REDUCED BLOOD FEEDING SUCCESS ON SQUIRRELS AND CHIPMUNKS BY OLDER *AEDES TRISERIATUS* FEMALES[1]

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ABSTRACT. Engorgement rates were determined for nulliparous and uniparous *Ae. triseriatus* enclosed with unrestrained chipmunks and grey squirrels in a large laboratory cage. Animals exposed singly permitted a lower proportion of full engorgements by nulliparios mosquitoes than did animals exposed as pairs, ranging from 6 to 24% for the former compared to 60–64% for the latter. Uniparous females were only about half as successful in obtaining complete blood meals from individually exposed animals as were nulliparous females and fewer than half as many uniparous as nulliparous non-engorged females enclosed previously with individually exposed hosts launched a post-test attack when a non-defensive human was offered. Implications relative to La Crosse virus epidemiology are discussed.

INTRODUCTION

Grimstad et al. (1980) reported increased probing and decreased ability to achieve a blood meal by *Aedes triseriatus* (Say) mosquitoes that were orally infected with La Crosse (LAC) encephalitis virus. Observations in our laboratory (L. Patrican, unpublished data) have shown that increasing age in both infected and uninfected mosquitoes may also increase probing behavior.

Chipmunks and tree squirrels are regarded as the major vertebrate amplifying hosts of La Crosse virus (Pantuwatana et al. 1972, Gauld et al. 1974). Miller et al. (1979) demonstrated that orally infected *Aedes triseriatus* do not become vertical transmitters until the second post-infection oviposition, and studies to date have indicated that fewer than 10% of females in nature become biparous (DeFoliart 1983).

Therefore, we initiated experiments designed to determine whether uniparous and nulliparous *Ae. triseriatus* are equally successful in obtaining full blood meals from unrestrained squirrels and chipmunks. Although the probing response is influenced by age, and not parity, the average age of blood-seeking uniparous females in nature is greater than that of nulliparous blood-seeking females. Reduced blood feeding success by uniparous females would be expected to adversely affect the efficiency of vertebrate amplification of LAC virus.

METHODS AND MATERIALS

Experiments were done in a 1.8 × 1.8 × 1.9 m screened cage reinforced with hardware cloth, situated in a small enclosed laboratory. Laboratory conditions were kept constant at 21±1°C, 75±10% RH, and 16:8 LD with 2 hours crepuscular light at both ends of the photoperiod. The cage floor was lined with leaf litter and scattered logs to simulate a natural woodland setting. Five medium-sized corn plants were included to provide 'habitat' heterogeneity and improve microclimatic conditions for mosquitoes. Aerial resting perches for squirrels were also provided. Most of the test mosquitoes were from eggs collected from the Burkholder population of *Ae. triseriatus* (Iowa Co., Wisconsin) and reared as previously described (Mather and DeFoliart 1983). Observations of host behavior were made for 30 min following introduction of mosquitoes, and for 30 min each during some other daytime and crepuscular period during each day.

In preliminary experiments, a recently trapped grey squirrel was exposed to *Ae. triseriatus* at densities of 20, 40 and 80 unmated 6–7 day old females plus an equal number of males released into the cage at the same time. Tests at each density were replicated twice. Surviving females were collected at the end of 48 hr and the numbers recovered and the numbers having evidence of partial (<1/2 engorgement) or complete blood meals were recorded and averaged for each density level. Blood digestion by *Ae. triseriatus* is slow at 21°C, making it possible to recognize the remnants of a full blood meal for at least 48 hr after ingestion. It was possible to score older blood meals as full using a combination of blood remnants and amount of ovarian development. This was compared with mosquitoes held at 21°C whose blood meal quantity was known. Older partial engorgements (between 1/4 and 1/2) could be distinguished in the same manner. It is possible that some mosquitoes may have been scored as non-engorged, which had, in fact imbibed a very small volume of blood, i.e., less than ¼ engorged, soon after the beginning of a test.

