Pre-mating Blood Feeding by *Anopheles pharoensis* (Diptera: Culicidae) and Its Effects on Mating, Longevity and Egg Production[1]

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ABSTRACT Field observations and laboratory studies were conducted to determine the effects of a pre-mating blood meal on mating, ovarian development and oviposition in *Anopheles pharoensis* (Theobald). Approximately 24% of the females blood feed before mating; however, swollen abdomens of blood-fed females interfere with the mating process. In females which mated prior to first blood meal, first oviposition occurred at 6.6 days and required only a single blood meal. A second blood meal takes them to the second oviposition in an additional 2.5 days; the third oviposition required 1.4 blood meals and occurred 3 days after the second oviposition. Blood-fed unmated females reach first oviposition at 13.4 days and require an average of 4 blood meals prior to the first oviposition. At this age, the female is chronologically old and even if mating now occurs, egg production is greatly reduced and continues to decrease through subsequent ovipositions until death. The taking of a blood meal prior to mating greatly decreases the reproductive potential of this species.

KEY WORDS Blood-feeding, mating, oviposition, egg production, *Anopheles pharoensis,* mosquito.

Anopheles pharoensis is the most important species of mosquito involved in transmission of malaria in Egypt (Bates 1941, El-Said 1975, El-Akad 1981). Most eradication trials for this species rely on insecticides as a quick method for reduction of *An. pharoensis* regardless of side effects on plants, animals, beneficial insects, and man. As more knowledge is gained on the behavior, bionomics, and physiology of this species, perhaps less emphasis can be placed upon insecticides as a control method. Laboratory observations indicated that when this species blood-fed immediately after emergence, because of swollen abdomens, the females had trouble joining mating swarms.

The ordinary sequence of feeding/reproductive behavior for *An. pharoensis* and other mosquito species is as follows: the female seeks a sugar/nectar meal immediately after emergence (El-Akad and Humphreys 1988); swarming, mating, and insemination occur 2-5 days after emergence before the gonotrophic cycle activity begins and these are not repeated in later cycles (Seaton and Lumsden 1941, Lang 1965, Nielsen and Haegar, 1960, Siddiqui et al. 1978, Reinsen et al. 1979, El-Akad 1985); the first blood meal is taken 2-3 days after emergence (Seaton and Lumsden 1941, Lang 1956, El-Akad 1985); and oviposition of mated

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females occurs 3-5 days after the blood meal (El-Akad and Humphryes 1988). Under field conditions modification in the female behavior sequence may occur because of certain larval conditions (unpublished data, El-Akad and Humphreys). In these cases, the female takes the first blood meal before mating. Swarming is an important part of the sexual behavior of *An. pharoensis* and movement of the females into a mating swarm is necessary since mating occurs only during flight (El-Akad 1985). For all mosquitoes in the *Anopheles gambiae* complex, mating also is initiated during flight, the male being attracted to the female by her flight tones. Males do not mate with "grounded" females (Clements 1963, Charlwood & Jones 1979).

This study was undertaken to elucidate the effects of a premating blood meal upon mating and fecundity in *An. pharoensis*. A factor which would decrease fecundity should be examined as a possible control measure.

Materials and Methods

Laboratory studies were conducted in the Training and Research Center on Vectors of Diseases, Ain Shams University, Cairo, Egypt. The field observations were performed in the Abheet El-Hagar area, Fayioum Governorate, southeast of Cairo. The experimental mosquitoes were either wild-caught or from a 3-year-old lab colony. Observations recorded were the number of blood meals taken by an unmated female leading to the first oviposition, mating and insemination relative to female age and the longevity of unmated females. Nulliparous females (110) were collected via aspirators from human and donkey baits. Collections from humans occurred both inside and outside of dwellings; collections from donkeys were always outside. Some of the mosquitoes were blood feeding when collected and it is assumed that those not actively feeding were going to feed since they landed on the bait. Spermathecal dissection was performed to determine if mating had occurred (Anonymous 1975). The ovaries were dissected to determine if the females were nulliparous or parous. Stages of ovarian development were determined using the methods of Detinova (1962).

