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LABORATORY OBSERVATIONS ON THE TIME OF MATING OF *ANOPHELES CULICIFACIES* GILES

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ABSTRACT. Under insectary conditions the Sattoki laboratory strain of *Anopheles culicifacies* emerged during late afternoon and early evening. Mating occurred rhythmically, primarily during the simulated dusk with a few matings occurring during dawn. Females were sexually mature by the dusk of their 2nd day of life, whereas males required an extra day to attain maximum maturity. Maximum male mating activity on day 3 was followed by a rest interval, after which mating activity was again renewed on day 7.

Knowledge of the reproductive behavior of the target species is a vital aspect of any genetic control research. In 1975 *Anopheles culicifacies* Giles, the primary vector of human malaria in the Indo-Pakistan subcontinent, was successfully colonized at our laboratory (Ainsley 1976), and recently several chromosomal aberrations have been induced (Baker et al. 1978). As a prelude to planned field experimentation, studies on the reproductive behavior of this species have been initiated to develop protocols for the manipulation and release of genetically engineered adults.

Previous studies on the swarming and mating behavior of *An. culicifacies* have been restricted to observations on an outdoor insectary population by Russell and Rao (1942) and unconfined wild populations by Reisen and Aslamkhan (1976) and Reisen et al. (1977). In Pakistan pairing was commonly observed at male swarms during winter, but was rarely seen during summer when swarms occurred later in relation to sunset, leading Reisen et al. (1977) to suggest that mating may be occurring elsewhere. However, casual observations under "midsummer" insectary conditions indicated that mating pairs were always formed in flight during periods of male activity.

The purpose of the present series of experiments was to describe the time of mating in a laboratory-adapted colony of *An. culicifacies* under summer day-length conditions to ensure that mating was not occurring at other times of the diel. Additional observations on the time of emergence and the age of males and females at sexual maturity were also included and are of considerable importance in devising release procedures.

METHODS AND MATERIALS

STRAIN. The Sattoki, Pakistan, strain of *An. culicifacies* originally colonized by

Ainsley (1976), was used throughout and was considered well adapted to the continuous insectary conditions of 16:8 LL:DD with a 1.5 hr simulated dusk and dawn, 28 ± 2°C and 70% RH used during the present experiments. Photophase illumination was provided by fluorescent and 60 watt incandescent lights. Dusk was simulated by extinguishing the fluorescent lighting and then gradually dimming the incandescent bulbs over a 1.5 hr period; the procedure was reversed to simulate dawn. The Sattoki strain now readily mates in 1-gal (= 3.8 liter) paper carton cages; however, there seems to be a requirement for communal mating activity, as matings between single pairs rarely occur.

TIME OF EMERGENCE. Six cohorts of 100 larvae were reared from eclosion to pupation in enameled pans (surface area = 144 cm^2). Pupation lasted 5 to 6 days, with maximum pupation occurring 8 to 10 days from eclosion. In the present experiment, pupae picked from the pans on the mornings of days 8 and 9 were placed in separate plastic jars for emergence. Jars were observed throughout the diel, and, with the onset of emergence, the adults were removed at hourly intervals, sexed, and counted.

TIME OF MATING. Females and males were isolated into separate 1-gal carton cages at emergence and held for 2 nights. On day 3, 12 groups of 25 females and 12 groups of 25 males were counted into separate cartons. At hourly intervals starting at 1900 hrs, 1 group of 25 males was released into a 1-gal carton cage containing 25 females. To ensure sufficient time for mating to occur, males and females were kept together for 2 hrs after which all males were removed. The following day, all females were dissected to determine if insemination had occurred. Thus, each group overlapped the next one by 1 hr, (e.g., Group 1 males and females were together from 1900 to 2100 hrs; Group 2 from 2000 to 2200 hrs, etc). Adults were offered 3% sucrose continuously, and the entire experiment was repeated once. Vertical illumination was measured at 5 min intervals during the simulated dusk and dawn with a Kahlsico light meter accurate to 0.1 ft-candles (≈ 1.08 lux) (Kahl Scientific Instrument Corp., El Cajon, CA).