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Table 1. Bloodfeeding success of *Aedes triseriatus* on caged chipmunk and grey squirrel hosts.

	Column number										
	1	2	3	4	5	6	7	8	9	10	11
Host and sex	Population[a]	No. of trials	No. of ♀♀ released	No. of ♀♀ recovered	% engorgement[b] Fully	Partly[c]	Non-	% engaging in post-test attack on a human: Of partly engorged	Of non-engorged	Both	% fully engorged or in post-test attack
Nulliparous											
Human	—	8	320	289	64(184)	02 (7)	34 (98)	—	—	—	—
Squirrel	B	5	200	170	22 (37)	15 (25)	64(108)	68(17)	75 (81)	74 (98)	79(135)
	H	3	120	90	28 (25)	12 (11)	60 (54)	—	—	—	—
		8	320	260	24 (62)	14 (36)	62(162)				
Chipmunk ♀	B	11	440	373	12 (45)	07 (27)	81(301)	37(10)	63(188)	60(198)	65(243)
♂	B	8	320	266	07 (19)	42(111)	51(136)	63(70)	28 (38)	44(108)	48(127)
	H	5	200	165	03 (5)	49 (80)	49 (80)	—	—	—	—
		13	520	431	06 (24)	44(191)	50(216)				
♀ + ♀	B	5	200	173	63(109)	06 (11)	31 (53)	55 (6)	15 (8)	22 (14)	71(123)
♂ + ♂	B	5	200	163	64(104)	08 (13)	28 (46)	58 (7)	13 (6)	22 (13)	72(117)
♂ + ♀	B	5	200	173	62(107)	07 (12)	31 (54)	67 (8)	16 (5)	20 (13)	69(120)
	H	2	80	71	56 (40)	09 (6)	35 (25)	—	—	—	—
		7	280	244	60(147)	07 (18)	32 (79)				
Uniparous											
Human	—	3	120	112	38 (43)	10 (11)	52 (58)	—	—	—	—
Squirrel	B	5	200	169	12 (21)	04 (6)	84(142)	67 (4)	29 (41)	30 (45)	39 (66)
Chipmunk ♀	B	5	200	159	03 (5)	03 (5)	94(149)	60 (3)	28 (42)	29 (45)	31 (50)
Chipmunk ♂	B	5	200	166	04 (6)	27 (44)	70(116)	55(24)	10 (12)	23 (36)	25 (42)

[a] B = Burkholder population, H = Hanson.
[b] As a percentage of the number recovered (actual number of mosquitoes shown in parentheses).
[c] Abdomen <½ filled with blood.

Prior to the beginning of the tests the squirrel was allowed a 5-day acclimation period. Food and water for the squirrel were provided *ad libitum*. Mosquitoes had free access to a 10% sucrose solution prior to their introduction into the cage, but sugar was withheld during the testing period.

In the main series of experiments (Table 1), tests were conducted by introducing 40 female mosquitoes plus an equal number of males into the cage with one of either of two grey squirrels, or one or a combination of two chipmunks of the same or different sex. A pre-test cage acclimation period was provided for the rodents. Exposures were for 48 hr. Except in the earliest tests, following the 48 hr exposure, the collector entered the cage and collected and recorded the engorged mosquitoes. Partially engorged and non-engorged mosquitoes attracted to and biting the collector were collected and recorded separately during a 30-min period, after which the remaining survivors were collected and recorded. Separate recording of mosquitoes attracted to the collector was done to determine the proportion of mosquitoes that were willing and able to feed although they had not obtained a full blood meal from the rodent hosts during the preceding 48 hr. Before using this technique, several tests were conducted using human bait exclusively in order to determine biting rates on a known non-defensive host. In these, the human entered the cage 24 hr after release of the mosquitoes and bloodfeeding was permitted for 30 min, after which all mosquitoes were collected and engorgement rates recorded. Data from these 30-min exposures are included in Table 1, but they are not intended as a control for the 48-hr exposures of rodents.