Two cages (50 cm^3) covered with fine nylon netting were used to check differences in blood feeding between unmated and mated females. Newly emerged females and males (50:50, males:females) were placed in one cage. Previous studies had indicated this ratio of males: females adequate to insure insemination of all females (El-Akad and Humphreys 1988). The second cage held only 50 newly emerged females. Mosquitoes were fed a 10% glucose solution via a cotton wick. On day 4 mosquitoes were offered a blood meal via the senior author's arm inserted into each cage for 10 min. The percent of blood-fed females was recorded for each group. Spermathecal dissections were performed in cage 1 females to ensure that mating/insemination had occurred.

Three groups of newly emerged females (50/cage) were placed in cages (50 cm^3) with a 10% glucose solution to determine the effects of premating blood feeding upon mating. Group 1 females were blood-fed just after emergence and were isolated in the cage for 4 days until they became gravid. On day 4, three-day-old males (50) were placed in the cage and at 24 hr post-introduction. Observations were made on the mating behavior of the gravid females. Three-day-old males were used because peak mating activity occurs in 3-day-old males (El-Akad 1985). Group 2 females were held for 4 days after emergence and then blood-fed. Males

(50) were added after the females completed feeding and mating behavior observed. Group 3 females were maintained on glucose (10%) for 4 days, males (50) added to the cage, and mating behavior monitored. Group 3 females were never offered a blood meal.

Two groups of newly emerged females (50/group) were placed in cages (50 cm^3) to evaluate the effects of insemination and blood feeding upon the first oviposition of female *An. pharoensis*. Each cage was provided a 10% glucose solution. One cage was provided with males (50) to insure mating, the other cage was left mateless. On day 4, both batches were provided a blood meal. The females from each cage were isolated individually in plastic cups (5 × 7.5 cm) with distilled water (75 ml) used as an oviposition medium and covered with muslin. A glucose solution (10%) was available via a cotton wick. Blood meals were offered to females daily. Data collected included mortality, the number of blood meals required for first and subsequent ovipositions to occur, duration of the oviposition periods, and longevity.

Newly emerged and 2, 4, 6, or 8-day-old females (50 females/age group) were placed in a cage with three-day-old males (50) to determine the effects of female age on mating. After 24 hr, 20 females were removed and the spermathecae dissected to determine if mating had occurred.

Results and Discussion

Field collections of nulliparous female *An. pharoensis* yielded 27 unmated (25%) and 83 mated (75%) females. Because females were captured either feeding or attempting to feed on animal or human baits, the avidity of some *An. pharoensis* for the first blood meal is not affected by mating/insemination. Twenty-seven of the field-caught females had taken a blood meal prior to insemination. Twenty-one had ovaries developed to Stage I and 6 had ovarian development to Resting Stage II (Detinova, 1962). Because of the heavy swollen abdomens from taking a blood meal, these females were unable to join a mating swarm. Avidity for the first blood meal is the same in both mated and unmated females. In the laboratory, when mated and unmated four-day-old females were offered their first blood meal, 34 uninseminated females (68%) fed and 33 (66%) of the mated females took a blood meal. However, blood feeding prior to mating can be detrimental to population numbers as evidenced by results of the additional laboratory studies.

No mating was observed when newly-emerged females were blood-fed just after emergence and isolated 4 days until gravid, or when females were held 4 days after emergence and then blood-fed. In both experiments, spermathecal dissections of females from each group revealed not a single mating. Greatly distended abdomens of females in both gravid and blood-fed groups prevented the females from joining in mating swarms. However, females maintained on glucose for 4 days flew freely into the mating swarm. Spermathecal dissection showed that 54% of these females had mated. Presumably, empty stomachs and empty ovaries reduced the weight and permitted them to share the mating swarm. Reisen and Aslamkhan (1976) reported a replete midgut in mosquitoes was a mechanical hinderance to mating.

