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I am submitting herewith a thesis written by Nathan David Caldwell entitled "Seasonal abundance of mosquitoes (Diptera: Culicidae) at a high and low prevalence site for La Crosse encephalitis in eastern Tennessee." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Entomology and Plant Pathology.

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SEASONAL ABUNDANCE OF MOSQUITOES
(DIPTERA: CULICIDAE) AT A HIGH AND LOW PREVALENCE SITE
FOR LA CROSSE ENCEPHALITIS IN EASTERN TENNESSEE

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ABSTRACT

La Crosse (LAC), a California (CAL) serogroup bunyavirus, is the most prevalent pediatric arboviral disease in the United States and accounts for virtually all encephalitis cases associated with CAL serogroup arboviruses. In Tennessee, USA, prior to the drastic increase of confirmed La Crosse (LAC) encephalitis cases in 1997, the sum of documented cases reported to the Tennessee Department of Health totaled 9. During subsequent years in Tennessee (1997 to 2003), an average of 12 cases has been reported annually. Approximately 62% of these reported cases have occurred in the months of July and August. The native, container-inhabiting mosquito *Ochlerotatus triseriatus* (Say) is a proven vector of the LAC. Recently, the LAC virus was isolated from *Aedes albopictus* (Skuse) in Tennessee and North Carolina and it has been postulated that an increase in both incidence and distribution of LAC virus throughout Tennessee coincided with an expansion of the exotic and ubiquitous *Ae. albopictus* (Gerhardt et al. 2001, Erwin et al. 2002).

Weekly collections of container-inhabiting mosquitoes were conducted from 19 May to 24 November, 2003, in an attempt to quantify differences in seasonal patterns of *Oc. triseriatus* and *Ae. albopictus* populations between a high (Knox County) and low (Blount County) prevalence area for reported cases of LAC encephalitis in eastern Tennessee. When eggs found in ovitraps (n = 108,973) were utilized as an index of population density, the data showed that *Oc. triseriatus* populations were highest from 26 May to 23 June and are abundant during the early onset dates of LAC encephalitis cases in eastern Tennessee. Collections of *Oc. triseriatus* eggs declined throughout July with a smaller peak in August. In contrast, *Ae. albopictus* egg collections gradually increased in

density from May to July and sustained high levels until September. Egg densities of both *Oc. triseriatus* and *Ae. albopictus* were abundant during July and August and increased the opportunities for vertical and horizontal transmission throughout the mosquito season.

Adult females of *Oc. triseriatus* and *Ae. albopictus* were collected weekly with host-seeking and gravid traps from each sites in both Knox and Blount County. A total of 5,187 adult *Oc. triseriatus* and *Ae. albopictus* was collected from all adult trapping techniques. The proportion of parous females for each species was determined via dissection. Monthly parity averages were above 40% and based upon the general increase from May to October, opportunities for vertical and horizontal transmission by *Oc. triseriatus* and *Ae. albopictus* should have increased throughout the mosquito season.

The blood-engorged females ($n = 8$) successfully tested by polymerase chain reaction (PCR) contained mammalian blood. Furthermore, each site contained sufficient populations of sciurids and potentially allowed for LAC virus amplification. This research suggests that there are no differences between the high and low prevalence area for LAC encephalitis in regards to egg or adult collections, parity rates, host selection preferences.

Seasonal abundance, occurrence, and diversity of all mosquito species were evaluated between 19 May and 17 November, 2003, and 24 May and 5 July, 2004, at the two forested sites in Knox and Blount County. Nineteen species representing 7 genera were identified between the 2 collection seasons with an accumulation of 4,961 host-seeking and 296 gravid females. The overall abundance of adult mosquitoes collected and identified to species was similar between Knox ($n = 2590$) and Blount ($n = 2667$)

counties. Diversity of collected species was highest in June. *Aedes vexans* (Meigen), a potential bridge vector of West Nile virus (WNV), was the most commonly collected species in 2003 and accounted for approximately 28% of all host-seeking and gravid females collected. In 2004, *Ae. vexans* numbers were extremely low at both sites and was likely affected by lower amounts of precipitation in the early portion of the mosquito season. Neither a high abundance nor diversity of *Culex* species were found in gravid traps in either year. The proven and potential LAC vectors, *Oc. triseriatus* and *Ae. albopictus*, constituted 59.3% and 73.8% of all adult species collected in the 2003 and 2004 season, respectively. In Knox County, *Oc. japonicus japonicus* (Theobald) was collected during both seasons and was the first reported occurrence of this species in the state of Tennessee.

