OBSERVATIONS ON THE REPRODUCTIVE HISTORY OF *AEDES (STEGOMYIA) ALBOPICTUS* IN THE LABORATORY[1]

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During comparisons made by Gubler (1970) of the reproductive potentials of *A. albopictus* and *A. polynesiensis*, isolated *A. albopictus* females refused to take blood or oviposit in the small containers. Information on the individual gonotrophic cycles of this species is essential for evaluation of the relative reproductive potentials of these two species, a vital factor in competitive displacement (Gubler, 1970a). Also, information of this kind is required for observations now underway on the population dynamics of *A. albopictus* in nature. The purpose of this investigation, therefore, was to observe the gonotrophic activity of individual females and record their reproductive history.

MATERIALS AND METHODS. The *A. albopictus* were taken from a colony recently established from eggs collected in Calcutta. Eggs were hatched in deoxygenated water and the larvae reared in large aluminum pans. Larval food consisted of brewer's yeast tablets and small quantities of cut-up dog biscuits.

Newly emerged females were placed individually in small cages measuring 20 x 25 x 15 cm. Two to three newly emerged males were placed in the cage with each female to insure insemination since virgin *A. albopictus* do not normally oviposit (Gubler, 1970b). Each cage was provided with a 10 percent sucrose maintenance diet and an 80-mm-diameter crystallizing dish containing tap water and lined with paper toweling for oviposition.

The first blood meal was offered on the first day after emergence and daily thereafter until complete engorgement was achieved. Blood was not offered again until after oviposition, or until 6 to 7 days had elapsed without oviposition. In either case, blood was again offered daily until the female had fed to repletion. Only human blood was offered. The cages were observed daily for eggs and mortality.

All mosquitoes were maintained in an air conditioned room at about 26° C.

RESULTS AND DISCUSSION. The number of eggs laid by the average *A. albopictus* female in each gonotrophic cycle, the number of days per cycle, and the proportion of females requiring two or more blood meals in each cycle are presented in Table 1. The mean number of eggs in the first cycle was 63.1, a figure which is remarkably close to the 62.4 eggs observed by Gubler (1970) for this same species. Moreover, the mean number of eggs in subsequent cycles remained fairly constant until about the tenth cycle, although the individual female variation from cycle to cycle was considerable. For example, one female laid 113, 80, 68, 67, 20, 88, 79, 85, 26, 20, 80, 35 and 23 eggs in 13 gonotrophic cycles respectively. Most of the females exhibited such variability in fecundity; however, the fluctuations in cycles of individual females did not coincide. They were not apparent from the data presented in Table 1.

A fluctuating oviposition pattern does not agree with some observations on other mosquitoes. Generally there is a decreased fecundity with age because more follicles degenerate in each successive cycle. This has been found to be the case for *Aedes*

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TABLE 1.—Mean number of eggs laid by *A. albopictus* females per gonotrophic cycle, length of time required for each cycle, and the percent females requiring 2 or more blood meals to complete each cycle.

Cycle No.	No. ♀♀	Total eggs	$\bar{x}$ No. eggs	$\bar{x}$ No. days per cycle	% ♀♀ requiring 2 or more blood meals
1	38	2398	63.1	4.5	12.5
2	32	1826	57.1	4.4	20.0
3	29	1369	47.2	4.5	6.5
4	23	1150	50.0	4.5	0
5	17	935	55.0	5.0	10.5
6	16	1171	73.2	4.8	12.5
7	13	777	59.8	5.4	13.3
8	9	513	57.0	7.5	22.2
9	5	211	42.2	3.3	0
10	4	113	28.2	4.1	0
11	3	201	67.0	5.0	0
12	2	71	35.5	3.5	0
13	1	23	23.0	4.0	0
TOTALS	38	10,758	283.1	4.6	

aegypti and *A. polynesiensis* (Putnam and Shannon, 1934; Ingram, 1954; Gubler, 1970). Moreover, the latter author found that the average *A. polynesiensis* female laid 84.6 percent of her total eggs in the first 3 gonotrophic cycles. The average *A. albopictus* female, on the other hand, lays only 52.0 percent of her eggs in the first 3 cycles. Some *A. albopictus* females do show a progressive decrease in the number of eggs laid with increasing age, but the majority do not until they are quite old. Furthermore, it appears that females with a fluctuating oviposition pattern survive the longest; this was true of 12 of 13 females which oviposited 7 or more times.

Ovarian dissections of females collected in the field indicate that only rarely do most of the follicles develop in a particular gonotrophic cycle. A gravid female may have only a fourth of the follicles in Stage V, with the rest in Stage II and with one or more follicular relics on many of them. The lack of complete follicular development in each cycle is probably common among most mosquitoes, but it appears to be very pronounced in *A. albopictus*. Possibly there is some factor other than the amount of blood ingested which establishes the number of follicles that will develop in any one cycle. All females in the present study were nearly always fully engorged. Lowrie (personal communication) has found that the original weight of *A. albopictus* females was more important than the size of the blood meal in determining the size of the egg batch.

