1988

Intrinsic and extrinsic factors affecting the laboratory flight ability of Aedes triseriatus (Say)

John Lyell Clarke III
Iowa State University

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Intrinsic and extrinsic factors affecting the laboratory flight ability of *Aedes triseriatus* (Say)

Clarke, John Lyell, III, Ph.D.
Iowa State University, 1988
Intrinsic and extrinsic factors affecting the laboratory flight ability of *Aedes triseriatus* (Say)

by

John Lyell Clarke III

A Dissertation Submitted to the Graduate Faculty in Partial Fulfillment of the Requirements for the Degree of DOCTOR OF PHILOSOPHY

Department: Entomology  
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In Charge of Major Work

For the Major/Department

For the Graduate College

Iowa State University  
Ames, Iowa  
1988
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>GENERAL INFORMATION</td>
<td>1</td>
</tr>
<tr>
<td>Explanation of Dissertation Format</td>
<td>9</td>
</tr>
<tr>
<td><strong>SECTION I. LABORATORY FLIGHT ABILITY OF AEDES TRISERIATUS (SAY)</strong></td>
<td>10</td>
</tr>
<tr>
<td><strong>INTRODUCTION</strong></td>
<td>12</td>
</tr>
<tr>
<td><strong>MATERIALS AND METHODS</strong></td>
<td>14</td>
</tr>
<tr>
<td>Mosquitoes</td>
<td>14</td>
</tr>
<tr>
<td>Flight of Virgin Mosquitoes</td>
<td>14</td>
</tr>
<tr>
<td>Flight of Blood-Fed Mosquitoes</td>
<td>15</td>
</tr>
<tr>
<td>Flight of Parous Mosquitoes</td>
<td>15</td>
</tr>
<tr>
<td>Statistical Analysis</td>
<td>15</td>
</tr>
<tr>
<td><strong>RESULTS</strong></td>
<td>16</td>
</tr>
<tr>
<td>Flight of Virgin Mosquitoes</td>
<td>16</td>
</tr>
<tr>
<td>Flight of Blood-Fed Mosquitoes</td>
<td>19</td>
</tr>
<tr>
<td>Flight of Parous Mosquitoes</td>
<td>22</td>
</tr>
<tr>
<td><strong>DISCUSSION</strong></td>
<td>23</td>
</tr>
<tr>
<td><strong>REFERENCES CITED</strong></td>
<td>26</td>
</tr>
<tr>
<td><strong>SECTION II. CIRCADIAN FLIGHT ACTIVITY OF AEDES TRISERIATUS (SAY) IN RELATION TO INSEMINATION, BLOOD-FEEDING, AND OVIPOSITION</strong></td>
<td>28</td>
</tr>
<tr>
<td><strong>INTRODUCTION</strong></td>
<td>30</td>
</tr>
<tr>
<td><strong>MATERIALS AND METHODS</strong></td>
<td>32</td>
</tr>
<tr>
<td>Mosquitoes</td>
<td>32</td>
</tr>
<tr>
<td>Activity Chambers</td>
<td>32</td>
</tr>
<tr>
<td>Section</td>
<td>Page</td>
</tr>
<tr>
<td>------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>Statistical Analysis</td>
<td>33</td>
</tr>
<tr>
<td>RESULTS</td>
<td>34</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>41</td>
</tr>
<tr>
<td>REFERENCES CITED</td>
<td>45</td>
</tr>
<tr>
<td>SECTION III. THE EFFECT OF LA CROSSE VIRUS (CALIFORNIA SEROGRUP) ON THE SPONTANEOUS FLIGHT ACTIVITY OF <em>Aedes triseriatus</em> (Say) (DIPTERA: CULICIDAE)</td>
<td>47</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>49</td>
</tr>
<tr>
<td>MATERIALS AND METHODS</td>
<td>51</td>
</tr>
<tr>
<td>Mosquitoes</td>
<td>51</td>
</tr>
<tr>
<td>Infection of Mosquitoes</td>
<td>51</td>
</tr>
<tr>
<td>Activity Chambers</td>
<td>52</td>
</tr>
<tr>
<td>Virus Assay</td>
<td>53</td>
</tr>
<tr>
<td>Statistical Analysis</td>
<td>53</td>
</tr>
<tr>
<td>RESULTS</td>
<td>54</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>60</td>
</tr>
<tr>
<td>REFERENCES CITED</td>
<td>63</td>
</tr>
<tr>
<td>SUMMARY AND CONCLUSIONS</td>
<td>65</td>
</tr>
<tr>
<td>REFERENCES CITED</td>
<td>68</td>
</tr>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>74</td>
</tr>
</tbody>
</table>
GENERAL INTRODUCTION

*Aedes triseriatus* (Say), the eastern treehole mosquito, is widely distributed east of the Rocky Mountains in the U.S. (Zavortink, 1972). Under natural conditions, this species utilizes basal treeholes as a larval habitat. However, man-made containers, especially discarded tires, are readily colonized by this mosquito (Craig, 1983). *Aedes triseriatus* overwinters solely as a diapausing egg in the northern part of its range (Shroyer and Craig, 1983). Termination of egg diapause occurs after long-term exposure to cold temperatures. Initial hatching takes place in early to mid-March, following treehole thawing (Shroyer and Craig, 1981). *Aedes triseriatus* larvae develop slowly. The first adults appear in late May to early June (Parry, 1983). Under natural conditions, there are one to two generations per year, with an average of 100-200 females per hectare (Scholl and DeFoliart, 1977, 1978; DeFoliart, 1983). Adult male and female mosquitoes are long lived, and females are capable of taking several blood-meals (Scholl et al., 1979a; Sinsko and Craig, 1979; Beier et al., 1982; Walker et al., 1987). *Aedes triseriatus* feeds readily on humans and other mammals; however, its "preferred" hosts are chipmunks (*Tamias striatus*), tree squirrels (*Sciurus carolinensis*, Singer), and deer (*Odocoileus virginianus*) (Wright and DeFoliart, 1970; Haramis, 1981; Burkot and DeFoliart, 1982; Nasci, 1982b, 1985).

La Crosse (LAC) virus was initially isolated during necropsy from the brain of a 4-year-old girl (Thompson et al., 1965). Since the initial discovery, LAC virus has been isolated in 13 states east of, or contiguous with, the Mississippi river (Calisher, 1983). A majority of human cases of
La Crosse encephalitis occur in Ohio, Indiana, Illinois, Wisconsin, Minnesota, and Iowa (Kappus et al., 1983). In Iowa, cases of LAC encephalitis tend to be concentrated in the northeastern corner of the state where hardwood deciduous forests are found (Rowley et al., 1983). From 1966 to 1983, there were 113 serologically confirmed cases of LAC virus in Iowa, 62 cases in males and 51 cases in females. The infection occurred primarily in children between 3 and 12 years of age. Cases in Iowa, as in other areas, are rare in individuals more than 15 years old (Rowley et al., 1983; Kappus et al., 1983).

La Crosse virus is generally restricted to the deciduous forests of the north central states where *Ae. triseriatus* is relatively abundant. Experimental infection and transmission of LAC virus by *Ae. triseriatus* was initially demonstrated by Watts et al. (1972, 1973). *Aedes triseriatus* is a dynamic vector of LAC virus. Both vertical and horizontal transmission function to maintain this virus in nature (Watts et al., 1972, 1973, 1974; Pantuwatana et al., 1972). Horizontal transmission occurs when *Ae. triseriatus* bites an amplifier animal with an infective viremia. Venereal transmission occurs when transovarially infected males pass virus to females in infected seminal fluid (Watts et al., 1972, 1973; Thompson and Beaty, 1977; Kramer and Thompson, 1982; Patrican and DeFoliart, 1987). Chipmunks and tree squirrels are the major amplifying vertebrates of this virus (Moulton and Thompson, 1971; Pantuwatana et al., 1972; Gauld et al., 1974, 1975; Ksiazek and Yuill, 1977). Vertical (transovarial) transmission of virus occurs when an infected female mosquito passes the virus to her progeny through the egg (Watts et al., 1974). La Cross virus isolates from
larvae collected in early spring before emergence of adults provide conclusive evidence for transovarial transmission as an overwintering mechanism for LAC virus.

