THE LIFE CYCLE OF LABORATORY-BRED ANOPHELES ALBIMANUS WIEDEMANN

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The success of the Americans, under the leadership of Gorgas, in the fight against tropical diseases in Panama is now an old and well-known story. In the early construction days of the Panama Canal, the force of necessity directed the anti-mosquito campaigns against all of the Culicidae; there was no time to determine which were the important vectors of malaria, to study their habits, and to concentrate on them alone. Nevertheless, basic research was soon started; systematic studies were made by Jennings, Zetek, and Dyar; Darling (1910), in his infection experiments, found that *Anopheles albimanus* was the important malaria vector, while the Health Department of the Panama Canal began to accumulate a vast amount of data on the breeding habits of the Anophelines. The investigations on the mosquitoes have been maintained continuously by the Health Department; Dr. D. P. Curry has added to our knowledge of both the systematics and bionomics of the Isthmian Anophelines. Although we now know fairly well the breeding habits of the *Anopheles* larvae of Panama, a more thorough knowledge of the bionomics of the malaria vectors would be of aid in the solution of the malaria problem, and as a complete record of the life cycle of *Anopheles albimanus* bred in the laboratory might facilitate observations on its habits in nature, this report on the various stages in the life history of this mosquito is presented.

MATERIAL AND METHODS

The technique for breeding *A. albimanus* in the laboratory has been published in a separate paper (Rozeboom, 1936), but the essential points of the procedure would bear repetition.

The adults were kept in a screen cage 2 by 2 by 2 feet in size, and with a temperature of 80° to 86° F. and with a relative humidity of around 80 per cent. The temperature of the larval breeding water varied from 21° to 27° C., and that of the water containing the eggs and pupae from 27° to 30° C. The laboratory room temperature usually remained between 82° and 89° F., although occasionally it went down to 80° F., and at night, when the windows of the laboratory were closed, it rose at times above 89° F.

The eggs were removed from the breeding cage in the morning, and allowed to hatch in the laboratory. After a batch of eggs had hatched, the larvae were counted, and transferred to the breeding pans, which were kept out of doors. The breeding water consisted of well ripened alfalfa infusions, and the larvae were given fresh Fleischmann's yeast daily. Pupae were removed from the basins at least once a day, placed in cups of fresh tap water, and taken into the laboratory. Lantern globes were fitted above the cups, and after emergence, the adults were transferred to the breeding cage.

THE LIFE CYCLE

The Egg Stage.

Development of Ovaries and Oviposition.—Information concerning the egg-laying capacity of individual females, as well as evidence as to the period of time necessary for ovarian development, were obtained by the following experiment, which for convenience will be designated as *Experiment 1*. Ten engorged females and fifteen males, which had emerged about 12 hours previously, were placed inside a breeding cage similar to the one in which the stock colony is maintained. The mosquitoes were fed sugar-water and fruit, and the females were offered human blood daily. On the eighth day seven females were still alive; they were imprisoned singly in lantern globes, and each globe was placed above a cup of water. These globes were kept inside of the breeding cage, in order to keep the mosquitoes in an environment with a temperature of 80° to 86° F., and a relative humidity of around 80 per cent. Pads of cellucotton moistened with sugar-water were placed on the bobbinet tops of the globes, and each female was given an opportunity to suck human blood every day. A record was kept of the blood meals, the days when oviposition occurred, the number of eggs in each batch, and the number of larvae that hatched from the eggs. The females were dissected at the time of their death and the ovaries examined under a microscope.

Six of the seven mosquitoes deposited from two to six batches of eggs. Two oviposited for the first time during the night of the 9th day, three more during the night of the 10th day, and one during the night of the 12th day. Subsequent batches of eggs were usually deposited in intervals of two or three days, but occasionally a mosquito would wait a longer time between batches; one female laid her second batch eight days after the first. When the laboratory colonies were started, the first females to oviposit did so sooner than those in Experiment 1. Two separate laboratory colonies were established; the first eggs appeared after a period of seven days in one case, and after nine days in the other. It would seem, therefore, that a week, and usually a little more, is necessary for the maturation of the first eggs. It seems probable that the more unfavorable conditions accompanying imprisonment in a small container over a cup of water were the reasons why the females of Experiment 1 waited longer before ovipositing than did those in the laboratory colonies. This is shown most strikingly in the case of the female that refused to lay eggs. This insect lived for 24 days, and although her abdomen was distended with mature eggs, she refused to deposit them. She was also very reluctant to feed.

