OBSERVATIONS ON BITING BEHAVIOR OF *AEDES AEGYPTI* (L.)

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INTRODUCTION. Blood feeding behavior of *Aedes aegypti* (L.) has been studied by many workers and the literature has been reviewed by Christophers (1960) and Clements (1963). The conclusions drawn by various investigators regarding variation of the blood feeding behavior of *A. aegypti* with age, time of day, effect of mating or starvation are often different and sometimes contradictory. Much of this difference may be due to difference in experimental conditions. In view of the uncertainty of conditions required for consistent biting, we felt it necessary to quantitate the variables of age, time of day, mating and feeding under ambient laboratory conditions.

DESCRIPTION OF THE COLONY. Our strain of *Aedes aegypti* var. *queenslandensis* Theo. was originally obtained from the School of Tropical Medicine and Hygiene, Liverpool, and has been maintained for several years at the University of California at Davis and San Francisco. Female mosquitoes are fed on guinea pigs and are provided moist filter paper for oviposition. When adults are needed the filter paper is immersed in tap water in an open pan (29 x 18 x 5 cm.). The larvae are fed crushed dog food (Purina Dog Chow). The larval density is kept below 250 per liter of water per pan. Pupae are removed daily, transferred to clean water in beakers and placed in stock cages. As adults emerge, the beakers are moved to a fresh stock cage every 24 hours to get adults of the same day-age in one stock cage. The stock cages are 1 foot cubes made with wooden frames, covered with 40 mesh nylon, with an access sleeve on one side. The usual population of such a cage is less than 400 adults. The adults are supplied with 5 percent sucrose solution on cotton wicks. Laboratory temperature runs from 21–27° C. with a relative humidity of 40–50 percent.

VARIATION OF BITING AVIDITY WITH AGE. Marchoux *et al.* (1903) found that under favorable conditions female *A. aegypti* were ready to bite shortly after emergence, and in general did so readily after 24 hours. Gutzevich (1931) stated that at 28° C. the first blood meal occurred, usually 2–3 days after emergence. Seaton and Lumsden (1941) used completely starved virgin females up to 6 days old on a human host and found few mosquitoes feeding when 1–2 days old. The experiments were conducted between 24.5–25.5° C. and 4.5–5.5 mm. Hg saturation deficiency. Bishop and Gilchrist (1946) fed the mosquitoes in total darkness on chicken and used mosquitoes up to 4 days old only. They also found significantly few mosquitoes feeding when 1–2 days old and most mosquitoes fed when 3 or 4 days old. Their results with the 4-day-old mosquitoes were based on only one experiment. Christophers (1960) reports that in a cage of 100 female and 100 male mosquitoes offered an arm for 15 minutes on the day following emergence, 41 females fed. On the other hand, Gouck and Smith (1962) measuring bites per minute on repellent-treated arms

found increasing avidity to age 6 days. We decided to examine the effect of age on blood feeding in *A. aegypti* in the laboratory, where ambient conditions of temperature and humidity were monitored but not controlled, and also to extend our experiments up to age 10 days.

Female mosquitoes of the same age group were placed into cylindrical cages 14 cm. high and 5 cm. in diameter, covered with 40 mesh nylon net at each end. They were applied for 10 minutes to the shaved abdomen of a guinea pig in a room lighted with fluorescent light and diffused sunlight. Following application the mosquitoes were crushed, allowing us to see a blood spot if the mosquito had fed. No distinction was made between the fully gorged and partly gorged mosquitoes during counting. Ten mosquitoes were used for each experiment. Experiments were conducted at 21–27° C. with a relative humidity of 40–50 percent. One- through ten-day-old mosquitoes were used and experiments with each age were replicated 20 times. The results are given in Table 1.

TABLE 1.—Number of *Aedes aegypti* females age 1 through 10 days feeding on guinea pig.