TIME OF FEMALE SEXUAL MATURITY. At emergence 5 groups of 50 females were isolated in separate 1-gal carton cages. On nights 1 through 5 after emergence 50 3- to 4-day old, sexually mature males were added for a 1 night period. To determine if newly emerged females would mate, 100 pupae were allowed to emerge into a 1-gal cage containing 50 3-day old males. On the following morning, all females were dissected and the number of inseminations recorded.

TIME OF MALE SEXUAL MATURITY AND MATING ACTIVITY. At emergence 3 groups of 30 males were released into each of 3 1-gal cages. On days 1 through 13, each group of surviving males was offered 30 sexually mature, 2- to 4-day old virgin females; from day 14 until all the males died, 15 females were offered daily. On each morning the dead males were removed and counted, and the females' spermathecae dissected for the presence of spermatozoa.

RESULTS AND DISCUSSION

TIME OF EMERGENCE. The emergence of *An. culicifacies* males and females began 3.5 hr before the onset of the simulated dusk and continued throughout scotophase and the simulated dawn period, ending by 0700 hr shortly after light-on (Fig. 1). On 2 replicate days, the modes of the emergence peak occurred at 2400 and 2200 hr for females and at 0200 and 2200 hr for males. A total of 72 and 113 females and 101 and 86 males successfully emerged on each of the 2 nights, respectively.

Emergence occurred primarily during scotophase, although apparently not entrained by the transition in illumination from photophase to scotophase. Simila4 results have been reported for other anophelines, e.g., *An. bradleyi* King by Nayar and Sauerman (1970). In some

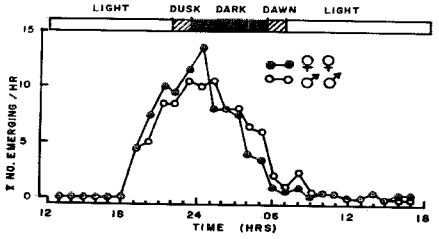

Fig. 1. The time of emergence of male and female *An. culicifacies* under insectary conditions. Mean number of individuals from the same cohort emerging during each of 2 consecutive nights.

Anopheles species pupation and emergence rhythms are entrained early in ontogeny and are truly circadian, persisting under free-running conditions of continuous light or dark, e.g., *An. gambiae* species A (Jones and Reiter 1975). Further research will be required to elucidate the entrainment mechanism and circadian nature of the time of emergence (or pupation) in *An. culicifacies.*

TIME OF MATING. Mating, as indicated by the successful transference of sperm to the female, occurred rhythmically during the simulated dusk and to a much lesser extent, dawn periods; i.e., mating only occurred within those groups which were together for at least a portion of the dusk and dawn (Fig. 2,3). Apparently the turning-off of the fluorescent lights in the insectary which caused a drop in illumination from 592.0 to 18.3 lux stimulated mating activity. Active swarming was also observed in *An. culicifacies* colony cages at this time. No females were inseminated in those periods occurring entirely in complete darkness or complete light. The progressive dimming of the incandescent lighting rapidly decreased the detectable illumination to 0.0 lux by 2210 hrs; however, maximum mating activity took place during the 2200 to 2400 hr period when the detectable illumination dropped from 2 to 0 lux (Fig. 2, 3). These observations agreed well with those of Russell and Rao (1942) who found that during summer in southern India most swarming and pairing activity occurred after sunset (below 22 lux illumination). In Pakistan, Reisen et al. (1977) failed to observe pairing at male swarms from late June through September and suggested that mating may be occurring elsewhere. The present observations, however, indicated that some pairing may have occurred at very low illuminations and thus gone undetected. Only 3 of 46 females were inseminated during the simulated dawn period. Similarly, Reisen et al. (1977) were not able to find dawn swarms or pairs of *An. culicifacies* at sites having considerable

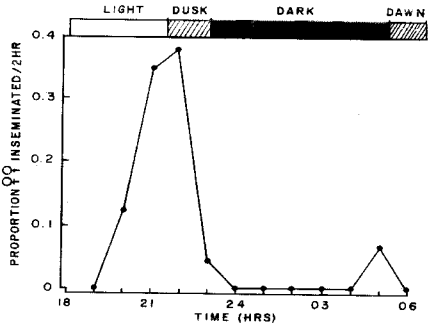

Fig. 2. Time of mating of sexually mature *An. culicifacies* under insectary conditions. Proportion of females inseminated during each consecutive 2 hr. test period presented; points are plotted at the start of each 2 hr. period.

