ASPECTS OF THE BIOLOGY OF LABORATORY-REARED FEMALE *AEDES FLUVIATILIS*[1]

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ABSTRACT. The biology of laboratory-reared *Aedes fluviatilis* (Lutz) is very similar to that of *Ae. aegypti* (L.). *Ae. fluviatilis* can serve as a substitute for *Ae. aegypti* in the parts of tropical America where laboratory colonies of the yellow fever mosquito are prejudicial to public heath interests.

INTRODUCTION

The escape of *Aedes aegypti* (L.) from laboratory colonies could create public health hazards in the tropical American countries where urban yellow fever has been eradicated or effectively controlled. It is necessary, therefore, to find alternative laboratory animals that can be colonized easily. From larvae and pupae collected from natural breeding sites in the city and environs of Belo Horizonte, we have maintained a closed colony of *Ae. fluviatilis* (Lutz) for more than 5 years. Routine methods for maintaining the colony, and an account of the biology of the immature stages have been described elsewhere (Cônsoli and Williams 1978). We now record observations on the blood-feeding behavior of the adult females and on egg production.

METHODS AND RESULTS

Unless otherwise stated, the mosquitoes were maintained in 40 cm. cubical cages of fine nylon netting and had continuous access to a supply of 10% aqueous glucose solution. Males and females were kept together until the females were offered the 1st blood meal but no attempt was made to determine if the females had, in fact, mated. Throughout most experiments, females were isolated from males. At the beginning of the experiments, females were taking their 1st blood meal or had not blood fed for a least 5 days. Blood meals on a human host were taken on the forearm of the senior author.

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FIRST BLOOD MEAL IN RELATION TO AGE. Eleven batches, each of 50 previously unfed females, were offered a single human blood meal. One batch was used on each day of adult life from day 0 (day of emergence) to day 10. The mosquitoes had opportunity to blood feed for 10 min, but those still feeding at the end of the 10 min period were allowed to finish the meal.

Results. Of the 550 mosquitoes used in the experiment, 165 (30%) ingested blood. None fed on day 0, but a proportion did so on each succeeding day (Fig. 1). The majority blood fed when they were 5 or 6 days old.

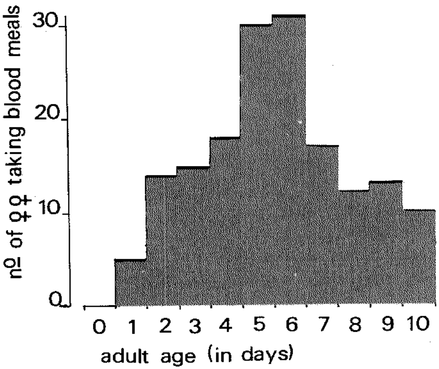

Fig. 1. Frequency distribution diagram of the ages at which 165 female *Ae. fluviatilis* took the 1st blood meal.

HOURLY BITING ACTIVITY. In the 1st of 2 experiments, 350 six-day-old female *A. fluviatilis* were placed in a 40 cm. cubical nylon cage. The mosquitoes were offered a human blood meal for the first 10 min of every hour between 15.00 hr on one day and 14.00 hr the next. The numbers feeding each hour were recorded; the proportions of unfed females which could have fed each hour but did not do so were calculated retrospectively.

In the 2nd experiment, 480 four-day-old mosquitoes were separated into 24 batches. Each batch of 20 females was confined in a glass cylinder (9.5 cm. long, 4 cm. in diameter) with each end covered with fine nylon net. A cylinder was applied to a human arm for the first 10 min of every hour between 20.00 hr on one day and 19.00 hr the next; each batch was offered only 1 blood meal.

In both experiments, the mosquitoes were maintained in a regime of natural light and darkness but, at night, it was necessary to illuminate the laboratory for the short period needed to count the numbers of fed specimens.

Results. Of the 350 mosquitoes used in the 1st experiment, 328 (93.7%) took blood meals. More than 50% blood fed in the first 3 hr; the mosquitoes were at the optimal age for taking the 1st blood meal. Some females blood fed in all but two of the remaining 21 hr of the experiment. There is no discernable pattern in biting activity (Fig. 2). More specimens (62.5%) fed in hours of daylight than at night.

