EGG FORMATION AND OVIPOSITION IN BLOOD-FED *AEDES AEGYPTI* L.

ROBERT C. WALLIS [1] AND CALVIN A. LANG [1, 2]

Investigation of the blood meal factor which is required by adult female mosquitoes for egg production necessitates the development of a suitable assay to evaluate test diets. Some of the conditions of such an assay have been studied, and it was noted that maximal egg deposition was related to mating. Such a relationship has been discussed by several investigators (MacGregor, 1931; Mellanby and Mellanby, 1937) but the recent studies of Gillett (1955) demonstrated this with a strain of *Aedes aegypti*. From more recent studies, Lang (1956) suggested that the blood meal is the primary stimulus for egg formation, whereas mating is concerned chiefly with oviposition. The results of further experimentation to elucidate these interrelationships are presented here.

EXPERIMENTAL PROCEDURE. Larvae of *Aedes aegypti* were reared on a commercial feed medium in enameled pans. Males and females were separated in the pupal stage. Adults were divided into two groups: one contained only virgin females, and the other contained females plus an excess number of males. Both groups of mosquitoes were maintained on a 10 percent sucrose solution for the remainder of the experiment. After 7 to 10 days from emergence, the mosquitoes were deprived of food and water for approximately 20 hours and then offered a blood meal from the forearm of a human host. Following this meal, engorged females from each test group were transferred to individual test cages, and two males were added to the cage of each "mated" female. The control series of both virgin and mated females was maintained on sucrose solution alone. For the next 14 days of the experimental period the cages were kept in a humid condition (greater than 70 percent R. H.) at a temperature of 23–25° C. Cages were examined daily, and the oviposition time of each female was noted. This experiment, which included a total number of 128 virgin and 46 mated females, was replicated 4 times.

In the first three experimental series each female was dissected on the 14th day post-blood meal and examined microscopically after oviposition to ascertain the number of eggs retained in the abdomen. In addition, every mated female was examined to determine the presence of sperm in the spermathecae.

To obtain information on ovarian development and egg formation, a series of mosquitoes from all groups were dissected at daily intervals from the time of emergence through a four-day period following engorgement.

[1] The Connecticut Agricultural Experiment Station, New Haven, Connecticut.

[2] Present address—Division of Medical Entomology, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Maryland.

RESULTS. Of a total number of 128 virgin females, 55 percent deposited eggs within seven days of the blood feeding. Of the 46 mated females, 89 percent oviposited during the same period. The average number of eggs from females in each group is shown in Table 1. The eggs per female ratios based on the observed data were significantly less than those ratios of corrected data in which the females laying less than 20 eggs or retaining eggs were excluded in the calculation.

A large percentage of mosquitoes retained eggs after 14 days as shown in Table 2. The incidence of retention in the virgin group was approximately three times greater than in the mated females.

Of these females retaining eggs, the number retained was compared with the number oviposited (Table 3). The sum of these two counts provided an index of the number formed. The 25 virgins oviposited an average of 26 eggs per female and retained an additional 56 eggs per female. Therefore, 31 percent of the total eggs formed were deposited and 69 percent were retained. The three mated females oviposited 20 percent and retained 80 percent of the total eggs formed.

Spermatozoa were observed in the dissected spermathecae of all but four of the 46 mated females.

Daily examination of ovaries dissected between the time of emergence and oviposition revealed marked changes in ovarian size and development. Up to the time of the blood meal, the ovaries of all females progressed to a stage of development similar to Christopher's Stage 2, which was characterized by the appearance of spherical, transparent follicles. No further development was observed in any of the mosquitoes which engorged only on sucrose. In the blood-fed females, however, rapid ovarian changes occurred on the second and third day post blood meal, resulting in a stage similar to Christopher's Stage 4 or 5. During this period the follicles became elongated and filled with dense, opaque granules. A clear polar region was apparent at one end. By the 4th post blood meal day the eggs appeared ready for oviposition. The only difference observed between the fed mated and virgin females was a slight delay in development of the latter through the second day following the blood meal. There appeared to be a greater variation in ovarian development among the blood-fed virgins in contrast to the uniform response in the mated group.

DISCUSSION. In studies of nutritional factors in the blood meal which stimulate ovarian activity and subsequent egg formation, the criterion commonly used for evaluation of the potency of test diets has been the number of eggs laid per female after the mosquito has engorged on a test fluid. Such an index is based on the assumption that only the blood meal is important in the subsequent oviposition response. However, recent evidence indicates that other factors influence oviposition (Woke, 1955) so that an egg count may not be an accurate indication of the nutritional potency of the meal.

Gillett (1955) has reported that one strain of *Aedes aegypti* failed to lay eggs unless mating took place. Furthermore, in a recent report, Lang (1956) indicated that there was a difference in the number of eggs oviposited by mated and virgin *Aedes aegypti*. The question arose as to whether mating played a role in stimulating egg formation or affected only the oviposition response.