Although the common name for *Ae. triseriatus* is the treehole mosquito, it readily and extensively colonizes discarded tires. This relatively new habitat (last two decades) has allowed *Ae. triseriatus* to move from forests and wood lots to backyards and suburban neighborhoods (DeFoliart, 1983). Unfortunately, the mosquito often brings with it LAC virus. Leiser (1981) used ovitraps to demonstrate that *Ae. triseriatus* was widely distributed in urban areas of South Bend, Indiana. The extent of the urban distribution of *Ae. triseriatus* (32 of 48 sections of South Bend had positive ovitraps) is of considerable significance to the epidemiology of LAC virus. In Ohio, Minnesota, and Wisconsin, *Ae. triseriatus* have been found breeding in small containers or tires close to houses in which cases of LAC encephalitis occurred (DeFoliart and Lisitza, 1980; DeFoliart, 1983; Craig, 1983; Hedberg et al., 1985). These data suggest that the presence of tire carcasses at or near a home may markedly increase the risk of exposure to LAC virus.

Although extensive research has been conducted on the biology of *Ae. triseriatus* and its relationship to the natural history of LAC virus, little information is available about the flight behavior of *Ae. triseriatus* or how changes in the physiological condition of the mosquito, such as insemination, bloodfeeding, or virus infection affect flight.
While *Ae. triseriatus* is somewhat restricted to wood lots, increasing evidence suggests that it flies across open terrain under certain circumstances. Thus, it is free to colonize and re-colonize habitats such as old tires in both rural and urban areas. Dispersal flights by *Ae. triseriatus* of 50-100 m or more from isolated wood lots into or across open terrain have been reported in several separate studies in Wisconsin (DeFoliart and Lisitza, 1980; Garry and DeFoliart, 1975; Scholl et al., 1979a; Mather and DeFoliart, 1984). Scholl et al. (1979a) recovered a marked female mosquito in a separate wood lot 425 m from the wood lot in which it was released. Sinsko and Craig (1979) found no evidence of interchange between two wood lots separated by 300 m of open terrain. However, Nasci (1982a) suggested that fence rows connecting two wood lots acted as corridors for movement between wood lots. He indicated that *Ae. triseriatus* flies extensively in search of oviposition sites and is not limited to wood lots. Beier and Trpis (1981) also suggested that *Ae. triseriatus* was not strictly confined to the forest and that the extent of dispersal may depend on the size and structure of a wood lot as well as on peripheral areas. Berry and Craig (1984) found that recently discarded tires in open terrain were readily colonized by *Ae. triseriatus*. In many of these studies, oviposition traps and mark-release-recapture methods were used to measure dispersal or distance traveled. Although oviposition traps and mark-release-recapture are good tools to measure dispersal in small, isolated areas, they do not provide critical information about the flight ability of this important mosquito.
No information exists on the circadian flight activity or changes in the behavior of *Ae. triseriatus* associated with different stages of the gonotrophic cycle. Demonstrable changes have been reported in the circadian flight activity of *Anopheles gambiae* Giles, *Culex quinquefasciatus* Say, and *Aedes aegypti* (L.) in relationship to the gonotrophic cycle (Jones and Gubbins, 1978, 1979; Jones, 1981). Jones (1981) found that *Ae. aegypti* females switched behavioral programs in response to essential activities of mating, host-seeking, and oviposition. The few studies that have examined activity patterns of *Ae. triseriatus* were limited to the host-seeking female. Loor and DeFoliart (1970) observed heavy biting in the early morning (5-9 a.m. CDT) and in the late afternoon; however, few collections were made in the late morning or at midday. Scholl et al. (1979b) and Novak et al. (1981) concluded that *Ae. triseriatus* is diurnal with regards to biting activity at ground level but bites during evening hours in the forest canopy. Biting in the forest canopy occurred from 6-11 p.m. CDT.

Studies by Berry et al. (1986, 1987) substantiated previous suggestions that a pathogen might modify the behavior (flight) of vector mosquitoes. The fact that many arboviruses, including LAC viruses, replicate in the cells of a wide range of tissues, but especially in thoracic and abdominal ganglia, suggests that viral infection could modify mosquito flight behavior (Beaty and Thompson, 1978; Tesh and Beaty, 1983). A few studies suggest that arbovirus infection may affect its arthropod host. Mims et al. (1966) and Lam and Marshall (1968) found extensive cytopathic changes in the salivary glands of Semliki Forest (SF) virus
infected *Ae. aegypti*. The degradation of the salivary glands prevented the transmission of Semliki Forest virus three weeks after exposure. Effects of arbovirus infection on the fecundity of infected females and the prolonged development time of transovarially infected larvae have been documented. *Culex pipiens* L. infected with Rift Valley Fever (RV) virus were less fecund, showed a 21% reduction in re-feeding, and all larvae inoculated with the virus failed to emerge as adults (Turell et al., 1985). Large cage studies where LAC virus infected and uninfected female *Ae. triseriatus* were forced to seek both a blood meal and oviposition site resulted in significantly fewer eggs recovered from the infected group (Miller as cited by Turell and LeDuc, 1983). *Aedes albopictus* (Skuse) transovarially infected with Kunjin (KUN) or San Angelo (SA) virus produced 14 and 12% fewer eggs, respectively, than uninfected controls (Tesh, 1980). Transovarially infected larvae take longer to develop than their uninfected siblings. This has been shown in several cases, including *Ae. aegypti* and yellow fever (YF) virus (Beaty et al., 1980), *Ae. albopictus* and KUN virus (Tesh, 1980), and *Ae. dorsalis* and *Ae. melanimon* and California encephalitis (CE) virus (Turell et al., 1982). Conversely, Patrican and DeFoliart (1985a) found no adverse effects on the duration of larval stage, sex ratio, hatching success, time to ovarian maturation, fecundity, or adult survival of *Ae. triseriatus* transovarially infected with LAC virus.

Grimstad et al. (1980) provided the only report of an arbovirus influencing the behavior of a mosquito. They showed that orally infected *Ae. triseriatus* probe more but engorge less than their uninfected siblings. However, studies with transovarially infected *Ae. triseriatus* and
uninfected controls did not detect a significant difference in probing or the ability to re-feed (Patrican and DeFoliart, 1985b). Although no relationship was demonstrated between infection status and re-feeding ability, a significant number of infected and uninfected, uniparous *Ae. triseriatus* females probed multiple times to obtain a second blood meal (Patrican and DeFoliart, 1985b). The conclusion may be drawn that chronological age influences mosquito feeding activities. Therefore, it seems reasonable that LAC virus infection may modify the behavior of older, uniparous mosquitoes and consequently could be important in estimating vectorial capacity.

A mosquito flight mill system (Rowley et al., 1968; Clarke et al., 1984) and an acoustic activity system (Jones et al., 1967; Rowley et al., 1987) were used to evaluate the flight activity of *Ae. triseriatus* and changes that occur in flight ability as a result of infections by LAC virus. The flight mill takes advantage of a positive tarsal reflex response by the mosquito, following removal of tarsal contact with a substrate (Fraenkel, 1939). Mosquitoes tethered to a flight mill fly around a stationary pivot in a fixed circle 1 m in circumference. Parameters measured include distance flown, duration, and the speed of flight.

The acoustic activity system provides an unobstructive method of monitoring spontaneous flight activity of mosquitoes. Individual mosquitoes are held in recording chambers made from the top half of 250 ml glass reagent bottles. The bottoms of the chambers are covered with tightly stretched filter paper and plastic wrap. Each chamber is placed
over a microphone sensitive to the wing beat frequency (approx. 400-450 BPS) of mosquitoes. Activity chambers provide information on the endogenous circadian activity pattern of mosquitoes by recording the number of flights and total flying time in each 30 min period. Both systems of monitoring flight were interfaced to microcomputers to enhance data acquisition (Clarke et al., 1984; Rowley et al., 1987). The microcomputer interface makes it possible to efficiently process information from the large numbers of mosquitoes needed to assess flight ability and the intrinsic and extrinsic factors that affect flight performance.