Before combining data from different animals of the same species and sex, it was ascertained that there were no significant differences in engorgement rates on the individuals (Fig. 1).

Tests were conducted separately with nulliparous and uniparous mosquitoes. The average age of nulliparous mosquitoes when introduced into the cage was 5 days. The uniparous mosquitoes, average age 14 days, had received their first blood meal from a suckling mouse on days 6–7 post-emergence. These were held separately and observed daily. Only females that had completed the first gonotrophic cycle, as confirmed by the absence of any new eggs for two consecutive days, were introduced into the cage. There were three or more replicates of each test.

RESULTS AND DISCUSSION

The preliminary experiments with mosquito densities of 20, 40 and 80 female *Ae. triseriatus* exposed to a single grey squirrel revealed little variation over this range of densities in the percentage of partial, complete and total engorgements. The average total engorgement rate (partial + complete engorgements) was 37% at a density of 20 mosquitoes, 39% at a density of 40 mosquitoes, and 47% at a density of 80 mosquitoes. Similarly, small variation was seen in the number of partial or complete engorgements when these data were taken independently. Subsequent tests were therefore conducted at a density level of 40 females.

In the tests to determine any differences in attraction and feeding success on different individuals of the same host species, total engorgement rates were similar when two individuals each of grey squirrel, female chipmunk or male chipmunk were exposed separately (Fig. 1). There were no significant differences ($\alpha = 0.01$) between average total or partial engorgement rates for mosquitoes fed on any of the paired test animals (i.e., squirrel A vs. squirrel B, etc.). The amount and nature of host activity appeared similar between host animals of the same species and sex.

We did not attempt to quantify chipmunk and squirrel behavior during exposure to mosquitoes but observations suggested that behavior differs between species. Mosquitoes tended to alight on the head region of both host species, with the most common feeding sites behind the ears, on the nose and eyelids. Mosquitoes also were successful on the forefeet of squirrels. The most commonly observed behavior exhibited by squirrels included face rub-

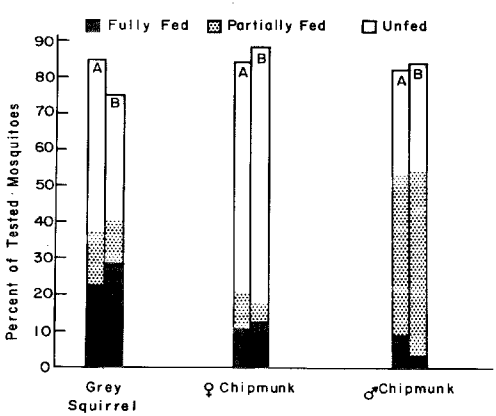

Fig. 1. Engorgement rates of *Aedes triseriatus* on 2 individuals of the same host species.

bing, foot shaking and biting at the attacking mosquito. Single chipmunks rarely displayed such behavior, instead they would frequently run across the cage in response to an attacking mosquito. There appeared fewer such actions when two chipmunks occupied the cage. Instead, these hosts appeared more preoccupied with the position of the other individual in the cage.

Single animals permitted a lower proportion of full engorgements by nulliparous females than did paired animals, ranging from 6 to 24% in the former compared to 60–64% in the latter (Table 1, Column 5). That this reduced feeding success was caused by increased defensive behavior on the part of single animals is indicated by the fact that, following the subsequent 30 min of additional exposure of a non-defensive human host, the final percentages of nulliparous mosquitoes either obtaining a full engorgement or engaging in a post-test attack on the human was similar for all host groups except the single male chipmunk. It was only 48% for this host compared to 65–79% for other groups (Table 1, Column 11). Of the nulliparous mosquitoes either fully engorging or engaging in a post-test attack on the human, 89% of those enclosed with two chipmunks obtained full blood meals on those hosts, whereas of the mosquitoes enclosed with a single squirrel, female, or male chipmunk, only 27, 19 and 15% were able to obtain full blood meals on those hosts, respectively. These data on chipmunks indicate that these animals concentrate less attention on host-seeking mosquitoes when a distracting factor is present, such as interraction with another chipmunk.