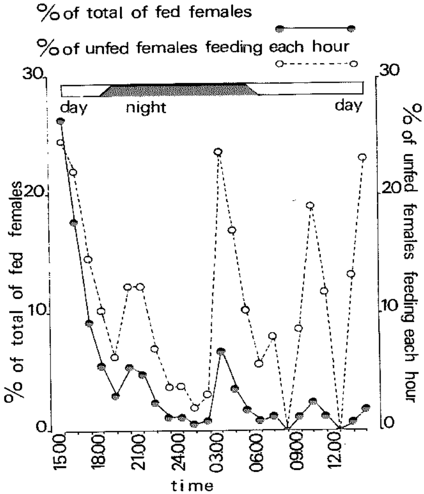

Fig. 2. Biting activity of a cohort of 350 six-day-old female *Ae. fluviatilis* offered a human blood meal for 10 min in every hour for 24 hr; 328 of the mosquitoes (93.7%) took blood meals.

In the second experiment, only 134 (27.9% of the 480 specimens offered blood meal) fed. The low proportion of blood feeders could be related to age, but confinement in small containers, access to a limited area of skin and feeding through a fine mesh might have inhibited the blood feeding drive. Some specimens fed in every hour but, again, there is no discernible pattern in biting activity (Fig. 3). The majority (53.7%) fed at night.

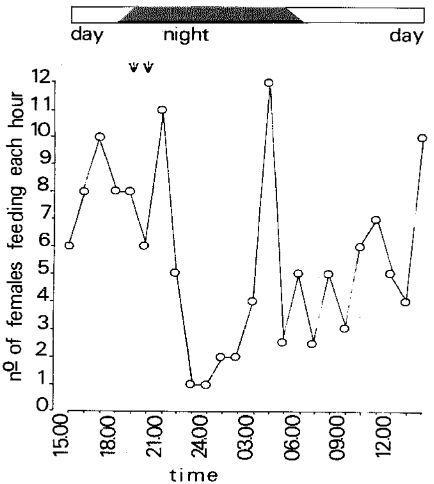

Fig. 3. Biting activity of 480 four-day-old female *Ae. fluviatilis*, divided into 24 batches of 20 mosquitos, a batch of unfed specimens being offered a human blood meal every hour for 24 hr. Results are arranged for comparison with Fig. 2, arrows marking the times that the experiment began and ended. Only 134 of the mosquitos (27.9%) took blood meals.

DURATION OF BLOOD MEALS. Fifty female mosquitoes blood fed on a human host. The duration of each blood meal was assessed by timing the interval between insertion of mouth parts into the skin and their final withdrawal.

Results. Fig. 4 shows the frequency distribution diagram of blood feeding times. Forty-eight mosquitoes completed their meal in 103 ± 20.5 seconds. Two others had their mouth parts inserted in the skin for 230 ± 14.1 seconds, and one of these ingested only a very small volume of blood. The difference in the mean feeding times of the 2 groups is statistically significant. The larger group of rapid feeders presumably took blood directly from vessels, whereas the smaller group were pool feeders.

HOST PREFERENCE TRIALS. The mosquitoes were offered blood meals on man, a marmoset, 3 standard laboratory animals (white mouse, white rat, guinea pig), a 3-week-old chick, a pigeon, a quail and a lizard in 2 series of experiments. In the 2nd series, only, a horse was exposed to the bites of the mosquitoes.

In the 1st experiment, batches of 50 female mosquitoes were confined in standard cubical nylon cages and the host was placed in the cages. Non-human hosts were within the mosquito cages for 3 hr, from 14.00 to 17.00 hr. The lizard was tied to a metal place with the ventral surface uppermost; the birds and small mammals were confined in small cages of 1 cm. wire mesh. Only one host was placed in each mosquito cage. For meals on the human host, an arm was placed inside a mosquito cage for the first 10 min of each hr between 14.00 and 17.00 h. The observations were replicated using

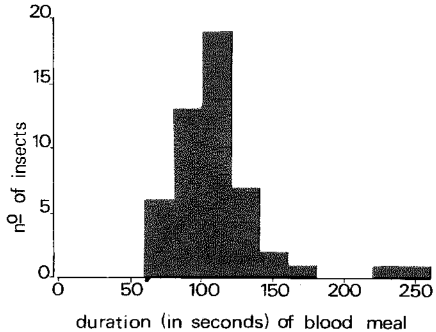

Fig. 4. Frequency distribution diagram of the duration of blood feeding by 50 female *Ae. fluviatilis*.

separate batches of 4, 5 and 6-day-old females.