The smallest number of eggs in a single batch was 29; the largest number was 262. A total of 2,610 eggs were obtained in 20 separate batches, an average of 130 eggs per batch. The smallest number of eggs from any one female was 184, while the largest number was 788. As the 2,610 eggs were deposited by six insects, an average of 435 eggs were obtained per female. However, dissections showed that the only mosquito that appeared to have deposited all of the eggs she was capable of developing was the female that laid 788 eggs. At the time of her death her ovaries were entirely depleted. All of the remaining mosquitoes were found to have ovaries filled with eggs in various stages of development at the time they died. Although the number of insects used in this experiment was small, the results show that the egg-laying capacity of *A. albimanus* is similar to that of *A. tarsimaculatus* and *argyritarsis*. Moore (1929) demonstrated that females of both of these species can lay approximately 1,000 eggs apiece. Earle (1932) found that *A. albimanus* females averaged 160 eggs each, but this number would appear to be too small.

Of the 2,610 eggs, only 1,188, or 45.5 percent, hatched. This low percentage of fertility was probably due to the fact that after the females had been imprisoned in the lantern globes, they no longer had access to males; copulation was possible only during the period before the first eggs had matured. Thus the mosquito (#4) that deposited 788 eggs, did so in six batches; only 290 of these eggs were fertile, none of the eggs belonging to the last two batches, and only 11 of the fourth batch, being viable. On the other hand, two mosquitoes deposited nothing but non-viable eggs at first, while their second batches were fertile.

Immediately after laying eggs, these females were ravenous. Not a single mosquito in Experiment 1 failed to feed readily after oviposition. One blood meal was sufficient for the development of the second and subsequent batches, but occasionally two or three meals were taken between layings.

Oviposition was actually observed only once. One morning a gravid female belonging to the stock colony was found stranded in the egg trap. At 8:45 A. M. it was noted that this female was depositing eggs, and the process was watched under a lens. The eggs followed one another in intervals of 10 to 15 seconds. The first part of an egg would come out slowly; then, when almost half of the egg was protruding, there would be a sudden abdominal contraction, causing the egg to shoot out rapidly. Immediately after an egg was expelled, the tip of the next could be seen between the cerci. The female finished ovipositing at 8:55 A. M., having deposited a total of 129 eggs. These freshly-laid eggs were almost pure white in color; the exochorion and the floats were entirely colorless. The eggs were too small to fill in the space between the floats; later they swelled until the floats fitted snugly. By 10:30 A. M. the eggs had turned almost completely black, and at 10:50 A. M. were entirely so.

Observations made in the evening showed that oviposition first began around 6:30 P. M., at which time the egg trap never contained more than just a few light-colored eggs. At times the females did not begin to deposit their eggs until after 9:00 P. M. By 8:00 A. M.

oviposition had ceased. This was the earliest observation made in the morning, and it is quite certain that the females had stopped laying an hour or two before this. The stranded female that began to oviposit at 8:45 A. M. was the only mosquito, during several months of observation, that was ever seen to deposit eggs during the day.