Following the development of these microcomputer-interfaced data-acquisition systems, studies were designed to evaluate the influence of gonotrophic status and infection with LAC virus on flight behavior of *Ae. triseriatus*. The objective of the first study was to evaluate the ability of virgin, gravid, and parous *Ae. triseriatus* to fly under controlled laboratory conditions using the flight mill system previously described (Rowley et al., 1968; Clarke et al., 1984). The second study examines the circadian flight activity of *Ae. triseriatus* in relationship to insemination, blood-feeding, and oviposition using the acoustic activity chamber system (Jones et al., 1967; Rowley et al., 1987). Establishing activity patterns of *Ae. triseriatus* and any changes associated with gonotrophic status were crucial prior to conducting studies that involved subtle changes in behavior of mosquitoes infected with arboviruses or other pathogens. The third study was designed to assess the spontaneous flight activity of *Ae. triseriatus* infected with LAC virus. The acoustic activity
system was used to determine changes in circadian rhythm and/or amounts of spontaneous flight associated with viral infection.

Explanation of Dissertation Format

This dissertation is in three sections. Section I is concerned with the tethered flight of *Aedes triseriatus*. This section will be in the December, 1987 issue of the *Bulletin of the Society of Vector Ecologists*. Section II evaluated the daily circadian flight activity of *Ae. triseriatus* and Section III examined the influence of La Crosse virus infection on the spontaneous flight activity of *Ae. triseriatus*. Sections II and III will be submitted to the *Journal of Medical Entomology* for publication.

The senior author is responsible for the design and performance of experiments in all three sections of the dissertation. Stock virus was prepared and titered by the Virology Division of the University Hygienic Laboratory of the University of Iowa, Iowa City, Iowa. All potentially infectious specimens generated by the experiments were processed at the University Hygienic Laboratory. Results from these experiments were assembled, interpreted, and written into manuscript form by the senior author. Conclusions and recommendations in the three sections of the dissertation are those of the senior author.
SECTION I. LABORATORY FLIGHT ABILITY OF

Aedes Triseriatus (Say)
Laboratory flight ability of *Aedes triseriatus* (Say)

John L. Clarke III
Wayne A. Rowley

From the Department of Entomology, Iowa State University, Ames, IA 50011
INTRODUCTION

Numerous studies have examined the dispersal of Aedes triseriatus (Say), particularly in relation to wood lots. Dispersal flights by Ae. triseriatus of 50-100 m or more from isolated wood lots into or across open terrain have been reported in four separate studies in Wisconsin (DeFoliart and Lisitza, 1980; Garry and DeFoliart, 1975; Scholl et al., 1979; Mather and DeFoliart, 1984). Scholl et al. (1979) recovered a marked female mosquito in a separate wood lot 425 m from the wood lot in which it was released. Sinsko and Craig (1979) found no evidence of interchange between two wood lots separated by 300 m of open terrain. However, Nasci (1982) suggested that fence rows connecting two wood lots functioned as corridors for movement between wood lots. He indicated that Ae. triseriatus flies extensively in search of oviposition sites and is not limited to wood lots. Beier and Trpis (1981) also suggested that Ae. triseriatus was not strictly confined to the forest and that the extent of dispersal may depend on the size and structure of a wood lot as well as on peripheral areas. Berry and Craig (1984) found that recently discarded tires in open terrain were readily colonized by Ae. triseriatus. Although this species seems to be somewhat reluctant to leave wood lots, increasing evidence suggests that it does fly across open terrain under some circumstances.

Oviposition traps and mark-release-recapture studies have contributed very little information on the flight ability of this important vector mosquito. This study examines the ability of virgin, gravid, and parous
Ae. triseriatus to fly under controlled, laboratory conditions using a flight-mill system similar to that described by Rowley et al. (1968) and interfaced with a microcomputer (Clarke et al., 1984).
MATERIALS AND METHODS

Mosquitoes

*Aedes triseriatus* eggs from a 3-year-old laboratory colony were hatched in deoxygenated water, and larvae were reared in white enamel 25 x 42 x 7 cm rearing trays (250 larvae/tray). Larvae were fed a mixture of ground Tetramin® and dog biscuits.

Pupae were harvested on developmental day 10, and females were separated from males on the basis of size. Female pupae were divided into lots of 45 and placed in 0.5 l paper cans. Adult mosquitoes were afforded access to cotton pads soaked in 0.3 M sucrose. Both adult and immature mosquitoes were maintained at 26.5 ± 1°C and 70-80% RH in LD 16:8 (alternating 16 hrs light : 8 hrs dark) cycle without crepuscular periods.

Flight of Virgin Mosquitoes

A single carton of 45 virgin female mosquitoes was randomly selected for flight studies each day during a six-week period. Twelve individual mosquitoes were removed from this carton, inactivated by chilling, weighed, and flown to exhaustion each day for six weeks. Mosquitoes that did not fly a minimum of 1,600 m in their initial flights were rejected as nonfliers.

Nonfliers were replaced with other mosquitoes from the same carton. After exhaustive flight, mosquitoes were reweighed to determine weight lost during flight.
Flight of Blood-Fed Mosquitoes

Unmated *Ae. triseriatus* females were blood-fed on day 5 postemergence (PE). Twelve blood-fed mosquitoes were flown for a 24-hr period on days 0-4 postfeeding (days 5-9 PE).

Flight of Parous Mosquitoes

Six-day-old (PE) females were mated by induced copulation and held without sucrose for 24 hrs before being fed on a restrained rabbit. Individual engorged mosquitoes were placed in 0.5 l cartons with an oviposition cup (Mather and DeFoliart, 1983) and a balsa ovistrip (Novak and Peloquin, 1981). After blood-feeding, mosquitoes were provided continuous access to 0.3 M sucrose in cotton pads.

On days 10-12 after the initial blood meal (17-19 days PE), randomly selected parous and virgin mosquitoes were flown for 24 hrs. Another nonflown group of parous mosquitoes was given a second blood meal and allowed to oviposit. These biparous and similarly aged virgin mosquitoes were flown for 24 hrs on days 20-22 after the initial blood meal (27-29 PE).

Statistical Analysis

Analysis of variance was used to test transformed data \([\log(n + 1)]\), and correlation coefficients were calculated to assess relationships between variables. A priori comparisons were executed to test differences in flight performance between weeks.
RESULTS

Flight of Virgin Mosquitoes

Statistically significant differences (P<0.001) occurred in the distance flown, the duration, and the speed of flight of virgin *Ae. triseriatus* from one week to another. However, differences were not evident for any of the flight parameters between days within weeks. Figure 1 shows the mean distance flown weekly by 60 mosquitoes. A total of 416 mosquitoes were flown during this study, of which 264 (63%) flew more than 1,600 m and were treated as fliers. During week 1, mosquitoes averaged 5,805 m (36% flew less than 4,000 m, and 15% flew 9,000 m or more). In week 2 (days 8-12 PE), *Ae. triseriatus* females flew an average of 9,552 m. Forty-one percent flew more than 9,000 m during this week, and five mosquitoes flew more than 19,000 m. During week 3 (days 15-19 PE), the mean distance flown was 9,910 m. One 3-week-old mosquito flew 25,460 m, and a second mosquito flew 22,212 m. During week 3, 50% of the mosquitoes flew more than 9,000 m. A decline in flight ability occurred in the fourth week. The mean distance flown was 6,739 m, a decline of 32% from week 3. Flight performance remained near this level through week 6.

The duration of sustained, exhaustive flights during week 1 was 268 min. By week 3, the duration of sustained flights almost doubled to 478 min or just under 8 hrs. The length of flights decreased considerably during weeks 4 and 5. As expected, there was a correlation between how far mosquitoes flew and the duration of their flights.
Figure 1. Mean distance flown by virgin female *Aedes triseriatus* mosquitoes. Sixty mosquitoes were flown each week for six weeks.
Mosquitoes flew at speeds between 24 and 31 m/min during the first 35 days of this study. During week 6, mosquitoes flew only half as fast as in week 5. The correlation between how far mosquitoes flew and their flight speeds was low, and there was a negative correlation between the length of flights and the average speeds of individual flights.

