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PROMPT MATING OF RELEASED *ANOPHELES DARLINGI* IN WESTERN AMAZONIAN BRAZIL

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ABSTRACT. To identify times and sites of mating, 1- and 2-day-old virgin female *Anopheles darlingi* were marked with fluorescent dusts and released at dusk, midnight or dawn in a village on the outskirts of Porto Velho, Rondônia State, Brazil. Dissections of marked females captured at human baits revealed that mating occurred in less than 2 h after dusk releases near houses, and among these early recaptures, older females were significantly more likely to be inseminated. We suggest that mating shortly after peridomestic releases occurred without swarming outside houses.

KEY WORDS Insemination, malaria, mark-release-recapture, mating, *Nyssorhynchus,* peridomestic

INTRODUCTION

Surprisingly little is known about the sites and times of mating of species of *Anopheles* (*Nyssorhynchus*), the subgenus that includes most of the important vectors of human malaria in the Neotropics. Nearly all *Anopheles darlingi* Root captured at human baits have been inseminated (Giglioli 1947; Charlwood and Wilkes 1979), a fact that contributes to the presumption that mating may occur near emergence sites before host seeking. Although swarm formation by this species has been observed (J.B.P. Lima, personal communication), to our knowledge mating in nature has not.

The most complete account of mating in nature among anophelines of this subgenus was by Senior White (1951) who noted and sampled swarms of *Anopheles aquasalis* Curry in Trinidad. These swarms occurred over grass in the waning light shortly after sunset and were composed of more than 99% males. Although females flew into such male aggregations, Senior White (1951) inferred that insemination occurred outside swarms.

Anopheles darlingi from South America were successfully colonized in large outdoor cages (Bates 1947, Freire and Faria 1947, Giglioli 1947) more than 50 years ago. Although high frequencies of insemination were recorded, no investigator observed swarming in cages, and Bates (1947) remarked on this difference compared to several species of Old World malaria vectors colonized in Europe. Galvão et al. (1944) reported on cage mating without swarming for *Anopheles albitarsis* Lynch Arribalzaga and *Anopheles argyritarsis* Robineau-Desvoidy, and Rozeboom (1936) noted that caged *Anopheles albimanus* Wiedemann paired around dusk without swarming. Thus, swarming is clearly not a prerequisite for mating among species of *Anopheles* (*Nyssorhynchus*), as has been appreciated for other mosquito genera (Nielsen and Haeger 1961).

The current study was done to shed light on the time and place of mating of *An. darlingi* in nature. Successful rearing of progeny of field-collected females was coupled with simple mark–recapture techniques in an area of Rondônia State, Brazil, where this species is highly anthropophilic (Oliveira-Ferreira et al. 1992) and the principal vector of human malaria (Lourenço-de-Oliveira et al. 1989). We also report an age-specific difference in sexual receptivity of *An. darlingi.*

MATERIALS AND METHODS

Naturally inseminated females of *An. darlingi* were captured at human baits in the vicinity of Porto Velho, Rondônia State, Brazil, and bloodfed in an insectary on mice or quail. Eggs were oviposited in cages on trays of distilled water, or were collected from individual females confined in vials with damp filter paper. Larvae were reared in trays and fed a finely ground mixture of flower pollen, Tetramin® (Tetrawerke, Melle, Germany) baby fish food, Tetramin® (Tetrawerke, Melle, Germany) fish flakes, and dog chow combined 1:1:2:1 parts by weight. Mortality was uniformly low in the larval stages, and resultant adults were similar in size to wild-caught individuals from this same site (Lounibos et al. 1995). Pupae were harvested daily to ensure cohorts of synchronized ages. Adults were segregated by sex and age at emergence. Dissections of females that remained with males in laboratory cages prior to each release revealed no inseminations.

Approximately 1 h before release, females were confined in 4-liter, cardboard ice cream containers and dusted through screened lids with a fluorescent powder applied as a mist with an insufflator. Five distinguishable colors of powders, obtained from Day-Glo (Cleveland, OH) and BioQuip (Santa Monica, CA) permitted recognition of females of different ages during recapture periods.