The single male chipmunks produced a high proportion of partial engorgements at the end of 48 hr, 44% of nulliparous females and 27% of uniparous females (Table 1, Column 6). By comparison, the single squirrel produced 14% partial engorgements among the nulliparous females recovered at the end of 48 hr, while the single female chipmunk and all chipmunk pairs produced less than 10% partial engorgements. It is not possible, however, to determine from these data the total extent of interrupted feeding. Undoubtedly, some initially partial bloodmeals were converted to full engorgements during the course of the 48 hr exposure periods. For most groups, although not all, mosquitoes that had a partial bloodmeal at the end of the 48 hr period were more aggressive in attacking the non-defensive human than were mosquitoes in which no blood could be detected (Table 1, Columns 8 and 9).

The major finding was that uniparous females were only about half as successful in obtaining complete blood meals from single animals as were nulliparous mosquitoes (Table 1, Column 5), and fewer than half as many uniparous as nulliparous non-engorged females enclosed previously with single hosts launched a post-test attack when a non-defensive human host was offered (Column 9). Totally, only about half as many uniparous as nulliparous females either successfully obtained a full blood meal or launched a post-test attack on the human (Column 11).

Miller et al. (1979), Burkot and DeFoliart (1982), Mather and DeFoliart (1983) and DeFoliart (1983) have previously identified and discussed factors that appear to diminish the efficiency of vertebrate amplification in the case of La Crosse virus. This finding of reduced blood feeding success by uniparous females, if it holds true in nature, appears to be another factor that reduces the efficiency of vertebrate amplification of LACV. Because orally infected females do not become vertical transmitters until completing the second post-infection oviposition (Miller et al. 1979), reduced blood feeding success by uniparous females would reduce the number of vertically infected egg masses produced by orally infected mosquitoes. The phenomenon of reduced blood feeding success by uniparous females also may offer at least a partial explanation for the finding that freshly uniparous females captured in specially designed ovitraps outnumber freshly biparous females by a ratio of approximately 12:1 (S. V. Landry, unpublished data).

References Cited

Burkot, T. R. and G. R. DeFoliart. 1982. Bloodmeal sources of *Aedes triseriatus* and *Aedes vexans* in a southern Wisconsin forest endemic for La Crosse Encephalitis virus. Am. J. Trop. Med. Hyg. 31:376–381.

DeFoliart, G. R. 1983. *Aedes triseriatus:* vector biology in relationship to the persistence of La Crosse virus in endemic foci, pp. 89–104. In C. H. Calisher and W. H. Thompson (eds.), California serogroup viruses, New York: Alan R. Liss, Inc.

Gauld, L. W., R. P. Hanson, W. H. Thompson and S. K. Sinha. 1974. Observations on a natural cycle of La Crosse virus (California group) in southwestern Wisconsin. Am. J. Trop. Med. Hyg. 23:983–992.

Grimstad, P. R., Q. E. Ross and G. B. Craig, Jr. 1980. *Aedes triseriatus* (Diptera: Culicidae) and La Crosse virus. II. Modification of mosquito feeding behavior by virus infection. J. Med. Entomol. 17:1–7.

Mather, T. N. and G. R. DeFoliart. 1983. Effect of host blood source on the gonotrophic cycle of *Aedes triseriatus.* Am. J. Trop. Med. Hyg. 32:189–193.

Miller, B. R., G. R. DeFoliart and T. M. Yuill. 1979.

Aedes triseriatus and La Crosse virus: lack of infection in eggs of the first ovarian cycle following oral infection of females. Am. J. Trop. Med. Hyg. 28:897–901.

Pantuwatana, S., W. H. Thompson, D. M. Watts and R. P. Hanson. 1972. Experimental infection of chipmunks and squirrels with La Crosse and Trivittatus viruses and biological transmission of La Crosse virus by *Aedes triseriatus*. Am. J. Trop. Med. Hyg. 21:476–481.