When nonblood-fed female *An. pharoensis* were exposed to males, peak mating occurred in the 2-day-old females and decreased as females grew older (Fig. 1). No

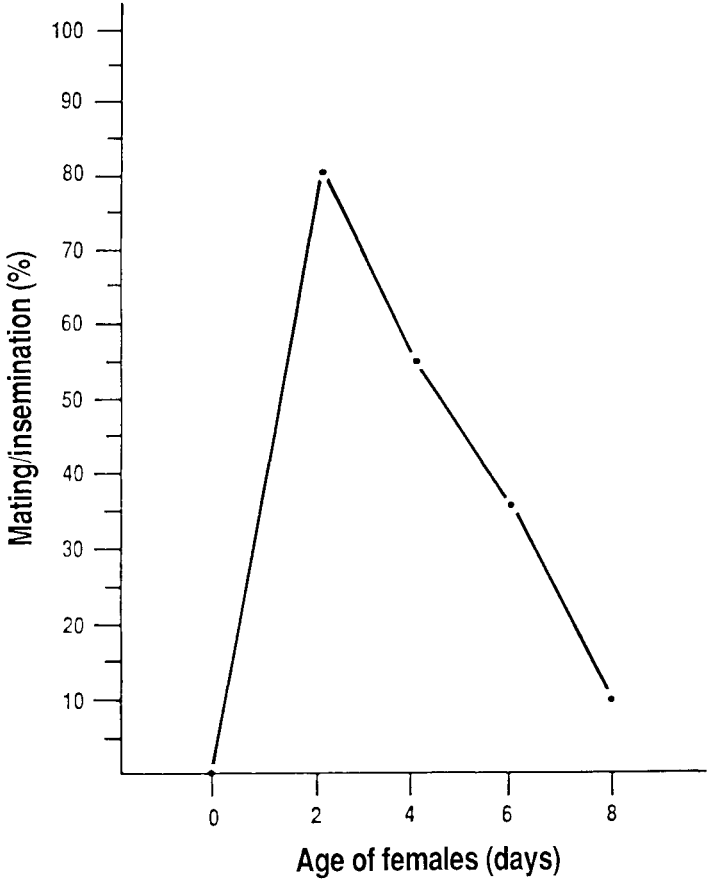

Fig. 1. Natural mating/insemination of *Anopheles pharoensis* relative to female age.

mating was observed in females younger than 2 days of age. Only 10% of the females 8 days of age succeeded in mating/insemination. Sebastian and DeMellon (1967), Lea (1967), Gwadz and Craig (1968), Spielman et al. (1969), Edman et al. (1972) and Reisen et al. (1979) reported insemination problems with female mosquitoes less than 48-hr-old possibly because young females cannot keep semen in an effective condition or the flight tone of young females is not attractive to males.

In comparing effects of mating on longevity, unmated females became gravid after taking 1 or 2 blood meals and oviposited the first batch of eggs after taking an average of 4 subsequent blood meals. Mortality rate was fairly high among uninseminated females with only 12 of 50 (24%) surviving to the first oviposition (Fig. 2). The survivors averaged 13.5 days to the first egg laying. Five of the 12 survived to a second oviposition period which occurred on an average of 16 days, 4 to a third oviposition averaging 22 days, and 3 to a fourth averged 21 days. Females averaged 4 blood meals prior to the first oviposition, but required only a

