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LOW RATES OF MULTIPLE FERTILIZATION IN PAROUS *ANOPHELES ALBIMANUS*

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ABSTRACT. We determined how frequently parous female *Anopheles albimanus* fertilize their eggs with sperm from more than one male. To establish paternity we relied on 2 phenotypically distinct laboratory strains. Nulliparous females were allowed to mate freely with males from one strain, and after oviposition they were offered a 2nd mating with males of the other strain. Fertilization patterns were determined by the phenotypes of offspring. Only 0.6% of females ovipositing for a 2nd time (n = 312) used sperm from the 2nd male, as did 4% of females completing a 3rd gonotrophic cycle (n = 25). In this species receptivity is not routinely renewed following oviposition.

INTRODUCTION

In Central America, *Anopheles albimanus* Wiedemann is a vector of malaria that is amenable to control by release of sterile males (Weidhass et al. 1974). Genetic control of mosquitoes is receiving renewed attention due to novel applications of genetic engineering (e.g., James 1992), and *An. albimanus* is a potential target for such an approach. If such introductions are to be successful, the sexual biology of the target species must be well understood. Of particular importance is the question of female receptivity. If females of a target species mate more than once, it is crucial to establish which males in the population achieve most matings, and to endow released males with competitive advantages. Conversely, if females are strictly monogamous, releases must be timed so as to intercept the largest number of virgin females.

Male mosquitoes are adept at controlling the reproductive output of females with whom they successfully copulate. Male ejaculate contains a mass of accessory gland secretions that in the short term form a physical barrier (mating plug) against further inseminations (Giglioli and Mason 1966). The ejaculate also contains a peptide (matrone) that effectively changes female behavior by rendering her nonreceptive to further inseminations (Craig 1967, Young and Downe 1987). There is a difference, both semantic and biological, between copulation, insemination, and fertilization, three successive events that do not automatically follow each other (Jones 1973). Nevertheless, there is evidence that under some conditions, females copulate more than once, and subsequently fertilize their eggs with sperm of more than one male.

Females may copulate and be reinseminated either immediately after their first copulation (e.g., Gwadz and Craig 1970), or following oviposition (e.g., Williams and Berger 1980; Young and Downe 1982, 1983). A different mechanism may underlie each of these scenarios. In nulliparous females, the first male may fail to impose monogamy due to interrupted copulation or lack of accessory gland material (Gwadz and Craig 1970, Mahmood and Reisen 1980, Gomulski 1990). Conversely, the experiments of Young and Downe (1982, 1983) demonstrated that mated, parous *Aedes aegypti* (Linn.) and *Culex tarsalis* (Coq.) commonly accept sperm from a 2nd male. Though no evidence of fertilization by 2nd mates was provided, their findings suggest that receptivity may be routinely renewed by females as an oviposition-dependent response to sperm depletion. Nevertheless, Reisen et al. (1984), using more rigorous criteria, found no evidence for renewed receptivity in *Cx. tarsalis.*

In the experiments reported here we used genetic markers to determine, in the laboratory, whether parous, mated female *An. albimanus* fertilize their 2nd and 3rd egg batches with sperm from a 2nd mate.

MATERIALS AND METHODS

Experiments were performed in the laboratory of the Malaria Research Center, Tapachula, Mexico. We used 2 phenotypically pure distinct strains originated from a long-established colony of *An. albimanus* (Rodriguez et al. 1992). These strains express different 4th-instar larval and pupal markings. In the first, designated cafe-claro (henceforth *cc*), larvae are uniformly brown colored. Larvae of the 2nd strain, designated franja-blanca (henceforth *fb*), exhibit a white stripe along their dorsum. The white stripe trait is dominant (Rabbani and Seawright 1976), and both strains have been selected to homozygosity (Rodriguez, unpublished). Mosquitoes were maintained at 26–27°C and 80% RH.

To determine whether females mated multiply

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Table 1. Rates of multiple fertilization in successive gonotrophic cycles of *Anopheles albimanus* females.