The average weight of *Ae. triseriatus* flown in these experiments was 4.6 mg. Figure 2 shows the mean live weights of mosquitoes and the amount of weight lost during exhaustive flights. There seems to be a threshold below which weight loss does not occur. After flight, weights were remarkably similar, with most mosquitoes weighing approximately 3 mg. The before-flight weight of mosquitoes increased slightly as they aged. One-week-old mosquitoes weighed 4.06 mg, whereas 6-week-old mosquitoes weighed 5.15 mg. The weight of an individual mosquito did not affect how far it flew.

**Flight of Blood-Fed Mosquitoes**

One-week-old (postemergence) blood-fed mosquitoes flew more than 10,000 m in 762 min of flying (Table 1). Blood-fed mosquitoes flew more slowly than virgin mosquitoes of the same age, probably because of the burden of the blood meal. The speed (15.0 m/min) of blood-fed mosquitoes was almost identical to flight speeds of 6-week-old virgin mosquitoes that had not blood-fed.

Developing follicles of gravid mosquitoes four days after blood-feeding contained fully formed eggs with visible chorionic structures and were in stage V (five) (Christophers, 1911).
Figure 2. Weekly pre- and postflight weights during exhaustive flights by virgin female *Aedes triseriatus* mosquitoes
Mosquito Wt. (mg)

Preflight Wt.
Postflight Wt.

Weeks

1 2 3 4 5 6
Table 1. Mean distance (m), duration (min), and speed (m/min) flown by virgin, blood-fed (virgin), uniparous, and biparous *Aedes triseriatus* mosquitoes

<table>
<thead>
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<th>Parity</th>
<th>N</th>
<th>Distance (SEM)</th>
<th>Duration (SEM)</th>
<th>Speed (SEM)</th>
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<td>60</td>
<td>10780 (422)</td>
<td>763 (36)</td>
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<td>Uniparous</td>
<td>15</td>
<td>7343 (789)</td>
<td>599 (93)</td>
<td>14 (1.4)</td>
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<td>Biparous</td>
<td>12</td>
<td>7355 (1258)</td>
<td>563 (112)</td>
<td>14 (1.0)</td>
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<td>Virgin</td>
<td>27</td>
<td>11717 (773)</td>
<td>905 (61)</td>
<td>14 (0.8)</td>
</tr>
</tbody>
</table>

^aGravid mosquitoes were 0-4 days old (postblood meal) and 5-9 days old (postemergence).

^bVirgin mosquitoes represent two groups: one group was 15-17 days old flown as controls with the uniparous mosquitoes and the other was 20-22 days old flown with the biparous mosquitoes.

Flight of Parous Mosquitoes

Statistically significant differences occurred in the distance flown and duration of flight of virgin, parous, and biparous mosquitoes. Parous mosquitoes were 17-19 days old (PE), and biparous mosquitoes were 27-29 days old (PE) (Table 1). Virgin mosquitoes averaged 11,717 m. Uniparous and biparous mosquitoes flew an average of 7,734 m and 7,355 m, respectively. In this study, virgin females flew 55.7% farther than parous mosquitoes. Virgin females also flew longer (905 min) than either uniparous or biparous mosquitoes (599 and 563 min, respectively). The mean number of eggs laid by uniparous mosquitoes was 90. Biparous mosquitoes laid an average of 72 eggs.
DISCUSSION

Generally, *Ae. triseriatus* is considered to be an extremely limited flier, more or less restricted to wood lots (its natural habitat). These studies indicate that *Ae. triseriatus* is a strong flier capable of flying much farther than the 50 to 100 m generally considered to be the extent of its flight ability. *Aedes triseriatus* flew well for the entire six weeks tested; however, there was a characteristic decline in flight ability beginning in the fourth week. A similar decline has been observed in other species of mosquitoes. Age-related changes in flight performance of virgin mosquitoes have been reported by Rowley and Graham (1968), Rowley (1970), and Nayar and Sauerman (1972, 1973). Maximal flight performance in *Ae. aegypti* (L.) and *Culex tarsalis* Coquillet occurred during the first 14 days of adult life (Rowley and Graham, 1968; Rowley, 1970). Three-week-old *Cx. tarsalis* and *Ae. aegypti* flew only about 60% as far as younger (1-2-week-old) mosquitoes. *Aedes triseriatus* had a similar decrease in flight ability, but it did not occur until the fourth week of adult life.

Rowley (1970) found that gravid *Cx. tarsalis* mosquitoes flew substantially farther than virgins but did not find an age-related loss of flight ability in gravid mosquitoes. For some reason, the physiological changes that occur during the gonotrophic cycle allow *Cx. tarsalis* to maintain its ability to fly long distances. Lea (1975) and Klowden and Lea (1979) concluded that changes in mosquito activity during the gonotrophic cycle are under endocrine control mediated by the neurosecretory system and the ovaries. Jones and Gubbins (1978), Jones (1981), and Clarke and Rowley
(1987) all found increased levels of spontaneous flight activity in gravid mosquitoes. Beier et al. (1982) did not find a correlation between the distribution of eggs collected from ovitraps and the horizontal resting distribution of marked or natural populations of *Ae. triseriatus*. They suggested that *Ae. triseriatus* flies out of resting areas in search of oviposition sites to ensure efficient use of treehole resources throughout the forest. Undoubtedly, this search for oviposition sites would also result in some dispersal out of wood lots. Beier and Trpis (1981) and Nasci (1982) suggest that variations in habitat may influence dispersal. Assuming that *Ae. triseriatus* does not undergo migratory flights, the appetential stimulus associated with being gravid combined with limited oviposition sites probably represents a primary dispersal mechanism for this mosquito.

The ability of *Ae. triseriatus* to disperse is important because a majority of LAC encephalitis cases occur in urban or rural environments. Often these areas are well-removed from large wood lots considered to be the natural habitat of *Ae. triseriatus*. Man-made containers, especially discarded tires, are colonized by *Ae. triseriatus* emigrating from wood lots, and transovarial transmission of LAC virus to progeny provides an immediate focus of infection near human habitation (DeFoliart and Lisitza, 1980; Mather and DeFoliart, 1984). In a retrospective study in Ohio, approximately half of the 71 LAC encephalitis cases examined from 1979 to 1981 were associated with old tires (Craig, 1983). Fourteen isolates of LAC were obtained from 4,903 *Ae. triseriatus* larvae taken from a discarded tire in the backyard of a sick child's home (Craig, 1983). Leiser (1981)
demonstrated the magnitude of the urban distribution of *Ae. triseriatus* when she found that 34 of 48 sections (66%) of South Bend, Indiana, had positive ovitraps.

The flight ability of this species, particularly in urban environments, may be substantially greater than the literature suggests. The ability of *Ae. triseriatus* to fly significant distances probably plays a role in the epidemiology of LAC and is significant in the ability of *Ae. triseriatus* to colonize discarded tires in both rural and urban environments. The natural flight range of this mosquito needs to be evaluated along with the influence that infection with LAC virus has on its flight ability.

These studies have demonstrated that *Ae. triseriatus* has the ability to fly substantial distances and for considerable lengths of time. It is not known if such flights occur under natural conditions and, if they do, what factors or conditions mediate such flights. Additional studies, especially in the field and designed to determine how species fly in urban environments, are needed to evaluate the role of *Ae. triseriatus* as an urban vector of LAC virus.
REFERENCES CITED


SECTION II. CIRCADIAN FLIGHT ACTIVITY OF *Aedes triseriatus* (SAY) IN RELATION TO INSEMINATION, BLOOD- FEEDING, AND OVIPOSITION
Circadian flight activity of *Aedes triseriatus* (Say) in relation to insemination, blood-feeding, and oviposition

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Wayne A. Rowley

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INTRODUCTION

The reproductive state (gonotrophic status) of female mosquitoes produces significant changes in their activity. These changes can be observed in the level of flight activity and in the circadian pattern of activity. Mosquitoes where reproductive tissue mediated changes in activity are known include *Anopheles gambiae* Giles (Jones and Gubbins, 1978), *Culex pipiens quinquefasciatus* Say (Jones and Gubbins, 1979), and *Aedes aegypti* (L.) (Jones, 1981). Jones (1981) found that female *Ae. aegypti* switched between distinct behavioral activity patterns in response to essential activities such as mating, host-seeking, and oviposition. Behavioral modifications similar to those induced by insemination were induced in *Culex pipiens quinquefasciatus* by injection of male accessory gland extract (Jones and Gubbins, 1979). They suggested that a pheromone transferred during copulation was responsible for mosquitoes switching from one behavioral program to another.