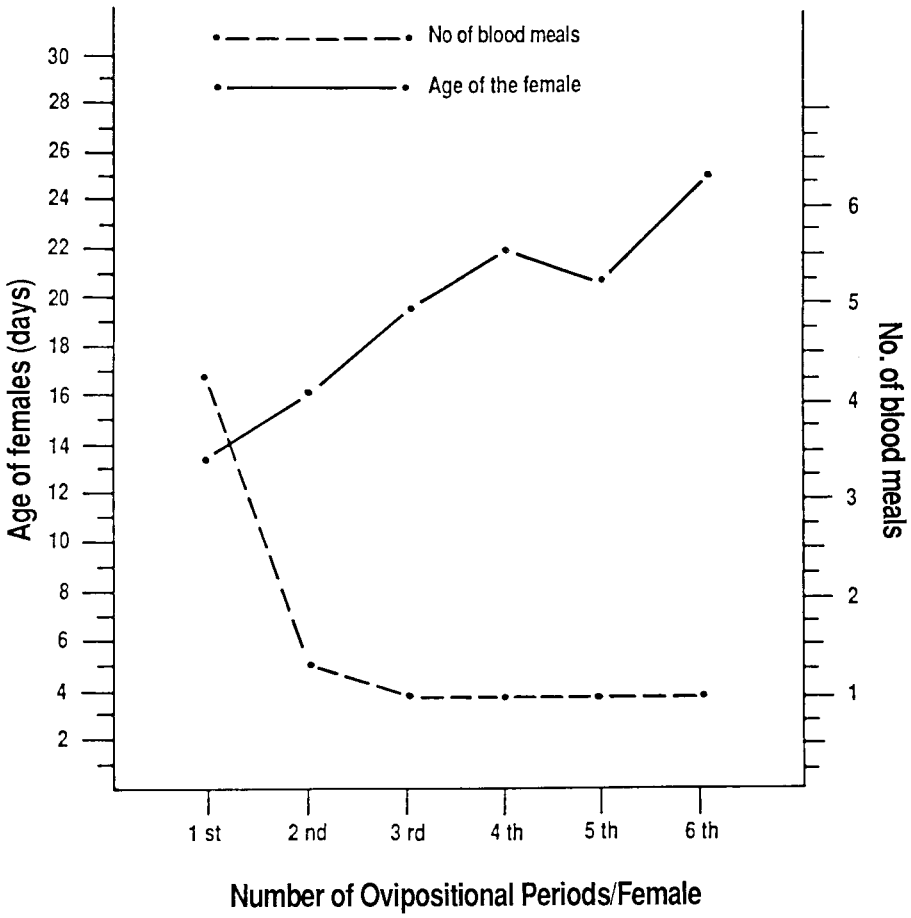

Fig. 2. Average number of days and blood meals required for successive ovipositional periods in *Anopheles pharoensis* females.

single blood meal prior to each subsequent ovipositon periods. Inseminated females (100%) survived and oviposited at 6.6 days and required only a single blood meal. Twenty-six (52%) survived to second egglaying that occurred 2.5 days after the first and required 2 blood meals. Twenty-one (42%) survived to oviposition three, 1.4 blood meals were required, and the third oviposition occurred 3 days after the second. At the fourth oviposition, 16 (32%) were still living.

Blood feeding as the first food source following emergence decreases longevity of the female and reduces egg production efficiency. When a female feeds on blood following emergence, the huge abdomen swollen with blood prevents joining in mating swarms. As the blood is utilized for egg production, the female becomes gravid, but due to the size of the abdomen still cannot join a mating swarm. By

the time the female oviposits the unfertilized eggs, the mosquito is now approximately 2-weeks-old. This is chronologically old for an *An. pharoensis* and if mating now occurs, the number of eggs produced is fewer than would be observed at the first oviposition and will decrease in subsequent ovipositions. By taking a blood meal prior to mating, the female *An. pharoensis* has greatly decreased the potential for offspring production. Nayar and Sauerman (1971) reported a decrease in life span for female *Aedes taeniorhynchus* fed on blood alone. Eggs began maturing immediately after the blood meal, trapping energy and protein from the blood with very little available for general maintenance, and thus the females soon died. It appears that the same process occurs in *An. pharoensis* females that feed only upon blood.

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