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CHAPTER I

INTRODUCTION

i - California Serogroup Viruses

The first of the California (CAL) serogroup arboviruses identified, California encephalitis (CE) virus, (Bunyaviridae: Bunyavirus spp.) was isolated in California from 2 mosquito species, *Ochlerotatus malanimon* Dyar and *Culex tarsalis* Coquillett, in 1943 (Reeves and Hammon 1952). California encephalitis virus (CEV) was implicated with human disease and subclinical infection after serological evidence was obtained from 3 patients suffering from encephalitis in the San Joaquin Valley of California in 1945 (Henderson and Coleman 1971). Cases of natural human infection with CAL-serogroup viruses were documented from Wisconsin (Thompson et al. 1965), Minnesota, Indiana, Ohio, Ottawa, Ontario (Monath et al. 1970), North Carolina (Kelsey and Smith 1978) and Florida (Chamberlain et al. 1964, Quick et al. 1965, Taylor et al. 1971) throughout the 1960's. The reported expansion of geographical range and increase in human cases caused by CAL-serogroup arboviruses may reflect an increase in clinical awareness and improvements in laboratory facilities and testing procedures rather than through geographic expansion, ecological changes, or evolution of more virulent strains (Monath et al. 1970). A multitude of CAL-serogroup virus types, subtypes, and varieties have currently been isolated throughout the Americas, Europe, Asia, and Africa (Calisher and Karabatsos 1988). Five viruses are endemic to North America and include CE, Jamestown Canyon (JC), La Crosse (LAC), snowshoe hare (SSH), and trivittatus (TVT).

With the exceptions of LAC, and to a lesser extent CE, viruses of the CAL-

serogroup have not been sufficiently studied to allow for accurate descriptions of transmission cycles (Grimstad et al. 1986). However, mosquitoes (Diptera: Culicidae), mainly within the genera *Aedes* and *Ochlerotatus*, are the primary arthropod vectors of CAL-serogroup viruses (Sudia et al. 1971, Reinert 2000). These specific arboviruses are generally transmitted transovarially from female to offspring and allow mosquitoes to be both vector and reservoir host (LeDuc et al. 1975, Eldridge 1990). Long term maintenance of CAL-serogroup viruses is dependant upon this form of transgenerational transmission, although vertebrate amplification of the virus to mosquitoes may be required to maintain virus prevalence in local populations (Eldridge 1990).

Virus neutralizing antibodies of the CAL serogroup have been found in numerous vertebrates including the following: ground and arboreal squirrels (*Tamias spp.* and *Sciurus spp.*), cotton rat (*Sigmodon hispidus*), raccoon (*Procyon lotor*), opossum, *Didelphis virginiana*), snowshoe hare (*Lepus americanus*), eastern cottontail (*Sylvialagus floridanus*) and marsh rabbit (*S. palustris*), groundhog (*Marmota momax*), and domesticated animals (cows, pigs, horses) (Henderson and Coleman 1971, Taylor et al. 1971, Yuill 1983). However, sciurids support the natural transmission cycle of CAL-serogroup viruses and are considered the primary vertebrate hosts because they are susceptible to infection and maintain high viremias capable of infecting subsequent feeding mosquitoes (Sudia et al. 1971, Pantuwatana et al. 1972, Yuill 1983). The virus circulating within the infected amplification host does not produce high pathogenicity or mortality and suggests a protracted history of association between the viruses and the primary hosts in nature and a balanced virus-host relationship (Sudia et al. 1971, Moulton and Thompson 1971). However, the primary vector(s) and host(s) associated with each

CAL-serogroup virus may differ and is postulated to be influenced by differences in geography and topography (Gauld et al. 1974).