The number of gonotrophic cycles completed by *A. albopictus* females ranged from 0 (2 females not included in Table 1) to 13, and the total lifetime egg production from 0 to 784 eggs. The average female, however, oviposited 4.8 (5) times and laid a total of 283.1 eggs, or 58.9 eggs per batch. The total average egg production in these experiments is somewhat less than that obtained by Gubler (1970) for this same species in larger cubic foot cages. However, it is nearly identical to the figure recorded when the females were kept in the smaller gallon carton cages. The latter had about the same area as the cages used in the present experiments. Thus, 283 eggs per female is probably a low estimate and possibly could be increased if larger cages were used. On the other hand, it is probably an over-estimate of the actual fecundity of female *A. albopictus* in nature. Dissections of wild females from our Calcutta study area suggest that the average female may oviposit only once or twice in her lifetime.

Macdonald (1956) found that in *A. aegypti* the first gonotrophic cycle was the longest because in subsequent cycles the ovaries were already in Stage II of development when the blood meal was taken. This was not the case for *A. albopictus*, as shown in Table 1. The number of days for each cycle ranged from a low of 3.3 days in cycle number 9 to a high of 7.5 days in cycle number 8. Moreover, there was no trend of either increased or decreased cycle time with age. On the contrary, the time between ovipositions remained relatively constant throughout the life of the female, with an average time of 4.6 days. Thus, those females that survived several cycles fed and oviposited eggs at the same rate as younger females.

A few females required two or more blood meals to develop their eggs; however, none of the females surviving 9 or more cycles exhibited this need. This was probably because it was only the most healthy and vigorous individuals that survived 9 cycles. The majority of those females requiring two or more blood meals to develop their eggs died before the next cycle was complete. Thus, 5 out of the 38 females required more than one blood meal for the first batch of eggs, and none survived through the second cycle. During the entire period of the experiment, females required two or more blood meals 22 times, and of these, only 6 lived to complete the next cycle. However, 4 of these 6 females required only one blood meal for the next cycle. The factor determining the number of blood meals required to develop eggs was probably not the amount of blood taken, as all of the females were offered blood until they had completely engorged or had taken at least two partial meals consecutively. Two blood meals were considered to be separate ones only if there was an intervening period of at least 5 days (the average cycle time). Macdonald (1956) found that larval rearing conditions influenced the number of meals required by *A. aegypti* to develop the first batch of eggs.

The *A. albopictus* used in the present experiments were reared in uncrowded pans with as much food as could be given without causing a scum on the water surface. Therefore, larval rearing conditions should not have been the determining factor. Had it been a lack of larval food, the proportion of females requiring two or more blood meals to develop the first batch of eggs should have been higher than for subsequent cycles. This was not the case. Possibly in a certain proportion of the females there is some inherent requirement for additional blood meals. It could be a characteristic of females about to die. This is suggested by the fact that the majority of these females did not survive to complete another gonotrophic cycle. Still another possibility is that the oviposition container and media were not attractive enough to stimulate oviposition, and had these females been placed in shell vials on a moist substrate, only one blood meal would have been required.

Of the 38 females, the mean time from emergence to the first blood meal was 2.0 days. There was 0.5 day difference between the females requiring one blood meal (1.9 days) and those requiring two or more blood meals (2.4 days). However, only 5 females required two or more blood meals for the first cycle, and the difference apparently is not significant ($t=1.28$; $p>0.05$).

The average female in these experiments survived only 38.0 days as compared to 30.3 days for males. Maximum survival was 73 and 68 days respectively. This LT_{50} value for females was only about half that observed by Gubler (1970), and no explanation can be offered for this difference, other than that the former study involved the Poona strain of *A. albopictus*, which had been colonized for a longer period of time and may have been better adapted to laboratory conditions. The relative humidity in the laboratory was only 50 to 60 percent, but this factor has been shown not to affect survival of *A. albopictus* (Hylton, 1969; Gubler, 1970).

The results of these experiments support the conclusion of Gubler (1970) that

A. albopictus has a reproductive advantage over *A. polynesiensis,* which should operate in its favor should these species occur in the same area, where they would compete with one another. However, additional observations are necessary on reproductive potential of natural populations, in view of the suggestion given above that wild *A. albopictus* females oviposit only once or twice.

SUMMARY AND CONCLUSIONS. Blood feeding and oviposition cycles were determined for *A. albopictus* females taken from a colony established in Calcutta, India. The number of cycles ranged from 0 to 13, with an average of 4.8, and the average number of eggs per female was 283.1, or 58.9 per batch. The numbers of eggs produced in successive batches by individual females were erratic. The number of days for each cycle ranged from 3.3 to 7.5, with a mean of 4.6. Some females required two or more blood meals to develop a batch of eggs; this did not appear to be correlated with the size of the blood meal. Although these laboratory reared females feed and oviposit repeatedly, the suggestion is made that in nature *A. albopictus* females may oviposit only once or twice.

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