Circadian flight activity of *Aedes triseriatus* (Say) is largely unstudied, and there are no data on the relationship, if any, between insemination and/or blood-feeding and activity. Hayes and Morlan (1957) found that mating occurred in a laboratory colony of *Ae. triseriatus* in semidarkness while oviposition occurred principally at night. Wright et al. (1966) and Loor and DeFoliart (1970) reported that swarming began 1–2 hrs prior to sunset and lasted until dark. Loor and DeFoliart (1970) observed heavy biting in the early morning (5–9 a.m. CDT) and in the late afternoon; however, few collections were made in the late morning or
mid-day. Scholl et al. (1979) and Novak et al. (1981) concluded that \textit{Ae. triseriatus} is diurnal with biting activity at ground level but bites during evening hours in the forest canopy. Biting in the forest canopy occurred from 6-11 p.m. CDT.

This study examined the spontaneous flight activity of female \textit{Ae. triseriatus} mosquitoes following insemination, blood-feeding, and oviposition. The spontaneous flight activity of individual mosquitoes was monitored using an acoustic activity chamber system similar to that described by Jones (1964) and interfaced with a microcomputer (Rowley et al., 1987).
MATERIALS AND METHODS

Mosquitoes

*Ae. triseriatus* from a 3-year-old colony were reared at 26.5 ± 1°C and 70-80% RH in LD 16:8 (alternating 16 hrs light : 8 hrs dark) cycle with a sharp transition between light and dark. To minimize variation during experiments, a single egg cloth containing eggs produced during a one-week period was cut into several pieces which were hatched as needed (Grimstad et al., 1977). Larvae were reared in lots of 200 in 2.0 l of deionized water in 25 x 42 x 7 cm white enamel trays. Larvae were fed a slurry of finely ground Tetramin® (0.125g/48 hrs) (Mather and DeFoliart, 1983). Male and female pupae were separated by size, and adult mosquitoes were afforded access to cotton pads soaked in 0.3 M sucrose. Female mosquitoes were inseminated by induced copulation five days after emergence. Sucrose pads were removed from cartons 24-36 hrs prior to blood-feeding. Mosquitoes were blood-fed on a rabbit 2-4 hrs before they were placed in activity chambers. To obtain parous mosquitoes, engorged mosquitoes were transferred to individual 0.5 l paper cartons containing oviposition cups (Mather and DeFoliart, 1983). Oviposition cups were fitted with balsa ovistrips (Novak and Peloquin, 1981). Ten days after the blood meal, mosquitoes that had completed a single gonotrophic cycle were placed in activity chambers, and their activity was monitored for five days.

Activity Chambers

Mosquitoes were isolated individually in recording chambers made from modified 250 ml glass reagent bottles (Jones et al., 1967). The bottom of
each chamber was covered with tightly stretched filter paper and plastic wrap. A 1.5 ml microcentrifuge tube stuffed with absorbent cotton soaked in 0.3 M sucrose solution was suspended from the lid of each chamber to provide carbohydrate and moisture.

Spontaneous flight activity of individual mosquitoes was recorded using an acoustic actograph interfaced with a microcomputer (Jones, 1964; Jones et al., 1967; Rowley et al., 1987). The flight parameters measured included an activity score (number of minutes in each 30 min period during which a mosquito made at least one flight of any duration), flying time, and the number of flights within each period. The LD 16:8 (43 lux) regime in the activity chambers was synchronized with the rearing regime and also included sharp changes between light and dark. Mosquitoes were allowed to acclimate for 16 hrs before activity was measured. The temperature was maintained at 27 ± 2°C, and RH was approximately 70%.

Statistical Analysis

Activity scores from consecutive 30 min periods were combined into hourly scores. Hour 1 consisted of the two 30 min periods following lights on. Analysis of variance was used to compare the mean activity of inseminated versus virgin and blood-fed virgin versus blood-fed inseminated mosquitoes. Additionally, comparisons were made of the circadian activity patterns of inseminated, virgin, blood-fed inseminated, and blood-fed virgin mosquitoes.
RESULTS

Virgin females *Ae. triseriatus* have a bimodal activity pattern, with one peak of activity occurring during the hour immediately prior to lights off and a second, smaller peak occurring at lights on (in the morning) (Figure 1). Spontaneous activity began 4 hrs before lights off and increased steadily until lights off. Low levels of activity continued through the scotophase. After the initial morning peak, mosquitoes were inactive through all but the last 4 hrs of the photophase.

Inseminated mosquitoes also have a bimodal activity pattern (Figure 1). However, the level of activity of inseminated mosquitoes was only 50% that of virgin females (Table 1, Figure 2). The lower flight activity is reflected in a reduction in the magnitude of the principal activity peaks. A dramatic shift in peak activity also occurred in inseminated mosquitoes with peak flight activity occurring during the first hour of the scotophase as opposed to the last hour of the photophase in virgin females (Figure 1). Almost no spontaneous flight activity occurred between the 2nd and 13th hrs of each photophase.

Blood-fed virgin *Ae. triseriatus* were considerably less active in the 24 hrs immediately following a blood meal (Table 2, Figure 2). However, the activity pattern 2-5 days following blood-feeding returned to one similar to that of virgin mosquitoes (Figure 1). Activity increased in the latter hours of the photophase with peak activity occurring during the hour immediately before lights off. The smaller peak at lights on was also maintained.
Figure 1. Circadian flight activity of virgin, inseminated, blood-fed virgin, blood-fed inseminated, and parous *Aedes triseriatus* in a 16:8 light/dark cycle. On day 1, virgin and inseminated mosquitoes were 5 days old (postemergence). Blood-fed virgin and blood-fed inseminated mosquitoes were 6 days old and parous mosquitoes were 16 days old (postemergence)
Table 1. Mean total daily activity of virgin (n = 31) and inseminated (n = 30) *Aedes triseriatus* in a LD 16:8 regime

<table>
<thead>
<tr>
<th>Day</th>
<th>Activity score</th>
<th>Number of flights</th>
<th>Flying time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Virgin</td>
<td>Insem</td>
<td>F</td>
</tr>
<tr>
<td>1</td>
<td>183</td>
<td>107</td>
<td>9.1*</td>
</tr>
<tr>
<td>2</td>
<td>226</td>
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<td>3</td>
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<td>108</td>
<td>28.2*</td>
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<tr>
<td>5</td>
<td>294</td>
<td>133</td>
<td>24.6*</td>
</tr>
</tbody>
</table>

*Pooled standard errors of the mean were: Activity score = 16, no. of flights = 47, flying time = 400.

*Virgin and inseminated mosquitoes were 5 days old (postemergence) on day 1.

*F* value with (1, 59) degrees of freedom.

*P* < 0.05.

Blood-fed inseminated mosquitoes were only 50% as active as their virgin counterparts during the first 48 hrs after taking a blood meal (Table 2, Figure 2). However, once the blood meal was digested, the activity pattern was bimodal and resembled the pattern of inseminated, nulliparous mosquitoes (Figure 1). Activity peaked during the first hour of the scotophase but was 37% below that of similarly aged inseminated, nulliparous mosquitoes.

