ASPECTS OF THE BIOLOGY OF LABORATORY-REAED FEMALE Aedes FLUVIATILIS

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

METHODS AND RESULTS

We now record observations on the blood-feeding behavior of the adult females and on egg production.

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 males were kept together until the females were offerred 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.
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.

Hourly Biting Activity. In the 1st of 2 experiments, 350 six-day-old female A. fluctuata were placed in a 40 cm. cubic 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.
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. flavusita*, divided into 24 batches of 20 mosquitoes, 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 mosquitoes (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 ± 1.41 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
separate batches of 4, 5 and 6-day-old females.

In the 2nd series, groups of 20 females were confined in 9.5 cm. \( \times \) 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 anesthetized with "Nembutal."

Results. Blood meals were taken on all the tests hosts (Table 1) except the chick. (Later studies by Tasón de Camargo and Kretzli (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 priamate 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 were 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 penicillata 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 Consoli 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 5.8 days after blood feeding (Consoli and Williams, I.c.). We erroneously stated that the mosquitoes produced a mean of 64.3 eggs per batch; mean clutch size was, in fact, 67.0 \( \pm \) 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 \( \pm \) 18.1 eggs per batch) when compared with egg production following blood meals on man, pigeon (73.8 \( \pm \) 16.8), white mouse (89.9 \( \pm \) 43.1) and lizard with laboratory-reared female *Ae. fluviatilis*.

<table>
<thead>
<tr>
<th>Host</th>
<th>Experiment 1</th>
<th></th>
<th>Experiment 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of mosquitoes</td>
<td>No. fed</td>
<td>% fed</td>
<td>No. of mosquitoes</td>
</tr>
<tr>
<td>Man</td>
<td>150</td>
<td>92</td>
<td>61.3</td>
<td>20</td>
</tr>
<tr>
<td>Marmoset</td>
<td>150</td>
<td>31</td>
<td>20.7</td>
<td>20</td>
</tr>
<tr>
<td>Horse</td>
<td></td>
<td></td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>150</td>
<td>2</td>
<td>1.3</td>
<td>20</td>
</tr>
<tr>
<td>White rat</td>
<td>150</td>
<td>12</td>
<td>6.8</td>
<td>20</td>
</tr>
<tr>
<td>White mouse</td>
<td>150</td>
<td>0</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>Pigeon</td>
<td>150</td>
<td>10</td>
<td>6.7</td>
<td>20</td>
</tr>
<tr>
<td>Quail</td>
<td>150</td>
<td>0</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>Chick</td>
<td>150</td>
<td>0</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>Lizard</td>
<td>150</td>
<td>29</td>
<td>19.3</td>
<td>20</td>
</tr>
<tr>
<td>Total</td>
<td>1350</td>
<td>176</td>
<td>13.0</td>
<td>200</td>
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</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Host</th>
<th>No. of egg batches</th>
<th>Incubation period in days (mean)</th>
<th>Total no. of eggs</th>
<th>Mean batch size (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marmoset</td>
<td>14</td>
<td>4–11 (5.8)</td>
<td>567</td>
<td>40.5 (16–80)</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>3</td>
<td>4–6 (5.3)</td>
<td>257</td>
<td>79.0 (36–144)</td>
</tr>
<tr>
<td>White rat</td>
<td>9</td>
<td>3–9 (4.4)</td>
<td>765</td>
<td>85.0 (45–139)</td>
</tr>
<tr>
<td>White mouse</td>
<td>2</td>
<td>3–4 (3.5)</td>
<td>179</td>
<td>89.5 (59–120)</td>
</tr>
<tr>
<td>Pigeon</td>
<td>8</td>
<td>3–6 (4.4)</td>
<td>590</td>
<td>73.7 (54–100)</td>
</tr>
<tr>
<td>Quail</td>
<td>1</td>
<td>3</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>Lizard</td>
<td>14</td>
<td>3–4 (3.7)</td>
<td>1466</td>
<td>104.7 (14–157)</td>
</tr>
</tbody>
</table>

(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 anthropophagic 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, 165 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, frequency of blood meals and fecundity of 12 laboratory-reared female *Ae. fluviatilis*.

<table>
<thead>
<tr>
<th>Female no.</th>
<th>Longevity (in days)</th>
<th>No. of blood meals</th>
<th>No. of eggs/meal</th>
<th>Total no. of eggs</th>
<th>No. of eggs per batch</th>
<th>No. of eggs per blood meal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15</td>
<td>5</td>
<td>0</td>
<td>1</td>
<td>71</td>
<td>71.00</td>
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<td>2</td>
<td>10</td>
<td>1</td>
<td>1</td>
<td>71</td>
<td>71.00</td>
<td>71.00</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>2</td>
<td>0</td>
<td>8</td>
<td>8.00</td>
<td>1.60</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>8</td>
<td>8.00</td>
<td>1.60</td>
</tr>
<tr>
<td>5</td>
<td>35</td>
<td>5</td>
<td>1</td>
<td>8</td>
<td>8.00</td>
<td>1.60</td>
</tr>
<tr>
<td>6</td>
<td>7</td>
<td>2</td>
<td>0</td>
<td>8</td>
<td>8.00</td>
<td>1.60</td>
</tr>
<tr>
<td>7</td>
<td>32</td>
<td>8</td>
<td>1</td>
<td>289</td>
<td>289.00</td>
<td>36.13</td>
</tr>
<tr>
<td>8</td>
<td>16</td>
<td>3</td>
<td>1</td>
<td>103</td>
<td>103.00</td>
<td>34.33</td>
</tr>
<tr>
<td>9</td>
<td>79</td>
<td>13</td>
<td>8</td>
<td>627</td>
<td>78.38</td>
<td>48.23</td>
</tr>
<tr>
<td>10</td>
<td>36</td>
<td>6</td>
<td>2</td>
<td>261</td>
<td>130.50</td>
<td>45.50</td>
</tr>
<tr>
<td>11</td>
<td>72</td>
<td>12</td>
<td>8</td>
<td>596</td>
<td>74.25</td>
<td>49.50</td>
</tr>
<tr>
<td>12</td>
<td>70</td>
<td>12</td>
<td>2</td>
<td>129</td>
<td>64.50</td>
<td>10.75</td>
</tr>
</tbody>
</table>

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 (Consoli 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 anthropophagic 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 peritrrophic 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 *Ae. 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 105.5 ± 20.5
seconds. The difference in the blood
feeding times of the 2 species is statisti-
cally significant. A comparative study of
the functional morphology of the mouth
parts of the 2 species, with special refer-
cence to the sensilla on the mouth parts,
would probably explain the differences in
blood feeding behavior.

From these and our previous observa-
tions (Cónsoli and Williams 1978) on Ae.
fluviatilis, we think it is a convenient sub-
stitute for Ae. aegypti in laboratories in the
American tropics. Colonies of Ae.
fluviatilis can be established and main-
tained at little expense, without elaborate
or sophisticated equipment and without
special techniques at any phase of the in-
ssect's life cycle.

Although Ae. fluviatilis is a widely dis-
tributed Neotropical mosquito, little is
known about the biornomics of wild
populations and we cannot be certain of
the extent to which our laboratory observa-
tions mirror the field biology of the
mosquito. Our observations were made in
ambient conditions, with an annual varia-
tion 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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