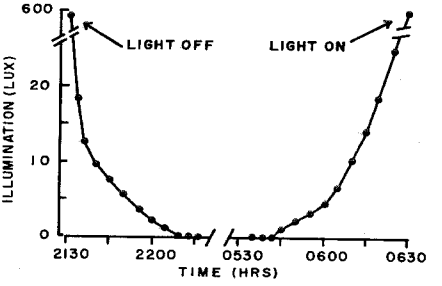

Fig. 3. Change in insectary illumination (lux) during the simulated dusk and dawn crepuscular periods.

swarming and pairing activity during dusk.

TIME OF FEMALE SEXUAL MATURITY. Female *An. culicifacies* confined with sexually mature males were able to mate by their first night of life, i.e. during the dusk following the night they emerged (Fig. 4). None of the females mated the same night that they emerged. Maximum mating activity occurred on nights 2 to 4. Although not actually measured, the quantity of sperm present in the spermathecae of the females mating on night 1 appeared to be less than in the spermathecae of older females.

TIME OF MALE SEXUAL MATURITY. A total of 185 females were inseminated during the life time of the 90 males tested; mean (±S.E.) = 2.06±0.19 females inseminated per male in each cohort of 30 males. This mean agrees well with reported observations on other *Anopheles*, e.g. *An. gambiae* species A = 2.5♀ (Cuellar et al. 1970). Males were capable of successfully inseminating females during the dusk following their night of emergence, however, maximum mating activity occurred when the males were 3 days of age (Fig. 5).

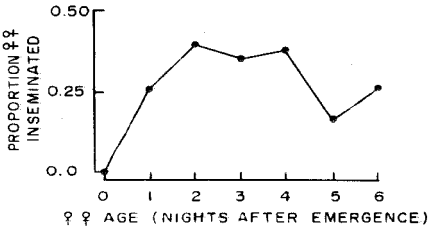

Fig. 4. Time of sexual maturity of female *An. culicifacies* under insectary conditions. Proportion of 50 females progressively aged that were inseminated by sexually mature males during a 1 night test period presented.

Although the actual performance of individual males could not be ascertained, the communal mating patterns summarized in Fig. 5 indicated that the initial increase in mating activity (mode = day 3) was followed by a rest period on day 6 with renewed mating activity on day 7. This pattern was consistent for the 3 cohorts as indicated by fairly low standard

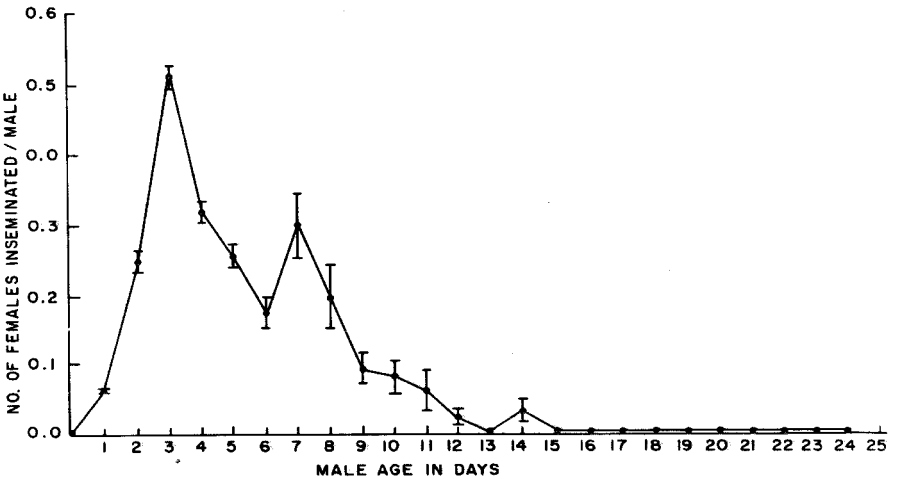

Fig. 5. Time of sexual maturity and mating activity of male *An. culicifacies* under insectary conditions. Mean (S.E. brackets) number of females inseminated per surviving male in each of three cohorts on each day presented.