Age of mosquitos in days	1	2	3	4	5	6	7	8	9	10
No. fed [a]	0.9 [b] ±0.02	6.65 [b] ±0.4	8.3 ±0.3	8.8 ±0.02	9.2 ±0.1	8.3 ±0.3	8.2 ±0.3	7.8 ±0.3	7.7 ±0.3	8.3 ±0.2

[a] Each figure is a mean of 20 replicates of 10 mosquitoes each ± S.E.
[b] Significantly low: $P < .01$.

Significantly few mosquitoes fed when one or two days old but from the third day on biting avidity remained high until the tenth day. We conclude that mosquitoes between 3 and 10 days old may be used to obtain consistent results in experiments involving blood feeding.

VARIATION OF BITING AVIDITY WITH TIME OF DAY. The observation that *A. aegypti* is principally a day biter is based on free flying mosquitoes (Dutton, 1903; Goeldi, 1905; McClelland, 1959, 1960). In the laboratory the species feeds readily at all times of the day, in light or in dark (Christophers, 1960). Bishop and Gilchrist (1946) could feed *A. aegypti* on chickens in darkness and up to 94 percent gorged in 30 minutes. Seaton and Lumsden (1941), found that the effect of light (illumination 0.5 meter candles) was to reduce the number of mosquitoes biting by almost half. The previously cited studies of Gouck and Smith (1962) measuring biting rates on repellent-treated arms showed much higher rates in the afternoon than in the morning. Besides these observations, which do not fully substantiate that mosquitoes feed equally at night or during the day, nothing is known about the biting potential of this species over a 24-hour period.

We conducted biting experiments in the laboratory, in natural light during the day and in darkness at night, twice on one human subject and once on each of three others, a total of 5 replicates. The occurrence of blood feeding was determined by crushing the mosquitoes. Results are given in Table 2. No significant difference was found in the number of mosquitoes feeding at different times of the day or night. We conclude that laboratory biting experiments need not be restricted to any particular time of day.

VARIATION OF BITING AVIDITY WITH STARVATION. Most workers who have used mosquitoes for biting experiments have fed them 5–10 percent sugar solution up to the time of feeding them blood. Lums-

TABLE 2.—Biting avidity of *Aedes aegypti* females with time of day.

Hour of biting	Day						Night					
	0600	0800	1000	1200	1400	1600	1800	2000	2200	2400	0200	0400
Number fed [a]	7.4 ±1.6	6.8 ±1.4	8.6 ±0.9	9.4 ±0.5	9.6 ±0.2	9.5[c] ±0.4	8.2 ±0.7	7.5 ±0.9	9.4 ±0.2	9.2[b] ±0.5	9.6[c] ±0.4	9.2 ±0.5

[a] Each figure is a mean of 5 replicates of 10 mosquitos each ± S.E.
[b] N=4.
[c] N=6.

den and Bertram (1940) fed 40 percent cane sugar for the first 2 days and then gave nothing. Seaton and Lumsden (1941) and Bishop and Gilchrist (1946), in their experiments fed nothing to their mosquitoes until they were used. Christophers (1960) says that some effect upon biting potential was thought to result when insects had been previously allowed to feed on sugar, fruit, and even water, shortly before the experiment. We wanted to compare the biting potential of sugar-water fed mosquitoes with that of completely starved ones.

Mosquitoes were divided into two groups on emergence. One group was allowed free access to 5 percent sucrose solution up to the time of biting, while the other was given nothing. Ten mosquitoes of the same age group were placed in a biting cage and applied to the shaved abdomen of a guinea pig for 10 minutes. Biting was determined by finding blood in crushed mosquitoes, as mentioned previously. Each day five such replicates were run with the sugar-water fed and the starved groups. The experiments were terminated after 5 days because of the attrition rate of starved mosquitoes at this time. We found that the starved mosquitoes bite at a rate identical to that of sugar-water fed mosquitoes (Table 3). We conclude that starvation up to 5 days does not diminish biting vigor and also that sugar-water does not appear to affect the biting potential.

TABLE 3.—Number of sugar-water fed and starved *Aedes aegypti* females feeding on guinea pig.