The results presented here confirmed the previous finding that the oviposition time of virgin females is longer and more variable than that of mated females. In addition, a higher percentage of the virgins retained eggs, and in these individuals the number of eggs retained was sizable—amounting to approximately 70 percent of the number of eggs formed—despite the fact that they developed more eggs than mated females.

While mating affected the number of eggs deposited, and to some extent the number developed in the female, dissection of individual specimens at 24-hour intervals during the experimental period

TABLE 1.—Egg deposition by *Aedes aegypti* 14 days post blood meal

	Virgin			Mated		
		Eggs/Female			Eggs/Female	
Experiment	Number	Observed	Corrected*	Number	Observed	Corrected*
1	88	68.5	87.7	31	54.6	73.6
2	40	56.9	72.9	15	57.7	65.2
Combined	128	64.9	83.1	46	55.6	70.5

* Corrected values exclude those females laying less than 20 eggs or retaining eggs.

TABLE 2.—Number of *Aedes aegypti* retaining eggs 14 days post blood meal

Replicate	Total Number	Number Retaining	Total Number	Number Retaining
1	29	5	10	0
2	30	12	11	2
3	29	8	10	1
Combined	88	25	31	3

TABLE 3.—Egg formation by *Aedes aegypti* retaining eggs

	Virgin				Mated			
		Eggs/Female				Eggs/Female		
Replicate	Number	Oviposited	Retained	Formed	Number	Oviposited	Retained	Formed
1	5	19.6	50.5	70.2	0	....	....	....
2	12	25.5	63.3	88.8	2	17.0	46.0	63.0
3	8	30.3	48.8	79.0	1	0	53.0	53.0
Combined	25	25.8	56.1	82.0	3	11.3	48.3	59.7

revealed little influence of mating on ovarian development. Rapid changes took place within three days post blood meal in individuals of both the virgin and mated groups. In comparison, no such stimulation of activity appeared in females engorged only on sucrose. It is possible, therefore, to evaluate a test diet by examination of dissected ovaries two to three days after administration of the test preparation. This provides a rapid assay devoid of the variation associated with the oviposition response.

Clements (1956) concluded that gonadotrophic hormone from the corpora allata is not responsible for the stimulation of rapid ovarian development. He summarized his discussion thus:

"It seems unlikely that, where ovary development takes place despite ligation within a very few minutes of the start of feeding, sufficent hormone can pass into the abdomen. If hormones are secreted before feeding takes place, some chemical factor in the food or some behavioural stimulus from feeding may trigger ovary growth."

In the experimental series of females which engorged on sucrose solution alone, dissection and examination of the ovaries at daily intervals revealed no stimulation of ovarian activity, so that it seems un-

likely that a behavioural stimulus such as abdominal distension following feeding is involved. Rather, it is suggested that stimulation is associated with a chemical factor in the blood meal, and further work is essential for the identification of this factor.

SUMMARY. Laboratory experiments were conducted using blood-fed virgin and mated *Aedes aegypti* to determine the effect of mating on ovarian development and oviposition. The average oviposition time of the virgin females was considerably greater than that of the mated ones. Furthermore, the oviposition time of the virgins was variable compared to a relatively uniform response of the mated females. The average number of eggs per female in both groups was increased significantly by excluding from the calculation those females which retained or laid few eggs. Females retaining eggs deposited only 20–30 percent of their total eggs formed. In both virgin and mated females ovarian development was arrested at Christopher Stage 2 unless a blood meal was ingested. Therefore, mating affects the oviposition response but has little effect on ovarian development and egg formation. Abdominal distension from engorgement with sucrose solution failed to stimulate development comparable to that following engorgement with blood. Dissection and examination of the ovaries of blood-fed females two to three days post blood feeding provides a rapid assay for the presence of an ovarian stimulating factor in an experimental preparation.

Bibliography

CHRISTOPHER, S. R., STINTON, J. A., and COVELL, G. 1936. How to do a malaria survey. Hlth. Bull. Malar. Inst. India, No. 14 (4th ed.), iv and 147.

CLEMENTS, A. N. 1956. Hormonal control of ovary development in mosquitoes. J. Exp. Biol. 33:211–223.

GILLETT, J. D. 1955. Behavior differences in two strains of *Aedes aegypti*. Nature 176:124–125.

LANG, CALVIN A. 1956. The influence of mating on egg production by *Aedes aegypti* L. Am. J. Trop. Med. and Hyg. 5:909–914.

LANG, CALVIN A., and WALLIS, ROBERT C. 1956. An artificial feeding procedure for *Aedes aegypti* L. Am. J. Trop. Med. and Hyg. 5:915–920.

MACGREGOR, M. E. 1931. The nutrition of adult mosquitoes: Preliminary contribution. Trans. Roy. Soc. Trop. Med. Hyg. 24:465–473.

MELLANBY, H., and MELLANBY, K. 1937. Rearing Tsetse flies in captivity. Proc. Roy. Ent. Soc. Lond. A. 12:1–3.

WOKE, PAUL A. 1955. Deferred oviposition in *Aedes aegypti* (Linn.) Ann. Ent. Soc. Amer. 48: 39–46.