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BEHAVIORAL INTERACTION OF *AEDES TRISERIATUS* AND SCIURID HOSTS; WITH A SURVEY FOR CALIFORNIA SEROGROUP VIRUSES IN WESTERN MASSACHUSETTS

A Dissertation Presented

By

Edward Dixon Walker

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To my parents and brothers

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ABSTRACT

Behavioral Interaction of Aedes triseriatus and Sciurid Hosts; with a Survey for California Serogroup Viruses in Western Massachusetts

(February 1984)
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The behavioral interaction of Aedes triseriatus with eastern chipmunk (Tamias striatus) and gray squirrel (Sciurus carolinensis) hosts was studied, to evaluate its importance to the La Crosse virus cycle. Additionally, a survey was conducted from 1980-1982 to determine prevalence of California serogroup virus infections in mosquito and wild mammal populations of western Massachusetts.

Processing of 44,247 mosquitoes (761 pools) grouped by species yielded one isolate of snowshoe hare virus from Aedes stimulans group, and three isolates of Jamestown Canyon virus (one from Aedes abserratus-punctor, two from Aedes intrudens-sticticus). Neutralizing antibody to La Crosse virus was found at low levels in deer, chipmunk, and gray squirrel populations.

Searching behavior of Aedes triseriatus on chipmunks and squirrels consisted of three hierarchic levels: (1) non-discriminant initial landing on hosts, (2) random foraging on the hosts for a probing site, and (3) non-random, fast probing for a blood vessel. Feeding site selection was limited by host pelage to ears, eyelids, noses, and feet. Blood feeding was rapid.

Sciurids exhibited defensive behaviors against attacking *Aedes triseriatus*. Squirrels were very defensive, with head shake, eye blink, and forepaw scratch as effective behaviors. Chipmunks were less defensive, but head shake was an effective behavior. Mosquito feeding success was high on chipmunks and low on squirrels. Field estimates of attraction densities of *Aedes triseriatus* to these rodents allow the prediction that, in nature, *Aedes triseriatus* will feed successfully on chipmunks but with difficulty on gray squirrels.

Aedes triseriatus behaviorally gave up attempting to feed on a defensive host. Giving-up time (biting persistence) varied with nutritional state and experience of mosquitoes. Giving-up behavior, mediated by host defensive behavior, provides a mechanism whereby *Aedes triseriatus* will have multiple host contacts, thus increasing vectorial capacity of the mosquito for La Crosse virus.

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CHAPTER I

INTRODUCTION

Literature review

The California serogroup viruses. The California serogroup of arboviruses (genus Bunyavirus, family Bunyaviridae) consists of 4 virus complexes: prototype California encephalitis, Melao, trivittatus, and Guaroa (Calisher 1983). These viruses characteristically infect mosquitoes, which act as vectors in transmitting the viruses to mammalian hosts. Each of the California viruses is associated with particular species of mosquitoes and mammals (LeDuc 1979). Many of the California viruses cause encephalitis-type illnesses in humans. Because of this, the virus group was the subject of a recent sypmposium, the proceedings of which have been published (Calisher and Thompson 1983). The following brief review of the history and ecology of the California serogroup is based on these proceedings, the thorough review of LeDuc (1979), and other more specific references (mentioned below). I have not attempted to review those California serogroup viruses that occur outside of North America (including Guaroa, which forms its own complex separate from the 3 other more closely related viruses); LeDuc (1979) reviewed them in detail.

The prototype California encephalitis virus was originally isolated from mosquitoes in 1943. At least 11 additional subtypes and varieties of California serogroup viruses have since been serologically identified, forming the California encephalitis complex (Calisher 1983): Inkoo, La Crosse, snowshoe hare, San Angelo, Tahyna, and Lumbo (all subtypes or varieties of California encephalitis virus); Melao with its subtypes and varieties (Jamestown Canyon, Keystone, and Serra do Navio); and trivittatus. Seven of these occur only in North America: California encephalitis, La Crosse, snowshoe hare, San Angelo, Jamestown Canyon, Keystone, and trivittatus.

The prototype California encephalitis virus, as mentioned above, was first isolated from a pool of *Aedes melanimon* Dyar mosquitoes collected in Kern County, California, in 1943 (Hammon and Reeves 1945). This virus is now known to occur in Utah, New Mexico, and Texas as well as California. Reeves et al. (1983) reviewed the extensive studies on the host-relationships of this virus. *Aedes melanimon* and *Aedes dorsalis* (Meigen) appear to be principal vectors; these mosquitoes transmit the virus orally and transovarially. Serologically surveys of wild animals in California have implicated jackrabbits (*Lepus californicus* Gray), cottontail rabbits (*Sylvilagus audubonii* [Baird]), California ground squirrels (*Citellus beecheyi* [Richardson]), and kangaroo rats (*Dipodomys* spp.) as vertebrate hosts of the virus. Turrell et al. (1982a,b) hypothesized that transovarial transmission of California encephalitis virus in *Aedes melanimon* and *Aedes dorsalis* is the major route of transmission of this virus, and that virus amplification in mosquitoes by feeding on infected vertebrates is only an incidental or supplemental route of transmission. The relative importance of vertical and horizontal transmission to endemicity of California encephalitis and other California viruses is currently in dispute (Turrel et al. 1982c, DeFoliart 1983).

Snowshoe hare virus was originally isolated from the blood of a snowshoe hare (*Lepus americanus* Erxleben) trapped in Montana in 1958 (LeDuc 1979). This virus is generally boreal and northern in distribution in North America, occurring in most of Canada and Alaska, Montana, Minnesota, Wisconsin, Ohio, Pennsylvania, New York State, and Massachusetts (Calisher 1983). The virus has been isolated mainly from *Culiseta inornata* (Williston) in the western extension of its range, and from *Aedes* spp. in the east. Vertebrate hosts of snowshoe hare virus in the west are snowshoe hares and ground squirrels (LeDuc 1979). Vertebrate hosts in the eastern part of the range of the virus are not known, but are probably lagomorphs.

San Angelo virus was first isolated from *Anopheles punctipennis* (Say) in Texas in 1958 (LeDuc 1979). The virus has also been isolated from mosquitoes in New Mexico, Arizona, and Colorado. The ecology of the virus is poorly known.

Jamestown Canyon virus was originally isolated from *Culiseta inornata* in Colorado in 1961 (LeDuc 1979). This virus is widely distributed in North America, having been isolated from mosquitoes collected in Alaska, Alberta, Saskatchewan, Ontario, Wisconsin, Ohio, Maryland, New York, Connecticut, California, Utah, Colorado, Arizona, and Texas (Calisher 1983). Chapter II of this dissertation reports isolation of Jamestown Canyon virus in Massachusetts. In the west, Jamestown Canyon virus has been isolated mainly from *Culiseta inornata*, which Turrell and LeDuc (1983) prematurely identified as the major vector. In the east, this virus has been isolated from a large number of *Aedes* spp. and tabanids (Turrell and LeDuc 1983); vector incrimination studies with Jamestown Canyon virus are badly needed. Several studies have strongly implicated white-tailed deer (*Odocoileus virginianus* Miller) as the vertebrate host of Jamestown Canyon virus (Issel et al. 1972, Issel 1973, Issel et al. 1973, Watts et al. 1982).

Keystone virus was first isolated from mosquitoes collected in Florida in 1962 (LeDuc 1979). This virus has been isolated mainly from mosquitoes collected along the eastern seaboard of the United States. The vectors of Keystone virus are apparently *Aedes atlanticus* Dyar and Knab, *Aedes tormentor* Dyar and Knab, and *Aedes infirmatus* Dyar and Knab. LeDuc et al. (1975) demonstrated transovarial transmission of Keystone virus in *Aedes atlanticus* collected in the DelMarVa penninsula. Field studies in this area implicate gray squirrels (*Sciurus carolinensis* Gmelin) and cottontail rabbits (*Sylvilagus floridanus* [Allen]) as vertebrate hosts (Watts et al. 1982). Studies in Florida and Texas have incriminated cottontail rabbits and cotton rats (*Sigmodon hispidus* Say and Ord) as vertebrate hosts (Taylor et al. 1971, Roberts and Scanlon 1975). Fine and LeDuc (1978) constructed a quantitative model of the Keystone virus cycle which takes into account transovarial transmission and summertime vertebrate amplification to explain viral endemicity.

Trivittatus virus was first isolated from Aedes trivittatus (Coquillett) collected in North Dakota in 1948 (LeDuc 1979). The virus has also been isolated from other mosquitoes; Aedes infirmatus is probably the vector in the southeastern United States, where Aedes trivittatus does not occur. Trivittatus virus has been isolated from the Dakotas, Minnesota, Iowa, Wisconsin, Illinois, Indiana, Michigan, Ontario, Ohio, Pennsylvania, New York State, Utah, Texas, Florida, Alabama, and Georgia (Calisher 1983). Andrews et al. (1977) isolated trivittatus virus from Aedes trivittatus larvae in Iowa, confirming transovarial transmission of the virus by this mosquito. Studies in Iowa implicate cottontail rabbits as vertebrate amplifier hosts (Pinger et al. 1975).

La Crosse virus was first isolated in 1964 from the brain tissue of a child who died of encephalitis in 1960 (Thompson et al. 1965). La Crosse virus has been isolated mainly from Aedes triseriatus (Say), but also other mosquitoes (particularly Aedes canadensis [Theobald]), in Minnesota, Iowa, Wisconsin, Illinois, Indiana, Ohio, New York State, North Carolina, Georgia, Alabama, Arkansas, Louisiana, and Texas (Calisher 1983, Turrell and LeDuc 1983). However, most isolates have come from the upper mid-west and New York, probably because of vigilant surveillance programs in these regions.

The ecology of La Crosse virus has been greatly studied, especially in Wisconsin, allowing the following summary. La Crosse virus overwinters in the diapausing eggs of Aedes triseriatus (Watts et al. 1974). Virus-infected adults emerge from water-filled treeholes and tires (the habitats of immature stages of the mosquito) from late June through the end of summer (Beaty and Thompson 1975). Male and female adult *Aedes triseriatus* can be transovarially infected, and males may transmit virus to females upon insemination (Thompson and Beaty 1978). Female mosquitoes transmit La Crosse virus by bite to sciurid rodent hosts, particularly eastern chipmunks (*Tamias striatus* [L.]) and gray squirrels. These rodents circulate a viremia of short duration (2 to 4 days) but of sufficient titer to infect previously non-infected *Aedes triseriatus* which blood feed on them (Pantuwatana et al. 1972). Field studies in Wisconsin have revealed high antibody rates in sciurid populations (100% in some areas), and have shown rapid seroconversion rates in chipmunk populations by September (Moulton and Thompson 1971, Gauld et al. 1974). La Crosse virus has been isolated from wild chipmunks (Gauld et al. 1975) and sentinel chipmunks and gray squirrels (Ksiazek and Yuill 1977) in the summer.

California serogroup viruses do not appear to cause illness in their natural vertebrate hosts (Pantuwatana et al. 1972, Issel et al. 1972). However, humans (particularly children) that have been bitten by infected mosquitoes may become ill. The first 3 human cases of California encephalitis occurred in California in 1943; these were later attributed to infection with California encephalitis virus (Hammon et al. 1952). No further cases were reported until Quick et al. (1965) documented a human case occurring in Florida in

1963; the specific virus causing this infection was not determined. Thompson et al. (1965) showed that a child that had died of encephalitis in 1960 in La Crosse, Wisconsin had been infected with a California virus, the (then) newly described La Crosse virus. Kappus et al. (1983) noted that the first case of California encephalitis was reported to the Centers for Disease Control in 1963, and that to 1982, 1,456 cases were reported, making California encephalitis viruses second to St. Louis encephalitis virus as a cause of arthropod-borne encephalitis in the United States. The majority of cases have occurred in the eastern United States, particularly in the upper mid-west and in New York State (Henderson and Coleman 1971, McGowan et al. 1973, Kappus et al. 1983). Nearly all (92.4%) of cases have been children 14 years old and younger (Kappus et al. 1983). Symptoms include fever, vomiting, diarrhea, headache, stiff neck, seizures, lethargy, and coma (Gundersen and Brown 1983). Death is rare, having been reported twice in Wisconsin, 5 times in Ohio, and once in New York State (Gundersen and Brown 1983, Kappus et al. 1983, Berry et al. 1983, M.A. Grayson, New York State Department of Health, personal communication). Probably because of poor surveillance and misdiagnosis of clinical symptoms, many California encephalitis cases go unreported.

La Crosse virus has undoubtedly been the causative agent of most California encephalitis cases in the United States (Kappus et al. 1983). However, recent studies indicate that other California serogroup viruses also cause human disease. Deibel et al. (1983)

reported that Jamestown Canyon virus was the cause of encephalitis in a case in Michigan in 1980 (see also Grimstad et al. 1982), another in Ontario in 1981 (see also Artsob 1983), and 10 in New York State in 1981 and 1982. Fauvel et al. (1980) reported 3 cases of snowshoe hare virus encephalitis in children in Quebec. The role of California serogroup viruses other than La Crosse in causing human illness in the United States and Canada may be clarified as state and provincial health authorities increase surveillance, modify diagnostic procedures, and include more California viruses in serologic tests.

Biology of Aedes triseriatus. The mosquito *Aedes triseriatus* is the most abundant of the tree-hole developing mosquitoes of eastern North America (Jenkins and Carpenter 1946, Carpenter and LaCasse 1955). This species has a widespread distribution, from Texas north to Manitoba, and in all states east, as well as Ontario and Quebec (Wood et al. 1979, Darsie and Ward 1981). Sympatric over the range of *Aedes triseriatus*, but with its own distribution extending further westward, is the sibling species *Aedes hendersoni* Cockerell (Darsie and Ward 1981). This treehole-developing species is nearly identical to *Aedes triseriatus* in the adult stage. Craig (1983) reviewed the biology of *Aedes triseriatus* with reference to sampling and control.

The habitats of immature *Aedes triseriatus* are mainly water-filled treeholes and discarded tires (Craig 1983). Eggs pass the winter in diapause; in southern parts of its range, late instar larvae of Aedes triseriatus also overwinter in diapause condition (Sims 1982). Typically in the north, Aedes triseriatus eggs hatch in March in response to flooding and changes in photoperiod and temperature (Shroyer 1978). Larvae pass through 4 instars in a rather long developmental period. Rate of larval development, leading to pupation, depends on intrapsecific competition for food (McCoombs 1979, Fish and Carpenter 1982) and temperature (Shelton 1973). Larval food includes bacteria, fungal mycelia, nematodes, rotifers, and cyclopoid crustaceans (Jenkins and Carpenter 1946, Fish and Carpenter 1982).

Pupation begins in northern areas in June and continues throughout the summer (Sinsko and Craig 1981). Adult Aedes triseriatus begin to emerge from treeholes in northern states from mid-June to fall (Sinsko and Craig 1979, Scholl and DeFoliart 1978). Males emerge 2-3 weeks before females; this differential may prolong oviposition of females to the end of July, thereby limiting Aedes triseriatus to 1 generation per year (Scholl and DeFoliart 1978).

Sites of assembly for mating of Aedes triseriatus are not known. Males form mating swarms in the laboratory and in the field, and some mating may take place near hosts (Wright et al. 1966, Loor and DeFoliart 1970, Foster and Lea 1975). Scholl et al. (1979a) showed that mating need not precede host-seeking in this species.

Adult population dynamics of Aedes triseriatus have only recently received quantitative study. Estimates of adult population densities in woodlots in Indiana and Ohio have ranged from

23-205/hectare (Sinsko and Craig 1979, Haramis and Foster 1983). Thus, *Aedes triseriatus* is not an abundant mosquito. Estimates of daily survival rates, based on mark-recapture studies, have ranged from 0.80 to 0.97 (Sinsko and Craig 1979, Beier et al. 1982, Haramis and Foster 1983). Haramis and Foster (1983) concluded that *Aedes triseriatus* has an "...extraordinarily high survival rate...", but cautioned that no studies have considered adult fitness, particularly adult size, as a factor affecting adult survival. Parity studies indicate that females complete the first gonotrophic cycle and begin to seek hosts again by 15.6 (Scholl et al. 1979b) or 17.5 (Haramis 1981) days, under field conditions.

Very little is known of the sugar-feeding behavior of *Aedes triseriatus*. Grimstad and DeFoliart (1975) observed a female feeding on goldenrod before sunset. Haramis (1981) detected nectar in the crops of wild-caught females.

Host feeding patterns of *Aedes triseriatus* have only recently been studied. Nolan et al. (1965) observed an *Aedes triseriatus* feeding on a turtle. Wright and DeFoliart (1970) exposed a variety of mammals, birds, and reptiles in Magoon traps to mosquitoes in Wisconsin, and found that *Aedes triseriatus* was attracted to and blood fed on raccoon, red fox, opossum, domestic and cottontail rabbits, woodchuck, gray squirrel, chipmunk, ground squirrel, domestic chicken, and painted turtle. This was the first attempt to determine host references of *Aedes triseriatus*. Magnarelli (1977) collected 14 blood-fed *Aedes triseriatus* in Connecticut, and found that 5 had fed on dogs, 4 on mice, 2 on sciurid rodents, 1 one a raccoon, and 2 on unidentified mammals. Burkot and DeFoliart (1982) identified the host sources of blood-fed *Aedes triseriatus* collected in a La Crosse virus-enzootic woods in Wisconsin. These authors found that *Aedes triseriatus* had a predilection to feed on eastern chipmunks and gray squirrels (in comparison with the mosquito *Aedes vexans* Meigen), with 24% of 218 mosquitoes having fed on sciurids. *Aedes triseriatus* had fed mostly (65%) on deer in this study. Nasci (1982) identified the hosts of *Aedes triseriatus* and *Aedes hendersoni* collected in rural, suburban, and urban areas in Indiana. He found that *Aedes triseriatus* fed predominantly on chipmunks and tree squirrels (species not identified) in urban and suburban woodlots, but on deer and chipmunks in rural woodlots. In contrast, *Aedes hendersoni* fed predominantly on tree squirrels and raccoons in the three habitats, and fed little on deer or chipmunks.

The studies reviewed above, particularly those of Nasci (1982) and Burkot and DeFoliart (1982), indicate that *Aedes triseriatus* blood feeds to a considerable extent in nature on sciurid rodents. Selection by mosquitoes of rodent hosts is unusual in nature (Edman 1971, 1979), probably because of the defensiveness of rodents towards mosquitoes attempting to feed (Edman et al. 1974, Day and Edman 1983, Day et al. 1983). Other examples of mosquitoes showing a predilection for feeding on rodents are *Anopheles dureni millecampsi* Gillies and DeMeillon (a vector of rodent malaria) feeding on African murine rodents (Killick-Kendrick 1978), *Aedes* *atlanticus* feeding on cotton rats (Taylor et al. 1971), and *Culex taeniopus* Dyar and Knab feeding on *Sigmodon* and *Peromyscus* rodents (Edman 1979). Choice of sciurid rodents as hosts by *Aedes triseriatus* has clear relevance to the epizootiology of La Crosse virus, because chipmunks and gray squirrels are vertebrate hosts of the virus (discussed above).

Studies of temporal and vertical patterns of host-seeking behavior of *Aedes triseriatus* have shown that this species tends to seek hosts during the day (particularly in the afternoon) near the ground, but extends host-seeking into the canopy in evening crepuscular periods (Loor and DeFoliart 1970, Scholl et al. 1979a, Novak et al. 1981). Day biting activity of *Aedes triseriatus* coincides with activity of chipmunks and gray squirrels (Elliot 1978, Thompson 1977b). Other aspects of blood feeding behavior of this species, including simple description of the process and the behavioral interaction of *Aedes triseriatus* with sciurid rodents leading to successful blood feeding, have not been studied.

Oviposition behavior of *Aedes triseriatus* has been studied in the laboratory and the field. Wilton (1968) showed that gravid *Aedes triseriatus* prefer dark-colored containers with horizontal openings and textured walls for ovipostion. Chemicals in treehole water are attractive to gravid females (Bentley et al. 1982). Loor and DeFoliart (1970) noted that egg-laying occurred at dusk. Sinsko and Grimstad (1977) and Scholl and DeFoliart (1977) showed that *Aedes triseriatus* lays eggs in treeholes at ground level whereas *Aedes* *hendersoni* lays eggs in treeholes in the canopy. Eggs laid after the beginning of August assume diapause and do not hatch until the following spring in Wisconsin (Scholl and DeFoliart 1978) and Indiana (Sinsko and Craig 1981).

DeFoliart (1983) developed a quantitative, deterministic model of La Crosse virus transmission, predicated mainly on the biology of *Aedes triseriatus*. The purpose of the model is to explain how La Crosse virus remains enzootic from year to year without disappearing from host populations. The model assumes that transovarial transmission is the most important route of transmission, and that oral infection of mosquitoes (i.e. horizontal amplification) is only a means of recruiting new vertical (transovarial) transmitting females. The model includes the following parameters: (1) low minimum field infection rates of La Crosse virus in overwintered *Aedes triseriatus* in the spring (Lisitza et al. 1977), (2) virus-attritional transovarial transmission rates in *Aedes triseriatus* (Miller et al. 1977), (3) survival of adult female *Aedes triseriatus* (Sinsko and Craig 1979), (4) host utilization patterns of *Aedes triseriatus* (Burkot and DeFoliart 1982), (5) effect of blood source on duration of gonotrophic cycles of *Aedes triseriatus* (Mather and DeFoliart 1983), and (6) relative availability of immune and non-immune sciurid amplifier hosts (Gauld et al. 1974). A single run of the model calculates the number of virus-infected, diapusing eggs laid at the end of the transmission season. If the model simulates reality accurately, then this number should equal or exceed the number of virus-infected larvae observed the previous spring. Simultaneously, the model must achieve a high rate of seroconversion in the sciurid rodent populations. However, the model fails to approach the field estimates of these factors, instead underestimating them both. Apparently, some essential information about the La Crosse virus cycle is lacking in the model.

Mosquito-host interaction. Mosquitoes have evolved to exploit a protein-rich food resource, vertebrate blood, for egg development and consequent reproduction (Waage 1979). Blood feeding is the culmination of a complex behavioral process by which mosquitoes locate a vertebrate host, tap the host′s circulatory system, and ingest the host′s blood. An enormous literature exists on this process (reviews of Kalmus and Hocking 1960, Hocking 1971, and Friend and Smith 1977) which contains conflicting opinions and is difficult to summarize meaningfully. However, general patterns are clear. Mosquitoes seeking hosts initially search for host habitats, and then respond orientatively to host-produced or host-related stimuli, such as carbon dioxide, humidity, heat, host odors, and visual factors (Hocking 1971, Gillies 1972, Gillies 1980, Gillies and Wilkes 1982). Mosquitoes eventually find and land on a host, and probe with the fascicle of the mouthparts into the skin (Griffiths and Gordon 1952). When the fascicle pierces a blood vessel, mosquitoes ingest the blood (Friend and Smith 1977). Blood feeding ceases at a critical volume when abdominal stretch receptors are activated (Klowden and Lea

1979b). Several studies contain detailed descriptions of blood feeding by mosquitoes (Gordon and Lumsden 1939, Griffiths and Gordon 1952, O'Rourke 1956, Clements 1963, Gillett 1967, Service 1971, Reisen and Emory 1976, Magnarelli 1979, Mellink et al. 1982), but blood-feeding behavior of *Aedes triseriatus* has neither been described nor analyzed.