Dusted females were released outdoors among a cluster of houses in Cristo Redentor (Fig. 1), a resettlement village in the outskirts of Porto Velho (8°49′S, 63°54′W) with approximately 100 inhabitants. The area around houses was cleared except

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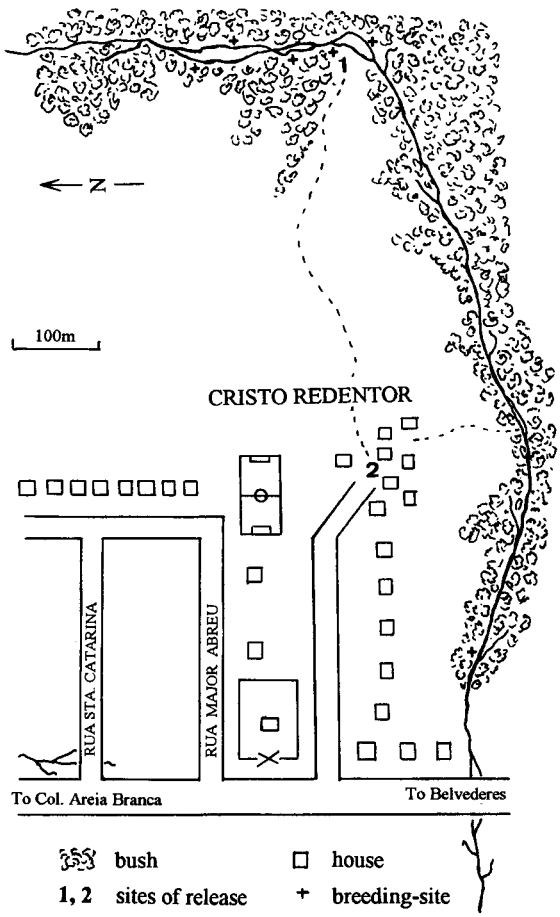

Fig. 1. Map of the study village showing release sites of marked *Anopheles darlingi*. Larval collection sites of this species beside stream and pool margins are shown by crosses. Rectangular area north of release point 2 is a football field.

for vegetable and ornamental plantings. Larval *An. darlingi* have been collected from a stream that borders the village to the south and west (Fig. 1), and malaria is common among village inhabitants (Fundação Nacional da Saude, Porto Velho). The village was a source of previous collections used to demonstrate size variation in *An. darlingi* (Lounibos et al. 1995).

At the inception of this study, a single release of dusted *An. darlingi* was attempted beside a pool in the stream approximately 0.5 km from the nearest houses (Fig. 1). All subsequent releases were in the village; most were conducted at dusk at 1745 h, followed by human bait collections by 4 persons seated outdoors within 100 m of release sites from 1800 to 1930 h. For releases in July 1995 females ranged in age from 9 to 54 h, but in October 1995 and May 1996, dusted individuals were more uniform in age, either 20 to 28 or 44 to 52 h post-emergence, hereinafter referred to, respectively, as 24-h- or 1-day- and 48-h- or 2-day-old females. In the final experimental period when more reared females were available (Table 1), releases were also done at 2400 h for both age groups and at 0500 h for 24-h individuals. The latter releases were followed by human bait collections from 0530 to 0630 h, the interval just before and after dawn. The initial hours after sunset are the favored host seeking times of *An. darlingi* in Rondônia State (Lourenço-de-Oliveira et al. 1989, Klein and Lima 1990).

Mosquitoes captured at human bait were transported in humidified containers to the laboratory where they were lightly anesthetized and separated by species under a dissecting microscope. All specimens identified as *An. darlingi* were examined with a long-wavelength ultraviolet light to detect fluorescent dusts. The spermatheca of each dusted *An. darlingi* was dissected in a mosquito saline (Hayes 1953) and examined under high power of a compound microscope for the presence of sperm.

RESULTS

Of 245 *An. darlingi* released near the pool, no specimens were recovered in 3 subsequent collection nights nearby or 6 nights in the village. A total of 4,921 *An. darlingi* was dusted and released in the village during 3 periods in 1995 and 1996 (Table 1). Eighty-three marked females were recovered, for an overall recapture rate of 1.7%. During recapture periods, *An. darlingi* accounted for 84.9% and *Anopheles triannulatus* (Neiva and Pinto) accounted for 13.2% of all anophelines collected at human baits; 3 other *Anopheles* species and unidentifiable specimens accounted for the remainder (Table 1).

For both 24- and 48-h-old females, recaptures were made less than 2 h after releases, and 60% (3/5) of the recovered 48-h females were inseminated,

Table 1. Releases and recaptures of *Anopheles darlingi* in Cristo Redentor, Porto Velho, Rondônia State, Brazil.

