer eggs with each subsequent gonotrophic cycle (Clements 1992). Overall, the fecundity of *An. oswaldoi* females (61.1 ± 32.3) was much lower than that observed for 3 other Caribbean anopheline species, *An. albimanus* (71.1 ± 12.0), *An. albitarsis* (104 ± 18.40), and *An. aquasalis* (89.80 ± 21.32) (Chadee et al. 1993, Chadee 1995, Chadee and Mohammed 1996).

Among other features of the results deserving comment, the distribution in Table 1 illustrates 2 points. First, the periodicity of *An. oswaldoi* observed under laboratory conditions coincided

with the peak landing times of this species in Venezuela (i.e., before midnight) (Rubio-Palis and Curtis 1992). Second, both activities were observed during the scotophase (2000–0200 h), suggesting that host-seeking, malaria transmission, and oviposition occurred just before and just after midnight (Downs et al. 1943, Rubio-Palis and Curtis 1992).

The present collection of this potential vector is probably linked to environmental changes caused by deforestation in this area of Trinidad and the consequent decrease in the population of sylvatic animals, which have led to a greater level of contact of this vector species and humans. In addition, these results demonstrate the importance of entomological information in understanding vector-borne disease transmission by man–vector contact, in planning control strategies, and in the planning of parks, camp sites, and other recreational facilities. Although, this species is considered a potential vector of malaria, its role in the maintenance of malaria transmission cannot be underestimated throughout the Neotropics.

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