Age of mosquitoes in days	Number of mosquitoes feeding [a]	
	Sugar-water fed	Starved
1	1.8±0.6	1.4±0.5
2	5.2±0.7	4.9±0.8
3	7.6±0.5	7.6±0.6
4	8.7±0.7	8.7±0.3
5	9.0±0.5	8.9±0.5

[a] Each figure is a mean of 10 replicates of 10 mosquitoes each ± S.E.

VARIATION IN BITING AVIDITY WITH MATING. Howard (1923) reported that virgin females would feed, but that fertilized females were more greedy. Seaton and Lumsden (1941) showed that the presence of males made no difference in the number of females feeding on blood, but they used only 3–4 day old mosquitoes in their tests. Their mosquitoes were also previously starved before feeding. Lang (1956) found biting rates to be the same for virgin and mated females, but used only 4–5 day old mosquitoes. Burgess (1959) showed a 4–6 day rhythm in probing activity of virgin female *A. aegypti* who were fed 10 percent sugar solution. He found very low probing activity on day 6 in response to a current of warm moist air. We have examined this question using sugar-water fed females and carrying the experiment up to age 10 days.

To obtain unfertilized females, sexes were separated at the pupal stage. Mated females were obtained from a cage of mixed sexes. In each experiment ten females in a cylindrical cage were applied to the shaved abdomen of a guinea pig for 10 minutes. Ten replicates were run with each age group from 1–10 days. Experiments with mated and unmated mosquitoes were carried out simultaneously.

TABLE 4.—Number of mated and unmated *Aedes aegypti* females 1 through 10 days old feeding on guinea pig.

Age of mosquitoes in days	Number of mosquitoes feeding [a]	
	Mated	Unmated
1	0.0	0.0
2	7.9±0.2	8.2±0.2
3	9.0±0.2	9.2±0.2
4	9.4±0.2	9.0±0.3
5	9.4±0.2	9.4±0.1
6	9.3±0.2	6.5±0.5 [b]
7	9.3±0.3	8.9±0.2
8	9.3±0.3	8.8±0.2
9	7.9±0.2	7.9±0.3
10	7.8±0.2	7.9±0.3

[a] Each figure is a mean of 10 replicates of 10 mosquitoes each ± S.E.

[b] Significantly low: $P<.01$.

It was found that biting rates for virgin and mated *A. aegypti* were identical except for a significant decrease in biting by the virgin mosquitoes on day 6 (Table 4). Interestingly, this low biting avidity in virgin females on day 6 falls on the same age-day on which Burgess had noted low probing activity in virgins.

SUMMARY. Observations were made on the biting avidity of adult *Aedes aegypti* females with regard to age of the adult, time of day of feeding, effect of feeding sugar-water and starvation, and effect of mating. Few mosquitoes fed when one and two days old but from the third day on biting avidity was found to be high. No significant difference was found in the number of mosquitoes feeding at different times of the day. Sugar-water feeding or starvation up to five days did not affect avidity of mosquitoes for blood. The biting avidity of females was equally high whether mated or unmated, except that unmated females bit significantly less when six days old.

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LABORATORY STUDIES OF OVIPOSITIONAL PREFERENCES OF *AEDES AEGYPTI*[1]

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In programs such as the *Aedes aegypti* eradication campaign in continental U.S.A., Puerto Rico, and the Virgin Islands (Schliessmann, 1964), a surveillance method for detecting the pertinent species at low population levels is a necessity. The data obtained would assist in determining the extent of area to be treated and the point in time for termination of the field applications.

One method for surveillance is the detection of adult female mosquitoes through their egg-laying activities. Although this approach samples the females only at intervals when oviposition occurs, the specificity of the egg-laying sites, *i.e.*, artificial containers or tree holes, limits the environment to be sampled and provides sensitivity in the method. Evidence of egg laying coupled with the limited flight range of the females enables quite accurate delineation of the field area requiring detailed examination. Moreover, confusion of *A. aegypti* eggs with those of other species is limited probably to those of *Aedes triseriatus*.

The present paper describes laboratory studies conducted to secure an attractive egg-laying site based on female *A. aegypti*

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