Host-seeking and blood-feeding behaviors by mosquitoes is one facet of mosquito-host interaction; another is host behavior. Mosquito feeding success is largely a function of host behavioral reactions to the presence of the mosquitoes (Edman and Kale 1971). Such "defensive" behavior has risky consequences for mosquitoes, by preventing them from taking full blood meals, or by injuring or killing mosquitoes. Defensive behavior of animals toward mosquitoes is now well documented for a variety of birds and mammals (Edman and Kale 1971, Webber and Edman 1972, Kale et al. 1972, Klowden and Lea 1979a, Waage and Nondo 1982, Edman et al. 1983). The effectiveness of defensive behavior in limiting mosquito feeding success varies with many host-related factors, including host species (Webber and Edman 1972, Edman et al. 1974), individual hosts within a species (Kale et al. 1972), host age (Blackmore and Dow 1958, Kale et al. 1972), host health (Day and Edman 1983a), and mosquito density (Edman et al. 1972, Waage and Nondo 1982). Reeves (1971) and Klowden and Lea (1979a) cogently argued the epidemiological importance of host defensive behavior, by pointing out that such behavior would tend to multiply the number of host contacts of vector mosquitoes, thereby increasing the transmission rate of pathogens. There is serological evidence for this "multiple feeding" in nature (Tempelis 1975, Washino and Tempelis 1983).

Scope and Purpose

The behavioral aspects of the ecological cycle of La Crosse virus are poorly understood. Transmission of this virus, from mosquito to vertebrate host and back to mosquito, depends on the seemingly highly tenuous behavioral interaction of *Aedes triseriatus* females and sciurid rodents. A better understanding of this interaction may lead to a better understanding of the enzootic nature of La Crosse virus. Yet, the literature of La Crosse virus ecology, and biology of *Aedes triseriatus*, is nearly devoid of specific behavioral information on this interaction. The purpose of this dissertation is to resolve this by (1) describing and analyzing the blood-feeding behavior of *Aedes triseriatus* on sciurid hosts (Chapter III); (2) evaluating the effect of defensive behavior of chipmunks and gray squirrels on the blood-feeding success of *Aedes triseriatus* (Chapter IV); and (3) investigating the behavioral response of *Aedes triseriatus* to host defensive behavior (Chapter V). The scope of this dissertation is not limited to behavioral studies, but also includes a survey of occurrence of California serogroup virus infections in mosquito and wild mammal populations of western Massachusetts (Chapter II). There has been no previous systematic survey of this kind in the western part of the state.

CHAPTER II

OCCURRENCE OF CALIFORNIA SEROGROUP VIRUS INFECTIONS IN MOSQUITOES AND WILD MAMMALS OF WESTERN MASSACHUSETTS

Introduction

California encephalitis prototype virus was first isolated from the mosquito Aedes melanimon in California in 1943 (Hammon et al. 1952, Reeves et al. 1983). Subsequently 3 human cases of encephalitis, caused by this virus, were discovered in California (Hammon and Reeves 1952). Since these original studies, California serogroup viruses have been intensively studied because of recognition of their role as etiologic agents of human disease (Henderson and Coleman 1971, Kappus et al. 1983). Interest in these viruses led to an international symposium on the California serogroup, the proceedings of which have been published (Calisher and Thompson 1983).

The California encephalitis virus complex (California serogroup, genus Bunyavirus) consists of three viruses serologically distinguishable into at least 12 distinct subtypes and varieties (Calisher 1983): California encephalitis, Inkoo, La Crosse, snowshoe hare, San Angelo, Tahyna, Lumbo, Melao, Jamestown Canyon, Keystone, Serra do Navio, and trivittatus. Calisher (1983) proposed South River virus as an additional variety of Melao subtype. Seven of these viruses (California encephalitis, La Crosse, snowshoe hare, San

Angelo, Jamestown Canyon, Keystone, and trivittatus) occur only in North America. Each of these has a distinct ecological cycle (LeDuc 1979), existing at low infection rates in mosquito populations with seasonal amplification in wild mammals (Turrell and LeDuc 1983, Yuill 1983, Reeves et al. 1983). These viruses tend to be highly focal in regions where they occur (LeDuc 1979).

La Crosse virus, which is distributed in the midwestern United States, New York State, and patchily in other areas of the eastern United States (Calisher 1983), is medically the most important of the California serogroup viruses. This virus has very likely been the cause of most of the 1,456 reported human cases of California encephalitis in the United States (Kappus et al. 1983). The mosquito _Aedes_ _triseriatus_ is the major vector of this virus; sciurid rodents are summertime amplifying hosts (Thompson 1983, Yuill 1983). Snowshoe hare virus in Canada (Fauvel et al. 1980) and Jamestown Canyon virus in the United States have also been linked to human encephalitis (Grimstad et al. 1982, Kappus et al. 1983, Deibel et al. 1983).

There has been no systematic attempt to determine occurrence and prevalence of California serogroup viruses in western Massachusetts, although snowshoe hare, Keystone, and several untyped California serogroup viruses have been isolated from mosquitoes during routine surveys for eastern equine encephalitis virus in eastern Massachusetts (H.K. Maxfield, Massachusetts Department of Public Health, personal communication). The purpose of this part of the study was to survey mosquito, sciurid rodent, and white-tailed deer populations of western Massachusetts for infection with California serogroup viruses. A special attempt was made to locate La Crosse virus in the region.

Materials and methods

Mosquitoes. Mosquitoes were collected from town and state forest lands of Franklin, Hampshire, Hampden, and Berkshire counties in western Massachusetts in 1981 and 1982. Adult females were collected by hand at human bait and with CDC light traps. A small number were collected from resting areas with a battery-powered aspirator (Appendix II). Larvae were collected from treeholes and tires using a turkey baster or dipper. Mosquitoes were brought alive to the laboratory. Adults were killed by freezing, sorted (by species into pools of 100 or less) on a chill table, and stored at -85° C in an ultra-low freezer. Larvae were reared to fourth instar, identified, and frozen at -85° C. Mosquitoes were identified using keys in Darsie and Ward (1981) and Wood et al. (1979).

Virus isolation and identification. Mosquito pools were triturated with mortar and pestel in 7.5% bovine plasma albumin (in phosphate-buffered saline supplemented with 1000 units of penicillin and 1000 µg of streptomycin), and centrifuged at 2000 rpm for 60 min at 4° C. Each of eight 3-day-old mice were intracerebrally (i.c.) inoculated with approximately 0.025 ml of each supernatant.

Remainders of supernatants were quick-frozen in dry ice-alcohol baths and stored at -70° C. Mice were observed daily for at least 10 days for signs of illness. Brains of sick mice (e.g., those laying on their side, unable to right themselves, quivering, dark of color) were harvested, triturated in 0.75% bovine plasma albumin to make 10% (brain weight:diluent volume) seed, centrifuged at 2000 rpm for 30 min at 4° C, and 0.025 ml of supernatants passed i.c. to 3-day-old mice. Brains of sick mice of first and subsequent serial passages were harvested, and either 20% (weight:volume) stock virus (procedure as with 10% seed) or crude antigen was prepared. Crude antigens were made by incubating triturated brains overnight at 4° C in borate-buffered saline (pH 9.0), centrifuging mixtures at 10,000 rpm for 60 min at 4° C, and quick-freezing supernatants. Above procedures followed Lennette and Schmidt (1969).

Virus isolates were assigned to the California serogroup using neutralization tests in 3-day old mice. These tests employed a broadly reactive hyperimmune serum from rabbits vaccinated with California strain 74-32813 (a New York State La Crosse virus strain) under a constant-serum, varying-virus dilution protocol (Lennette and Schmidt 1969).

California serogroup virus isolates were subtyped using complement-fixation (CF) tests (Kent and Fife 1963, Sprance and Shope 1977). These tests employed crude antigens of the California serogroup isolates reacted against immune mouse ascitic fluids prepared with New York State isolates of snowshoe hare (SSH), La

Crosse (LAC), Keystone (KEY), Jamestown Canyon (JC), and trivittatus (TVT) viruses. Immune fluids were inactivated for 1 hour at 56° C prior to use. In tests, appropriate antigen, serum, red blood cell, and homologous antigen-immune fluid controls were done. Triethanolamine-buffered saline solution was used for diluent. Final volume in tubes was 2.0 ml. Complement was titrated with each test, to ensure adequate complement titer. A test was considered positive if 30% or fewer of red blood cells hemolyzed. The "box" titration method, with 2-fold serial dilutions of antigens and immune fluids, was used to approach complement-fixation endpoints.

Virus reisolations were attempted from positive mosquito suspensions 4-5 months after original isolations. Procedures described above were used for reisolation and identification of reisolates. Positive mosquito suspensions were titrated by i.c. inoculation of approximately 0.025 ml of serial ten-fold dilutions of suspensions into 3-day-old mice. Titration endpoints were calculated as "suckling mouse intracerebral 50% lethal doses" ($SMICLD_{50}$) per 0.025 ml by the method of Reed and Muench (1938).

Wild mammals. Eastern chipmunks (Tamias striatus) and gray squirrels (Sciurus carolinensis) were trapped in town and state forest lands in Franklin, Hampshire, and Berkshire counties in western Massachusetts in 1980 and 1981. Blood was drawn from the orbital sinus of chipmunks and from the heart of squirrels. Blood of white-tailed deer (Odocoileus virginianus) was obtained by aspirating from pools of blood in the abdominal cavities of shot deer brought to checking stations in Berkshire County during the 1980 shotgun season (December 1-6).

Sentinel rabbits. Hardware cloth cages, each containing a white New Zealand rabbit, were hung from trees in town and state forest lands in Franklin, Hampshire, and Berkshire counties in 1981 and 1982. In 1981, rabbits were set out during the first two weeks of July and taken in during the last week of September in the following areas: 5 in the area between Granby Notch and Mount Norwottuck in the Holyoke Range, Amherst, Hampshire County; and 9 in Pittsfield State Forest in Pittsfield and Hancock, Berkshire County. In 1982, rabbits were set out during the first two weeks of May and taken in during the first week of July in the following areas: 2 in Warwick State Forest, Warwick, Franklin County; 3 in Wendell State Forest, Wendell, Franklin County; 3 in Lawrence Swamp Conservation Area in Amherst, Hampshire County; 1 in Granby Notch woods, Amherst, Hampshire County; and 1 in Windsor State Forest, Windsor, Berkshire County. Rabbits were bled weekly from an ear vein or from the heart. All rabbits were bled prior to placement in the woods.

Blood samples. Blood samples of wild mammals and sentinel rabbits were centrifuged at 1000 rpm at room temperature for 30 min or more, the serum decanted and frozen at -40° C. Sera were screened for antibody to California viruses in neutralization tests. Wild mammal sera were diluted 1:5 with phosphate-buffered saline; sentinel rabbit sera were not diluted. Wild mammal sera were tested with California strain 74-32813 (a New York State La Crosse virus strain). Sentinel rabbit sera were tested with this antigen and California strain 78-30641 (a New York State Jamestown Canyon virus strain). Positive sera were titrated in neutralization index tests with a constant-serum, varying-virus method (Lennette and Schmidt 1969). Sera neutralizing at least 1.7 logs of virus were considered positive for California viruses.

Results

Mosquitoes. Table 1 shows results of mosquito collections for 1981 and 1982 in western Massachusetts. One thousand, seven hundred and thirty-two mosquitoes in 92 pools were collected in 1981, and 42,515 mosquitoes (669 pools) in 1982. Collections consisted mainly of spring-brood *Aedes* spp. and *Aedes triseriatus* larvae and adults, in order to maximize chance of isolating California serogroup viruses. Certain species were so similar that they were either inseparable (as adult females) or were impractical to separate during mass processing. Thus the following species groups were formed: *Culex pipiens* L./*restuans* Theobald; *Aedes abserratus* (Felt and Young)/*punctor* (Kirby); *Aedes stimulans* (Walker) group (which probably included mainly *stimulans*, *excrucians* [Walker], and *fitchii* [Felt and Young]); *Aedes dianteus* Howard, Dyar, and Knab/*decticus* Howard, Dyar, and Knab; and *Aedes intrudens* Dyar/*sticticus* (Meigen).

Table 1. Mosquito collection records from Franklin, Hampshire, Hampden, and Berkshire counties, Massachusetts, 1981-1982.

Species	No. Mosquitoes	No. Pools
Aedes abserratus/punctor	3844	68
Aedes aurifer	95	13
Aedes canadensis	1222	54
Aedes cinereus	151	19
Aedes communis	6001	87
Aedes dianteus/decticus	370	26
Aedes implicatus	3	1
Aedes intrudens/sticticus	3060	57
Aedes provocans	2497	45
Aedes stimulans group	2264	53
Aedes triseriatus adults	1552	40
Aedes triseriatus larvae	22557	243
Aedes trivittatus	88	12
Aedes vexans	461	23
Anopheles quadrimaculatus	2	2
Anopheles punctipennis	12	7
Anopheles walkeri	1	1
Coquillettidia perturbans	17	6
Culex pipiens/restuans	47	2
Culiseta morsitans	3	2
Total	44247	761

The latter two species are normally separable except when the scutum is rubbed, which was often the case in collections. No Aedes hendersoni larvae were found. If any Aedes hendersoni adults were collected, they would have been pooled with Aedes triseriatus. Few Anopheles, Culex, or Culiseta were collected.

Table 2 shows mosquito collection records by county in western Massachusetts. Few mosquitoes (all Aedes triseriatus) were collected from Hampden County. Large numbers of Aedes triseriatus larvae were collected from Hampshire and Berkshire counties, primarily from the area between Granby Notch and Mount Norwottuck in Hampshire County; and from woods around Race Brook Trail in Sheffield, and woods in Pittsfield State Forest in Hancock and Pittsfield, in Berkshire County. Large numbers of spring Aedes were collected in Franklin, Hampshire, and Berkshire counties.

Processing of mosquito pools yielded 4 California serogroup virus isolates (Table 3). Snowshoe hare virus was isolated once from Aedes stimulans group mosquitoes collected June 9, 1982 in Lawrence Swamp Conservation Area, Amherst, Hampshire County. The $SMICLD_{50}$ of this isolate was $10^{-2.6}$. Jamestown Canyon virus was isolated three times: from Aedes intrudens/sticticus collected June 10, 1982 in Lawrence Swamp ($SMICLD_{50}$ not reached, as isolate was of low titer); from Aedes abserratus/punctor collected June 22, 1982 in Lawrence Swamp ($SMICLD_{50}=10^{-3.0}$); and from Aedes intrudens/sticticus collected June 29, 1982 in Warwick State Forest, Warwick, Franklin County ($SMICLD_{50}$ not reached, as isolate was of

Table 2. Mosquito collection records by county in western Massachusetts, 1981-1982.

County	No. Mosquitoes	No. Pools
Franklin	11836	112
Hampshire	13501	356
Hampden	392	5
Berkshire	18518	288
Total	44247	761

Table 3. California serogroup virus isolates from mosquitoes collected in western Massachusetts, 1981-1982.

Species	Pool no.	No. Mosquitoes	Site	Date	Subtype	MFIR*1:
Aedes stimulans group	235	100	Lawrence Swamp, Amherst, Hampshire County	9VI82	snowshoe hare	2264
Aedes intrudens/sticticus	247	55	Lawrence Swamp, Amherst, Hampshire County	10VI82	Jamestown Canyon	1530
Aedes abserratus/punctor	471	17	Lawrence Swamp, Amherst, Hampshire County	22VI82	Jamestown Canyon	3844
Aedes intrudens/sticticus	555	100	Warwick St. Forest, Warwick, Franklin County,	29VI82	Jamestown Canyon	1530

*MFIR, minimum field infection rate. Assumes 1 infected mosquito per pool.

low titer). No virus was isolated from Aedes triseriatus larvae or adults; La Crosse virus was not isolated.

Tables 4 and 5 show results of complement-fixation box titrations used to subtype the four California serogroup isolates. Table 4 shows positive reaction endpoint titers of the 5 reference immune mouse ascitic fluids at 4 or 5 dilutions of isolate antigen. Isolate 235 (see Table 3) reacted strongly with SSH fluid, and less strongly with LAC and JC fluids, and not with KEY or TVT fluids. Cross-reactivity with this isolate occurred at 1:4 dilutions of antigen with LAC and JC immune fluids, but this cross-reactivity ceased at 1:8 and higher dilutions. Isolate 235 reacted positively with SSH immune fluid at 1:32 dilution of antigen. Isolates 247, 471, and 555 (see Table 3) showed patterns similar to each other, and different than isolate 235. Each of these isolates reacted positively at 1:32 dilution of antigen with JC immune fluid, and did not cross-react at 1:4 dilution with SSH, LAC, KEY, and TVT immune fluids.

Table 5 shows positive reaction endpoint titers of the 4 antigens at various dilutions of the immune fluids. Snowshoe hare immune fluid reacted positively at 1:16 dilution of fluid with isolate 235, but did not react with other antigens at even 1:4 dilution. La Crosse immune fluid reacted at 1:4 fluid dilution with isolate 235, but not with the other isolates. Keystone and TVT immune fluids did not react with any antigens. Jamestown Canyon immune fluid reacted positively at 1:4 fluid dilution with isolate

Table 4. Results of complement-fixation box titrations with California group isolates. Endpoint titers of immune mouse ascitic fluids for dilutions of antigen isolates.

		Immune Fluids				
Isolate #	Dil.	SSH	LAC	KEY	JC	TVT*
235	4**	16**	4	<4	4	<4
	8	4	<4	<4	<4	ND***
	16	4	<4	<4	<4	ND
	32	4	<4	<4	<4	ND
	64	<4	<4	<4	<4	ND
247	4	<4	<4	<4	16	<4
	8	ND	ND	<4	8	ND
	16	ND	ND	<4	8	ND
	32	ND	ND	<4	8	ND
471	4	<4	<4	<4	4	<4
	8	ND	ND	<4	8	ND
	16	ND	ND	<4	8	ND
	32	ND	ND	<4	16	ND
555	4	<4	<4	<4	16	<4
	8	ND	ND	<4	8	ND
	16	ND	ND	<4	8	ND
	32	ND	ND	<4	16	ND

*SSH, snowshoe hare; LAC, La Crosse; KEY, Keystone; JC, Jamestown Canyon; TVT, trivittatus.
**Numbers are reciprocals of dilutions.
***ND, not done.

Table 5. Results of complement-fixation box titrations with California serogroup isolates, continued. Endpoint titers of virus isolates for dilutions of immune fluids.

		Isolate #			
IMAF*	Dil.	235	247	471	555
SSH	4**	32**	<4	<4	<4
	8	8	ND	ND	ND***
	16	8	ND	ND	ND
	32	<4	ND	ND	ND
LAC	4	4	<4	<4	<4
	8	<4	ND	ND	ND
KEY	4	<4	<4	<4	<4
	8	<4	<4	<4	<4
	16	ND	<4	<4	<4
JC	4	4	≥32	≥32	≥32
	8	<4	≥32	≥32	≥32
	16	<4	4	≥32	≥32
	32	<4	<4	<4	<4
	64	<4	<4	<4	<4
TVT	4	<4	<4	<4	<4

*IMAF, immune mouse ascitic fluid.
**Numbers are reciprocals of dilutions.
***ND, not done.

235, and at 1:16 fluid dilutions with isolates 247, 471, and 555. Jamestown Canyon immune fluid reacted strongly with antigens of these three isolates. Results in Tables 4 and 5 definitively show that isolate 235 is snowshoe hare virus, and isolates 247, 471, and 555 are Jamestown Canyon viruses.

Sentinel rabbits. No sentinel rabbits seroconverted to La Crosse or Jamestown Canyon virus in either 1981 or 1982.

Wild mammals. Table 6 shows results of serosurvey of chipmunks, squirrels, and deer. One of 178 (0.6%) eastern chipmunks had neutralizing antibody to LAC virus. This animal was trapped in Granby Notch woods in the Holyoke Range, Amherst, Hampshire County on June 9, 1980. Five of 31 (16%) gray squirrels had neutralizing antibody to LAC virus. These squirrels were trapped in the following areas: (1) Wendell State Forest, Wendell, Franklin County on June 20, 1980; (2) Lulu group camp , Pittsfield State Forest, Pittsfield, Berkshire County, on July 6, 1980; (3) Doll Mountain, Pittsfield State Forest, Hancock, Berkshire County, on July 9, 1980; (4) Mount Washington State Forest, Mount Washington, Berkshire County, on August 23, 1980; and (5) Granby Notch in the Holyoke Range, Amherst, Hampshire County, on September 9, 1980. Eight of 144 (6%) white-tailed deer had neutralizing antibody to LAC virus. These animals were shot in Monroe, Franklin County; and in Sheffield (2 deer), Washington (2 deer), Lee, West Stockbridge, and Richmond, all

Table 6. La Crosse virus seropositive sciurid rodents and deer trapped or shot in western Massachusetts, 1980-1981, by county.

County	Chipmunk	Squirrel	Deer
Franklin	0/10	1/3	1/1
Hampshire	1/72	1/9	0/0
Berkshire	0/96	3/19	7/143
Total	1/178	5/31	8/144
Percent	0.6%	16%	6%

in Berkshire County.

Discussion

Jamestown Canyon virus was originally isolated from Culiseta inornata in Colorado in 1961, and since then has been isolated from a large number of mosquito and tabanid species collected in 12 states of the United States (LeDuc 1979, Turrell and LeDuc 1983, Calisher 1983). In the northeastern United States, JC virus has been isolated mainly from the "dark-legged" spring Aedes, including Aedes communis DeGeer group in New York State (Grayson et al. 1983) and from Aedes abserratus in Connecticut (Main et al. 1979). Isolation of JC virus in Massachusetts, reported here, is the first record of this virus in the state. Also, JC virus has not been previously isolated from Aedes intrudens/sticticus. The minimum field infection rates of JC virus in mosquitoes collected in Massachusetts are comparable with those in New York State (Grayson et al. 1983) but are lower than those reported in Connecticut (Main et al. 1979).

Snowshoe hare virus was originally isolated from a snowshoe hare (Lepus americanus) in Montana in 1958 (LeDuc 1979), and has since been isolated from 7 states and 8 provinces and territories in Canada (Calisher 1983). The virus has been isolated from over 17 species of mosquitoes (Turrell and LeDuc 1983). Snowshoe hare virus was isolated from a pool of Aedes canadensis collected by Massachusetts Department of Public Health workers on August 12, 1968, in Easton, Bristol County (H.K. Maxfield, Massachusetts Department of

Public Health, personal communication). In New York State, SSH virus has been isolated several times from mainly Aedes mosquitoes, particularly the Aedes stimulans group and Aedes canadensis (Grayson et al. 1983). Isolation of SSH from Aedes stimulans in this study is in agreement with results of other studies.

The vertebrate host of Jamestown Canyon virus in the eastern United States is probably white-tailed deer (Issel 1973, Issel 1974, Issel et al. 1972, Issel et al. 1973, Watts et al. 1982), although experiments of transmission of JC virus by mosquito bite to deer have not been done. Grayson et al. (1983) noted a correlation between increase in prevalence of JC virus in New York State and a rise in white-tailed deer populations. Vertebrate hosts of SSH virus are probably small mammals, including hares, rabbits, and rodents (LeDuc 1979).