Hatching.—Table I records the numbers of larvae that hatched from 48 batches of eggs. Collections of the newly-hatched larvae were made at 8:00 A. M., 11:00 A. M., 2:00 P. M., 5:00 P. M., and 7:00 P. M. The eggs usually began to hatch a little before 11:00 A. M. on the second day after the *beginning* of oviposition, about 40 hours after the first eggs had been found in the trap. It is assumed that the earliest larvae to hatch out came from the first eggs deposited. An examination of Table I

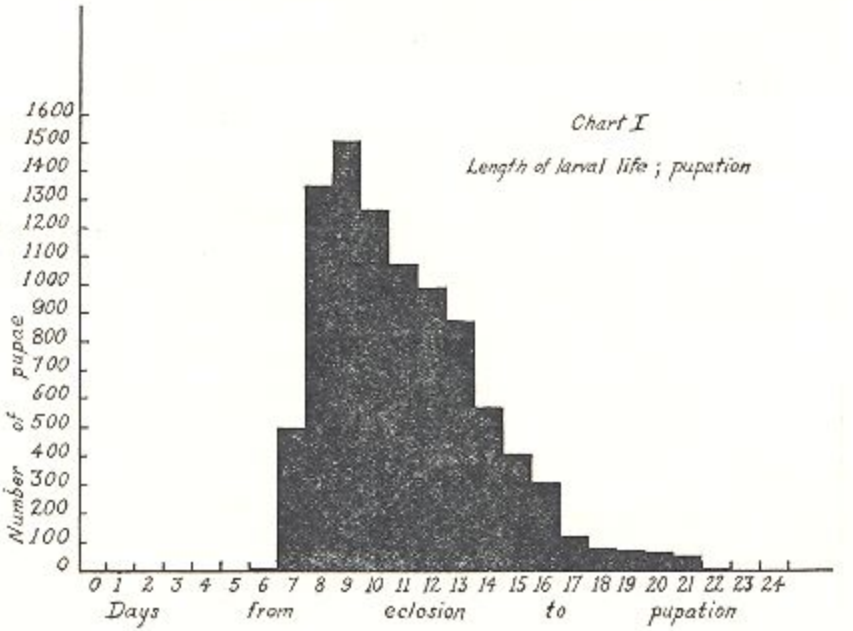
TABLE I

PERIOD OF TIME NECESSARY FOR HATCHING

OVIPOSITION		Days Following Oviposition	HATCHING				
			TIME OF COLLECTION Hours Following Oviposition; numbers of Larvae				
Started	Completed		8:00 A. M.	11:00 A. M.	2:00 P. M.	5:00 P. M.	7:00 P. M.
6:30 P. M.	8:00 A. M.	1st day	0–13½ hrs.				
		2nd day	24–37½ hrs.	27–40½ hrs. 312	30–43½ hrs. 5047	33–46½ hrs. 5907	35–48½ hrs. 6049
		3rd day	48–61½ hrs. 11,307	51–64½ hrs. 545	54–67½ hrs. 230	57–70½ hrs. 249	59–72½ hrs. 118
		4th day	72–85½ hrs. 68				

will show that the majority of the larvae were collected on the second day, at 2:00 P. M., 5:00 P. M., and 7:00 P. M., 30 to 35 hours after the eggs had been removed from the cage, or 43½ to 48½ hours after the beginning of oviposition. There is no reason to believe that the eggs hatching before the 2:00 P. M. collection had been deposited shortly before 8:00 A. M., and so these eggs were more than 30 hours old when they hatched. The larvae collected at 7:00 P. M. certainly did not come from eggs that had been deposited at 6:30 P. M., as only a few eggs were seen in the trap at that time; these ova, therefore, were less than 48½ hours old when they hatched. The majority of the larvae having been collected before or at 7:00 P. M. on the second day, the egg stage generally lasted less than 48 hours. On the other hand, some of the larvae collected at 8:00 A. M. on the third day, and all of those of the 11:00 A. M. collection, hatched from eggs that were more than 48 hours old, and the

few eggs hatching after the 7:00 P. M. collection on the third day were more than 59 hours old. The most accurate observation on the length of time necessary for hatching was that made on the eggs from the stranded female mentioned above, which were kept in a separate container and watched closely. This mosquito had deposited all of her ova within a few minutes, finishing at 8:55 A. M. Two days later, at 8:00 A. M., 27 larvae had already hatched out. At 8:30 A. M. 17 more were found, at 9:00 A. M. 11, and at 11:00 A. M. 8; two more appeared after 11:00 A. M. Thus the eggs of this batch did not all hatch at the same time. The first larvae to break out of the shell did so after a little less than 48 hours of development; the last came out after remaining in the egg for more than 50 hours.