Humans are considered secondary, incidental, or dead-end hosts for CAL-serogroup arboviruses because humans produce an insufficient viremia to infect proboscis-probing mosquitoes (Campbell et al. 1992). Seroprevalence of CAL-serogroup antibodies in humans throughout the United States show an increase in antibody prevalence associated with an increase in age (Chun et al. 1968, Henderson and Coleman 1971, Kappus et al. 1982). In Indiana, the prevalence of CAL-serogroup antibodies between the ages of one to nine years was 5%, whereas 18 to 34% of persons over 10 years of age possessed antibodies (Henderson and Coleman 1971). The comparative rarity of reported clinical cases in adults and the high prevalence of neutralizing antibodies suggest the rate of mild or asymptomatic infections with CAL-serogroup viruses is more abundant in comparison to the rate of clinical cases (Chun et al. 1968, Acha and Szyfres 1987).

ii - La Crosse Virus

La Crosse (LAC), a CAL-serogroup bunyavirus, is the most prevalent pediatric arboviral disease in the United States and accounts for virtually all encephalitis cases associated with CAL-serogroup arboviruses (Kappus et al. 1982, Calisher et al. 1988, Jones 1999). Children and adolescents under 15 years of age are particularly susceptible to contracting LAC encephalitis (Estrado-Franco et al. 1995) with symptomatic cases taking place mostly in late summer and early fall (Chun et al. 1968, Jones et al. 1999). Human manifestation of LAC virus infection varies in severity and ranges from asymptomatic to acute central nervous system disease with symptoms including febrile

headache, vomiting, seizure, aseptic meningitis, encephalitis, virus induced coma, and decreased lifelong neurological function (Chun et al. 1968, Woodruff et al. 1992, Erwin et al. 2002). Infections resulting in death are rare but do occur in less than 1% of reported cases (Kappus et al. 1983, Estrado-Franco et al. 1995). Although clinical signs can suggest LAC infection, antibody titers that exhibit a four-fold change between acute and convalescent sera confirm the diagnosis (Jones et al. 1999). Economic and social impacts have been studied in North Carolina and have shown that medical costs associated with LAC encephalitis ranges between \$7,521 to \$175,586 (n=24) and the projected costs of individuals with lifelong neurological sequelae (n=5) would be between \$48,775 to \$3,090,798 over 89.6 life years (Utz et al. 2003).

La Crosse was discovered in and named after La Crosse, Wisconsin, where the disease agent was originally isolated from the brain of a child who died of encephalitis in 1960 (Thompson et al. 1965, DeFoliart et al. 1986). In a ten-year period between 1960 and 1970, approximately 100 human cases in Wisconsin were serologically confirmed as LAC virus infection (Watts et al. 1972). Reported cases of LAC encephalitis increased by the apparent geographical range expansion of the viral agent to neighboring mid-western states, primarily Illinois, Indiana, Iowa, Minnesota, and Ohio (Thompson et al. 1965, Calisher 1994). From 1964 to 1986, a total of 1,726 LAC encephalitis cases was reported to the CDC with a mean of 75 cases per year; most of these cases were reported from the previously mentioned mid-western states (Nasci et al. 2000). Between 1987 and 1997, approximately 744 LAC cases were reported nationwide with 36% of total cases ranging throughout the southern portion of West Virginia (Nasci et al. 2000). La Crosse is endemic to 29 states and ranges from Wisconsin eastward to New York and south to

Georgia, Alabama, and Texas (Nasci et al. 2000). Due to nonspecific diagnostic testing as well as differences in individual reactions to LAC virus, distribution and the number of human cases of LAC encephalitis have been suggested to be more prevalent than reported (Henderson and Coleman 1971, R. Nasci pers. comm.).