Results of serosurvey of gray squirrels, chipmunks, and deer in western Massachusetts (Table 6) showed low rates of exposure of these mammals to California serogroup viruses. Use of La Crosse virus as antigen in neutralization tests on sera from these wild mammals would detect antibody specifically to La Crosse virus or snowshoe hare virus, and possibly to heterotypic viruses (e.g. Jamestown Canyon virus). It cannot be concluded definitively that positive animals had been exposed to La Crosse virus specifically. Considering that La Crosse virus was not isolated in areas where seropositive sciurids were trapped, it would seem more likely that the positive animals had been exposed to another California serogroup virus, probably snowshoe hare.

Sentinel rabbits have been used extensively and successfully in surveillance studies of California serogroup viruses (eg. McKiel et al. 1966, Jennings et al. 1968, Pinger et al. 1975, LeDuc 1978). No sentinel rabbits seroconverted in this study, despite exposure to mosquitoes in areas where JC and SSH viruses were isolated. I often observed mosquitoes feeding on the sentinel rabbits; apparently none were bitten by virus-infected mosquitoes.

There have been no documented autochthonous human cases of California encephalitis in Massachusetts. Low, stable antibody to LAC virus was detected in a ten-year-old boy in Worcester in 1974 (R.F. Gilfillan, Chief, Virology Laboratory, State Laboratory Institute in Jamaica Plain; personal communication). Also, on August 22, 1978, a four-year old boy from Billerica became ill with encephalitis, which was later confirmed as California encephalitis (B. Rosenau, Virology Laboratory, State Laboratory Institute, Jamaica Plain, personal communication). However, this boy was camping in Michigan, Ohio, and Pennsylvania in the three weeks prior to onset of illness and actually became ill in Pennsylvania (personal communication from the boy's mother and from Dr. B.D. Roseman, Medical Associates, Chelmsford), so it is very unlikely that the boy was exposed to infected mosquitoes in Massachusetts.

Western Massachusetts would seem a likely area for LAC virus to occur. Recent forest growth and maturation (MacConnell 1975a,b,c,d) has created good habitat for treehole-developing mosquitoes, chipmunks, and squirrels, all hosts of the virus. Also, western Massachusetts abuts the LAC virus-endemic area in New York State, where LAC virus has been isolated and where many human cases have occurred (Deibel et al. 1979, Grayson et al. 1983). Yet, LAC virus was not isolated in this survey, despite extensive collections of _Aedes triseriatus_, the principal vector of the virus (Thompson 1983). It is entirely possible that La Crosse virus exists in Massachusetts, but was simply missed in this survey.

Recently, Jamestown Canyon virus in New York (Deibel et al. 1983) and Michigan (Grimstad et al. 1982), and snowshoe hare virus in Quebec (Fauvel et al. 1980) have been implicated as etiologic agents of human encephalitis. Jamestown Canyon and snowshoe hare viruses are now known to exist in western Massachusetts; these viruses should be included in the battery of antigens used in tests on sera from encephalitis and meningitis patients in the region.

Conclusions

1. Jamestown Canyon virus was isolated 3 times from mosquitoes collected in western Massachusetts in June, 1982; once from _Aedes abserratus/punctor_ (MFIR = 1:3844) and twice from _Aedes intrudens/sticticus_ (MFIR = 1:1530). This confirmed the presence of Jamestown Canyon virus in Massachusetts. Lawrence Swamp in Amherst, Hampshire County, and Warwick State Forest in Warwick, Franklin County, were identified as foci of Jamestown Canyon virus activity.

2. Snowshoe hare virus was isolated once from Aedes stimulans group mosquitoes collected in Lawrence Swamp June 1982 (MFIR = 1:2264). This confirmed presence of snowshoe hare virus in western Massachusetts.

3. California serogroup virus infections were detected by serosurvey in 1980 and 1981 in 0.6 % of chipmunks and 16.0% of gray squirrels, and 8.0% of white-tailed deer. These infections may have been caused by La Crosse virus, but more likely were caused by snowshoe hare or another California virus.

4. California serogroup viruses infected mosquitoes, sciurid rodents, and deer at low levels in western Massachuetts during the periods studied.

CHAPTER III

FEEDING SITE SELECTION AND BLOOD-FEEDING BEHAVIOR OF AEDES TRISERIATUS ON CHIPMUNKS AND GRAY SQUIRRELS

Introduction

Stable maintenance of the tripartite (mosquito - parasite - vertebrate) cycles of mosquito-borne diseases hinges largely on the host selection and utilization patterns of mosquito vectors. Host feeding patterns, as revealed by serological identification of blood meal sources of wild-caught, blood-fed mosquitoes (Tempelis 1975, Washino and Tempelis 1983), depends on the interaction of a large number of stochastic and deterministic factors (Reeves 1971, Edman 1971, Edman and Kale 1971, Edman et al. 1972, Gillies 1972) These include innate host-specific tendencies, flight patterns, population density, and ecology of mosquitoes; and availability, population density, ecology, and defensive behavior of hosts.

The factor which most directly affects transmission of pathogens between mosquito vectors and vertebrates is blood feeding behavior. Blood feeding is the end result of a highly evolved, complex behavioral process by which mosquitoes find a vertebrate host, tap the host's circulatory system, and imbibe blood. Clements (1963), Hocking (1971), and Friend and Smith (1977) reviewed details of blood feeding by mosquitoes and factors affecting the process.

Initially, mosquitoes seeking a host fly with orientation to the wind direction in search of host habitats and host-related stimuli (Gillies 1972). Such stimuli may include carbon dioxide, humidity, heat, host odors, and visual cues (Hocking 1971, Gillies 1980, Gillies and Wilkes 1982). The relative and sequential importance of these stimuli in guiding mosquitoes to hosts is actually not known, despite the morass of literature on the subject (Hocking 1971).

Host-seeking mosquitoes responding to host stimuli eventually find and land on a host. Factors inducing landing may be odor, color and texture of substrate, or other cues already mentioned (Khan and Maibach 1966, Hocking 1971). After landing, mosquitoes search for a probing (penetration) site on the host, and then probe with the fascicle of the mouthparts into the skin. During probing, mosquitoes salivate (Griffiths and Gordon 1952), which may assist them in finding a blood vessel for feeding (P. Rossignol, Harvard School of Public Health, personal communication) or may inhibit blood coagulation thus facilitating blood ingestion (Hudson 1964). Mosquitoes with arbovirus infections in salivary glands transmit virus particles to hosts while salivating (Hurlbut 1966, McLintock 1978, Mellink 1982).

Blood ingestion begins after a mosquito has pierced or lacerated a blood vessel with the fascicle (Friend and Smith 1977). Phagostimulants in the blood, perceived by sensilla on the fascicle, activate the cibarial and pharyngeal pumps in the mosquito. This results in negative pressure, thereby causing blood to flow from the tip of the fascicle through the food channel and esophagus to the midgut. Blood ingestion ceases at a critical volume when abdominal stretch receptors are activated (Gwadz 1969, Klowden and Lea 1979b). Gordon and Lumsden (1939), Griffiths and Gordon (1952), O'Rourke (1956), Clements (1963), Gillett (1967), Service (1971), Reisen and Emory (1976), Magnarelli (1979), and Mellink et al. (1982) provide descriptive, detailed accounts of blood feeding by mosquitoes.

The biology of the mosquito Aedes triseriatus has received considerable study since incrimination of this species as a vector of La Crosse virus (Watts et al. 1972, Craig 1983). Burkot and DeFoliart (1982) in Wisconsin and Nasci (1982) in Indiana recently studied host utilization patterns of Aedes triseriatus in nature, and found that this mosquito feeds to a considerable extent on eastern chipmunks (Tamias striatus) and tree squirrels. Previous studies showed that these rodents become infected with La Crosse virus, following bite by an infected mosquito, at sufficient viremia to infect other mosquitoes which feed upon them (Pantuwatana et al. 1972). Field studies in Wisconsin demonstrated high rates of transmission of La Crosse virus to chipmunks and squirrels (particularly gray squirrels, Sciurus carolinensis) from mid-summer to fall (Moulton and Thompson 1971, Gauld et al. 1974, Gauld et al. 1975). Thus, the enzootic cycle of La Crosse virus involves Aedes triseriatus as vector and sciurid rodents as summertime amplifier hosts (LeDuc 1979).

Rodents are uncommonly fed upon in nature by mosquitoes (Edman 1971, Taylor et al. 1971, Killick-Kendrick 1978, Edman 1979) probably because of the defensive behavior of rodents (Edman et al. 1974, Day and Edman 1983, Day et al. 1983). The selection of sciurid rodent hosts by Aedes triseriatus warrants detailed investigation.

Currently, there exists little quantitative information on the blood feeding behavior of Aedes triseriatus, despite the importance of this behavior in transmission of La Crosse virus to natural vertebrate hosts and to humans. Loor and DeFoliart (1970), Scholl et al. (1979a), and Novak et al. (1981) demonstrated that Aedes triseriatus tends to seek hosts in the afternoon near ground level. Other detailed aspects of host-seeking and blood-feeding behaviors of this species remain uninvestigated.

The purpose of this study was to describe and quantitatively analyze the searching and blood feeding behavior of Aedes triseriatus on chipmunk and gray squirrel hosts. The study was designed to evaluate the efficiency with which Aedes triseriatus obtains blood from sciurid hosts, thereby providing insight into the behavioral aspects of La Crosse virus transmission. Observations were confined to those behaviors occurring from initial contact of the mosquitoes with these hosts until cessation of blood feeding. Aspects of host-seeking behavior of Aedes triseriatus prior to contact with sciurid rodents were not studied here, although results of field attraction experiments of Aedes triseriatus to sciurid hosts are given in chapter IV.

Materials and methods

Mosquitoes. *Aedes triseriatus* mosquitoes were from the F-14 generation of a colony founded in October 1979 from mosquitoes collected in Amherst. Eggs were hatched under vacuum, and larvae reared at 27° C and 85% RH in 27.9 x 21.6 x 5.1 cm enamel pans with 750 ml distilled water and 100 larvae per pan. Larvae were fed 1:1 Brewer's yeast and lactalbumin at approximately 2.7 mg per larva. Pupae were pipetted from pans and adults allowed to emerge in cages 36 cm on a side. Adults were provided 5% sucrose solution-soaked cotton pledgets until the day before experiments, when only water was provided. Adults were 6-21 days old when used.

Rodents. Eastern chipmunks (*Tamias striatus* [L.]) and gray squirrels (*Sciurus carolinensis* Gmelin) were trapped in Amherst and Goshen (Hampshire County, Massachusetts) and held in captivity until used in experiments. Prior to experiments, animals were anesthatized by intraperitoneal injection of dilute Nembutal^{R} (6.5 mg/ml of 10% ethanol) at 0.8 ml per 100 g body weight. Anesthatized animals were lain ventral surface down on the floor of an observation cage, with limbs splayed out. Five adult chipmunks and 4 adult gray squirrels were used as experimental hosts for studying mosquito behavior. Behavior of mosquitoes was assumed not to be affected by the minor variation among different chipmunks, or among different squirrels.

Hair length and density were recorded from ears, eyelids, backs, noses, and feet of each of 4 squirrels and 4 chipmunks as follows: a 9 mm^2 square was cut into stiff paper, the square placed on a body region, and held in place with tape. Hair originating from skin framed by the square was teased through the square as a tuft. Twenty-five guard hairs were pulled from this tuft with forceps, and their length measured with an ocular micrometer. The remainder of the hairs in the tuft (including underfur) was pulled out or cut, placed in a petri dish, and the number counted.

Observation methods. The observation arena was a 53.3 x 40.9 x 34.1 cm wood, pressboard, screen, and plexiglas cage. Three plexiglas sides allowed viewing into the cage. An anesthatized rodent was placed on the bottom of the cage as described above, and then a mosquito was gently aspirated into the arena through a hole in a cage wall. Observations were done in late morning and afternoon, when Aedes triseriatus is normally active, at 27° C and 85% RH under flourescent light.

Behavior of mosquitoes was observed and recorded verbally on cassette tape. No magnification was used during observations of mosquito behavior. Behaviors recorded were "land", "fly", "forage", "probe", "stop probe", "feed", and "stop feed" (descriptions of these behaviors are in the following results section). Cassette tapes were transcribed with the aid of a stop watch. Frequency and duration of each behavior was recorded. Additionally, the initial landing site, transitional movements among body regions, and the feeding site of each mosquito on the host was recorded. The host body was, for analytical purposes, divided into 3 regions: back, which included all of the body (including the tail) behind the head except legs; head, which included the body anterior to an imaginary line connecting the posterior extension of the pinnae; and the legs, which included the limbs from the thighs distally. The hosts′ bodies were partitioned in this manner to allow analysis of movements of mosquitoes on the host. This partitioning was not artificial, because the three body regions represented true anatomical features which mosquitoes could encounter. Preliminary observations of non-anesthatized sciurids indicated that mosquitoes do not search over or feed on the underside of these hosts, so this area of the hosts′ bodies was not exposed to mosquitoes.

Data analysis. Data were analyzed in order to describe the organization and efficiency of the searching behavior of *Aedes triseriatus* on chipmunks and squirrels, from initial landing of the mosquito until encountering a feeding site and completion of blood feeding.

Transitional movements of mosquitoes among the 3 pre-defined host body regions were subjected to 3-way contingency table analysis and model fitting with the BMDP4F program (Brown 1981) following procedures of Brown (1976), Benedetti and Brown (1978), and Colgan and Smith (1978). The levels of the tables were initial landing site ("land site"), region on which a mosquito foraged before making a transition ("before"), and region on which a mosquito foraged after making a transition ("after"). Each of these levels was categorized by the regions back, head, and leg. Transitions among body regions occurred mainly by flight; therefore a transition from a body region to that same body region was considered a true transition and included in the contingency table. In other words, the contingency tables did not contain logical zeroes because of self-transitions (Colgan and Smith 1978).

Time allocation budgets of _Aedes triseriatus_ during searching on the host were constructed by measuring the following: foraging time on back, head, and leg; amount of time a mosquito flew (=flight time) while searching about the host, but after having landed initially; time spent probing; duration of the last probe before blood feeding commenced; feeding time; and total bout time (time from initial landing until blood feeding ceased).

Fifty individual bouts of _Aedes triseriatus_ on chipmunks and 50 on gray squirrels were studied.

Statistical analyses, besides the contingency table analyses, followed methods of Sokal and Rohlf (1969) and Conover (1980).

Results

Behaviors. The following mosquito behaviors were observed and scrutinized:

1. Land: a mosquito landed on one of the 3 pre-defined body regions of the host. A land was initial if it was the first contact with the host, or transitional if the mosquito had previously landed

on the host.

2. Foraging: after landing, a mosquito commenced walking while simultaneously rapidly tapping the labella on the host (tapping was too fast to count frequency of taps). I call this combination of behaviors foraging. Direction of walking during foraging was usually anterior with reference to the mosquito, but slight lateral inclinations did occur during walking.

3. Fly: a mosquito took flight from the host. Most transitions among the three body regions were by flight; a few occurred directly during foraging. During fly, a mosquito either completely left the host or flew in a skipping fashion over the host's body.

4. Probe: a mosquito inserted the fascicle of the mouthparts into the skin of the host. During probe, the mouthparts either were held stationary in the skin or were drawn up and down.

5. Stop probe: probe was terminated, either when a mosquito withdrew the mouthparts from the skin or when the mosquito began ingesting blood.

6. Feed: a mosquito began to blood feed. This was visually apparent when a red color (the blood) appeared in the pleural membrane of the abdomen.

7. Stop feed: a mosquito withdrew its mouthparts from the host's skin and often immediately flew from the host.

General description of sequence of behaviors. Typically, after a mosquito was released into the observation cage, it flew about, perhaps landed on a wall momentarily, and eventually flew to and landed on the host. Upon landing, the mosquito immediately began foraging, then would fly, hover above or skip on the host, and land again on the same or a different body region. The mosquito would then immediately resume foraging. This process continued until the mosquito arrived at a probing site and probed. In this, the legs appeared to function as anchor and fulcrum on the host as the mosquito's abdomen tipped away from the host, its head tipped toward the host, and its mouthparts penetrated the skin. Mosquitoes that withdrew the mouthparts (i.e. stopped probing) tended to forage in the vicinity of the last probe. Initiation of blood ingestion was readily apparent as described above. Tiny, clear droplets of fluid often exuded from the anus of mosquitoes while feeding; these droplets accumulated on the host's fur.

Initial landing sites. Table 7 shows the initial landing sites of *Aedes triseriatus* on chipmunks and squirrels. Mosquitoes landed most frequently on the back of the hosts, and less often on the head or a leg (X^2 tests, $P<0.01$). Few mosquitoes landed initially on a leg. There were no differences between chipmunks and squirrels in initial landing sites (X^2 tests, $P>0.05$). Thus most mosquitoes began the process of searching for a feeding site on the back of the sciurid hosts, the body region which offered the largest surface area.

Table 7. Initial landing sites of *Aedes triseriatus* on chipmunk and squirrel. Data are from 50 observations on squirrel and 50 on chipmunk.

	Landing site			
Host	Back	Head	Leg	X^2 test
Chipmunk	36	13	1	38.0**
Squirrel	29	15	6	14.6**
X^2 test	0.8ns	0.1ns	3.6ns	

**P<0.01; ns, not significant.

Transitional movements. Table 8 shows the 3-way contingency table of transitional movements of Aedes triseriatus on chipmunks. Nine of the 50 mosquitoes observed landed directly on the head and made no movements from the head, so Table 8 does not contain data on these mosquitoes. It is clear by simple inspection of the table that most transitional movements were back-back, back-head, head-back, and head-head. The transitions were biased by initial landing sites, which were predominantly on the back (Table 7). Screening for significant effects in Table 8 and model fitting to the data give the following interpretation of the table: land site and before interacted and were therefore dependent; land site and after were independent; and before and after were independent (results of X^2 tests given in Table 8). The best fitting model to these data is "after independent of the interaction of land site and before". This means that a transitional movement was biased by initial landing site, but that a single movement to a body region did not depend on where the mosquito was foraging before it made the movement.

Table 9 shows the 3-way contingency table of transitional movements of Aedes triseriatus on gray squirrels. Twelve of the 50 mosquitoes observed did not move from initial landing sites (9 on head, 3 on leg), so the table does not include data on these mosquitoes. As with chipmunks, most transitional movements on squirrels were back-back, back-head, head-back, and head-head. Initial landing sites, which were mainly the back (Table 7), biased these transitions. Screening for effects and model-fitting to data

Table 8. Three-way contingency table of mosquito transitional movements among back, head, and leg of chipmunk. Total movements=203.*

Land site	Before	After		
		Back	Head	Leg
Back	Back	65	60	5
	Head	23	19	0
	Leg	1	2	1
Head	Back	3	4	3
	Head	5	7	0
	Leg	3	0	0
Leg	Back	0	1	0
	Head	0	0	0
	Leg	1	0	0

*Effects: Landsite-before interact (X^2=18.38,P=0.0025); landsite-after are independent (X^2=4.73,P=0.4498); before-after are independent (X^2=9.03, P=0.1078). Nine of the 50 mosquitoes observed initially landed on the head and made no transitional movement from the head. Best fit model is model of independence of after from the interaction of land site and before (liklihood-ratio X^2=22.58,P=0.0674).

Table 9. Three-way contingency table of mosquito transitional movements among back, head, and leg of squirrel. Total movements=141.*

Land site	Before	After		
		Back	Head	Leg
Back	Back	19	25	16
	Head	4	10	6
	Leg	4	3	3
Head	Back	6	5	2
	Head	8	7	0
	Leg	1	1	0
Leg	Back	1	5	1
	Head	3	4	1
	Leg	3	3	0

*Effects: Landsite-before interact (X^2=14.42,P=0.0061); landsite-after interact (X^2=10.13,P=0.0382); before-after are independent (X^2=1.31,P=0.8590). Nine mosquitoes (of 50 observed) landed initially on the head and did not make a transitional movement; 3 landed on a leg and did not make a transitional movement. Best fit model is model of independence of after from the interaction of land site and before (liklihood-ratio X^2=19.21,P=0.2580).

in Table 9 show that land site-before and land site-after effects interacted, but that before-after were independent (results of X^2 tests in Table 9). Thus mosquito movements on squirrels were similar to those on chipmunks (Table 8): initial landing site affected the transitional movements, but where a mosquito foraged prior to a movement did not affect where the mosquito moved next. The best fitting model to the data was identical to the model in Table 8 ("after independent of the interaction of land site and before"). The interaction of after and land site can be ascribed to the effects of the before-land site interaction. Thus, following a rule of parsimony (Benedetti and Brown 1978), this interaction was not included in the final model.

Time allocation on the hosts. Table 10 shows time allocation of *Aedes triseriatus* to different activities during searching on the sciurid hosts. These activities included (1) foraging on back, head, and leg; (2) total foraging; (3) flight during transitions; (4) total probing (sum of all probe times); (5) final probing; (6) feeding; and (7) total bout (from initial land to stop feed). On chipmunks, mosquitoes foraged equally on back and head, and little on legs (paired t-test results in Table 10). On squirrel, mosquitoes foraged predominantly on head and to a lesser extent on the back or legs (paired t-test results shown in Table 10). Mosquitoes spent significantly less time flying in relation to total time spent foraging (t-tests, $P<0.001$). Total probing time was the same on

Table 10. Time allocation (in seconds) of _Aedes triseriatus_ for foraging, flight, probing, and feeding on rodent hosts. Fifty observations on squirrels and 50 on chipmunks.

	Foraging on:			Total	Flight After	All	Last	Feed	Total
Host	Back	Head	Leg	Foraging	Contact	Probes	Probe	Time	Bout
Chipmunk X	25.3a*	39.0a	2.6b	66.9	13.4	55.3	25.9	97.1	227.8
SE	4.5	7.3	1.4	9.5	2.8	7.9	2.5	7.2	17.5
Squirrel X	16.8b	45.3a	16.8b	77.3	5.5	59.8	26.4	94.5	231.5
SE	3.2	8.5	2.6	10.5	0.4	6.1	2.9	7.2	20.6
	ns**	ns	P<0.01	ns	ns	ns	ns	ns	ns

---

*Means of foraging on back, head, and leg of chipmunk or squirrel followed by the same letter are not different by t-test at P>0.05.

**T-test or Mann-Whitney U test comparisons of columns. ns, not significant.

chipmunks and squirrels (Mann-Whitney U test, $P>0.05$), averaging 55.3 seconds for the former and 59.8 seconds for the latter. Similarly, duration of the final probe before feeding was the same on chipmunks and gray squirrels (Mann-Whitney U test, $P>0.05$), averaging 25.9 seconds for the former and 26.4 seconds for the latter. Feeding time of *Aedes triseriatus* was not different on chipmunks and gray squirrels (Mann-Whitney U test, $P>0.05$), averaging 97.1 seconds for the former and 94.5 seconds for the latter. Despite the obvious difference in body size between chipmunks and gray squirrels, the total bout time for *Aedes triseriatus* on these rodents was not different (Mann-Whitney U test, $P>0.05$), averaging 227.8 seconds for the former and 231.5 seconds for the latter.