In the 2nd series, groups of 20 females were confined in 9.5 cm. × 4 cm. glass cylinders with both ends covered with fine nylon netting. One end of a cylinder was applied directly to the skin of a host, each batch of mosquitoes having access to only one of the test hosts. Feathers were plucked from selected parts of the bodies of the birds and belly hair was shaved off the guinea pig, white rat and white mouse. The rat and mouse were lightly anaesthetized with "Nembutal."

Results. Blood meals were taken on all the tests hosts (Table 1) except the chick. (Later studies by Tasón de Camargo and Krettli (1978) revealed that female *Ae. fluviatilis* can be induced to blood feed on chicks). In both experiments, more than 80% of the mosquitoes fed on the 2 primate hosts and, because they had access to the human host for only 30 min in comparison to the 3 hr exposure of the other hosts, the mosquitoes displayed very strong anthropophilic tendencies.

In the 1st series of experiments, there was some evidence that host preference might change with age. Of 33 four-day-old females that blood-fed, about 42% fed on man, about 21% on the marmoset (*Callithrix penicillate jordani*) and about 30% on the lizard (*Tropidurus torquatus*). Of 65 five-day-olds that ingested blood, 46% did so on man, 22% on the marmoset and 15% on the lizard. Of 74 six-day-old mosquitoes, 62% fed on man, 12% on the marmoset and 12% on the lizard.

FECUNDITY AND THE SOURCES OF BLOOD MEALS. Mosquitoes that blood fed in the host preference trials were subsequently kept, individually, in the oviposition jars described by Cônsoli and Williams (1978). The jars were examined daily for eggs until the contained mosquito died. A record was kept of the number of eggs produced by each female and the interval between blood feeding and oviposition.

Results. After a single blood meal on a human host, female *Ae. fluviatilis* produced eggs 3–13 days later, the mode being on the 4th day but the mean being 5.6 days after blood feeding (Cônsoli and Williams, *l.c.*). We erroneously stated that the mosquitoes produced a mean of 64.3 eggs per batch; mean clutch size was, in fact, 67.0 ± 28.3.

Table 2 summarizes the egg production of the 51 mosquitoes surviving long enough to lay eggs. Apart from those blood feeding on the marmoset, mosquitoes that blood fed on non-human hosts tended to produce larger clutches of eggs earlier than did the insects that fed on man. There is a statistically significant difference between the mean clutch size after blood meals on the marmoset (40.5 ± 18.1 eggs per batch) when compared with egg production following blood meals on man, pigeon (73.8 ± 16.8), white mouse (89.5 ± 43.1) and lizard

Table 1. Results of host preference trials with laboratory-reared female *Ae. fluviatilis*.

	Experiment 1			Experiment 2		
Host	No. of mosquitoes	No. fed	% fed	No. of mosquitoes	No. fed	% fed
Man	150	92	61.3	20	10	50.0
Marmoset	150	31	20.7	20	7	35.0
Horse	—	—	—	20	4	20.0
Guinea pig	150	2	1.3	20	4	20.0
White rat	150	12	6.8	20	1	5.0
White mouse	150	0	0	20	3	15.0
Pigeon	150	10	6.7	20	4	20.0
Quail	150	0	0	20	1	5.0
Chick	150	0	0	20	0	0
Lizard	150	29	19.3	20	3	15.0
Total	1350	176	13.0	200	37	18.5

Table 2. Egg production by female *Ae. fluviatilis* after blood feeding on different hosts.