Gonotrophic cycle	No. of females	Mean no. of offspring ± SE	Number mated multiply
II	312	30.2 ± 24.5	2
III	25	21.0 ± 14.4	1
IV	3	35.6 ± 15.5	0

and used sperm from the 2nd mating event to fertilize eggs, we performed the following experiment: Emerging males and females of the *cc* strain were allowed to cohabit in an emergence cage (45 cc^3) for 3 days. When females were 3–4 days old they were offered a blood meal. Engorged females were removed from the mating cage and kept in individual oviposition containers. Following oviposition, eggs were examined daily until they hatched. Females that laid egg batches that did not hatch were discarded from the experiment. Females whose eggs hatched normally were offered a 2nd mating opportunity, this time with 3–7-day-old males of the *fb* strain. Subsequently (after 3 days), they were offered a 2nd blood meal and oviposition opportunity. Egg batches were maintained singly, and the phenotype of developing larvae noted. We considered the appearance of larvae with the *fb* trait as unequivocal evidence of remating and fertilization with sperm from the 2nd mate.

Next, the effects of aging and sperm depletion on renewal of receptivity were established. We took 25 females that, after mating as nullipars with *cc* males, had already oviposited twice, and allowed them a mating opportunity with *fb* males prior to their 3rd blood meal. An additional 3 3-parous females (that had produced 3 *cc* cohorts of offspring) were confined with *fb* males prior to their 4th blood meal. The larval offspring of these females were examined and their phenotypes determined. As above, appearance of the *fb* trait was considered evidence of multiple mating.

RESULTS AND DISCUSSION

Of the 312 parous females offered a 2nd mate prior to the 2nd blood meal, 2 (0.6%) fertilized their 2nd batch of eggs with sperm from a 2nd mate (Table 1). Subsequently, 1 of 25 2-parous females (4%) produced offspring with the *fb* trait (Table 1), a significant increase ($Z = 1.87$; $P = 0.03$). None of the 3-parous females evidenced multiple mating, but the small sample size precludes definite conclusions.

Table 2. Offspring phenotypes of multiply fertilized female *Anopheles albimanus*.

Female no.	No. of offspring	% of offspring with phenotype	
		cc	*fb*
1	93	66.6	33.3
2	11	36.3	63.7
3	12	50.0	50.0

Our results indicate that receptivity is not routinely renewed following oviposition in this species. Nevertheless, the increase in multiple mating from 0.6% in 1-parous to 4% in 2-parous females indicates that sperm depletion or temporal decay of male-imposed restraints may affect the propensity of females to remate. All the females that remated produced a high proportion (33.3–66.6%) of *cc* offspring (Table 2). These distributions suggest that sperm depletion alone cannot acount for renewed receptivity.

Bryan (1972) demonstrated that accessory gland material from the first mate is crucial in controlling subsequent fertilization in *Anopheles gambiae* Giles females. Furthermore, circadian activity patterns of mated, parous *Anopheles stephensi* Liston females are similar to those of mated nullipars (Rowland 1989). Together these findings suggest that the accessory glands of anopheline males contain peptides similar in function to the matrone produced by *Ae. aegypti* males (Craig 1967). The renewal of receptivity in our experiments may have resulted from a decay of the inhibition imposed by the females first mate. However, the low rate of multiple fertilizations indicates that males are very effective in controlling female receptivity and ultimately, fertilization.

Our data conform with reports of low levels of multiple mating of anopheline females. In laboratory studies using an insecticide-resistant strain of *An. gambiae,* Goma (1963) concluded that multiple mating is rare. In more definitive experiments Mahmood and Reisen (1980) and Gomulski (1990) demonstrated that approximately 15% of females (*Anopheles culicifacies* Giles and *An. gambiae,* respectively) fertilized their first batch of eggs with the sperm of at least 2 males. These observations were not extended to successive gonotrophic cycles. Furthermore, Mahmood and Reisen (1980) showed experimentally that interrupted matings were the main cause of these multiple fertilizations. Their observations are in agreement with the work of Gwadz and Craig (1970), who noted that multiple fertilization in *Ae. aegypti* results from "inadequate semen transfer" during the first copu-

lation. In addition to evidence from laboratory studies, field studies show that multiple fertilization is absent or rare in nature. Baimai and Green (1987), relying on polymorphism of the Y chromosome, could find no evidence of multiple fertilizations in field populations of *Anopheles maculatus* Theobald and *Anopheles dirus* Peyton and Harrison. Similarly, Yuval and Fritz (1994), using polymorphisms of several enzyme loci, found a very low incidence of multiple fertilizations in a field population of *Anopheles freeborni* Aitken.

In the present study, the number of matings of nulliparous females were not determined, as we focused on renewal of receptivity in parous females. Our results indicate that multiple fertilization is quite rare in parous *An. albimanus*. We conclude that female receptivity is not routinely renewed in this species, and seems to be firmly controlled by the female's first mate.

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