Probing behavior. Figure 1 shows the frequency distribution of final probing times of *Aedes triseriatus* on chipmunks and squirrels. Frequency distributions of total probing times are not given, because most mosquitoes probed only once. Both distributions are clumped toward the lower end of the scale (i.e. most mosquitoes had short probing times, $G_2=4.0$ for chipmunks, $G_2=12.6$ for squirrels, $P<0.001$ for both G_2 values). Consequently, each distribution has a significant skew to the right ($G_1=1.3$ for chipmunks, $G_1=2.9$ for squirrels, $P<0.001$ for both G_1 values).

The frequency distributions of number of probes before feeding are shown in Figures 2 (chipmunks) and 3 (squirrels). From inspection of both distributions, it is obvious that most mosquitoes

Figure 1. Histograms of frequency distribution of final probing time of *Aedes triseriatus* on chipmunks and gray squirrels. G_1, moment of skewness; G_2, moment of kurtosis.

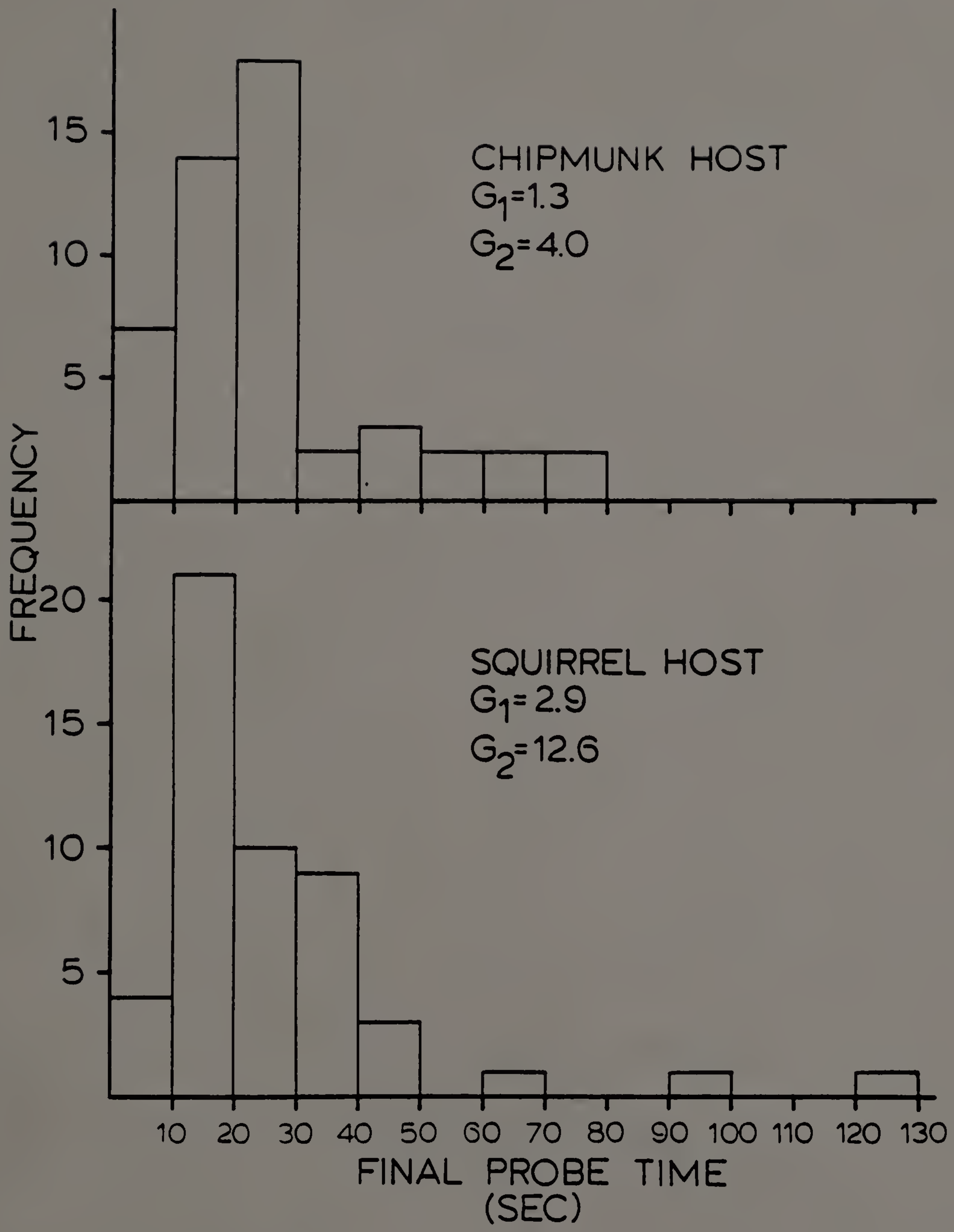

CHIPMUNK HOST
G_1=1.3
G_2=4.0
SQUIRREL HOST
G_1=2.9
G_2=12.6
FREQUENCY
5
10
15
20
10 20 30 40 50 60 70 80 90 100 110 120 130
FINAL PROBE TIME
(SEC)

Figure 2. Histogram of frequency of probing before feeding by _Aedes triseriatus_ on chipmunks.

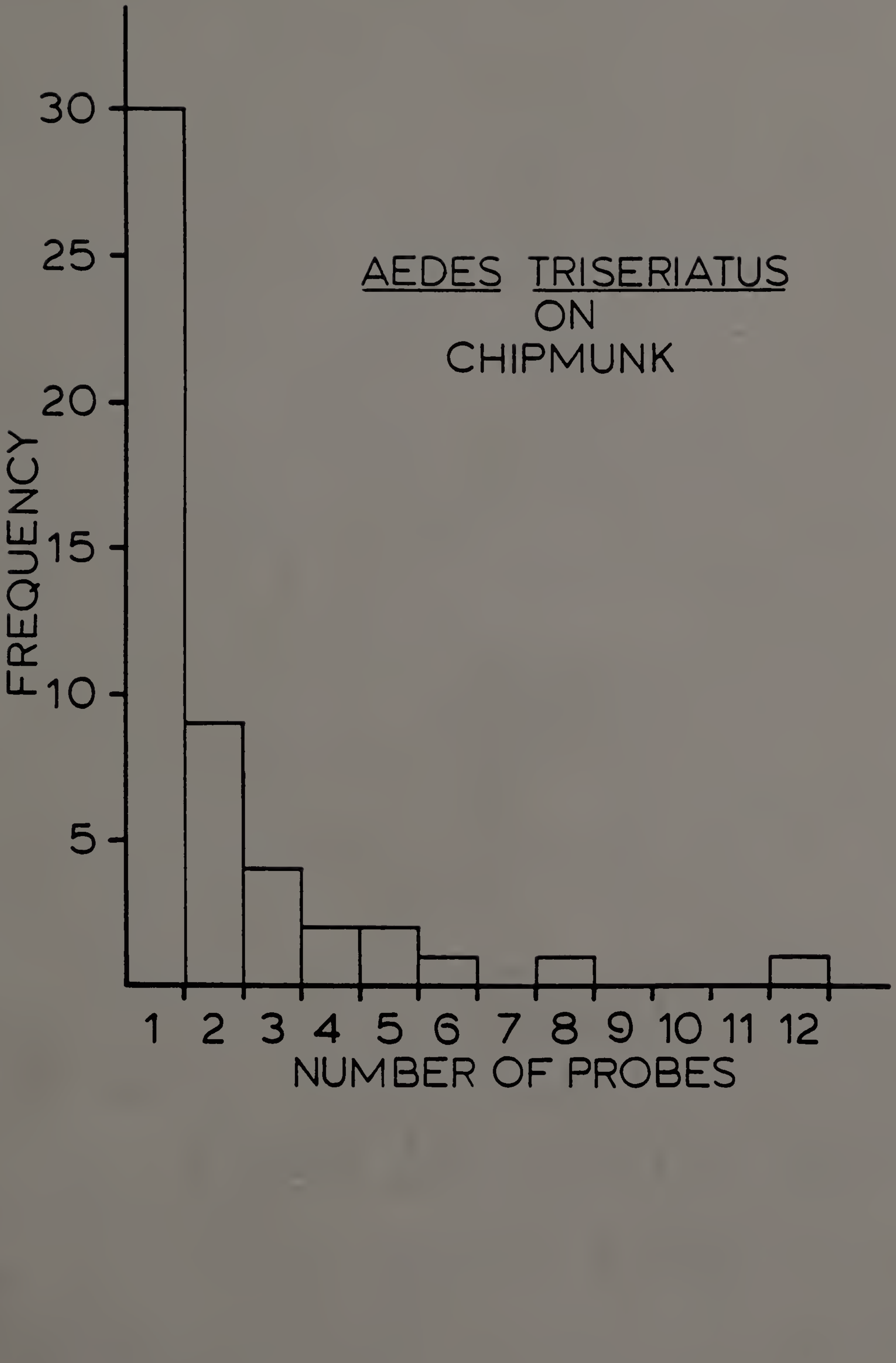
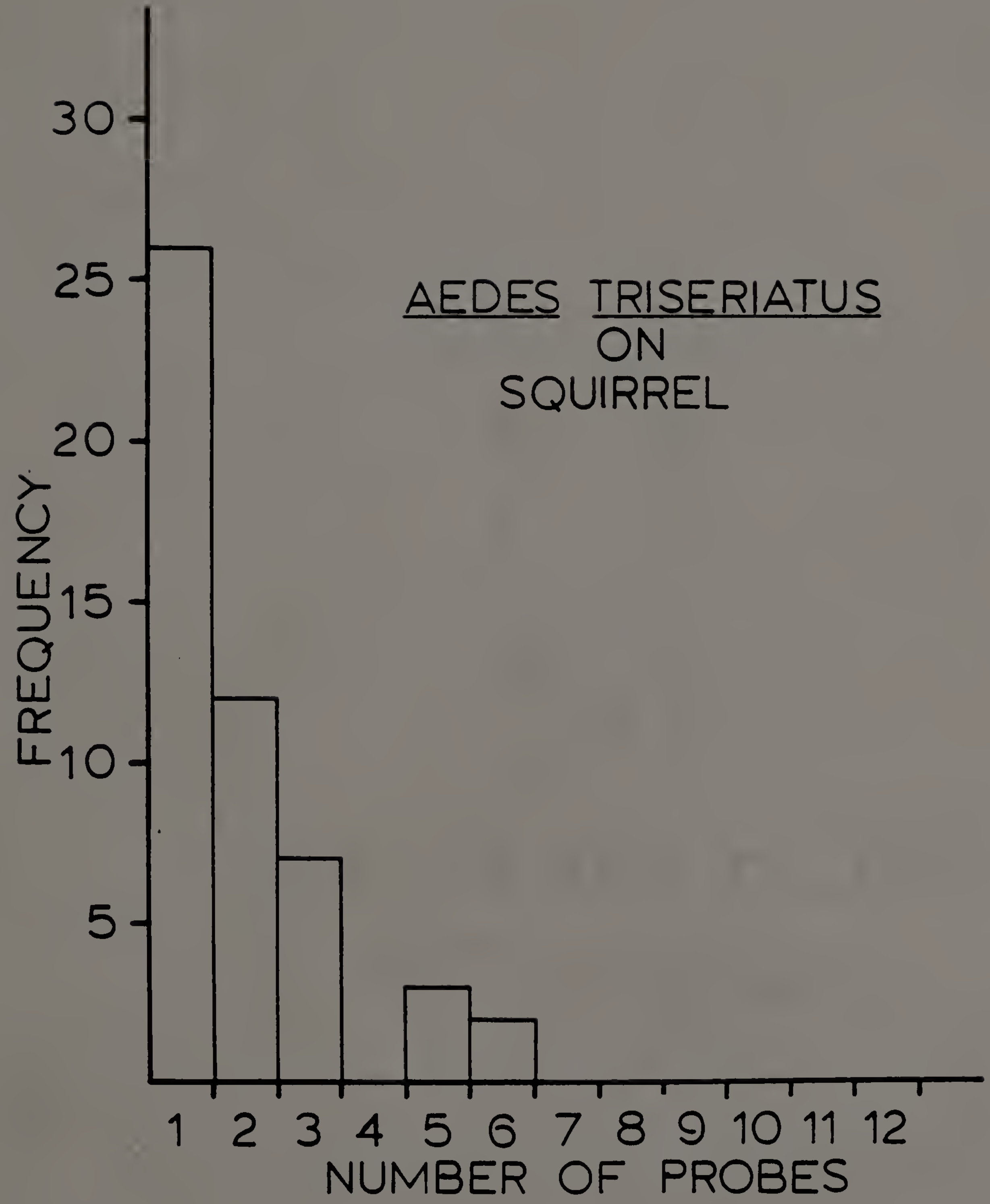

AEDES TRISERIATUS
ON
CHIPMUNK
FREQUENCY
30
25
20
15
10
5
1
2
3
4
5
6
7
8
9
10
11
12
NUMBER OF PROBES

Figure 3. Histogram of frequency of probing before blood feeding by <u>Aedes</u> <u>triseriatus</u> on gray squirrels.


AEDES TRISERIATUS
ON
SQUIRREL
FREQUENCY
30
25
20
15
10
5
1
2
3
4
5
6
7
8
9
10
11
12
NUMBER OF PROBES

probed only once before blood ingestion commenced. The median number of probes was 1 on chipmunk and 1 on gray squirrel. One mosquito on a chipmunk probed 12 times before feeding; 2 mosquitoes on gray squirrels probed 6 times each before feeding. There was no difference in number of probes of Aedes triseriatus on chipmunks or gray squirrels (Mann-Whitney U test, $P>0.05$). Frequency distributions of number of probes were fitted by X^2 goodness-of-fit tests to truncated Poisson distributions (Cohen 1960), in order to test if probing was a discrete, random process. Neither distribution fit a Poisson distribution ($P<0.001$ for both); each distribution had significantly more single probes than predicted by a Poisson (i.e. random) process.

Blood feeding. Table 11 shows feeding sites of Aedes triseriatus on chipmunks and gray squirrels. Mosquitoes fed on ears (both medial and lateral surfaces of the pinnae), the eyelids (rims around the eye), the nose, and the feet (dorsal surface near or on toes). Significantly more mosquitoes fed on the ears than on the other sites (X^2 tests, $P<0.01$). There were no differences between chipmunks and gray squirrels in feeding site selection by Aedes triseriatus, except that mosquitoes fed more on the feet of squirrels than chipmunks (X^2 test, $P<0.01$).

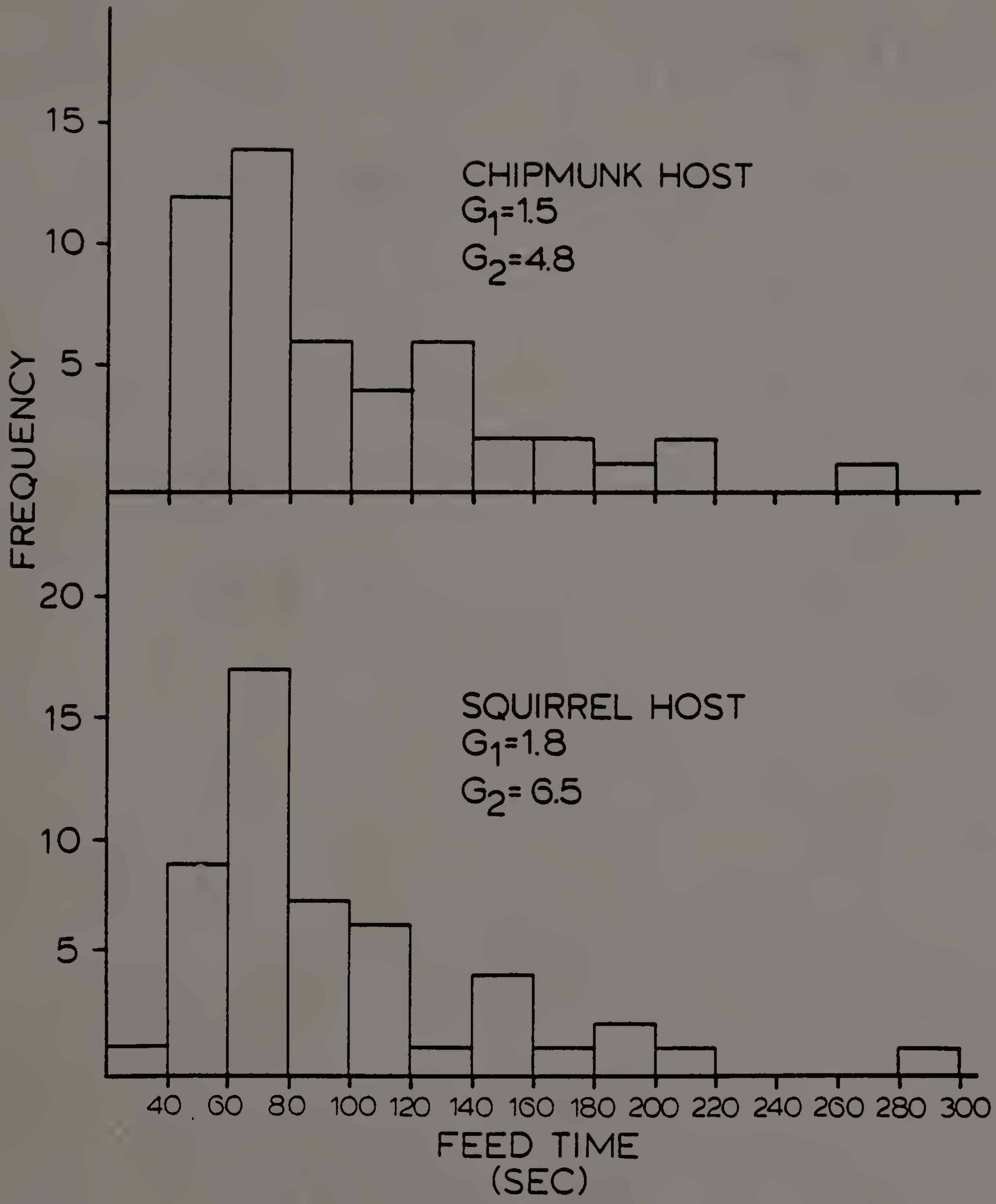
Figure 4 shows the frequency distributions of feeding times of Aedes triseriatus on chipmunks and gray squirrels. One mosquito on a squirrel interrupted its first feeding attempt, and later probed

Table 11. Feeding sites of *Aedes triseriatus* on chipmunks and squirrels. Fifty observations on chipmunks and 50 on squirrels.

	Feeding Site				
Host	Ear	Eyelid	Foot	Nose	X^2 test
Chipmunk	27	13	2	8	27.3**
Squirrel	19	11	16	4	9.9**
X^2 Test	1.4ns	0.1ns	10.9**	1.3ns	

**P<0.01; ns, not significant.

Figure 4. Histograms of frequency distribution of feeding times of <u>Aedes triseriatus</u> on chipmunks and gray squirrels. G_1, moment of skewness; G_2, moment of kurtosis.


15
10
5
FREQUENCY
CHIPMUNK HOST
G_1=1.5
G_2=4.8
20
15
10
5
SQUIRREL HOST
G_1=1.8
G_2= 6.5
40
60
80
100
120
140
160
180
200
220
240
260
280
300
FEED TIME
(SEC)

and initiated feeding again. Data from this mosquito are not included in the analysis. Both frequency distributions are clumped toward the lower end of the scale (most mosquitoes had shorter feeding times, $G_2=4.8$ for chipmunks, $G_2=6.5$ for squirrels, $P<0.001$ for both values) with a consequent skew to the right ($G_1=1.5$ for chipmunks, $G_1=1.8$ for squirrels, $P<0.001$ for both values).

Probing time and feeding time. Correlation analysis (Spearman's rank correlation coefficient rho) of total probe time or final probe time with feeding time was done, in order to determine if mosquitoes that had long probing times also had long feeding times. Results of this analysis are shown in Table 12. There was a positive correlation between total probing time and feeding time (Spearman's rho=0.33, $P<0.05$) but no correlation between final probing time and feeding time (Spearman's rho=0.22, $P>0.05$) of *Aedes triseriatus* on chipmunks. There were positive correlations between total probing time and feeding time (Spearman's rho=0.38, $P<0.01$) and final probing time and feeding time (Spearman's rho=0.60, $P<0.01$) of *Aedes triseriatus* on gray squirrels.

Hair density and length. The density (per 9 mm^2) of hair and length of guard hairs, on back, ear, eyelid, nose, and foot of gray squirrels and chipmunks are shown in Table 13. Comparisons of hair density and length among these areas of each host species by analysis

Table 12. Correlation of total probe time and last probe time with feeding time of _Aedes_ _triseriatus_ on rodent hosts, with Spearman's rank correlation coefficient.

Host	Variable	Correlation
Chipmunk	Last probe	0.22ns
	Total probe	0.33*
Squirrel	Last probe	0.60*
	Total probe	0.38*

*P<0.05. ns, not significant.

Table 13. Hair density (per 9 mm^2) and length (mm) on back, ear, eye, nose, and foot of 4 chipmunks and 4 gray squirrels.*

Rodent	Hair:	Back	Ear	Eye	Nose	Foot
Squirrel	Density**	454.0±11.53e	120.0±10.98b	58.7±12.50a	118.8±15.20b	80.8±7.48a
	Length***	13.6±0.54e	2.2±0.09b	3.0±0.15c	1.4±0.07a	3.8±0.17d
Chipmunk	Density	1582.8±497.00b	152.8±34.49a	253.5±44.20a	57.8±22.37a	49.2±4.50a
	Length	10.5±0.17e	1.6±0.03b	2.9±0.10d	0.9±0.03a	2.1±0.04c

*Values are means ± standard error. Means within each row followed by the same letter are not different by 1-way analysis of variance and Student-Newman-Keuls test at $P>0.05$.

**Based on 4 repetitions per area.

***Length of 100 guard hairs (25 per rodent) measured from each area.

of variance and Student-Newman-Keuls test showed that hair on the back was significantly longer and more dense than at the other areas.

The variation in hair length and density between the feeding sites and the back (which is taken to represent the remainder of the bodies of the hosts in terms of pelage) suggested that feeding site selection by Aedes triseriatus on chipmunks and gray squirrels is mediated by hair length and density. To test this hypothesis, the following experiment was done. A chipmunk was placed into a hardware cloth restrainer (see chapter IV) and completely covered with wrapping paper except for a 6.25 cm^2 area of the back. The hardware cloth above this area was cut away, and the hair in the area either not shaved or shaved. Each of these treatments was exposed (in the observation cage) to 15 Aedes triseriatus for 15 minutes in 4 separate trials. At the end of the 15 minute exposure, the mosquitoes were collected from the cage, frozen, and the presence of blood in the gut determined by dissection under magnification. This experiment was not done with a gray squirrel.

Results of this experiment are shown in Table 14. No mosquitoes fed on the chipmunk when the hair of the back was not shaved, while more than half blood fed when the hair was shaved. This experiment shows that hair on the back of chipmunks is an impediment to blood feeding by Aedes triseriatus, and supports the hypothesis that hair density and length are determinants of feeding site selection.

Table 14. Feeding success of *Aedes triseriatus* on a covered chipmunk with 6.25 cm^2 of the back either shaved or unshaved, and this area exposed to mosquitoes.