Host	No. of egg batches	Incubation period in days (mean)	Total no. of eggs	Mean batch size (range)
Marmoset	14	4–11 (5.8)	567	40.5 (16–80)
Guinea pig	3	4–6 (5.3)	237	79.0 (36–144)
White rat	9	3–9 (4.4)	765	85.0 (45–139)
White mouse	2	3–4 (3.5)	179	89.5 (59–120)
Pigeon	8	3–6 (4.4)	590	73.7 (54–100)
Quail	1	5	19	19
Lizard	14	3–4 (3.7)	1466	104.7 (14–157)

(104.7 ± 43.6). There is a statistically significant difference in mean clutch size after blood meals taken on the lizard in comparison with those taken on man and the pigeon.

As well as low egg production, the mosquitoes that blood fed on the marmoset had a high mortality, mostly 2–3 days after ingesting blood, before they began egg production.

EGG PRODUCTION AND MULTIPLE BLOOD FEEDING. Although female *Ae. fluviatilis* have strong anthropophilic tendencies (Table 1), the mosquitoes tend to produce greater numbers of eggs when they blood feed on non-primate hosts (Table 2). Because man is the most convenient host for routine maintenance of *Ae. fluviatilis* in the laboratory, a short trial was devised to determine if fecundity could be enhanced by offering the mosquitoes more frequent blood meals on a human host.

Twelve recently-emerged female mosquitoes were confined, separately, in 24 cm.-sided cubical nylon cages and three newly emerged males were placed in each cage. For oviposition, a 9 cm. diameter glass dish containing tap water was placed in each cage. Each female was offered a blood meal daily by inserting an arm into each cage for a 10 min period at some time between 14.00 hr and 17.00 hr. Records were kept of the number and frequency of blood meals, the frequencies of oviposition, the intervals between blood-feeding and oviposition, and the numbers of eggs produced in each clutch.

Results. The results, summarized in Table 3, were conflicting and confusing.

Four mosquitoes produced no eggs. Three of these took two blood meals, the fourth fed five times; all but one survived longer than the mean time (5.6 days) between a single blood meal on man and oviposition.

One female took a single blood meal and produced a batch of eggs within the normal range of 67.0 ± 28.3 eggs per batch.

Seven mosquitoes took multiple blood meals but produced eggs irregularly and unpredictably. The 2 mosquitoes that each produced 8 clutches of eggs can serve to illustrate erratic egg production. Specimen No. 9 produced no eggs after its 1st, 2nd, 3rd, 6th, 7th, 9th or 11th blood meals. It produced 77 eggs on day 7 after the 4th meal, 255 eggs on day 5 after the 5th, 29 eggs on day 8 after the 8th, 163 eggs on day 6 after the 10th, and 38 eggs on day 4 after the 12th. After the 13th blood meal, this female laid 3 separate batches of 14, 32 and 18 eggs on, respectively, days 10, 14 and 16 after blood ingestion. Specimen No. 11 laid no eggs after its 1st, 2nd, 4th, 6th, 8th and 12th meals. It produced 58 eggs on day 6 after the 3rd blood meal, 115 eggs on day 4 after the 5th, 73 eggs on day 8 after the 7th. After the 9th meal, she produced 3

Table 3. Longevity, frequence of blood meals and fecundity of 12 laboratory-reared female *Ae. fluviatilis*.

Female no.	Longevity (in days)	No. of blood meals	No. of eggs batches	Total no. of eggs	No. of eggs per batch	No. of eggs per blood meal
1	15	5	0	—	—	—
2	10	1	1	71	71.00	71.00
3	9	2	0	—	—	—
4	4	2	0	—	—	—
5	35	5	1	8	8.00	1.60
6	7	2	0	—	—	—
7	32	8	1	289	289.00	36.13
8	16	3	1	103	103.00	34.33
9	79	13	8	627	78.38	48.23
10	36	6	2	261	130.50	43.50
11	72	12	8	594	74.25	49.50
12	70	12	2	129	64.50	10.75

separate batches of 14, 88 and 96 eggs on, respectively, days 7, 11 and 12 after blood ingestion. Thereafter, the mosquito produced 56 eggs on day 4 after the 10th blood meal, and 94 eggs on day 4 after the 11th. A 12th blood meal was taken but the mosquito died, 72 days after the 1st blood meal, before producing more eggs.