Parous mosquitoes were similar to nulliparous, inseminated females in spontaneous activity (Figure 1). The mean hourly activity score of parous
Figure 2. Mean total daily activity score, number of flights, and total flying time of sugar-fed virgin (sf/virgin), sugar-fed inseminated (sf/insem), blood-fed virgin (bf/virgin), and blood-fed inseminated (bf/insem) Aedes triseriatus in a LD 16:8 cycle. On Day 1, virgin and inseminated mosquitoes were 5 days old (post-emergence); blood-fed virgins and blood-fed inseminated mosquitoes were 6 days old (post-emergence).
Table 2. Mean total daily activity of blood-fed, inseminated (n = 31), and blood-fed virgin (n = 30) *Aedes triseriatus* in a LD 16:8 cycle

<table>
<thead>
<tr>
<th>Day</th>
<th>Activity score^a</th>
<th>Number of flights^a</th>
<th>Flying time^a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bf/Vir Bf/Ins</td>
<td>Bf/Vir Bf/Ins</td>
<td>Bf/Vir Bf/Ins</td>
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<td></td>
<td></td>
<td>FC</td>
<td>FC</td>
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<tr>
<td>1</td>
<td>136 75</td>
<td>273 143</td>
<td>1165 675</td>
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<tr>
<td></td>
<td>9.7*</td>
<td>9.1*</td>
<td>4.5*</td>
</tr>
<tr>
<td>2</td>
<td>209 99</td>
<td>408 206</td>
<td>2424 1110</td>
</tr>
<tr>
<td></td>
<td>15.4*</td>
<td>14.0*</td>
<td>10.3*</td>
</tr>
<tr>
<td>3</td>
<td>233 138</td>
<td>559 273</td>
<td>3007 1699</td>
</tr>
<tr>
<td></td>
<td>12.5*</td>
<td>9.0*</td>
<td>7.7*</td>
</tr>
<tr>
<td>4</td>
<td>272 153</td>
<td>641 284</td>
<td>3762 1890</td>
</tr>
<tr>
<td></td>
<td>24.5*</td>
<td>18.3*</td>
<td>14.0*</td>
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<tr>
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<td>262 193</td>
<td>581 373</td>
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<td>7.4*</td>
<td>8.7*</td>
<td>3.9*</td>
</tr>
</tbody>
</table>

^aPooled standard errors of the mean were: Activity score = 14, no. of flights = 47, flying time = 266.

^bMosquitoes were 16 hrs post-blood meal and 5 days old (postemergence) on day 1.

^cF value with (1, 59) degrees of freedom.

*P<0.05.

mosquitoes was 5.6 compared to 6.0 for inseminated, nulliparous females. The activity peaks were less defined in parous mosquitoes, and they were more active during the scotophase.
DISCUSSION

Behavioral changes in the pattern and amount of flight activity accompanying physiological changes that take place after insemination, during host-seeking, and following oviposition have been reported in a number of species (Jones and Gubbins, 1978, 1979; Jones, 1981). In this study, the activity pattern in *Ae. triseriatus* seems to change demonstrably in response to changes in the mosquito's gonotrophic status.

*Ae. triseriatus* is considered to be diurnal (Scholl et al., 1979; Novak et al., 1981) with biting occurring in late afternoon or early evening. The circadian activity observed in these studies is that of a crepuscular mosquito, such as *An. gambiae* (Jones and Gubbins, 1978). The activity pattern of *Ae. triseriatus* is bimodal, with peak activity in the hour preceding lights off. Another, smaller peak occurs at lights on. Jones et al. (1966) have suggested that this second peak at lights on may be a "startle" reaction mediated directly by the nervous system in response to the sudden switching on of the lights. The lights on peak in many cases disappears when the change of light intensity is made slowly (artificial dawn). *Aedes triseriatus* is inactive throughout the daylight hours until about four hours before lights out. Significantly, low levels of flight activity occur throughout the entire scotophase in all stages of the gonotrophic cycle. Foster (W. A. Foster, Ohio State University, personal communication, 1986) observed a similar pattern of activity in the sugar feeding behavior of *Ae. triseriatus*. Scholl et al. (1979) and Novak et al. (1981) both indicated that biting activity increases in late afternoon,
particularly in the forest canopy. They suggested that *Ae. triseriatus* bites during the day at ground level and during the crepuscular period in the canopy. In contrast, *Ae. aegypti*, a diurnal species, has peaks of activity at dusk and dawn but virtually no activity during the scotophase (Jones and Gubbins, 1979).

Flight activity of inseminated *Ae. triseriatus* was more than 50% lower than that of virgins. Inseminated females maintained a bimodal activity pattern; however, the activity peak switched from the hour before lights out to the hour after lights out (Figure 1). Changes in the amount of activity and the timing of the circadian rhythm following insemination are consistent with a switch from a mating behavior to one of host-seeking. Reduced spontaneous flight activity after insemination has also been reported in *Ae. aegypti*, *An. gambiae*, and *Cx. pipiens quinquefasciatus* (Jones and Gubbins, 1978, 1979; Jones, 1981). After insemination, *An. gambiae* and *Cx. pipiens quinquefasciatus* are less active at dusk and dawn and more active during the dark hours (Jones and Gubbins, 1978, 1979). Inseminated *Ae. aegypti* are only 20% as active as virgin females but maintain a similar daily activity pattern (Jones, 1981).

*Aedes triseriatus* are inactive for 48 hrs after a blood meal; two days after a blood meal, activity increases to a level consistent with oviposition behavior, with an activity peak in the hour following lights off. Blood-fed virgin females maintain a high level of spontaneous activity that begins 24 hrs after the blood meal. However, activity peaks in blood-fed virgins in the hour preceding lights off. This is consistent with what Jones (1981) found with blood-fed inseminated *Ae. aegypti*, where activity
decreased significantly while activity in blood-fed virgin mosquitoes remained high. Jones (1981) suggested that uninseminated blood-fed females continue a behavior pattern that maximizes the probability of insemination. Therefore, the inhibition of activity associated with egg maturation is overridden (Klowden, 1983; Klowden and Lea, 1979). Laviopierre (1958) found that uninseminated blood-fed females continued biting activity at high levels even when they contain fully developed oocytes. In contrast, biting activity in fertilized *Ae. aegypti* ceases when oocytes reach the third developmental stage (Laviopierre, 1958). Klowden and Lea (1979) showed that during egg maturation, blood-fed, inseminated *Ae. aegypti* females did not fly upwind in an olfactometer in response to host stimuli. However, the degree of inhibition was much less for gravid uninseminated females and did not exist at 144 hrs after a blood meal.

Jones and Gubbins (1979) suggested that insemination (through the action of a male accessory gland substance) raises the threshold for spontaneous activity in *Cx. pipiens quinquefasciatus*. A similar phenomenon seems to occur in *Ae. triseriatus*. Jones (1981) believed that, in the absence of maturing eggs, the effect of the male accessory gland was not sufficient to prevent a high level of spontaneous response to host stimuli. After feeding, the inseminated female remains quiescent, with minimal activity. After ovarian development is complete, a switch from the mating program (principally during the photophase) to the oviposition program (principally during the scotophase) occurs. Clarke and Rowley (1987) found that gravid females fly significantly slower than virgin females. A modification of the activity pattern of *Ae. triseriatus*, from being most
active during the hour before dark to the hour after dark, could reduce the chance of predation when flight speed is significantly reduced. Regardless of the reason, the time of the day when *Ae. triseriatus* is active changes in response to insemination, blood-feeding, and oviposition. It also seems that the spontaneous activity pattern of this mosquito is diurnal with activity continuing through the crepuscular period. Studies with field collected *Ae. triseriatus* should take into account differences in spontaneous flight activity in relationship to insemination, host-seeking, and oviposition. Scholl et al. (1979) studied the vertical biting activity of *Ae. triseriatus* from sunrise to sunset. Our data indicate that activity peaks switch in inseminated and blood-fed inseminated mosquitoes. Consequently, their data may relate more to virgin and blood-fed virgin mosquitoes whose activity peak occurs during the hour prior to lights off. It is clear that activity in *Ae. triseriatus* and other species "switches" to activity vs inactivity depending on the reproductive state of the mosquito. Exactly what mediates these changes in activity is unclear although Jones and Cubbins (1979) showed that male accessory gland extract would induce a switch in behavior similar to that found in inseminated females. Other factors (extrinsic and/or intrinsic) may also play a role in mediating mosquito activity.