		Blood fed	Not Blood Fed
Back Shaved	$\bar{X}$	8.3	6.7
	SE	1.2	1.2
Back Not Shaved	$\bar{X}$	0.0	15.0
	SE	0.0	0.0
Mann-Whitney U test		$P<0.05$	$P<0.05$

Discussion

Landing. Results showed that the searching behavior of *Aedes triseriatus* on sciurid hosts consisted of 3 hierarchic and sequential levels: (1) landing on the host (after host location in the observation cage); (2) foraging with short, intermittent, transitional flights; and (3) probing for a blood vessel. Mosquitoes showed no apparent discrimination of initial landing sites at the first level of searching. Most mosquitoes landed on the broad surface of the back of the hosts (Table 7). Few mosquitoes landed directly on or near a probing-feeding site. Apparently, feeding site selection does not occur during the initial landing process. This contrasts with behavior of some Tabanidae, which tend to land directly on feeding sites on cows (Mullens and Gerhardt 1979, Magnarelli and Anderson 1980). Few studies have quantitatively examined initial landing sites of mosquitoes on hosts. Magnarelli (1979) noted that mosquitoes (*Aedes* spp.) "... seemed to alight indiscriminately on raccoons and mice...". Kalmus and Hocking (1960) and Khan and Maibach (1966) considered that temperature, tactile cues (e.g. texture of substrate), odors, and visual cues (color and shape) were important in stimulating landing by mosquitoes on hosts, but did not relate these behaviors to specific landing behavior in any quantitative way.

Foraging. The second level of searching behavior of *Aedes* triseriatus was foraging with short flights. Foraging was actually a combination of behaviors, including walking on the host while simultaneously thrusting the proboscis toward the substrate and tapping the labella on the host. Foraging was often, though not always, interrupted by short transitional flights during which mosquitoes re-oriented to the host and landed again. Analysis of movements on the host, resulting from these transitional flights or rarely from walking, showed that mosquitoes moved in a random fashion prior to encountering a probing-feeding site. This conclusion is based on model-fitting (Tables 8 and 9), which showed that where a mosquito moved to after a transition was not dependent on where it had been foraging prior to the movement. Comparison of total foraging time with total flight time (Table 10) showed that mosquitoes spent considerably more time foraging then flying while searching for a probing site. This supports the earlier conclusion that feeding site selection does not occur directly by flight.

The second level of searching corresponds to the "exploratory phase" of mosquito behavior described by Service (1971) and to the "searching by walking" phase described by Kalmus and Hocking (1960). The stimuli initiating and maintaining foraging by Aedes triseriatus on the sciurid hosts probably include a tarsal reflex, and chemical and tactile cues perceived by tarsal and labellar sensilla. Such stimuli have been hypothesized and studied electrophysiologically and in behavioral assays (review of McIver 1982), but the sequence of stimulus-response patterns during foraging, and the role of the labella in finding a probing site, are unknown.

The behavior I call foraging included labellar tapping on the substrate, which has unfortunately fallen under the rubric "probing" (Kalmus and Hocking 1960, Clements 1963, Khan and Maibach 1966, Friend and Smith 1977), although Jones and Pillit (1973) suggested two specific terms ("directional proboscidal thrust" and "labellation"). Clearly, labellar tapping is not probing because the fascicle of the mouthparts does not penetrate the skin. Foraging, with labellar tapping, hierarchically preceded probing in the case of *Aedes triseriatus* on sciurids and formed a large part (about 30%) of the total bout time (Table 10). Labellar tapping was probably the means by which *Aedes triseriatus* located probing sites.

Probing. Most *Aedes triseriatus* probed only once before blood feeding. The frequency distributions of probing (Figures 2 and 3) indicated that finding a blood vessel was very efficient, not a random process. The frequency distributions of probing times (Figure 1) also shows that probing was very efficient, because most mosquitoes probed quickly. A normal distribution of probing time would indicate that variation in probing time was due to sampling and random error. The distributions of probing times in Figure 1 suggest that natural selection has favored rapid blood vessel location. My finding that probing by *Aedes triseriatus* on sciurids was efficient contrasts with recent findings by Mellink et al. (1982), who studied probing of *Aedes aegypti* (L.) on the ears of laboratory mice. These authors observed frequent aborted probes and a skewed distribution of probing time, and concluded that blood vessel encounter was a "fortuitous" process. Had these authors examined the frequency distribution of probes, and considered the skewed distribution of probing time in an analytical manner (rather than just transforming their data to normalize the frequency distribution of probing time), they might have arrived at a different conclusion of the efficiency of probing behavior by mosquitoes. Grimstad et al. (1980) noted that _Aedes triseriatus_ infected with La Crosse virus probed more frequently than did non-infected _Aedes triseriatus_. Probably, La Crosse virus affects normal salivation (perhaps by disrupting function of salivary apyrase, P. Rossignol, Harvard School of Public Health, personal communication), and thus lessens efficiency of probing behavior.

Feeding sites. Friend and Smith (1977) did not list host pelage or plumage among the factors affecting blood feeding by mosquitoes, but obviously these factors are important. Laboratory workers routinely shave experimental hosts prior to feeding mosquitoes on them (Gerberg 1970). Blackmore and Dow (1958), Shilova and Troitsky (1958), and Kale et al. (1972) noted that plumage affected feeding success (blood acquisition) of mosquitoes on birds. Mullens and Gerhardt (1979) observed species-specific landing-feeding sites of tabanids on cows, and measured a positive correlation between the labral lengths of the species and the hair depths of their landing-feeding sites. This suggests that the tabanid guild parasitizing the cows had evolved, in response to the environmental variable of hair thickness, toward spatial separation on the cow hosts as a mechanism of minimizing interspecific competition (e.g. interference) for feeding sites. Ornithophilic black flies (Simuliidae) have apparently evolved a special tarsal claw which enables them to grasp and climb through feathers (Crosskey 1973). There is no documented example of an evolutionary response of these types to hair or feathers in mosquitoes (Waage 1979). This could be because mosquitoes are generally vessel feeders, whereas black flies and tabanids feed from pools of blood formed after tissue laceration (Hocking 1971). Therefore, mosquitoes must find a site relatively bare of hair in order to probe effectively.

Feeding site choice (Table 11) by Aedes triseriatus was very restricted and appeared to be limited by hair density and length. Hair was short and sparse on ears, eyelids, nose, and feet (Table 13). This probably allowed the labella to contact bare or nearly bare skin which stimulated probing behavior. Feeding site selection may also be affected by distribution or abundance of capillaries near the skin's surface, but since probing only occurred at feeding sites, this possibility remains untested. Aedes triseriatus fed successfully on the shaved back of a chipmunk (Table 14), which supports the notion that hair limits feeding site selection in this species. This contrasts with the case of tabanids and simuliids mentioned above.

Feeding time. Feeding times of *Aedes triseriatus* on chipmunks and gray squirrels were contagious in distribution (Figure 4), with most mosquitoes having rapid feeding times and few mosquitoes having longer feeding times. This distribution bespeaks efficiency in blood feeding, because rapid feeders would have a selective advantage over slower feeders in avoiding risks of host defensive behavior (cf. Gillett 1967). For *Aedes triseriatus*, these risks when feeding on sciurid hosts are less of injury or death but more of limiting access to blood for reproduction (results of Chapter IV). The distribution of feeding times of *Aedes triseriatus* may be related in a physical sense to the phenomena of "vessel feeding" and "pool feeding". In the former, a mosquito pierces the lumen of a capillary, venule, or arteriole with the fascicle and draws up blood directly from the lumen. In the latter, a mosquito lacerates a vessel and draws blood from the ensueing hemorrhage. Both these types of feeding have been observed with *Aedes aegypti* (Gordon and Lumsden 1939, Griffiths and Gordon 1952, O'Rourke 1956), with the general consensus that pool feeding takes longer than vessel feeding. This differential results in a skewed or even bimodal distribution of feeding time (O'Rourke 1956, Magnarelli 1979). However, recent findings (Mellink et al. 1982, P. Rossignol, Harvard School of Public Health, personal communication) suggest that pool feeding rarely occurs, and that slow blood feeding is related to the size of blood vessels that mosquitoes pierce.

Mellink (1981) failed to select for slow and fast feeding Aedes aegypti over 14 generations of directional selection. It cannot be concluded definitively that feeding speed is not amenable to selection based on these experiments, however, because feeding speed may have been fixed in Mellink's experimental population, subject only to the physical constraints of vessel and pool feeding. Gillett (1967) found that wild mosquitoes (Aedes africanus Theobald) fed faster than a colonized strain of Aedes aegypti, which he attributed to relaxation of selection pressure on the Aedes aegypti for fast feeding. Unfortunately Gillett did not attempt to select for fast and slow feeders, so his hypothesis remains unconfirmed. Also, comparison of feeding times of 2 different species is questionable. The contagious distributions of feeding time of Aedes triseriatus (Figure 4) suggest that selection for fast feeding has occurred in this species.

Gillett (1967) noted a positive correlation of feeding time and probing time of Aedes africanus, as did Service (1971) for Mansonia richardii (Ficalbi). Neither author offerred explanations for this correlation. Probing time and feeding time of Aedes triseriatus were also positively correlated (Table 12). These correlations were weakly positive, however, and not significant in the case of final probing time and feeding time on chipmunks. Thus not all mosquitoes that probed for a long time fed for a long time, and vice versa. A plausible explanation for these correlations is that some mosquitoes probed in areas depauperate of vessels, and the vessels they eventually did encounter were difficult to feed from (e.g., because of small diameter). It is doubtful that the correlations were due to variation in the experimental mosquito population.

Mosquito behavior on chipmunks and gray squirrels. Searching behavior of *Aedes triseriatus* on chipmunks and gray squirrels was similar, despite the differences in size of the two host species. The only differences in behavior were that mosquitoes tended to forage more on the legs (Table 10) and feed more on the feet (Table 11) of gray squirrels than of chipmunks.

Mosquito behavior and transmission of La Crosse virus. My study of the searching and blood feeding behavior of *Aedes triseriatus* on chipmunks and gray squirrels has clear relevance to the epizootiology of La Crosse virus. Through blood feeding, La Crosse virus moves from mosquito to vertebrate host by probing, and from vertebrate to mosquito by blood ingestion. Thus this study is essentially an examination of the "vehicle" which transports La Crosse virus. Further, transovarial transmission of La Crosse virus (Thompson 1983) depends on mosquitoes acquiring blood for egg development. *Aedes triseriatus* use sciurid rodent blood extensively for this (Burkot and DeFoliart 1982, Nasci 1982). However, the behavioral sequences and parameters I have outlined in this chapter may not reflect the behavior of La Crosse virus-infected *Aedes triseriatus*. This virus is neurotropic in Aedes triseriatus (Tesh and Beaty 1983), infecting cerebral, thoracic, and abdominal ganglia. Such infection may disrupt search and feeding behaviors by impairing normal function and integration of the nervous, muscular, and alimentary systems. Currently there is no evidence for these effects because appropriate experiments have not been done. The only documented effect of La Crosse virus infection on Aedes triseriatus, mentioned above, is in causing increased frequency of probing prior to blood feeding (Grimstad et al. 1980).

In this study, rodents were anesthatized in order to observe mosquito behavior without the complicating factor of host behavior. The succeeding chapter deals with the effects of host defensive behavior on blood feeding of Aedes triseriatus. Also, aspects of host-seeking behavior of Aedes triseriatus prior to contact with sciurid rodents were not studied here. Results of field attraction experiments of Aedes triseriatus to chipmunks and gray squirrels are given in chapter IV, and accounts of field sightings of Aedes triseriatus attracted to chipmunks are given in Appendix I.

Conclusions

1. Searching behavior of Aedes triseriatus on anesthatized chipmunk and gray squirrel hosts consisted of 3 hierarchic levels: (1) landing on the host, (2) foraging with transitional movements about the host body, and (3) probing into the host skin for a blood vessel.

2. Mosquitoes did not select feeding sites on the hosts directly by landing on feeding sites. Most mosquitoes landed on the back of hosts and reached feeding sites by foraging and random movement among back, head, and legs of hosts.

3. Probing for a blood vessel by *Aedes triseriatus* on chipmunks and gray squirrels was rapid and efficient. Analysis of frequency distribution of number of probes before blood feeding showed that probing was not a random process. Distribution of probing time suggested that mosquitoes have been selected for rapid blood vessel location.

4. Feeding site choice by *Aedes triseriatus* on chipmunks and gray squirrels was restricted by hair density and length to ears, eyelids, nose, and feet, where hair was sparse and short.

5. Distribution of feeding times on chipmunks and gray squirrels suggested that *Aedes triseriatus* has been selected for rapid blood feeding, which would be adaptive in avoiding the effects of host defensive behavior.

CHAPTER IV

INFLUENCE OF CHIPMUNK AND GRAY SQUIRREL DEFENSIVE BEHAVIOR ON FEEDING SUCCESS OF *AEDES TRISERIATUS*

Introduction

Mosquitoes attempting to blood feed may elicit behavioral reactions from attacked hosts (Edman and Kale 1971). These "defensive" reactions may totally prevent mosquitoes from blood feeding, may prevent mosquitoes from taking a full blood meal, or may injure or kill mosquitoes. The importance of host defensive behavior in relation to epidemiology of vector-borne diseases is well recognized (Reeves 1971, Klowden and Lea 1979a, Waage 1979). Because defensive behavior limits mosquitoes (or other vectors) from feeding successfully (i.e. completely), there is heightened probability that a vector will contact more than one host before blood feeding to repletion. Klowden and Lea (1979a) argued that, because of defensive behavior, the "one blood meal per gonotrophic cycle" dogma should be replaced with the idea that mosquitoes may take several small blood meals within a gonotrophic cycle. Indeed, there is serological evidence for such multiple feeding by mosquitoes (Tempelis 1975). This means that the biting rate variable in models of vectorial capacity (Garrett-Jones and Grab 1964) needs to be discarded in favor of a more realistic concept. Epidemiologically, host defensive behavior would compound vectorial capacity.

Expression of defensive behavior by various vertebrate hosts toward mosquitoes can vary with several factors (cf. Day 1981):

1. Interspecific variation. Different species of hosts vary considerably in effectiveness of types of defensive behavior. Edman and Kale (1971) and Webber and Edman (1972) documented differential mosquito feeding success on seven species of ciconiiform birds, which was due to specific differences in the birds' defensive behaviors. These differences have been related to normal foraging strategies of these birds in nature (Day 1981, Edman et al. 1983). Edman et al. (1974) noted marked interspecific host variation in mosquito feeding success on birds and mammals, finding that passerines and rodents in particular tended to be very defensive towards mosquitoes while larger birds and animals were less defensive.

2. Intraspecific variation. Hosts may vary among and temporally within individuals of the same species in defensive behavior. Kale et al. (1972) found such variation in wading birds. Dow et al. (1957) also showed this variation among chickens and certain species of perching birds.

3. Age. Host age may affect expression of defensive behavior. Blackmore and Dow (1958) noted higher mosquito feeding success on nestling vs. adult birds which was probably due in part to differential defensive behavior. Kale et al. (1972) found lower mosquito feeding success on adult vs. nestling little blue herons and snowy egrets, because of behavioral changes during maturation and growth of plumage. This was not true with black-crowned night

herons, which were rather tolerant of mosquitoes as adults.

4. Mosquito density. The density of mosquitoes attacking a host profoundly affects the expression of host defensive behavior. High densities stimulate increased host defensive behavior, thus reducing consequent mosquito feeding success. This is supported by results of field studies of mosquito feeding on caged hosts (Dow et al. 1957, Reeves 1971, Fujito et al. 1971, Nelson et al. 1976, Klowden and Lea 1979a). Edman et al. (1972) found a negative relationship between mosquito density and mosquito feeding success on four species of ciconiiform birds, and a positive relationship between mosquito density and frequency of the birds′ defensive behavior. Waage and Nondo (1982) found similar relationships with laboratory rabbits.

5. Host health. Day and Edman (1983a) showed that malarious mice were more susceptible to attacking mosquitoes than healthy mice. Day (1981) suggested that arbovirus infections may similarly sicken hosts sufficiently to make them less defensive towards mosquitoes than healthy hosts, but this idea needs further study.

6. Host experience. Waage and Nondo (1982) suggested that hosts (rabbits in their study) that have previously experienced mosquito biting will be more defensive than hosts that have not had such experiences. This idea also needs further study.

La Crosse (LAC) virus, of the California serogroup of Bunyaviridae (Calisher 1983), is a significant cause of arthropod-borne encephalitis in the eastern United States (Kappus et al. 1983). The container-developing mosquito *Aedes triseriatus*, enzootic vector of LAC virus, transmits the virus "horizontally" by bite but also "vertically", i.e. transovarially and transeminally (Thompson 1983). In deciduous forests and woodlots, *Aedes triseriatus* feeds mainly on deer, squirrels, and chipmunks (Burkot and DeFoliart 1982, Nasci 1982). Chipmunks and squirrels (particularly gray squirrels) are suitable hosts for LAC virus (Yuill 1983). They may act as summer-time amplifier hosts for the virus, providing a source of virus-laden blood for infecting previously non-infected mosquitoes, thereby allowing the virus to remain enzootic in a particular area (Moulton and Thompson 1971, Gauld et al. 1974, Gauld et al. 1975, Ksiazek and Yuill 1977). La Crosse virus seroconversion rates reach high levels in sciurid populations in virus-enzootic areas (Gauld et al. 1974).

Many investigators (Miller et al. 1977, Turrell and LeDuc 1983, Tesh and Beaty 1983) have postulated, based on evidence of chronic virus infection of mosquito ovaries, that transovarial transmission of LAC virus alone can be responsible for year-round maintenance of LAC virus in nature. However, data on low (0.0029 to 0.0059) minimum field infection rates in *Aedes triseriatus* (Lisitza et al. 1977), attritional transovarial transmission rates in *Aedes triseriatus* (Miller et al. 1977), host feeding patterns of this mosquito (Burkot and DeFoliart 1982, Nasci 1982), and effect of blood source on egg production (Mather and DeFoliart 1983) in *Aedes triseriatus* contribute to a deterministic model (DeFoliart 1983) which fails to explain quantitatively how LAC virus remains enzootic at natural, low levels. The model also fails to explain how seroconversion rates in sciurid populations reach high (39 to 100%, Gauld et al. 1974) levels. An important assumption of this model is that Aedes triseriatus females have only one contact with a host per gonotrophic cycle, which ignores effects of host defensive behavior.

I believe that the behavioral interaction of sciurid hosts and Aedes triseriatus attempting to blood feed, is an important component of the LAC virus cycle. The defensive behavior of chipmunks and squirrels towards attacking mosquitoes may have significant effects on this cycle, but quantitative data are generally lacking. Edman et al. (1974) made a small number of observations on gray squirrel behavior, using two gray squirrels and the mosquito Culex nigripalpus Theobald, and concluded that squirrels were rather defensive towards mosquitoes. However, these experiments were done at night when squirrels are generally inactive. There are no published data on chipmunk behavior towards mosquitoes. Wright and DeFoliart (1970) presented limited data of mosquito feeding on squirrels and chipmunks exposed to mosquitoes overnight in Magoon stable traps.

The purposes of this study were to (1) categorize and document eastern chipmunk (Tamias striatus [L.]) and gray squirrel (Sciurus carolinensis Gmelin) defensive behaviors against mosquitoes; (2) measure the frequency of occurrence of defensive behaviors as a function of mosquito density; (3) evaluate the feeding success of

Aedes triseriatus on gray squirrels and chipmunks; and (4) extrapolate results of the above to the natural setting, and make inferences relative to the LAC virus cycle.

Materials and methods

Mosquitoes. *Aedes triseriatus* mosquitoes were from F-9 through F-11 generations of a colony founded in October, 1979, fom mosquitoes collected in Amherst. Eggs were hatched under vacuum, and larvae reared in 27.9 x 21.6 x 5.1 cm enamel pans with 750 ml distilled water and 100 larvae per pan. Larvae were fed a 1:1 mixture of Brewer's yeast and lactalbumin at approximately 2.7 mg per larva. Ambient conditions in the environmentally controlled rearing room were 27° C and 85% RH. Adults were held in 36^3 cm cages, and provided 5% sucrose solution for nutrient up until the day before experiments, when sucrose was removed and water provided. Mosquitoes were 6-21 days old when used.

Rodents. Eastern chipmunks (*Tamias striatus*) and gray squirrels (*Sciurus carolinensis*) were trapped in Amherst and Goshen, Hampshire County, Massachusetts, and held in captivity until use in experiments. Only adults were used.

Observation cages. Observation cages were constructed of wood, screen, hardware cloth, pressboard, and plexiglas. Two walls of each cage were plexiglas to allow viewing into the cages. The chipmunk observation cage was 78.7 x 55.9 x 62.2 cm; the squirrel observation cage was 91.4 x 99.1 x 96.5 cm. Sleeved ports on top of the cages provided access to the insides.

Rodent handling. A rodent was put into an observation cage at least 1/2 h prior to use in experiments, to allow it to acclimate. Chipmunks were put directly into the cage, but squirrels were first put inside a 73.7 x 35.6 x 35.6 cm weldwire cage (mesh size 5 x 5 cm) which was inserted into the larger observation cage. This was done to prevent squirrels from gnawing through screen or the sleeved ports.

In experiments, animals were either free or were restrained in hardware cloth envelopes. This method of restraint prevented rodents from exhibiting normal movement, although the animals could and occassionally did squirm within the restrainers.

Experimental design. Defensive behavior was studied by placing a rodent into an observation cage, and then inserting either 1, 5, 15, or 25 *Aedes triseriatus* females. An observer then sat behind a plywood blind (which left the observer's head and shoulders exposed) at a distance of 3 meters from the cage, and recorded on cassette tape the catalog of defensive behaviors displayed by the rodent. Frequency, but not duration, of behaviors were recorded. Each experiment lasted 1/2 h, after which the mosquitoes were recovered from the cage using an aspirator, and frozen. During the 1/2 h period, the observer marked down at five minute intervals a "+" if the rodent was moving and a "-" if it was still. The pluses (maximum of six) were summed to give an activity index, which was used as a measure of general agitation caused by the mosquitoes. Control observations, with no mosquitoes present, were done for 1/2 h with each rodent to provide baseline behavioral data. Each rodent used was also restrained and exposed to 1, 5, 15, and 25 mosquitoes for 1/2 h. This was done to compare mosquito feeding success on rodents free to defend themselves with rodents restrained from doing so. The sequence of experiments was: first, nonrestrained rodents were exposed to each of the 4 densities of mosquitoes in a random sequence of exposure; then the control observations on rodent behavior with no mosquitoes present were done. Afterwards, rodents were restrained and exposed to mosquitoes at the 4 densities. Mosquitoes collected after exposure to restrained or nonrestrained rodents were visually classed (Edman et al. 1975) with aid of a microscope into 4 categories: fully blood-fed, partially fed (including trace meal), not fed, or killed. All experiments were done out-of-doors, in late morning or afternoon, from June through September 1982. Fifteen chipmunks and 8 squirrels were used. Experiments were attempted with two additional squirrels, but these animals paced continuously in the observation cage so experiments could not be done with them.