errors (Fig. 5). In some culicine mosquitoes (e.g. *Cx. tarsalis*, Asman 1975; *Ae. aegypti*, Hausermann and Nijhout 1975) sexually depleted males are not able to remate due to a permanent loss of male accessory gland fluid and/or spermatozoa, or perhaps changes in behavior (e.g. Gwadz et al. 1971, Jones 1973). Although crudely studied in the present investigation, male *An. culicifacies* seemed able to remate following a rest period. Whether or not individual males actually mated to depletion was not detected by the present system; however, the trends depicted in Fig. 5, agreed well with our observations on *Cx. tritaeniorhynchus* where males were tested individually (Reisen et al. 1979). In *Ae. aegypti* the similar recovery of male fecundity after a rest period has been attributed to the maturation of additional spermatocysts (Jones 1967, 1973). Periods of increased mating activity followed by a rest period and renewed mating activity does suggest the recovery of mating ability in *An. culicifacies* males.

In the present experiment the mean (±S.E.) life expectancy of the *An. culicifacies* males at emergence (life table e_1) was 10.85 ± 0.65 days for the 3 replicate cohorts. After day 8 the number of females inseminated progressively declined to 0 by day 15. No further matings were detected after day 14, although maximum male longevity was 24 days. By day 14 only 6, 12 and 12 males remained alive in each cage, and it may be that this number was too few to stimulate swarming and/or mating. Older males appear to retain their fecundity, and spermatozoa are readily visible in both the testes, vas deferens and seminal vesicles (Mahmood et al. unpublished), suggesting that the failure to mate was behavioral rather than physiological. Similar conclusions for the lack of mating activity in depleted *Ae. aegypti* were presented by Jones (1973).

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THE USE OF COMPUTERIZED INFORMATION RETRIEVAL IN MOSQUITO CONTROL

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ABSTRACT. A computerized information retrieval system was designed for Marion County (Indianapolis), IN. Working on a data base of 4 years of site inspection records, the Mosquito Information Retrieval System identifies those sites which have a historical record of producing mosquitoes under conditions similar to the present. The system is designed to run on an interactive timesharing computer system available through most state universities or from commercial computer firms.

In August of 1978, the Division of Public Health-Mosquito Control of Marion County in Indianapolis, Indiana, began using the computerized Mosquito Information Retrieval System (MIRS). With MIRS, the control personnel will now be able to retrieve breeding site information easily, to predict potential breeding sites throughout the control season using past site histories, and to summarize the activities of the program.

Computerized information retrieval is not new to entomology. Foote and Zidar (1975) listed many pre-1970 projects where the computer had come to the aid of the entomologist. Information retrieval has even had a significant effect upon mosquito biology. Crovello (1972) described the Mosquito Data Bank at the University of Notre Dame (MODABUND) and White and Grodhaus (1972) described an off-line information retrieval system for California mosquito collection records. To our knowledge this is the first attempt to utilize computerized information retrieval in an ongoing mosquito control program. This paper will explain the reasons for developing a system such as MIRS, the concept of MIRS, and how MIRS operates.

THE PROBLEM

Across the United States there are areas which use the most sophisticated control techniques and equipment, and there are areas which have no mosquito control program at all. Between these extremes there is a range in sophistication.

The basic procedure begins with a mosquito control operator inspecting a circuit of potential breeding sites. Each circuit may take from 1 to 4 weeks to complete depending upon the number of operators that are employed by the mosquito control program. Each site is inspected for the presence of some stage of mosquito. If mosquitoes are found, a lar-