SECTION III. THE EFFECT OF LA CROSSE ENCEPHALITIS VIRUS (CALIFORNIA SEROGROUP) ON THE SPONTANEOUS FLIGHT ACTIVITY OF Aedes triseriatus (SAY) (DIPTERA: CULICIDAE)
The effect of La Crosse encephalitis virus (California serogroup) on the spontaneous flight activity of *Aedes triseriatus* (Diptera: Culicidae)

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William J. Berry
Norman S. Swack
William J. Hausler, Jr.

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INTRODUCTION

Arboviruses have a wide range of affect on mosquito vectors. They replicate in many mosquito tissues and organs including heart and pericardial cells, cerebral, thoracic and abdominal ganglia, fat body, ovarian tissue, and salivary glands (Beaty and Thompson, 1978; Tesh and Beaty, 1983). A few studies suggest that viral replication within mosquito cells may deleteriously effect the mosquito host. Mims et al. (1966) and Lam and Marshall (1968) reported cytological changes in the salivary glands of Semliki Forest (SF) virus infected Aedes aegypti (L.). The virus altered the salivary glands, preventing transmission of SF virus. Culex pipiens L. infected with Rift Valley (RV) fever virus were less fecund and had higher mortality rates than noninfected controls (Turell et al., 1985). Aedes albopictus (Skuse) transovarially infected with Kunjin (KUN) or San Angelo (SA) virus produced 14 and 12% fewer viable eggs, respectively, than uninfected controls (Tesh, 1980). Aedes aegypti larvae transovarially infected with yellow fever virus have a prolonged larval development period (Beaty et al., 1980). Similar observations of Ae. albopictus infected with KUN virus (Tesh, 1980) and Ae. dorsalis (Meigen) and Ae. melanimon Dyer with California encephalitis (CE) virus (Turell et al., 1982) suggest that arbovirus replication in mosquito larvae has some deleterious effects on the resultant adult mosquitoes.

Grimstad et al. (1977) found that Aedes triseriatus (Say), collected from regions where La Crosse (LAC) virus was not endemic, had higher infection and transmission rates than Ae. triseriatus from endemic areas.
In contrast, *Ae. triseriatus* from LAC endemic areas (Wisconsin) had transovarial transmission rates two to four times higher than strains from nonendemic areas (Northeastern U.S.) (Miller et al., 1982). Grimstad et al. (1980) also found that LAC infected *Ae. triseriatus* probe more but engorge less than noninfected siblings.

The objective of this study was to evaluate the spontaneous flight activity of LAC virus infected *Ae. triseriatus* 18 to 23 days after infection. At this time, the viral infection would be disseminated, and the mosquitoes would be capable of transmitting the virus during feeding.
MATERIALS AND METHODS

Mosquitoes

Mosquitoes from a 3-year-old colony of *Ae. triseriatus* were reared at 26.5 ± 1°C and 70-80% RH in LD 16:8 (alternating 16 hrs light: 8 hrs dark) cycle without crepuscular periods. To minimize variation between experiments, a single egg cloth representing eggs produced during a one-week period was cut into several pieces, and eggs on each piece were hatched as needed (Grimstad et al., 1977). Larvae were fed in lots of 200 in 2.0 l of distilled water in 25 x 42 x 7 cm white enamel trays. Larvae were fed a slurry of finely ground Tetramin® (0.125g per larva/48 hrs) to insure uniformity (Mather and DeFoliart, 1983). Pupae were separated by sex, on the basis of size, and placed in a 0.5 l emergence cartons (50 pupae/carton). Adult mosquitoes had continuous access to cotton pads saturated with 0.3 M sucrose. Female mosquitoes were inseminated by induced copulation five days after emergence.

Infection of Mosquitoes

The LAC virus strain used to infect female *Ae. triseriatus* was the prototype LAC virus strain kindly supplied by Dr. Wayne Thompson, University of Wisconsin, Madison. It had undergone two suckling mouse brain passages. One day after insemination (six days postemergence), mosquitoes were exposed to a virus suspension in mechanically defibrinated guinea pig blood (1 part stock virus to 3 parts blood). Control mosquitoes were fed a virus-free blood. All mosquitoes were fed on a water-jacketed membrane feeder (Rutledge et al., 1964) fitted with a natural lambskin membrane.
Aliquots of the virus-blood suspension were withdrawn before and after feeding for titration. No contamination was found in aliquots of sterile diluent or defibrinated guinea pig blood. Engorged mosquitoes were transferred to individual 0.5 l paper cans fitted with oviposition cups (Mather and DeFoliart, 1983) and balsa ovitrap strips (Novak and Peloquin, 1981).

Activity Chambers

Seventeen days after infection, 16 control mosquitoes and 16 mosquitoes exposed to LAC were individually placed in activity chambers. A 1.5 ml microcentrifuge tube filled with absorbent cotton soaked in 0.3 M sucrose was suspended from the mouth of the chamber to provide carbohydrate and moisture. The temperature of the chamber (27 ± 2°C) was controlled by regulating the temperature of the room in which the chambers were located.

Flight activity was monitored and data acquisition was accomplished with an acoustic actograph system (Jones, 1964) interfaced with a microcomputer (Rowley et al., 1987). The flying time and the number of flights made within each 30-min period by each mosquito were recorded, along with an activity score representing the number of minutes within a 30-min period during which a mosquito made at least one flight of any duration. The light/dark (LD 16:8) regime in the room where the chambers were located was synchronized with the rearing regime. Mosquitoes were allowed to acclimate to the chambers for 16 hrs before flight activity measurement was initiated. Activity was monitored from day 18 to day 23 postexposure (day 24 to day 30 postemergence).
Virus Assay

Following activity studies, individual mosquitoes were ground in 1.0 ml of 1.0% bovine albumin and centrifuged at 2,000 rpm for 20 min. A 0.025 ml aliquot of supernatant was inoculated intracerebrally (IC) into 1-day-old suckling mice. LAC virus was identified by the complement-fixation (LCFT) test (U.S. Public Health Service, 1965).

Statistical Analysis

The experiment was replicated four times, and the mean daily activity of infected and noninfected mosquitoes was compared with analysis of variance tests. In addition, the circadian activity patterns of infected and noninfected mosquitoes were compared. A representative 24-hr activity pattern was constructed from the six-day pattern.
RESULTS

*Aedes triseriatus* has a bimodal activity pattern. Adult females become active about 4 hrs prior to lights off (Figure 1) and remain active until 1 hr after lights off. Activity in the second hour after lights off was identical to activity in the 1-hr period 4 hrs before lights off when flight activity for the day began. *Aedes triseriatus* maintains some flight activity through the night (scotophase) and has a short burst (1 hr) of flight activity at lights on. Activity at lights on was at about the same level as that in the second hour of activity in the afternoon (3 hrs before lights off). *Aedes triseriatus* is essentially inactive through the next 11 hrs of the light cycle (Figure 1). Both control and infected mosquitoes were most active in the 2 hrs preceding and the first hour after lights off. The endogenous activity pattern (circadian rhythm) was identical in both uninfected control and in LAC infected mosquitoes (Figure 2); however, infected mosquitoes were less active. They flew fewer times than control mosquitoes. Table 1 shows the mean daily activity scores (total activity) of these mosquitoes in a 24-hr period. La Crosse infected mosquitoes were 24% less active than control mosquitoes. The LAC infected mosquitoes initiated 36% fewer spontaneous flights than uninfected controls (Table 1). The total flying time of control mosquitoes was 34% longer than that of LAC infected mosquitoes.