Mosquito attraction density to rodents. Attraction density (here defined as the number of mosquitoes attracted to a host in a set period of time) of Aedes triseriatus to squirrel and chipmunk was estimated in an approximately 4 acre woods in Pittsfield State Forest, Pittsfield, Massachusetts, on August 30-31 and September 1, 3, and 5, 1982. Red oak (Quercus borealis Michx.) predominated in the woods. Understory was sparse, consisting mainly of mountain laurel (Kalmia latifolia L.). This woods is located just east of the Lulu group camp area, south of the swimming pond, southwest of the ski jump, and north of the trailer meadow. The woods is bordered on the north by a thick stand of red spruce (Picea rubens Sarg.), hemlock (Tsuga canadensis [L.]), and white pine (Pinus strobus L.); on the east by a tarmac road beyond which is an oak woods; on the west by a dirt road beyond which is another evergreen stand; and on the south by a gravel road beyond which is an oak woods. A census in June,1982, revealed 30 water-filled treeholes with Aedes triseriatus larvae. Many of these treeholes dried up by July. Mammal trapping and mosquito collecting done in 1980 and 1981 showed that eastern chipmunks, gray squirrels, and Aedes triseriatus were co-residents of this woods. No Aedes hendersoni Cockerell larvae or adults were collected in this area.

Black Magoon stable traps (Magoon 1935), containing either a caged chipmunk or squirrel, were used to collect mosquitoes in the woods. These traps were constructed of wood, screen, and plexiglas, and were of 64.9 x 47.0 x 38.1 cm dimensions. Mosquito entry-ways were 2 adjustable plexiglas louvres, 53.3 cm long which were opened 2.5 cm during tests. Mosquitoes which entered a louvre were prevented access to the rodent in the trap by screening. An adult male chipmunk (110 g) and an adult male gray squirrel (453 g) were used. Rodents were kept in clean plastic cages, 33.0 x 27.9 x 17.8 cm, with tops covered with hardware cloth and bottoms covered with fresh woodchips, during experiments.

Mosquitoes were collected as follows. A 55^2 m square circuit was laid out in the woods, and the circuit's corners used as mosquito collecting sites. A collector set a rodent-baited box at one corner, set an empty (control) box at the next corner (counter-clockwise direction) and walked to the next corner. The collector, for a 1/2 h period, aspirated mosquitoes attracted to him, then returned to the baited and control Magoon traps. Mosquitoes hovering about or walking on the traps, and mosquitoes inside the louvres, were collected. Subsequent collections were identically done, but were shifted counter-clockwise to the next three points on the circuit. This shifting was done to reduce positional bias and the effects of the presence of a human near the Magoon traps.

Attraction density was measured in order to estimate the number of _Aedes triseriatus_ that might be attempting to feed on a chipmunk or gray squirrel under typical afternoon, late summer conditions. Attraction density can be related to experiments of mosquito feeding success on squirrels and chipmunks at different densities, to predict feeding success of _Aedes triseriatus_ on these rodents in nature.

Data analysis. Statistical methods followed procedures of Sokal and Rohlf (1969) and Conover (1980).

Results

Behavioral catalogs. Chipmunks displayed eight defensive behaviors:

1. Eyeblink. An eye was closed and opened rapidly.
2. Ear twitch. An ear was rapidly moved.
3. Head shake. The head was quickly and vigorously shaken.
4. Body shake. The entire body, including the head, was vigorously shaken.
5. Forefoot scratch. A forefoot was quickly brushed over the side of the head, generally contacting the ear, eye, and side of the face.
6. Hindfoot scratch. A hindfoot was rapidly and repeatedly scratched against the side of the head.
7. Face groom. Chipmunk sat up on its haunches, licked its forefeet, and rapidly rubbed the feet over the ears and face. Other grooming behaviors (e.g. licking genitals) were not recorded.
8. Bite. Chipmunk bit at a mosquito.

Squirrels displayed nine defensive behaviors:

1. Eye blink. Similar to chipmunk.
2. Ear twitch. Similar to chipmunk, but appeared more vigorous.
3. Head shake. Similar to chipmunk.

4. Face groom. Similar to chipmunk. This behavior often precluded other grooming behaviors (e.g. tail groom, Horwich [1972]), but these were not recorded.
5. Face hide. Forefeet were briefly placed over the face. This behavior had a measurable duration, but was generally short.
6. Forefoot scratch. Similar to chipmunk.
7. Hindfoot scratch. Similar to chipmunk.
8. Face rub. The face was rubbed on the substrate.
9. Bite. Similar to chipmunk.

Frequency of defensive behaviors. Table 15 shows the frequency of occurrence of chipmunk defensive behaviors at densities of 0, 1, 5, 15, and 25 mosquitoes. Eyeblink, head shake, and body shake were frequent behaviors; forefoot scratch, hindfoot scratch, and face groom were less frequent; ear twitch and bite were relatively rare.

Mosquito density affected frequency of most defensive behaviors displayed by chipmunks (Table 15). Table 15 also shows that general chipmunk activity increased as mosquito density increased, suggesting that mosquitoes agitated chipmunks during experiments. In general, chipmunk defensive behaviors increased in frequency with increasing mosquito density (Kruskal-Wallis and multiple comparisons tests of Conover [1980] used to establish relationships among means). A logical prediction about the nature of this increase in frequency of defensive behavior is that the increase

Table 15. Frequencies of defensive behaviors of 15 chipmunks, exposed for 30 minutes each, to 0, 1, 5, 15, and 25 mosquito densities.*

Behavior	Mosquito Density				
	0	1	5	15	25
Eyeblink	11.8 (8.3)a	20.8 (5.6)a	59.5 (9.1)b	81.7 (8.9)c	107.5 (8.8)c
Ear twitch	0a	0a	0.3 (0.3)a	0.5 (0.3)a	0.2 (0.4)a
Head shake	0a	2.3 (1.2)a	11.7 (2.6)b	22.7 (4.5)c	27.6 (4.8)c
Body shake	0a	0.9 (0.5)a	3.9 (1.3)b	14.5 (3.5)c	20.9 (3.4)c
Forefoot scratch	0a	0.5 (0.5)a	1.5 (0.7)b	2.8 (1.3)bc	5.3 (1.7)c
Hindfoot scratch	0a	0.1 (0.1)a	2.0 (1.0)b	2.1 (0.9)b	5.3 (1.6)c
Face groom	0.6 (0.2)a	0.7 (0.5)a	1.9 (0.5)b	2.5 (0.6)b	2.4 (0.4)b
Bite	0a	0a	0.2 (0.2)ab	1.3 (0.5)c	1.2 (0.7)b
Total	12.4 (2.2)a	25.3 (6.7)a	81.0 (11.2)b	128.1(12.6)c	171.9(12.8)d
Activity index	0.3 (0.2)a	0.4 (0.2)a	1.1 (0.7)b	2.3 (0.3)c	3.1 (0.8)d

*Table values are means with standard errors in parentheses. Within each behavior, means followed by different letters are significantly different (Kruskal-Wallis and multiple comparisons tests, $P<0.05$).

should be a directly proportional linear function of numbers of mosquitoes attacking. For example, the frequency of total behaviors a chipmunk displayed at a density of one mosquito should be multiplied by 25 when the density of attacking mosquitoes is 25. This reasoning leads to the generation of a straight line which is a predictive model of frequency of defensive behaviors as a function of mosquito density (dashed line in Figure 5). The line was constructed by connecting the average number of total behaviors expressed with 1 mosquito present through points 5, 15, and 25 times that number (these points fall on the same straight line). If the model is a good predictor of frequency of defensive behaviors, then the line should fall within 95% confidence intervals surrounding the means of the observed frequencies of total defensive behaviors (Grant 1962). Figure 5 shows the mean observed frequencies and confidence intervals (connected by a solid line). The model does not fit the observed data well; it predicts a much greater increase in frequency of defensive behaviors than actually occurred. Linear regression yields a line (equation $Y = 26.9 + 6.2X$, $R^2 = 0.69$, $r = 0.83$, $P<0.05$) which fits the data much better than the model.

Table 16 shows frequency of occurrence of squirrel defensive behaviors at densities of 0, 1, 5, 15, and 25 mosquitoes. Eye blink, head shake, and forefoot scratch were frequently displayed behaviors; ear twitch, hindfoot scratch, face groom, face rub, and face hide were infrequently displayed; and bite was rare, having occurred only 3 times. Frequency of occurrence of behaviors increased with

Figure 5. Mean total frequency of defensive behaviors of chipmunks with 95% confidence intervals, at 0, 1, 5, 15, and 25 densities of mosquitoes. Experimental mean values are connected by a solid line. Dashed line is a predictive model of increase of defensive behaviors as a function of mosquito density. Dash-dot-dash line is the linear regression line of total defensive behaviors on mosquito density ($Y= 26.9 + 6.2X$, $R^2 = 0.69$).

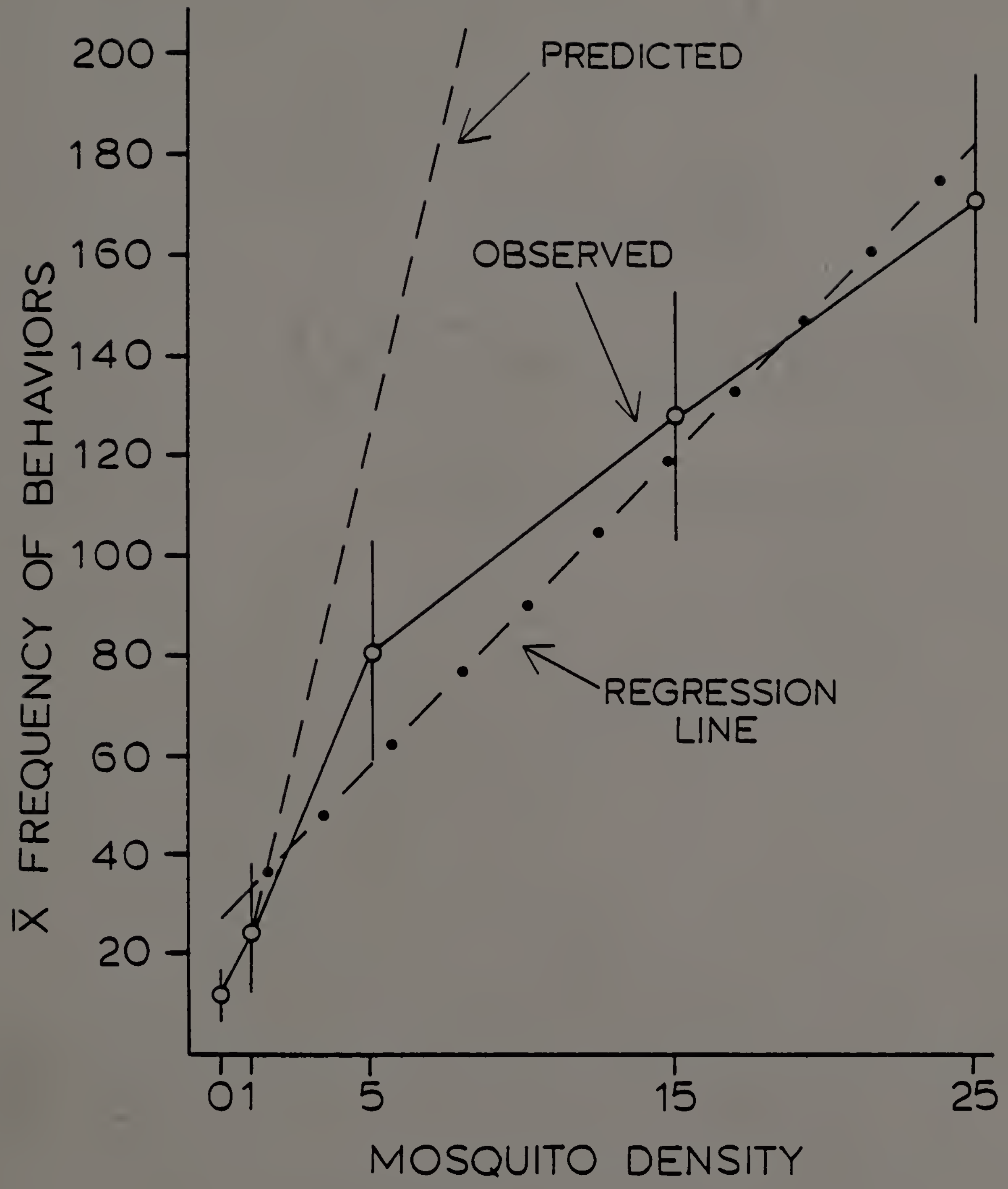

PREDICTED
OBSERVED
REGRESSION LINE
X̄ FREQUENCY OF BEHAVIORS
200
180
160
140
120
100
80
60
40
20
0
1
5
15
25
MOSQUITO DENSITY

Table 16. Frequencies of defensive behaviors of 8 grey squirrels, exposed for 30 minutes each, to 0, 1, 5, 15, and 25 mosquito densities.*

Behavior	Mosquito density				
	0	1	5	15	25
Eyeblink	10.4 (2.2)a	29.6 (7.2)b	37.0 (8.3)bc	48.5 (6.6)cd	57.8 (5.6)d
Ear flip	0a	1.5 (0.6)b	0.8 (0.4)ab	2.8 (0.6)c	8.4 (2.6)d
Head shake	0a	16.8 (4.8)b	34.9 (5.4)c	42.6 (7.4)d	70.1 (8.2)e
Face groom	0.2 (0.2)a	0.4 (0.3)a	0.4 (0.2)a	0.9 (0.2)b	1.5 (0.3)c
Face hide	0a	0a	0a	1.2 (0.4)b	2.0 (0.5)b
Forefoot scratch	0a	3.2 (1.2)b	14.0 (4.0)d	20.5 (4.0)d	25.9 (3.1)e
Hindfoot scratch	0.2 (0.2)a	0.1 (0.1)a	0.4 (0.3)a	1.0 (0.4)a	1.0 (0.4)a
Face rub	0a	0a	0.2 (0.2)b	1.2 (0.2)b	2.8 (0.5)b
Bite	0a	0a	0a	0a	0.4 (0.3)a
Total	10.9 (2.3)a	51.6 (12.4)b	87.5(15.1)c	118.8 (14.9)d	169.4 (16.9)e
Activity index	0.9 (0.3)a	0.8 (1.2)a	1.2 (0.4)a	1.5 (0.5)a	2.5 (0.5)a

*Table values are means with standard errors in parentheses. Within each row, means followed by different letters are significantly different (Kruskal-Wallis and multiple comparisons tests, $P<0.05$).

increasing mosquito density (results of Kruskal-Wallis and multiple comparisons tests presented in Table 16). A general increase in the activity index of squirrels is also apparent as mosquito density increased, but this trend was not significant (Kruskal-Wallis test, $P>0.05$).

Figure 6 shows a model which predicts a linear increase in average total squirrel defensive behavior as a function of mosquito density. The line was constructed as that in Figure 5. The model does not fit the observed data in Figure 6 well; it grossly overpredicts the increasing trend in defensive behavior. Linear regression yielded a better fitting line of more moderate slope ($Y= 37.4 + 5.5X$, $R^2 = 0.64$, $r = 0.80$, $P<0.05$).

<u>Mosquito feeding success</u>. Data of mosquito feeding success on chipmunks is shown in Table 17. A majority of mosquitoes fed completely on chipmunks at each density. There was, however, a trend for fewer mosquitoes to feed completely at higher densities, such that more mosquitoes obtained partial meals of blood or no blood. Mosquito feeding success on non-restrained and restrained chipmunks was compared at each density, in order to evaluate the effect of defensive behavior on feeding success (Table 18). At densities of 1 and 5 mosquitoes, frequencies of the 4 feeding success categories did not vary significantly (2x4 contingency tables, $P>0.05$). Thus, defensive behavior had little or no effect on mosquito feeding success at these densities. At densities of 15 and 25 mosquitoes,

Figure 6. Mean total defensive behaviors of squirrels, with 95% confidence intervals, at 0, 1, 5, 15, and 25 densities of mosquitoes. Means are connected by a solid line. Dashed line is a predictive model of increase of defensive behaviors as a function of mosquito density. Dash-dot-dash line is the linear regression line of total defensive behaviors on mosquito density ($Y= 37.4 + 5.5X$, $R^2 = 0.64$).

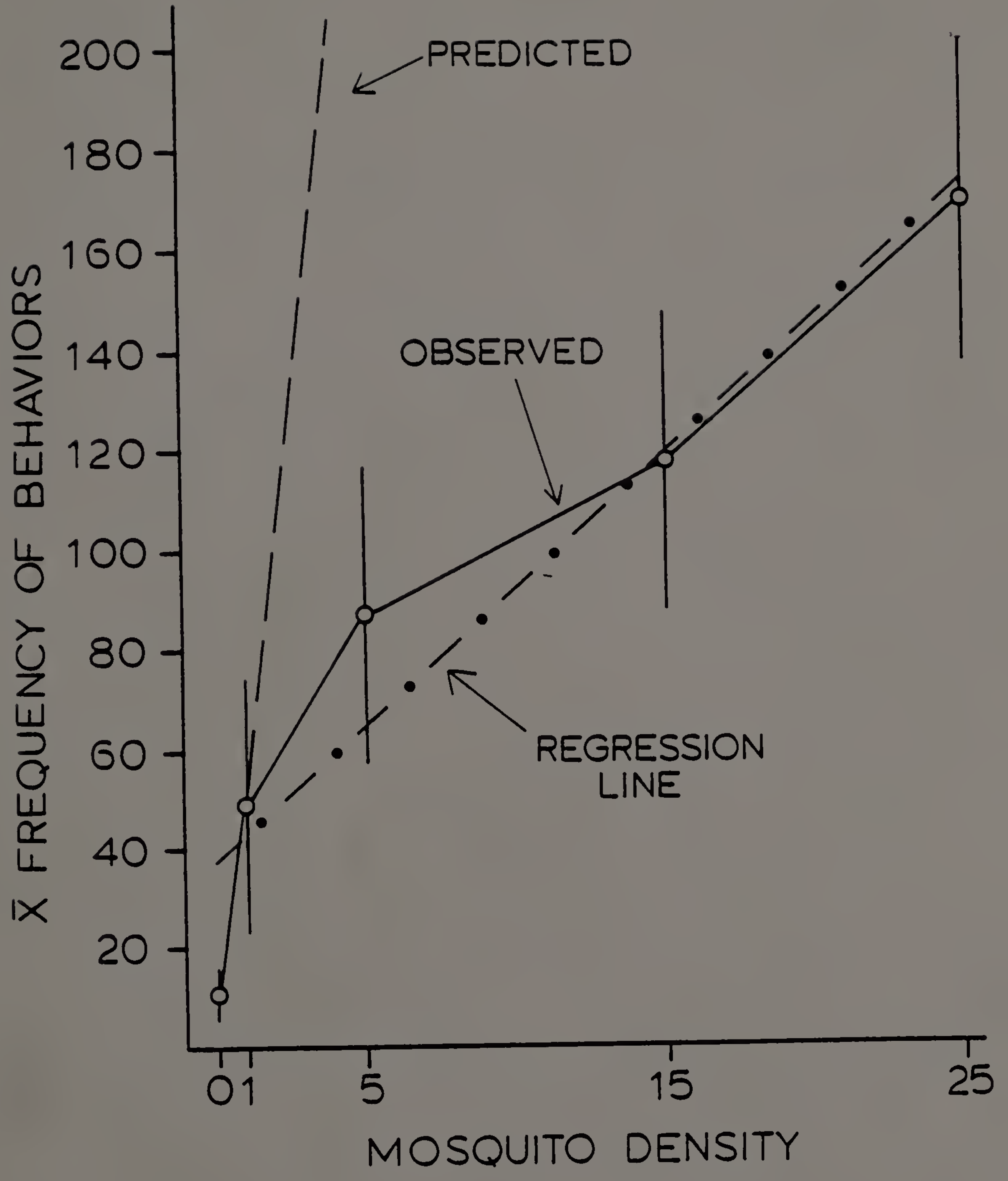

PREDICTED
OBSERVED
REGRESSION LINE
X̄ FREQUENCY OF BEHAVIORS
200
180
160
140
120
100
80
60
40
20
0
1
5
15
25
MOSQUITO DENSITY

Table 17. Percent feeding success of *Aedes triseriatus* on non-restrained chipmunks at various mosquito densities.

Mosquito Density	Feeding Success Category				Sample Size
	Full	Partial	Not fed	Killed	
1	80	7	13	0	15
5	67	12	20	1	75
15	57	14	27	1	225
25	64	15	20	1	375

Table 18. Comparison of feeding success of *Aedes triseriatus* on 15 restrained and non-restrained chipmunks at densities of 1, 5, 15, and 25 mosquitoes, in 30 minute exposures.

Mosquito Density	Treatment	Feeding Success Categories					X^2 Test*
		Full	Partial	Not fed	Killed	Total	
1	Non-restrained	12	1	2	0	15	X^2 =1.20ns
	Restrained	12	0	3	0	15	
5	Non-restrained	50	9	15	1	75	X^2 =2.39ns
	Restrained	57	6	12	0	75	
15	Non-restrained	128	33	61	3	225	X^2 =29.92**
	Restrained	180	12	33	0	225	
25	Non-restrained	240	58	75	2	375	X^2 =12.84**
	Restrained	315	19	41	0	375	

*X^2 tests were done on 2x4 contingency tables of numbers of mosquitoes in each feeding success category classified by non-restrained or restrained treatments. ns, not significant.

**$P<0.005$.

defensive behavior did affect the frequencies of the 4 feeding success categories (2x4 contingency tables, $P<0.05$). Thus at these densities defensive behavior had a significant effect.

Results in Tables 17 and 18 suggest that mosquitoes fed readily on chipmunks, but mosquito feeding success did vary among chipmunks. For example, at the density of 25 mosquitoes, fully fed mosquitoes varied from 4% to 96% (1 to 24 mosquitoes) among the 15 different chipmunks; partially fed mosquitoes varied from 0% to 48% (0 to 12 mosquitoes); not fed mosquitoes varied from 0% to 72% (0 to 18 mosquitoes); and killed mosquitoes varied from 0% to 4% (0 to 1 mosquitoes). This variation was due mainly to 3 very defensive individuals. Overall, mosquitoes fed with success on chipmunks.

Correlation analysis was done, at densities of 15 and 25 mosquitoes, between frequency of each defensive behavior and sums of partial, empty, and killed feeding success categories (which indicates unsuccessful feeding), in order to identify which chipmunk behaviors actually had a defensive effect. Results of these 3 feeding categories were used because it is within these categories that defensiveness would be apparent. Table 19 shows the correlation matrix. A high positive correlation indicates a defensive effect. Head shake had the highest correlation (Spearman′s rho = 0.54 at density of 15 and 0.73 at density of 25, $P<0.01$ for both values), indicating that this behavior was the most effective. Ear twitch also had a high correlation at density of 15 mosquitoes, but this behavior was infrequent. Body shake was significantly correlated

Table 19. Correlation of frequency of non-restrained chipmunk defensive behaviors (at densities of 15 and 25 mosquitoes) with unsuccessful mosquito feeding.*

Behavior	Spearman's Rho** Density 15	25
Eyeblink	-0.02ns	-0.25ns
Ear twitch	0.54***	0.16ns
Head shake	0.54***	0.73***
Body shake	0.63***	0.26ns
Forefoot scratch	0.24ns	0.38ns
Hindfoot scratch	0.59***	0.28ns
Face groom	0.38ns	0.18ns
Bite	0.28ns	-0.18ns
Total	0.40ns	0.26ns
Activity	0.42ns	0.31ns

*Unsuccessful feeding is sum of partially fed, not fed, and killed mosquitoes per chipmunk.
**Spearman's rank correlation coefficient rho.
***P<0.01; ns, not significant.

with unsuccessful feeding at density of 15 mosquitoes, but not at density of 25. Eye blink and body shake, despite frequent display, had low correlations, as did the less frequent behaviors. Overall, it is apparent that chipmunks displayed defensive behaviors that were not very effective in preventing mosquitoes from feeding. This observation is supported by the poor correlation between total chipmunks′ behaviors and unsuccessful feeding.