It can be concluded, therefore, that a period of from 40 to 48 hours is generally necessary for hatching, although some stragglers need more time. Hatching can be delayed by certain external factors; it can be held up for a day by desiccation. When stranded on moist filter paper and placed in the ice-box, eggs will remain dormant for over a week.

Hatching was watched several times under a microscope. In each case the cap was suddenly pushed open, the head of the larva emerged on the ventral side and hung downwards in the water; the larva worked its way out of the opening and in a few seconds kicked itself free.

The Larval Stage.—Larval development was observed in nine basins during a period of two months. A total of 18,935 newly-hatched larvae were placed in the breeding pans, and of this number only 9,327, or 49.3 percent, reached the pupal stage. These observations were made

during the elaboration of a technique for the laboratory rearing of *A. albimanus*, and the high mortality was due to improper care of the larvae.

Chart I illustrates the duration of larval life in the infusion water. Those larvae that developed the most rapidly molted to the second stage on the 1st or 2nd day after eclosion, to the third stage on the 3rd or 4th day, and to the fourth stage on the 5th or 6th day. The first larvae to pupate did so during the 6th day. Most of the larvae pupated from the 8th to the 13th day, and more pupation occurred on the 9th day than on any other day. Following this peak, the chart shows a steady decline, and a few stragglers reached the pupal stage as late as the 22nd day. Thus the minimum larval life was 6 days, and the maximum 22, while the usual length of time spent in the larval stage varied from 8 to 13 days.

TABLE II

COLLECTION OF PUPAE; EMERGENCE OF ADULTS

Collection of Pupae		Emergence—Days Following Collection of Pupae					Total Number of Adults
		1st Day Observation st				2nd Day Observation at	
Time of Collection	Number Collected	8:00 A. M.	3:30–5:00 P. M.	6:00–7:00 P. M.	9:00–10:00 P. M.	8:00 A. M.	
10:00 A. M.	3,097	585	335	1,246	65	6	2,237
1:00 P. M.	5,165	38	6	2,730	1,734	21	4,529
4:00 P. M.	3,938	.....	.....	398	2,365	474	3,237

The Pupal Stage.—The time of pupation and the duration of the pupal stage were determined as follows:

For two months the pupae were collected three times a day: at 10:00 A. M., 1:00 P. M., and at 4:00 P. M. These pupae were transferred to cups of fresh tap water, above which lantern globes were placed, and a record was kept of the number of adults that emerged at 8:00 A. M., 3:30 to 5:00 P. M., 6:00 to 7:00 P. M., and at 9:00 to 10:00 P. M. The results are summarized in Table II.

Sen (1935) states that in the case of certain Indian Anophelines, pupation occurs more often in the day than at night. This also took place in the laboratory colony of *A. albimanus*. Table II shows that of a total of 12,260 pupae, 3,097 were collected at 10:00 A. M. In other words, only a fourth of the mosquitoes pupated between the hours of 4:00 P. M. and 10:00 A. M., and of this number, no doubt a great many changed to the pupal stage shortly after 4:00 P. M. and a little before 10:00 A. M. Pupation always occurred most actively around noon.

The figures in Table II referring to the adults that emerged deal only with those mosquitoes that survived the process of emergence. The 585 and 38 adults in the 8:00 A. M. column of the first day do not

mean that these mosquitoes spent only about 24 hours as pupae; they emerged from the pupae that had been overlooked in previous collections.

Emergence usually commenced between the hours of 3:30 and 5:00 P. M., and, except for 6 individuals, always from pupae that had been collected at 10:00 A. M. on the previous day. It is not possible to determine the length of time spent in the pupal stage from the pupae collected in the morning, as pupation could take place throughout the night. A more accurate estimate can be made from the pupae collected at 1:00 P. M. and 4:00 P. M., as these pupae were not more than three hours old at the time they were taken out of the breeding pans. Table II shows that adults began to emerge from the 1:00 P. M. pupae shortly before 6:00 or 7:00 P. M. on the following day; the majority had already come out by 7:00 P. M., and with few exceptions, the remainder had emerged by 10:00 P. M. This gives a pupal stage lasting a minimum of about 30 hours, while the maximum could not be over 36 hours.