Figure 2 represents a 24-hr mean hourly activity pattern for both LAC infected and control mosquitoes. This figure shows the activity pattern for mosquitoes 18 to 23 days after infection. Flight activity for the 6-day period is combined into a single 24-hr representation to clearly show
Figure 1. Circadian flight activity patterns (mean hourly activity score) for *Aedes triseriatus* 18 to 23 days after infection with La Crosse (LAC) virus (23-29 days postemergence). A = noninfected controls, B = LAC infected mosquitoes, C = overlay of control and infected (shaded) activity patterns.
MEAN HOURLY ACTIVITY SCORE

DAYS POST-EXPOSURE

C  B  A
Figure 2. Hourly activity patterns of La Crosse virus infected and noninfected control *Aedes triseriatus* averaged from 18 through 23 days postexposure
Table 1. Mean total daily activity of *Aedes triseriatus* infected with La Crosse virus

<table>
<thead>
<tr>
<th>Day (PI)</th>
<th>Activity score(^a)</th>
<th>No. of flights(^a)</th>
<th>Flying time(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control(^b)</td>
<td>Infect(^c)</td>
<td>Control(^b)</td>
</tr>
<tr>
<td>18</td>
<td>170</td>
<td>141</td>
<td>290</td>
</tr>
<tr>
<td>19</td>
<td>153</td>
<td>126</td>
<td>255</td>
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<td>131</td>
<td>114</td>
<td>228</td>
</tr>
<tr>
<td>23</td>
<td>124</td>
<td>106</td>
<td>210</td>
</tr>
<tr>
<td>x (18-23)</td>
<td>146</td>
<td>118</td>
<td>250</td>
</tr>
</tbody>
</table>

\(^a\)Pooled standard errors of the mean were: Activity score = 13, no. of flights = 30, flying time = 234.

\(^b\)Control mosquitoes; \(n = 54\).

\(^c\)Infected mosquitoes; \(n = 36\).

how activity is partitioned during a 24-hr "day." This figure shows when *Ae. triseriatus* is active and the level of activity in each hour of a 24-hr day. Infected and control mosquitoes are active at exactly the same times. However, when they are active, the infected mosquitoes are clearly less active in almost every hour of the day.
DISCUSSION

The daily activity pattern in this study corresponds with observed biting activity of *Ae. triseriatus* which commences about 3 pm CDT and ends about dusk. The spontaneous flight activity of LAC infected *Ae. triseriatus* mosquitoes was examined at a time after exposure to virus when mosquitoes would be expected to transmit LAC virus in nature. Beaty and Thompson (1978) found that all infected *Ae. triseriatus* developed disseminated infections by at least 18 days of extrinsic incubation. A goal of this study was to see if LAC virus after replication in the mosquito's alimentary canal, heart, pericardial cells, ovaries and other reproductive tissues, abdominal, cephalic and thoracic ganglia, salivary glands, and fat body for at least 18 days altered or modified the activity of *Ae. triseriatus*.

The endogenous activity pattern of LAC infected and uninfected *Ae. triseriatus* was similar. Jones et al. (1967) originally used the activity score to evaluate spontaneous flight activity in mosquitoes. However, we feel it is important to measure at least three variables in virus infected mosquitoes because virus infection could change the length of individual flights and consequently modify the relationship between flight parameters.

La Crosse virus infection depresses by about 30% the spontaneous flight activity in *Ae. triseriatus* 18–23 days after they were infected; these were mosquitoes 24–30 days old (postemergence). When flight does occur, the flying time of individual LAC infected mosquitoes is markedly
reduced. In another study, *Ae. trivittatus* (Coquillett) infected with *trivittatus* virus (California serogroup) had almost identical levels of flight activity and circadian activity patterns as uninfected mosquitoes (Berry et al., 1987). The location of the circadian pacemaker in mosquitoes has not been documented; however, research with *Drosophilia* indicates the cephalic ganglion is the probable location of the pacemaker (Handler and Konopka, 1979). Beaty and Thompson (1978) found viral antigen in the cephalic ganglion of LAC infected *Ae. triseriatus*. If the cephalic ganglion is the location of the circadian pacemaker in mosquitoes, then LAC virus replication in this tissue does not disrupt the infected mosquito's circadian rhythm. Beaty and Thompson (1978) did not find LAC in the flight muscle of infected *Ae. triseriatus*. Consequently, virus infection would not directly influence metabolism in the flight muscle.

Reports of reduced feeding success in adult mosquitoes and increased development time of transovarially infected larvae indicate some neural involvement (Grimstad et al., 1980; Beaty et al., 1980; Tesh, 1980; Turell et al., 1982). Viral involvement in neurotrophic tissues of vector mosquitoes might be expected to disrupt activity patterns of virus infected mosquitoes. A study by Rowley and Jones (Dept. of Entomology, Iowa State University, unpublished data) found the normal endogenous circadian rhythm of SF virus infected *Ae. aegypti* was similar to noninfected controls until day 8 (postinfection). A modification of the normal circadian rhythm pattern began eight days after exposure to SF. Semliki Forest virus infected mosquitoes were more active than noninfected controls, particularly during periods of normal inactivity. However, infection with LAC
does not seem to change, in any way, the time of day that infected *Ae. triseriatus* mosquitoes are active. Consequently, LAC infection does not change the activity synchronization that exists between the mosquito and its natural hosts. Infected mosquitoes were 24–36% less active over the 6-day period on days 18–23 after infection. The time frame involved encompasses the time after LAC replication is complete and infected mosquitoes would transmit virus to a host on which they were feeding. A reduced level of activity in infected mosquitoes could be a significant factor in the natural history of LAC virus.
REFERENCES CITED


SUMMARY AND CONCLUSIONS

The tethered flight ability of virgin, gravid, and parous *Ae. triseriatus* was evaluated under laboratory conditions. Maximum flight distances were observed in 3-week-old virgin mosquitoes. The mean distance flown by these mosquitoes was 9,910 m. One 3-week-old mosquito flew a total of 25,460 meters. A 32% decline in flight ability occurred during the fourth week, and flight performance remained near this level through week 6. Virgin mosquitoes flew 50% farther than parous mosquitoes of a similar age. However, parous and biparous mosquitoes were able to fly farther than 7,000 m.

One-week-old blood-fed mosquitoes averaged more than 10,000 m but flew slower (15.0 m/min) than virgin mosquitoes of the same age. We suggested that the appetential stimulus associated with being gravid combined with limited oviposition sites probably represents a primary dispersal mechanism for this mosquito.

In addition, these experiments demonstrated that flight ability of *Ae. triseriatus* is much greater than the literature suggests. Further field studies designed to assess flight dynamics of this species, particularly in urban environments, are desperately needed.

The circadian flight activity of *Ae. triseriatus* in different physiological stages of the gonotrophic cycle was observed. Virgin, female *Aedes triseriatus* demonstrated a bimodal pattern of activity with a peak of flight activity at lights off and a second peak at lights on. A surprising amount of activity was observed throughout the scotophase, yet
virtually no activity was observed in the photophase until the 4 hrs prior to lights off.

Activity of inseminated mosquitoes was approximately half that of virgin females. Maximum activity occurred during the first hour of the scotophase as opposed to the last hour of the light phase in virgin females. The spontaneous flight activity of blood-fed virgins was depressed during the first 24 hrs following a blood meal. Thereafter, the spontaneous flight pattern closely resembled that of the virgin females, including a peak of activity in the hour prior to lights off. Blood-fed, inseminated females exhibited depressed activity for the first 48 hrs. However, from day 3 through day 5, blood-fed inseminated females had an activity pattern similar to inseminated mosquitoes with the peak of flight activity occurring during the hour after lights off.

*Aedes triseriatus* is often referred to as a diurnal mosquito; however, our studies indicate that its activity patterns more closely resemble a crepuscular mosquito such as *An. gambiae*. Furthermore, field studies on the flight dynamics of this species should take into account changes in behavior associated with the gonotrophic status of the mosquito.

Studies were conducted to determine the effect of La Crosse virus on the spontaneous flight activity of *Ae. triseriatus*. The daily activity pattern (circadian) of *Ae. triseriatus* infected with LAC was almost identical to that of noninfected mosquitoes. However, LAC infection depressed flight activity on days 18–24 postinfection. The LAC infected mosquitoes were 24% less active, took 36% less spontaneous flights, and their flying time was 34% shorter than individual flights of control
mosquitoes. The time frame involved (18-24 PI) encompasses the time after LAC replication is complete and infected mosquitoes would potentially transmit virus to a host on which they were feeding. A reduced level of activity in infected mosquitoes could be a significant factor in the natural history of LAC virus.
REFERENCES CITED


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