Dates of releases	Recapture efforts (range)		Total *Anopheles*[1]	No. of *An. darlingi*	
	No. nights	No. man-hours		Recaptured/released	%
July 26 and 28, 1995	3–6	10.5–28.5	623	19/497	3.8
Sept. 6, 9, and 11, 1995	3–7	18.0–42.0	501	21/1,468	1.4
May 8 and 9, 1996	1–2	10.0–16.0	199	43/2,956	1.5

[1] Other anophelines captured: *Anopheles triannulatus* (205), *Anopheles mediopunctatus* (Theobald) s.l. (7), *Anopheles nuneztovari* Gabaldón (3), *Anopheles braziliensis* (Chagas) (3), unidentified (17).

Table 2. Mating status of recaptured *Anopheles darlingi* related to age at, and time after, releases in October 1995 and May 1996.

Hours after release[1]:	No. inseminated/total recaptured									
	1	6	12	18	24	36	48	72	96	Total
Released at 24 h[2]	0/4	0/1	8/11	3/3	1/1	5/5	3/4	5/6	2/2	27/37
Released at 48 h	3/5	2/2	3/3	5/5	10/11	nd[3]	0/1	0/0	0/0	24/27

[1] Hours after release indicated as midpoint of recapture period.
[2] In May, 24-h-old females were released at 0500, 1745, and 2400 h, but 48-h-old females were released only at 1745 and 2400 h.
[3] nd, not done.

but none (0/4) of the recovered 24-h-old *An. darlingi* were inseminated (Table 2). When early recaptures included all dusted females recovered in the 1st 6 h after releases, the frequency of insemination was significantly higher for the 48-h group, when the 2 ages were compared by a *G*-test with Williams's correction for a 2 × 2 table (Sokal and Rohlf 1995) ($G_{adj} = 7.0$, df 1, $P < 0.01$). However, recapture of 48-h females shortly after a predawn release was precluded by the absence of a release at that time of day for this age group (Table 2). In collections more than 6 h after releases, most of the 24-h females had been inseminated (Table 2), and the insemination frequencies of total recaptures were not significantly different between the 2 age groups ($G_{adj} = 2.5$, df 1, $0.20 > P > 0.10$).

DISCUSSION

The most striking discovery of this study was the prompt mating of 2-day-old, virgin *An. darlingi* in less than 2 h after release. The circumstances of this release and recapture suggest that mating occurred close to houses, which would require the presence or entry of males in this area. Because swarms were never observed, we further infer that sexual encounters and copulation occurred through individual interactions without swarms. Although this explanation of the prompt inseminations is the most parsimonious, we cannot preclude alternatives such as that released females may have flown into the nearby vegetation (Fig. 1) where they were mated before returning to the vicinity of houses to seek blood. It is also possible that male swarms occurred in the village, but were too few, high, or dispersed to be detected.

This study adds support to the concept of flexible mating strategies of mosquitoes in general and of *An. darlingi* in particular. For the latter species, our interpretation of swarmless mating in nature would confirm such observations as Bates (1947) made of *An. darlingi* in cages, even though swarming may also occur under other circumstances. Cambournac and Hill (1940) determined that most matings of *Anopheles atroparvus* Van Thiel in nature occurred at rest under shelter, and concluded that swarming in this species had largely lost its functional character, although the ritual was preserved.

Our results also seem to indicate that 1-day-old female *An. darlingi* are less receptive to mating than are 2-day-old individuals. This confirms the general pattern known for other species of mosquitoes studied in confinement, that female sexual receptivity increases with age after emergence (Gwadz and Craig 1968, Lea 1968). The importance of the differences in insemination frequencies between early recaptures of the 2 age groups may be questioned because 2 early recaptures of 24-h-old females, but no 48-h-old females, occurred after a predawn release, and dawn is not as important as dusk for anopheline sexual arousal (e.g., Nijhout 1977, Charlwood and Jones 1979). However, among *An. darlingi* of both age groups released at midnight, only 48-h-old individuals caught at dawn were inseminated, indicating that mating did occur in the predawn hours of the morning. Wilton and Fetzer (1972) reported dusk, midnight, and dawn peaks in mating activity of caged *An. albimanus.*

Additionally, these experiments demonstrate that insights into the mating habits of important disease vectors, such as *An. darlingi,* can be achieved from simple methods, such as mark–recapture, applied in natural settings. Although our single attempt to follow the dispersal of marked adults from breeding to host-seeking sites failed, this procedure has proven useful for tracking mosquitoes by control programs (Morris et al. 1990). With intensive collection efforts that yielded 12–19% recaptures, Charlwood and Alecrim (1989) successfully traced the dispersal of marked *An. darlingi* in western Amazonian Brazil. We encourage further research with such techniques to elucidate features of vector life histories that may provide insights applicable to control.

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