TABLE III

VIABILITY AND MORTALITY OF PUPAE AND ADULTS

	Pupae			Adults		
	Collected	Lived	Died	Emerged	Lived	Died
Number.....	39,782	33,623	6,159	33,623	30,024	3,599
Percentage.....	100.0	84.5	15.5	100.0	89.3	10.7

The mosquitoes pupating between 1:00 P. M. and 4:00 P. M. began to transform to he adult stage at about 6:00 to 7:00 P. M. on the following day, and had mostly completed their emergence by 10:00 P. M., giving a minimum pupal period of less than 30 hours, and, since 474 individuals emerged after the 9:00-10:00 P. M. observation, a maximum of over 33 hours. Assuming that the mosquitoes emerging first had been the first to pupate, the estimate of the maximum pupal period is too high. No doubt the majority of the mosquitoes remained in the pupal stage from 30 to 33 hours.

It has been a matter of routine, in following the progress of the laboratory colony, to record the number of pupae collected daily, and the number of dead and living adults. The number of mosquitoes that emerged was determined by counting the pupal skins, and the number that survived emergence was determined by subtracting the dead adults from the pupal skins. The pupal mortality was determined by subtracting the number of pupal skins from the number of pupae collected. Table III summarizes the viability and mortality of the pupae and adults over a six-month period. During this time, a total of 39,782 pupae were collected, of which 6,159, or 15.5 percent, died, while 33,623 lived; that is to say, 84.5 percent of the pupae produced adults.

The Adult Stage.—As has been stated above, emergence began between 3:30 and 5:00 P. M., and was usually completed by 10:00 P. M. This also conforms with the results of Sen (1935); he found that Indian Anophelines emerged between the hours of six to ten in the evening. Earle (1932) also observed that the most favorable time for emergence was just after sunset, although he says that his *albimanus* did not emerge during the day.

Table III shows that during the six-month period, 33,623 adults emerged, and that 3,599 of them, or 10.7 percent, died during or shortly after emergence.

In Table II, a total of 10,003 adults emerged and lived. Of this number, 4,268, or 42.7 percent, were males, and 5,735, or 57.3 percent, were females. Sen (1935) also counted more females than males among his mosquitoes; his results showed two males to three females.

Females were ready to suck blood the morning after emergence. As has been said before, a number of blood meals are taken during the insects' adult life.

Adults were active during the short twilight, and copulation took place at this time, from about 6:30 to 7:00 P. M., and also around 6:30 A. M. It was never observed during the day or night. Pairing occurred during flight, and many couples would strike the paper-covered floor of the breeding cage, where they would hesitate for a second or two before separating and flying away. During this interval it was noted that the position of the mating pairs was a ventral-ventral one, similar to that pictured by Boyd, Cain, and Mulrennan (1935). Referring again to Experiment 1, the increasing percentage of non-viable eggs in the later batches from mosquito #4 indicates that copulation, when possible, will take place more than once. That females will mate before having taken blood was shown by placing a number of unfed males and females in a breeding cage, removing the males on the second day; then giving the females blood. A number of eggs deposited by these mosquitoes were fertile.

The longest life observed in the adult stage was that of one of the females in Experiment 1, which endured 31 days. The longest male adult life observed inside a breeding cage was 24 days.

DISCUSSION

At the temperatures and humidity given under *Material and Methods*, the complete life cycle of the laboratory-bred *A. albimanus* was as follows: The maturation of the ova within the female mosquito required a week or more; the incubation period of the eggs lasted from 40 to 48 hours; from 6 to 22 days, but usually 8 to 13 days, were spent in the larval stage; adults emerged in late afternoon and early night after a pupal period of about 30 to 33 hours. The shortest length of time from egg to egg, therefore, could be 16 days, but usually 18 to 24 days were necessary for one generation, an average of three weeks.