Mosquito feeding success on squirrels is shown in Table 20. The majority of mosquitoes did not feed fully on squirrels. At a density of one mosquito, only 25% (2 mosquitoes) among the eight trials obtained a full blood-meal. As density increased, the number of mosquitoes obtaining full meals tended to decrease, and the number of mosquitoes which did not feed tended to increase. Paradoxically, the number of mosquitoes which partially fed tended to decrease with increasing density. Squirrels killed a small number of mosquitoes at higher densities.

Mosquito feeding success on non-restrained and restrained squirrels was compared at each mosquito density (Table 21). As with chipmunks, this analysis was done to evaluate the effect of defensive behavior on feeding success. At mosquito density of 1, frequencies of the 4 feeding categories did not vary between non-restrained and restrained squirrels (2x4 contingency table, $P>0.05$). Thus at this density squirrel defensive behavior was ineffective (although data in the table suggest otherwise, and the X^2 test gave $0.05<P<0.1$). At higher densities, defensive behavior did affect frequencies in

Table 20. Percent feeding success of *Aedes triseriatus* on non-restrained squirrels at various densities of mosquitoes.

Mosquito Density	Feeding Success Category				Sample size
	Full	Partial	Not fed	Killed	
1	25	37	38	0	8
5	15	28	55	2	40
15	16	22	58	4	120
25	8	18	70	4	200

Table 21. Comparison of feeding success of *Aedes triseriatus* on 8 restrained and non-restrained squirrels at densities of 1, 5, 15, and 25 mosquitoes, in 30 minute exposures.

Mosquito Density	Treatment	Feeding Success Categories					X^2 Test*
		Full	Partial	Not fed	Killed	Total	
1	Non-restrained	2	3	3	0	8	X^2 =6.78ns
	Restrained	7	0	1	0	8	
5	Non-restrained	6	11	22	1	40	X^2 =39.29**
	Restrained	34	2	4	0	40	
15	Non-restrained	19	26	70	5	120	X^2 =109.23**
	Restrained	99	10	11	0	120	
25	Non-restrained	17	35	141	7	200	X^2 =229.43**
	Restrained	167	12	21	0	200	

---

*X^2 tests were done on 2x4 contingency tables of numbers of mosquitoes in each feeding success category classified by non-restrained and restrained treatments. ns, not significant.

**$P<0.005$.

feeding success categories (2x4 contigency tables, $P<0.005$). Thus, squirrel defensive behavior had an effect at densities of 5, 15, and 25 mosquitoes.

Results in Tables 20 and 21 suggest that mosquito feeding success was low on squirrels. There was variation, however, among the 8 squirrels in mosquito feeding success. For example, at the density of 25 mosquitoes, fully fed mosquitoes varied from 0% to 28% (0 to 7 mosquitoes); partially fed mosquitoes varied from (4% to 32%) (1 to 8 mosquitoes); not fed mosquitoes varied from 40% to 96% (10 to 24 mosquitoes); and killed mosquitoes varied from 0% to 8% (0 to 2 mosquitoes).

Correlation analysis was done (at densities of 15 and 25 mosquitoes) between frequency of each defensive behavior and sums of partial, empty, and killed feeding success categories to identify effective defensive behaviors. Table 22 shows the correlation matrix. Head shake and eye blink, which were frequent behaviors, correlated highly with unsuccessful feeding categories. Forefoot scratch, another frequently displayed behavior, was positively but not significantly correlated with unsuccessful feeding. Ear twitch and face groom were strongly correlated at density of 15 mosquitoes, but these behaviors were infrequent so the correlations are very likely spurious. Thus head shake, eye blink, and perhaps forefoot scratch were the most effective defensive behaviors of squirrels. Total behaviors correlated strongly with unsuccessful feeding, indicating that, overall, the gray squirrels were defensively

Table 22. Correlation of frequency of squirrel defensive behaviors (at densities of 15 and 25 mosquitoes) with unsuccessful mosquito feeding.*

Behavior	Spearman's Rho** Density 15	Spearman's Rho** Density 25
Eyeblink	0.64***	0.55ns
Ear twitch	0.70***	0.39ns
Head shake	0.78***	0.72***
Forefoot scratch	0.59ns	0.35ns
Hindfoot scratch	-0.08ns	-0.05ns
Face groom	0.68***	0.16ns
Face rub	0.39ns	0.30ns
Face hide	-0.21ns	-0.01ns
Bite	ND****	ND
Total	0.90***	0.58ns
Activity	0.54ns	0.08ns

*Unsuccessful mosquito feeding is sum of partially fed, not fed, and killed mosquitoes per squirrel.

**Spearman's rank correlation coefficient rho.

***$P<0.05$; ns, not significant

****ND, not done.

effective.

Tables 23-26 show comparisons (arcsine tests for the equality of 2 percentages) of results of mosquito feeding success on gray squirrels and chipmunks for full, partial, not fed, and killed categories, respectively. Table 23 shows that a consistently higher percentage of mosquitoes obtained complete blood meals from chipmunks than from squirrels. Table 24 shows a tendency for more mosquitoes to have obtained a partial blood meal from squirrels than chipmunks, but these observed differences were significant only at density of 5 mosquitoes (test for the equality of two percentages, $P<0.05$). Table 25 shows that, with the exception of density of 1 mosquito, significantly more mosquitoes did not obtain blood from squirrels than from chipmunks. Table 26 shows that few mosquitoes were killed by squirrels and chipmunks, and that there was no difference in mosquito mortality between chipmunks and squirrels except at mosquito density of 25, where squirrels killed more mosquitoes. Results in Tables 23-26 in general show that squirrels were more defensive towards mosquitoes than chipmunks, in particular by preventing mosquitoes from feeding at all. Mosquitoes fed equally well on restrained chipmunks or squirrels (Tables 18 and 21).

Attraction densities. Table 27 shows results of attraction density experiments. Aedes triseriatus females were exclusively collected in the experiments, except for 1 Aedes canadensis collected from a louvre of a chipmunk-baited trap. Observations during these

Table 23. Comparison of percent of _Aedes_ _triseriatus_ fully fed on squirrel and chipmunk at densities of 1, 5, 15, and 25 mosquitoes.

Mosquito Density	Percent of Total Mosquitoes Fully Fed		T-Test*
	Squirrel	Chipmunk	
1	25 (8)****	80 (15)	t=2.67**
5	15 (40)	67 (75)	t=5.73***
15	16 (120)	57 (225)	t=7.86***
25	17 (200)	64 (375)	t=11.47***

*Arcsine test or equality of 2 percentages. Ns, not signifcant.
**P<0.01.
***P<0.001.
****Percent of total mosquitoes with sample size in parentheses.

Table 24. Comparison of percent of *Aedes triseriatus* partially fed on squirrels and chipmunks at densities of 1, 5, 15, and 25 mosquitoes.

Mosquito Density	Percent of Total Mosquitoes Partially Fed		T-Test**
	Squirrel	Chipmunk	
1	38 (8)***	7 (15)	t=0.59ns
5	28 (40)	12 (75)	t=2.08*
15	22 (120)	14 (225)	t=1.85ns
25	18 (200)	15 (375)	t=0.92ns

*P<0.05.

**Arcsine test for equality of 2 percentages. ns, not significant.

***Percent of total mosquitoes with sample size in parentheses.

Table 25. Comparison of percent of *Aedes triseriatus* not fed on squirrel and chipmunk at densities of 1, 5, 15, and 25 mosquitoes.

Mosquito Density	Percent of Total Mosquitoes Not Fed		T-Test**
	Squirrel	Chipmunk	
1	38 (8)****	13 (15)	t=1.21ns
5	55 (40)	20 (75)	t=3.80*
15	58 (120)	27 (225)	t=5.65***
25	70 (200)	20 (375)	t=12.05***

*$P<0.05$.

**Arcsine test for equality of two percentages. ns, not significant.

***$P<0.001$.

****Percent of total mosquitoes with sample size in parentheses.

Table 26. Comparison of percent of *Aedes triseriatus* killed by squirrel and chipmunk at densities of 1, 5, 15, and 25 mosquitoes.

Mosquito Density	Percent of Total Mosquitoes Killed		T-Test**
	Squirrel	Chipmunk	
1	0 (8)***	0 (15)	t=0.0ns
5	2 (40)	1 (75)	t=0.60ns
15	4 (120)	1 (225)	t=1.88ns
25	4 (200)	1 (375)	t=2.01*

*$P<0.05$.

**Arcsine test for equality of 2 percentages.

***Percent of total mosquitoes with sample size in parentheses.

Table 27. Attraction density of *Aedes triseriatus* to a chipmunk and a squirrel in Magoon stable traps.*

Host		Baited Trap			Control Trap			Landing Count
		IL	AT	TOT**	IL	AT	TOT	
Chipmunk	$\overline{X}$***	2.4	1.4	3.9	0	0.5	0.5	8.0
	SE***	0.5	0.3	0.6	0	0.2	0.2	1.0
Squirrel	$\overline{X}$	3.8	1.9	5.7	0	0.8	0.8	8.4
	SE	0.5	0.4	0.8	0	0.3	0.3	1.2

*Eleven repetitions for chipmunk, 10 for squirrel.

**IL, in louvre; AT, around trap; TOT, total mosquitoes attracted.

***$\overline{X}$, mean; SE, standard error.

collections indicated that mosquitoes that had entered louvres tended to stay in the traps, probably because of an arrestment response to the hosts. Attraction density to the chipmunk averaged 2.4 mosquitoes in the louvres with 1.4 mosquitoes on or around the traps. Attraction density to the squirrel averaged 3.8 mosquitoes in the louvres and 1.9 on or around the traps. Total mosquitoes attracted to the squirrel (mosquitoes in louvres and around the trap) averaged 5.7, which was significantly higher (Mann-Whitney U test, $P<0.05$) than total mosquitoes attracted to the chipmunk, which averaged 3.8. Attraction density to the human collector averaged 8.0 during chipmunk experiments and 8.4 during squirrel experiments. It is important to note that attraction density of Aedes triseriatus to the chipmunk fell within the range where chipmunk defensive behavior had no effect (Table 18), whereas the attraction density of Aedes triseriatus to the squirrel fell within the range where squirrel defensive behavior had an effect (Table 21).

Linear interpolation was used to predict feeding success of Aedes triseriatus on chipmunks and squirrels, using feeding success observed at those densities in controlled experiments which bracket estimated total attraction densities. The interpolation assumes that these observed values reflect true feeding success rates in nature. The predictions for encounters of Aedes triseriatus with chipmunks are that 71% of mosquitoes will obtain a full bloodmeal during that encounter, 10% will partially feed, 17% will not get any blood, and 1% will be killed by the chipmunk. This assumes that the density of mosquitoes attempting to blood feed remains constant, and that the estimated densities using the Magoon traps are accurate. The predictions for encounters of <u>Aedes triseriatus</u> with gray squirrels are that 15% will obtain a full blood meal during the encounter, 28% will partially feed, 55% will not feed, and 2% will be killed by the squirrel.

<u>Discussion</u>

<u>Defensive behaviors</u>. Chipmunks and squirrels exhibited a diverse repertoire of defensive behaviors against mosquitoes. These behaviors, with the possible exception of body shake by chipmunk, were directed toward protecting the head, probably because mosquitoes blood feed primarily on the ears, eyelids, nose, and also feet of these rodents (Chapter III). Not all behaviors were defensively effective, however (Tables 19 and 22). Chipmunks blinked their eyes frequently in the presence of attacking mosquitoes, yet this behavior had no effect. Perhaps eyeblink of chipmunk, and other behaviors which correlated poorly with unsuccessful feeding, are chipmunks′ irritation reactions to mosquitoes; "defensive behavior" may be an anthropomorphism in these cases. Eyeblink of gray squirrels, unlike that of chipmunks, correlated rather strongly (Table 22) with unsuccessful feeding. This could be because this behavior is defensively effective, or because eyeblink was positively correlated with head shake in the case of gray squirrels (Spearman′s rho = 0.71 at density of 25 mosquitoes,$P<0.05$; 0.26 at density of 15,$P>0.05$).

Correlation of gray squirrels' eyeblinks with unsuccessful feeding may have been spurious.

Defensive behavior of chipmunks was considerably less effective than that of squirrels. This is apparent in data of mosquito feeding success on these rodents (Tables 23-26) and in the correlations of total defensive behaviors and unsuccessful feeding (Tables 19 and 22). Total squirrel behaviors had higher correlations than total chipmunk behaviors. It is difficult to compare chipmunk and squirrel behaviors statistically, because they are not strictly equivalent. However, this difference in defensive effectiveness is possibly due to more frequent use of head shake and forefoot scratch by squirrels than chipmunks (Mann-Whitney U tests show differences between chipmunks and squirrels in these behaviors at each density of mosquitoes, $P<0.05$). Edman et al. (1974) observed frequent head shaking by gray squirrels under attack by mosquitoes. It is puzzling that, despite obvious dexterity with forefeet, chipmunks did not frequently use the forefeet to bat mosquitoes away. Squirrels used forefeet regularly in this manner. These observations show that interspecific differences of hosts in mosquito feeding success can be due to differences in frequency and perhaps quality of defensive behaviors, as has been shown in the case of ciconiiform birds (Webber and Edman 1972).

Frequency of the rodents' defensive behaviors, as a function of mosquito density, did not increase as drastically as the multiplicative models predicted (Figures 5 and 6). Rather, the increase was more moderate (linear regression lines in figures). One explanation for this trend is that as mosquito density increased, the relative effectiveness of a single behavioral act increased, because proportionately more mosquitoes would be disturbed by one act at higher densities than at lower densities. Therefore, relatively fewer behaviors would be required at higher densities than at lower densities to accomplish more defensively. Thus, the actual increase in frequency of defensive behaviors as a function of mosquito density would not be as steep as predicted.

Chipmunk and gray squirrel defensive behavior must be viewed within the context of the routine behaviors these rodents exhibit in nature. A brief discussion of normal behavior of these rodents is therefore pertinent here. Eastern chipmunks are diurnally active, generally solitary, forest-dwelling, fossorial, larder-hoarders. Individual chipmunks occupy burrows and during active seasons spend approximately 1/3 of the diurnal cycle above ground, near (+15 meters) the burrow entrance (Elliot 1978, Yahner 1978a,b). The behavior of chipmunks varies with a variety of factors (Yahner 1978a,b), but in general chipmunks occupy most of their above-ground time budgets with "pause", "forage", "eat", and "locomotion" behaviors (Yahner 1978a). "Pause" occupies fully half of the above-ground time budget. This behavior is an alert state during which a chipmunk chips, monitors its core area surrounding its burrow for intrusion by other foraging chipmunks, and watches for and avoids predators. It is probably during this behavior that chipmunks are most susceptible to attacking mosquitoes. Chipmunks may "choose" to allow mosquitoes to feed in order to maintain the integrity of the paused state. However, Yahner (1978c) observed that "pause-groom" and "pause-scratch" transitions occurred, which indicates that chipmunks will disrupt "pause" with other stationary activities which resemble defensive behavior.

Gray squirrels are diurnally active, somewhat social, arboreal, dispersed, scatter-hoarders. Individual squirrels occupy home ranges which have distinct centers of activity and which broadly overlap home ranges of other squirrels (Thompson 1977a). Behavioral repertoires, time budgets, and frequency of occurrence of specific behaviors have not been as systematically studied in gray squirrels as in chipmunks, but Horwich (1972) and Thompson (1977a, 1977b, 1978) studied and reviewed several aspects of gray squirrel behavior. Two behaviors which gray squirrels display during agonistic interactions, "attentive posture" and "tooth chatter" (Thompson 1978, Horwich 1972), may predispose squirrels toward susceptibility to attacking mosquitoes because during these behaviors squirrels are stationary. Horwich (1972) observed head shaking behavior which occurred "...when dust, rain, a jet of air, or anything else gets on the head or shoulders" of squirrels. There is no evidence of a sustained pause behavior such as chipmunks display.

I attempted to observe defensive behavior of gray squirrels and chipmunks in nature, but was unsuccessful. Appendix I documents 2 field sightings of _Aedes_ _triseriatus_ attracted to chipmunks.

Mosquito feeding success. Mosquito feeding success tended to decrease, and unsuccessful feeding tended to increase, on squirrels and chipmunks as mosquito density increased. These results concur with those of other studies (Edman et al. 1972, Waage and Nondo 1982). At higher densities enhanced multiple feeding by Aedes triseriatus may occur, which has epidemiological importance in increasing rates of transmission of LAC virus to more sciurid amplifying hosts.

The relationship between mosquito density, host behavior, and mosquito feeding success in the case of Aedes triseriatus and sciurid rodents is mainly of academic interest unless compared with densities which these rodents experience in nature. This comparison gives predictions that feeding success of Aedes triseriatus will be high on chipmunks and low on gray squirrels in nature. However, the estimates of attraction density may be high in this study, because of the presence of a human setting up the box traps and because of the visual stimulus of the black boxes. Certainly, attraction density will vary seasonally as well. The estimates probably represent the higher densities that chipmunks and gray squirrels might experience. In the only other study of attraction of Aedes triseriatus to sciurids, Wright and DeFoliart (1970) found 6 and 9 Aedes triseriatus in Magoon traps baited with a gray squirrel and a chipmunk, respectively. Wright and DeFoliart (1970) also found low mosquito feeding success on the chipmunk and squirrel, except for rather high feeding success by *Aedes triseriatus*. Their experiments were done overnight (not during the afternoon activity periods of the rodents and *Aedes triseriatus*) and should be interpreted with caution.

Defensive behavior and the La Crosse virus cycle. Defensive behavior of squirrels and chipmunks can influence the LAC virus cycle in four ways. First, defensive behavior would disrupt blood feeding of virus-infected *Aedes triseriatus*. These mosquitoes would thus have heightened probability of multiple host contacts. Therefore, host defensive behavior would increase horizontal (oral) transmission to vertebrate amplifier species. Defensive behavior would interact with heightened probing and difficulty in feeding, a characteristic of virus-infected *Aedes triseriatus* (Grimstad et al. 1980), in increasing horizontal transmission. Defensive behavior is the logical explanation for the high observed seroconversion rates in sciurid populations in LAC enzootic areas (Gauld et al.1974), despite the low virus infection rates in *Aedes triseriatus* populations (Lisitza et al. 1977). As DeFoliart (1983) has noted, the assumption that *Aedes triseriatus* females bite once per ovarian cycle is an underestimation given the effects of defensive behavior (and I add, the phenomenon of giving-up behavior discussed in the next chapter). My studies of defensive behavior and mosquito feeding success confirm this. It would be difficult to estimate the true number of bites by *Aedes triseriatus* per gonotrophic cycle, but it is certainly more than one.

The second way defensive behavior can influence the LAC virus cycle is by limiting non-infected _Aedes triseriatus_ from being orally infected by feeding on viremic chipmunks or squirrels. There is no evidence that viremic sciurids become ill and therefore less defensive (cf. Day and Edman 1983). Defensive behavior could tend to prevent recruitment of orally infected mosquitoes, by limiting their access to virus-infected blood.

A third way defensive behavior will affect the LAC virus cycle is by lowering vertical transmission, through restricting access of virus-infected females to blood required for egg development. This restriction would affect total number of infected progeny per female, and duration of each gonotrophic cycle (the latter because females would devote more time to blood acquistion).

The fourth way defensive behavior could affect the LAC virus cycle is by killing mosquitoes, thus removing them from any potential role as vectors. This was rare in experiments, however, and is probably rare in nature. In no circumstance did chipmunks or squirrels ingest mosquitoes in experiments, as was the case with muroid rodents in experiments by Edman and Day (unpublished data). This supports Yuill′s (1983) conclusion that LAC virus transmission to sciurids by ingestion of infected mosquitoes is "...an unlikely event in nature."

Conclusions

1. Gray squirrels and eastern chipmunks displayed a similar

repertoire of defensive behaviors against Aedes triseriatus mosquitoes. Behaviors primarily protected the head. Effective defensive behaviors of gray squirrels were head shake, and perhaps eyeblink and forefoot scratch. Head shake was the most effective defensive behavior of chipmunks.

2. Frequency of defensive behaviors of chipmunks and gray squirrels increased with increasing mosquito density, but at a lower rate than predicted by a multiplicative linear model.

3. Mosquito feeding success was higher on chipmunks than on gray squirrels at densities of 1, 5, 15, and 25 mosquitoes in 1/2 hour exposures. In general, mosquitoes fed successfully on chipmunks but not on gray squirrels. This difference may be attributable to more extensive head shake and forefoot scratch movements of gray squirrels.

4. Field attraction densities of Aedes triseriatus to chipmunk and squirrel were low, measuring 5.7 and 3.8 mosquitoes per 1/2 h, respectively. Linear interpolation of feeding success at these densities gives the prediction that, in nature, mosquito feeding success will be high on chipmunk (71% of mosquitoes will obtain complete blood meals) and low on squirrel (15% of mosquitoes will obtain complete blood meals).

CHAPTER V

BITING PERSISTENCE OF AEDES TRISERIATUS: THE IDEA OF GIVING-UP TIME

Introduction

When attempting to blood feed, mosquitoes may encounter hosts which behaviorally defend themselves (Edman and Kale 1971, Chapter IV above). Such hosts may prevent mosquitoes from feeding, interrupt them during feeding, or even injure or kill them. I reviewed studies on host defensive behavior toward mosquitoes in the previous chapter. These studies have documented expression of defensive behavior by a variety of mammals and birds, and have shown that defensive behavior has a definite impact on the feeding success of mosquitoes. Klowden and Lea (1979) emphasized the epidemiological importance of host defensive behavior, by noting that defensive behavior interrupts blood feeding by mosquitoes and causes mosquitoes to have multiple host contacts (rather than just one) within a gonotrophic cycle. Implicit within this idea of multiple feeding (Tempelis 1975, Washino and Tempelis 1983) however, is that contact between mosquito and host is broken because of host behavior. No studies have examined how host defensiveness mediates this "contact-breakage". Nor have any studies addressed the role of host defensive behavior as a selection pressure on mosquito behavior.

There are numerous anecdotal references in the literature to "mosquito biting persistence". Carpenter and La Casse (1955) described Aedes stimulans (Walker), Aedes taeniorhynchus (Wiedemann), and Aedes trivittatus (Coquillett) as "persistent biters". These same authors quoted Thibault (1910) as stating that Anopheles barberi (Coquillett) "...is nervous and seldom finishes a blood meal at one sitting". These anecdotes suggest that biting persistence is a real behavioral phenomenon of mosquitoes. However, there has been no attempt to quantify biting persistence, nor to relate it to the foraging strategy or disease vector potential of mosquitoes.