DISCUSSION

We could not undertake a comparative study of *Ae. fluviatilis* and *Ae. aegypti* in our laboratories in Belo Horizonte and can, therefore, only relate our results to previously published reports on *Ae. aegypti*. Our studies were necessarily carried out without the aid of elaborate and sophisticated equipment and are best comparable to the earlier laboratory studies on *Ae. aegypti*, which were critically reviewed (and, in many cases, amplified) by Christophers (1960) and later examined by Clements (1963).

Our present and previously reported observations (Cônsoli and Williams 1978) show that the biology of *A. fluviatilis* and *Ae. aegypti* in the laboratory is similar. Females of the 2 species take the 1st blood meal at about the same ages; some females of both species will feed at any time blood meals are offered; females of both species are catholic blood feeders with strong anthropophilic tendencies; females of both species tend to produce more eggs after blood feeding on non-primate hosts and, for both species, lizards are the optimal hosts for maximal egg production.

The erratic egg production of *Ae. fluviatilis* taking multiple blood meals also probably occurs in *Ae. aegypti*. Madhukar and Jones (1974) recorded female *Ae. aegypti* taking 2–6 blood meals per week but producing only 4–7 egg clutches in 7 weeks. Erratic and unpredictable egg production following multiple blood meals is probably due, somewhat paradoxically, to starvation. If it is assumed that a peritrophic membrane is formed around each blood meal, digestion and absorption of the materials needed for oogenesis and vitellogenesis could have been impeded by the successive layers of membranes. We cannot ignore, however, that frequent distention of the abdominal body wall might have disrupted hormonal sequences controlling egg development.

There seems, however, to be a fundamental difference in the blood feeding mechanisms of *Ae. fluviatilis* and *Ae. aegypti*. O'Rourke (1956) deduced that 60% of female *Ae. aegypti* are vessel feeders. From similarly conducted trials, we conclude that 96% of female *A. fluviatilis* are vessel feeders. *Ae. aegypti* feeding from vessels take 131 ± 33 seconds to engorge (O'Rourke, l.c.); vessel feeding *Ae. fluviatilis* engorged in 103.5 ± 20.5 seconds. The difference in the blood feeding times of the 2 species is statistically significant. A comparative study of the functional morphology of the mouth parts of the 2 species, with special reference to the sensillae on the mouth parts, would probably explain the differences in blood feeding behavior.

From these and our previous observations (Cônsoli and Williams 1978) on *Ae. fluviatilis*, we think it is a convenient substitute for *Ae. aegypti* in laboratories in the American tropics. Colonies of *Ae. fluviatilis* can be established and maintained at little expense, without elaborate or sophisticated equipment and without special techniques at any phase of the insect's life cycle.

Although *Ae. fluviatilis* is a widely distributed Neotropical mosquito, little is known about the bionomics of wild populations and we cannot be certain of the extent to which our laboratory observations mirror the field biology of the mosquito. Our observations were made in ambient conditions, with an annual variation in room temperature of 22–31°C and of 61–73% R.H. Our results, therefore, might reflect the biology of populations of *Ae. fluviatilis* that have established themselves in urban areas of Belo Horizonte.

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References Cited

Christophers, S. R. 1960. *Aedes aegypti* (L.). The yellow fever mosquito, its life, history, bionomics and structure. 739 pp. London, Cambridge University Press.

Clements, A. N. 1963. The physiology of mosquitoes. 393 pp. Oxford, The Pergamon Press.

Cônsoli, R. A. G. B. and P. Williams. 1978. Laboratory observations on the bionomics of *Aedes fluviatilis* (Lutz) (Diptera: Culicidae). Bull. Entomol. Res. 68:123–136.

Madhukar, B. V. and J. C. Jones. 1974. How many blood meals does a mosquito take? Mosquito News 34:332–333.

O'Rourke, F. J. 1956. Observations on pool and capillary feeding in *Aedes aegypti* (L.). Nature (London) 177:1087–1088.

Tasón de Camargo, M. V. and A. U. Krettli. 1978. *Aedes fluviatilis* (Lutz): a new experimental host for *Plasmodium gallinaceum* Brumpt. J. Parasitol. 64:924–925.

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