The first question that comes to one's mind in a laboratory study of a dangerous mosquito is, to what extent can the results be applied to the insect in its natural environment? This can be answered only by detailed observations in the field. The many years of study on the Isthmian Anophelines has brought out that *A. albimanus* is highly

anthropophagous, that it is active after sundown and enters houses to feed on man; its breeding places are also well known. Nevertheless, there still are many gaps in our knowledge of the life history, particularly as regards the adults, and it is hoped that the results on the life cycle in the laboratory will point out methods for field investigations that will ultimately disclose all of this mosquito's habits and their relation to malaria transmission.

The natives of the Chagres River villages have assured the writer that they are painfully aware of the fact that mosquitoes become active at sundown, and there is no doubt that a large percentage of these pests are *albimanus*. The crepuscular activity of the writer's colony indicates that laboratory conditions have not changed this habit. The author has spent a great deal of time in collecting larvae and pupae of Isthmian mosquitoes, and on those occasions when he has collected continuously through the morning and into the afternoon, it was invariably noted that around noon and during the afternoon many more pupae were found than during the morning hours. It seems, therefore, that in nature also the majority of the mosquitoes pupate during noon and afternoon. This was true of *A. albitarsis* and *bachmanni* as well. If crepuscular activity and the time of pupation of "wild" and the laboratory mosquitoes are similar, it is not improbable that there is a close correlation between the remaining steps in the life cycle of the insects raised in the laboratory and those in nature, allowing, of course, for variations due to differences of temperature and humidity.

The voraciousness of females that have just completed oviposition has a direct bearing on the ability of *A. albimanus* to transmit malaria, as an infected mosquito, were she to deposit several batches of eggs, in her flights between breeding waters and source of blood meals, might have opportunities to inoculate sporozoites into several persons.

SUMMARY

A period of seven days, or a little more, was necessary for the development of the ovaries of *A. albimanus*, in a breeding cage with a temperature of 80° to 86° F. and a relative humidity of around 80 percent. In an experiment to determine the egg-laying capacity of the females, an average of 435 eggs were obtained from six mosquitoes, but only one of these insects appeared to have deposited all of the eggs she was capable of developing, judging from the depleted condition of her ovaries as compared with the many partially developed ova in the remaining five mosquitoes at the time of death. This female deposited 788 eggs. Each female oviposited from two to six times, and blood meals were always taken between layings. The smallest number of eggs in a batch was 20, the largest number was 262; in 20 batches there was an average of 130 eggs per batch. Oviposition took place during the night.

The eggs usually hatched after an incubation period of 40 to 48 hours, in water with a temperature of 27° to 30° C., although some needed a longer time. Hatching began at about 11:00 A. M. on the second day after oviposition, and occurred throughout the remainder of the day and night, during the third day, and some hatched as late as the night of the third day.

From 6 to 22 days, and usually 8 to 13 days, were spent in the larval stage, in hay infusion water in which the temperature varied from 21° to 27° C.

The majority of the larvae pupated during the day; pupation was most active around noon. In water with a temperature of 27° to 30° C., the mosquitoes usually remained in the pupal stage for 30 to 33 hours. Of 39,782 pupae, 15.5 percent died, and 84.5 percent produced adults.

With a few exceptions, emergence took place from 3:30 to 10:00 P. M., and 10.7 percent of 33,623 adults died during or shortly after emergence. Of 10,003 adults that survived emergence, 42.7 percent were males and 57.3 percent were females. Females were ready to suck blood the morning following emergence. Adults were active during the short twilight, from about 6:30 to 7:00 P. M., and around 6:30 A. M.; copulation took place during this time. Pairing occurred during flight, and the mosquitoes assumed a ventral-ventral position. Females were willing to copulate before sucking blood, and in nature copulation probably takes place more than once. The longest observed adult life of a female in a breeding cage was 31 days, of a male, 27 days.

In general, 18 to 24 days were necessary for the development of one generation, an average of three weeks.

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