I define mosquito biting persistence as the propensity of a mosquito to attempt to blood feed despite host defensive behavior. My reasoning is that if a mosquito encounters a defensive host, then it should persist in attacking (i.e. attempting to feed on) that host for a limited time. If the host is too defensive, the mosquito should terminate attacking by giving-up. Giving-up behavior would be adaptive for mosquitoes; otherwise a mosquito risks injury or death inflicted by the host or wastes energy trying to get blood from a behaviorally inaccessible host. Natural selection, mediated by host defensive behavior as a selective force, should favor mosquitoes that limit biting persistence with giving-up behavior.

The possibility that giving-up behavior and biting persistence are related to host behavior is suggested by two anecdotal field observations. Stamm (1958) watched as heron nestlings "...actively resisted mosquitoes and drove them away". Whether the mosquitoes had given up attacking the nestlings is not known. Service (1971) observed mosquitoes feeding on himself in nature, and noted the following: "It was observed in all species that when a mosquito alights on a host it immediately 'freezes' for a very short time (less than 5 seconds), and during this time any movement of the host usually results in it flying off. When disturbed, adults of M[ansonia]. richardsii and A[nopheles]. plumbeus usually rest on nearby vegetation for a minute or more before returning to the host, but when Aedes spp. are disturbed directly after alighting they usually hover in the immediate vicinity of the host and resettle within a few seconds." Service did not examine these behavioral reactions in more detail, but his account suggests that disturbed mosquitoes actually left the host "voluntarily".

The mosquito Aedes triseriatus (Say), enzootic vector of La Crosse encephalitis virus (Thompson 1983), feeds mainly on deer, squirrels, and chipmunks in deciduous forests and woodlots (Burkot and DeFoliart 1982, Nasci 1982). Chipmunks (Tamias striatus) and gray squirrels (Sciurus carolinensis) exhibit defensive behavior towards attacking mosquitoes (see results of previous chapter). Squirrels are particularly effective in preventing mosquitoes from feeding, but chipmunks are not so effective. The fact that defensive behavior limits feeding success of Aedes triseriatus, and may kill mosquitoes, provides a setting for testing the giving-up hypothesis. The defensive behavior of sciurid rodents may be sufficient selection pressure on Aedes triseriatus for this species to have evolved giving-up behavior.

Materials and methods

Mosquitoes. Aedes triseriatus mosquitoes were from F-11 and F-14 generations of a colony founded in October 1979 from mosquitoes collected in Amherst. Eggs were hatched under vacuum, and larvae reared at 27° C and 85% RH in 27.9 x 21.6 x 5.1 cm enamel pans with 750 ml distilled water and 100 larvae per pan. Larvae were fed 1:1 Brewer's yeast and lactalbumin at approximately 2.7 mg per larva. Pupae were pipetted from pans and adults allowed to emerge in 4 liter cardboard ice cream containers with gauze tops. Adults were provided 5% sucrose solution-soaked or water-soaked cotton pledgets. This rearing method yielded large, uniform-sized females.

Mosquitoes were either provided sucrose solution up until the day before experiments, when water was provided (the non-starved group); or were deprived of sucrose for 6 or 11 days (i.e. only water was provided the starved group). Mosquitoes in the non-starved group were 6, 11, or 16 days old; those in the starved group were 11 (starved 6 days) or 16 (starved 11 days) days old. This scheme allowed comparison of differently aged and differentially sucrose-starved mosquitoes.

Experimental design. The experimental design to test the giving-up hypothesis was simple. A mosquito of known age (days) and history of access to sucrose solution or water was put into a 36^3 cm cage which had a plexiglas side and top, and one side screen, for viewing into the cage. The mosquito was allowed 10 minutes to acclimate. An observer placed a hand into the cage via a sleeved port, and exhaled once into the cage to stimulate the mosquito to host-seek. The mosquito was allowed to land on the hand and then was gently dislodged with a finger. Typically, the mosquito would fly off the hand, hover briefly, and land again; the observer then dislodged the mosquito again. This was continued until the mosquito gave up attacking. The criterion for giving-up behavior was when the mosquito, having been dislodged, took flight, turned away from the hand, flew about the cage, and landed on a wall.

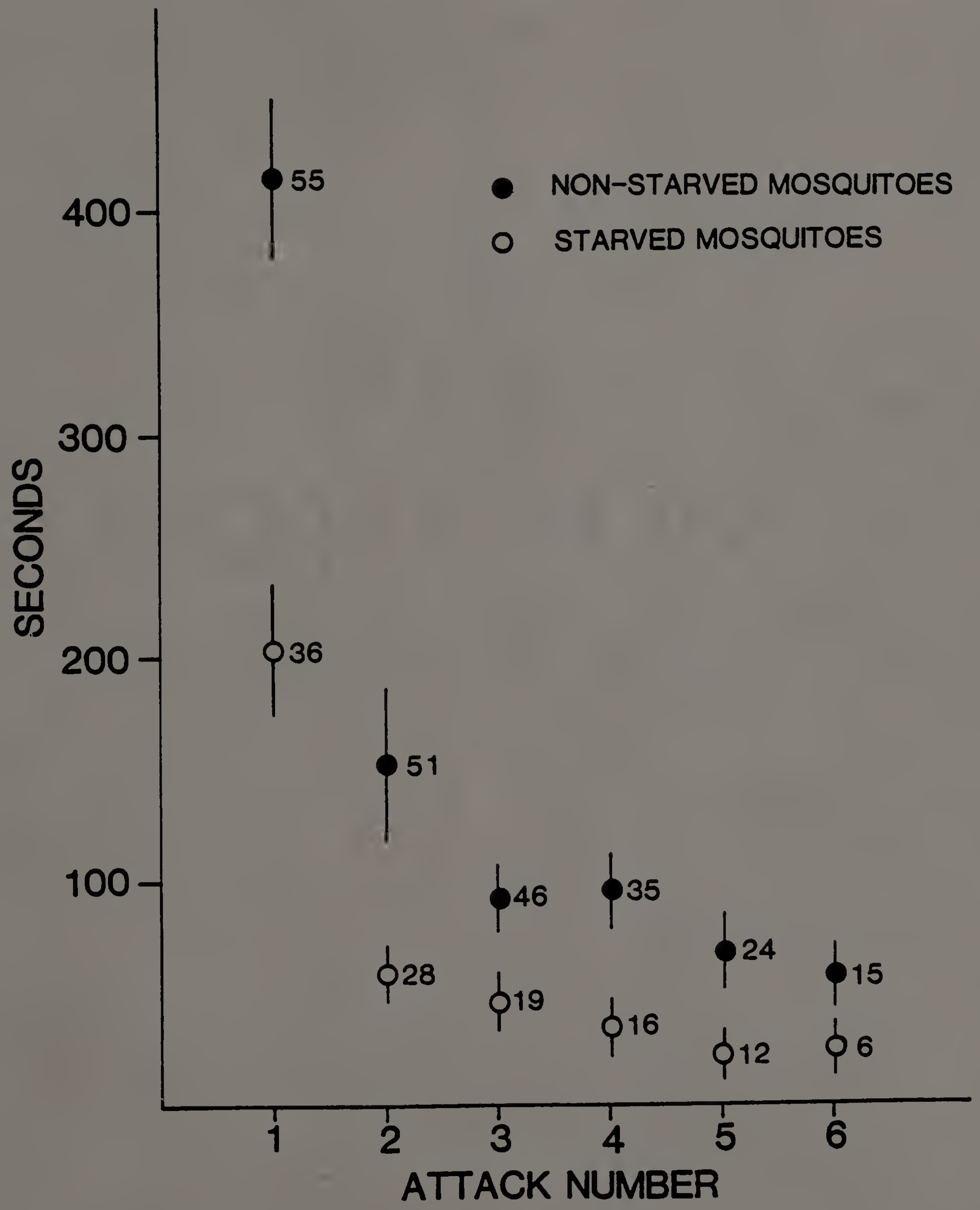
During experiments, the observer verbally recorded on cassette tape the mosquitoes′ behaviors "attack", "fly", and "land". Duration of each experiment was 45 minutes, during which a mosquito might attack and give up several times. Tapes were transcribed using a stopwatch. Duration of each attack in an experiment was recorded as time between initial landing of the mosquito on the hand until the mosquito gave up attacking. Attack durations are called "giving-up times" (McNair 1982) because each attack duration was the period of time a mosquito attacked until giving-up. Ninety-one experiments were done, all at 27° C and 85% RH under flourescent light. Experiments were done in late morning and afternoon, when _Aedes triseriatus_ is normally active.

Results

During experiments, each mosquito gave up every time it attacked the hand in each experiment. The number of times mosquitoes attacked the hand ranged from 1-13 (mean=4.01). There was no difference in the giving-up times of each successive attack by 6, 11, and 16 day old non-starved mosquitoes (i.e. off sucrose only one day). The data from these groups were therefore pooled into one "non-starved" group (n=55). There was no difference in giving-up times of 11 day old (starved 6 days) and 16 day old (starved 11 days) mosquitoes (Mann-Whitney U tests, P>0.05). Data from these mosquitoes were pooled into one "starved" group (n=36).

Figure 7 shows giving-up times of non-starved and starved groups for the first six successive attacks in the experiments. Few mosquitoes attacked 7 to 13 times, so these data are not presented in the figure. Two patterns are apparent in Figure 7. First, both non-starved and starved mosquitoes had consistently longer giving-up times during their initial attacks than in subsequent attacks (Kruskal-Wallis and multiple comparisons tests of Conover [1980], P<0.05). Thus, mosquitoes were more persistent in their first encounter with the defensive hand than in subsequent encounters. Correlation of giving-up times with successive attack numbers was negative for non-starved (Spearman's rank correlation coefficient rho=-0.62, P<0.01) and starved (Spearman's rho=-0.61, P<0.01) mosquitoes. This reinforces the observation (Figure 7) that giving-up times decreased with increasing attack number. The second

Figure 7. Giving-up times (seconds) of _Aedes triseriatus_. Solid circles are means of mosquitoes not sucrose-starved; open circles are means of sucrose-starved mosquitoes. Vertical bars are standard errors. Number to the right of each mean is the number of attacks which contribute to that mean.


● NON-STARVED MOSQUITOES
○ STARVED MOSQUITOES
SECONDS
400
300
200
100
1
2
3
4
5
6
ATTACK NUMBER
55
36
51
28
46
19
35
16
24
12
15
6

pattern apparent in Figure 1 is that non-starved mosquitoes averaged longer giving-up times than starved mosquitoes, for the first 5 attacks (Mann-Whitney U tests, $P<0.05$). This indicates a nutritional influence on giving-up behavior.

Discussion

Results of the experiments confirm the giving-up hypothesis by showing that mosquitoes give up attacking a defensive host. The giving-up times varied (Figure 7). Mosquitoes had longer giving-up times during their first attack than in subsequent attacks. An explanation for this could be that mosquitoes were inexperienced in their first encounter with the defensive hand and so attacked longer, but in later encounters mosquitoes rapidly assessed the hand as defensive and gave up sooner. Sugar-starved mosquitoes had shorter giving-up times than non-starved mosquitoes, which indicates that biting persistence is influenced by energy for flight. This influence hints at the mechanism underlying giving-up behavior, which could be related to depletion, to some critical level, of carbohydrate reserve immediately available for flight (Nayar and van Handel 1971). The energetics of biting persistence and the mechanism of giving-up time deserve study. It is possible that mosquitoes merely ran out of fuel for flight (i.e. got tired) and for this reason gave up attacking. Three observations during experiments work against this possibility:

(1) Mosquitoes often flew about the cage between attacks.

(2) Mosquitoes, after having given up, occassionally closely approached the hand but instead of landing turned away. This observation possibly indicates mosquitoes were inhibited from landing or responding to the hand in the period after exhibiting giving-up behavior.

(3) Mosquitoes often groomed themselves between attacks while resting on walls, indicating that mosquitoes had energy for activity.

Two mechanisms, abdominal distention (Klowden and Lea 1979b) and ovarian humoral feedback (Klowden and Lea 1979c) have been shown to inhibit host seeking behavior by mosquitoes. These mechanisms operate after a mosquito has blood-fed. Giving-up behavior (exhibited by *Aedes triseriatus*) also apparently temporarily shuts down mosquito attraction or response to a host, but contrasts with the two mechanisms mentioned above in that giving-up behavior may operate before a mosquito imbibes blood. This is important in relation to multiple feeding by mosquito vectors. Host defensive behavior may interrupt mosquito feeding, such that a mosquito only obtains a partial blood meal and still seeks a host (Klowden and Lea 1978). Giving-up behavior provides the mechanism by which contact between *Aedes triseriatus* and defensive sciurid hosts may be broken.

Foraging theory predicts that an animal foraging for a resource will make decisions which tend to optimize its chance of obtaining the resource while minimizing risks and energy expenditure during foraging (Pyke et al., 1977, Hassell and Southwood 1978, Krebs 1978). Such decisions, according to theory, are molded by natural selection and comprise the foraging strategy of the animal. I suggest that giving-up behavior is an important component of the foraging strategy of mosquitoes which feed on defensive hosts. Giving-up behavior delimits biting persistence; giving-up time is a measure of biting persistence because it reflects the effort a mosquito makes to obtain blood. In foraging theory terms, giving-up behavior determines foraging time allocation (and therefore energy investment) of a mosquito at the environmental patch level of host encounter (Hassell and Southwood 1978). Further, giving-up behavior provides a means whereby mosquitoes can avert the risk of injury or death associated with host defensive behavior (cf. foraging and risk-sensitivity concepts of Caraco et al. [1980], Sih [1980], and Stephens [1981]).

Gillett (1967) advanced a theory of mosquito feeding strategy which took no account of giving-up behavior. He argued that to blood feed mosquitoes must rely on (1) catching the host unaware of their presence, and (2) blood feeding as quickly as possible. Gillett postulated a "safe period" during which a mosquito might feed before the irritation of mosquito saliva alerted the host. However, there is evidence that feeding speed is not amenable to selection (Mellink 1981, discussion in Chapter III). Also, studies of host defensive behavior (see review and results in Chapter IV) have shown that defensive hosts not only interrupt mosquito feeding but prevent it as well. Gillett's (1967) ideas need modification in light of giving-up behavior.

Conclusions

1. *Aedes triseriatus* exhibited giving-up behavior when attempting to feed on a relentlessly defensive host. Thus, this species limits its biting persistence.

2. Giving-up times (the amount of time *Aedes triseriatus* attacked the hand until giving up) of sugar-starved mosquitoes were shorter than non-starved mosquitoes, indicating a nutritional effect on biting persistence.

3. Giving-up time was longer in the mosquitoes' first encounter with a defensive host than in subsequent encounters, indicating that mosquitoes that had previously experienced a defensive host gave up more quickly than inexperienced mosquitoes.

4. Giving-up behavior provides a mechanism whereby mosquito-host contact can be broken, thereby allowing multiple host contacts (multiple feeding) within a gonotrophic cycle by mosquitoes.

CHAPTER VI

SUMMARY

1. California serogroup viruses were found to infect *Aedes* mosquito, white-tailed deer, eastern chipmunk, and gray squirrel populations at low levels of incidence, in 1980-1982, in western Massachusetts. Lawrence Swamp in Amherst, Hampshire County, was identified as a focus of California serogroup viruses. At that site, Jamestown Canyon virus was isolated twice in June, 1982, from *Aedes intrudens*/*sticticus* and from *Aedes abserratus*/*punctor*. Snowshoe hare virus was isolated once in June, 1982, from *Aedes stimulans* group mosquitoes collected in Lawrence Swamp. Jamestown Canyon virus was also isolated in June, 1982, from *Aedes intrudens*/*sticticus* collected in Warwick State Forest, Warwick, Franklin County. Neutralizing antibody to La Crosse virus was found at low levels in wild mammal populations; but no focus of California serogroup viruses was identified by this serosurvey.

2. Analysis of searching and blood-feeding behavior of *Aedes triseriatus* on chipmunks and gray squirrels showed that these mosquitoes did not discriminately land directly on feeding sites on these hosts, but instead landed predominantly on the broad surface of the back. Foraging behavior of *Aedes triseriatus*, leading to encounter of feeding sites on the rodents, consisted of walking while tapping the labella on the host body, with occassional short,

intermittent, transitional flights. Encounter of feeding sites was a random process. Feeding site selection was restricted by host hair length and density to ears, eyelids, noses, and feet. Probing for a blood vessel at feeding sites was a quick, non-random process. Blood feeding was also rapid, indicating that Aedes triseriatus had been selected for fast blood feeding times. Behavior of Aedes triseriatus was similar on gray squirrels and chipmunks.

3. Eastern chipmunks and gray squirrels exhibited diverse defensive behaviors against Aedes triseriatus attempting to feed on them. Most behaviors were directed towards protecting the head. Frequency of defensive behaviors of the rodents increased with increasing density of mosquitoes, but at a lower rate than predicted by a multiplicative linear model. Effective defensive behaviors of gray squirrels were head shake, and perhaps eyeblink and forepaw scratch. Effective defensive behaviors of chipmunks were head shake and perhaps body shake. Gray squirrels were considerably more defensive than chipmunks.

4. Feeding success of Aedes triseriatus was high on chipmunks and low on gray squirrels. Estimates of field attraction densities of Aedes triseriatus to these rodents allow the prediction that, in nature, Aedes triseriatus will usually feed successfully on chipmunks, but not on gray squirrels.

5. *Aedes triseriatus* behaviorally gave up attempting to blood feed on a persistently defensive host (a human hand). The giving-up time, a measure of mosquito biting persistence, varied with nutritional state and experience of the mosquitoes: sugar-starved mosquitoes, and mosquitoes that had previously given up attacking the defensive host, gave up more quickly than did non-starved or inexperienced mosquitoes. It is suggested that defensive behavior of sciurid rodents, particularly gray squirrels, effects giving-up behavior in *Aedes triseriatus*. This provides a mechanism for multiple host contacts (within a gonotrophic cycle) by *Aedes triseriatus* in nature, thus increasing the vectorial capacity of *Aedes triseriatus* for La Crosse virus.

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APPENDIX I

FIELD EVIDENCE AGAINST BURROW ENTERING BEHAVIOR BY *AEDES TRISERIATUS*

Presumably, the site of interaction of *Aedes triseriatus* with chipmunks is on or near the forest floor, and with gray squirrels is the forest floor up to the canopy. However, it is possible that *Aedes triseriatus* enter chipmunk burrows to blood feed. I made 2 observations in the afternoon of August 31, 1982 (while carrying out attraction density experiments in Pittsfield State Forest, see Chapter IV) that mitigate against this possibility. In one instance, while walking in a woods I disturbed a foraging chipmunk which ran a short distance to its burrow entrance. The chipmunk entered the burrow but did not descend deeply; I could see its head in the burrow and hear it chipping. A mosquito, which I later collected and identified as *Aedes triseriatus*, was hovering above the burrow entrance at a distance of about 8 cm. The mosquito may have been attempting to feed on the chipmunk when I disturbed it. Notably, this mosquito did not descend into the burrow during the approximately 5 minutes that I watched. Apparently the mosquito was attracted to the chipmunk in the burrow. Later in the same afternoon I disturbed another chipmunk which ran into a hole, probably its burrow entrance, at the base of a fallen tree. Again I saw an *Aedes triseriatus* hovering close to and above the burrow entrance. The mosquito did not enter the hole during the approximately ten minutes that I watched it. If _Aedes triseriatus_ do normally enter chipmunk burrows to gain access to these rodents to blood feed, then the _Aedes triseriatus_ I saw should have descended into the burrows after the chipmunks; yet, I did not observe this.

The behavior of these 2 _Aedes triseriatus_ females sharply contrasted with flies of the family Heleomyzidae (species not determined) that I saw clustered around and actively diving into the burrow entrances. These flies were apparently entering the burrows to oviposit.

APPENDIX II

BLOOD-MEAL SOURCES OF *AEDES TRISERIATUS* IN THE HOLYOKE RANGE, 1981

The mosquito *Aedes triseriatus* blood feeds to a considerable extent on sciurid rodents (Nasci 1982, Burkot and DeFoliart 1982). This has relevance to the epizootic cycle of La Crosse virus (Chapter I). The following study was done to determine the host feeding patterns of *Aedes triseriatus* in a wooded section of the Holyoke Range in Amherst and Granby, Hampshire County, Massachusetts.

The study area was the south slope of the stretch of the Holyoke Range from east of the power line right-of-way at Granby Notch to Mount Norwottuck (distance of approximately 0.6 mile). During August and September 1981, adult female mosquitoes were collected from low vegetation and the ground with a battery-powered aspirator. A small number of collections were made on the north slope of the study area by following stream beds and logging roads to Bay Road in Amherst. Previous scouting trips and trapping studies showed that *Aedes triseriatus* and sciurid rodents, particularly chipmunks, were abundant in the collection area. In order to maximize collection efficiency, an effort was made to aspirate around chipmunk burrows.

During collections, the collecting bags were removed at 1/2 hour intervals, bound with a rubber band, and stored on wet ice. At the laboratory, mosquitoes in the bags were killed by freezing, sorted and identified on a cold table, and stored at -40° C. Blood fed Aedes triseriatus, which appeared to have fresh blood meals and no egg development, were mailed frozen to Dr. Roger Nasci, Department of Biology, University of Notre Dame, for processing with precipitin tests. Blood meals of some mosquitoes were tested by Dr. John Edman, Department of Entomology, University of Massachusetts. Precipitin test procedures followed those of Edman (1971) and Nasci (1982).

Results of precipitin tests are shown in Table 28. Twenty-nine blood fed Aedes triseriatus were collected. Twenty-seven positive reactions showed that this mosquito had fed primarily on chipmunks (52%), but also on tree squirrels (10%), rabbits (7%), cow, deer, and raccoon (3% each), and unidentified mammals (14%). Two blood meals did not react in tests. Four Aedes vexans had fed on rabbits, and 1 on an unidentified mammal. One Aedes cinereus Meigen had fed on an unidentified mammal. One Culex pipiens/restuans had fed on a passerine bird.

Although the sample size was small, results of this study concur with results of those in the midwest that Aedes triseriatus blood feeds to a considerable extent on sciurid rodents in nature. A variety of mammalian hosts were undoubtedly available in the study. Deer were common on the north slope of the study area, but not on the south. I had placed four sentinel rabbits in the study area, to monitor for La Crosse virus transmission. The rabbit-fed Aedes triseriatus and Aedes vexans had probably fed on these. The single

Table 28. Blood meal sources of Aedes triseriatus and other mosquitoes in the Holyoke Range, Amherst and Granby, Hampshire County, Massachusetts, during August and September 1981.

	Host Source									
Mosquito species	Deer	Cow	Rabbit	Chipmunk	Squirrel	Raccoon	Unidentified Mammal	Passerine Bird	Total Tested	Total Reacting
Aedes triseriatus	1	1	2	15	3	1	4		29	27 (93%)
Aedes vexans			4				1		5	5(100%)
Aedes cinereus							1		1	1(100%)
Culex pipiens/restuans								1	1	1(100%)

cow feeding of _Aedes triseriatus_ is perplexing; there is a dairy herd north of Bay Road in Amherst, north of the study area, but the mosquito would have had to fly a long way from that farm for me to collect it. This feeding may have actually been on a deer.