Although LAC has been diagnosed infrequently in southern states, human cases of LAC encephalitis have been documented in both Tennessee and North Carolina since 1964 (Kappus et al. 1982, Jones et al. 1999). In Tennessee, prior to the drastic increase of confirmed LAC cases in 1997, the sum of documented cases totaled nine (Jones et al. 1999). During subsequent years in Tennessee, 10 cases were reported in 1997, nine in 1998, six in 1999, 16 in 2000, nine in 2001, 15 in 2002, and 19 in 2003 (Jones et al. 1999, Smith 2001, Erwin et al. 2002, Morton 2002, K. Gottfried pers. comm.). The seroprevalence of LAC antibodies in humans in Tennessee (0.5%) suggests that the disease is relatively new to the state in comparison to western North Carolina (9.6%) (Jones 2000). It has been postulated that an increase in both incidence and distribution of LAC virus throughout Tennessee coincides with an expansion of the exotic and ubiquitous *Ae. albopictus* (Gerhardt et al. 2001, Erwin et al. 2002).

The natural transmission cycle for LAC involves container-inhabiting mosquitoes and small mammals, especially the eastern chipmunk (*T. striatus*), eastern gray squirrel (*S. carolinensis*) and fox squirrel (*S. niger*) (Moulton and Thompson 1971, Gauld et al. 1974, Yuill 1983). Transovarial and venereal transmission by mosquitoes and horizontal transmission to mosquitoes, through mammalian amplification, increases the dissemination potential of LAC. The potential for viral transmission from mosquito to man is influenced by human association with sylvatic habitats where the natural vector(s)

and amplification hosts are present (Sudia et al. 1971, Henderson and Coleman 1971) as well as the average number of hours spent outdoors (Erwin et al. 2002). Environmental factors including the presence of natural and artificial containers for mosquito larval development further amplifies the risk of LAC infection throughout peridomestic areas (Hedberg et al. 1979, Woodruff et al. 1992, Erwin et al. 2002). Thus, human behavioral factors and housing preferences increase the risk of becoming infected. Incidence of infection with LAC virus differs by time of year. Date of onset for confirmed cases of LAC encephalitis in eastern Tennessee, between 1997 and 2003, ranged from May 26 to October 13; over 75% of these cases occurred after the month of June. These data coincide with previous studies in North Carolina and Ohio that determined 70% of human LAC virus infections have disease onset dates in August and September (Kappus et al. 1983, Berry et al. 1983).

iii - Container-Inhabiting Mosquitoes

The eastern tree-hole mosquito, *Ochlerotatus triseriatus* (Say), is a native container-inhabiting culicid that is often considered to be a rural, sylvatic species associated with eastern deciduous forest and woodlots (Hanson et al. 1988, Nasci 1982). This diurnal species (Loor and DeFoliart 1970) is most active at ground level but has been collected 21 meters up in the canopy (Nasci 1982). *Ochlerotatus triseriatus* utilize natural tree-holes as larval habitats, especially oak (*Quercus spp.*) and hickory (*Carya spp.*), which are formed between root buttresses and arboreal crotches, or as a result of tree decay after a branch has broken off (Bradshaw and Holzapfel 1983). Tree holes provide a unique ecological niche for container-inhabiting mosquitoes and differ from the larval habitats of other species of mosquitoes. *Ochlerotatus triseriatus* has further

adapted to take advantage of discarded tires and other man made water collecting containers (Grimstad and Haramis 1984, Hanson and Hanson 1970). The most influential ecological determinate for a high population of *Oc. triseriatus* is the number of tree-holes capable of supporting large numbers of larvae (Gauld et al. 1974).

The presence of tree-holes in LAC endemic areas significantly increases the risk of LAC infection (Woodruff et al. 1992, Erwin et al. 2002). *Ochlerotatus triseriatus* is considered the natural vector and over winter reservoir of the LAC virus and has had the viral agent repeatedly isolated from naturally infected adult populations (Watts et al. 1973, Pantuwatana et al. 1974, Kappus et al. 1982, Grimstad and Haramis 1984, Szumlas et al. 1996, Gerhardt et al. 2001). Incrimination of this indigenous vector species is further corroborated by the observation that disease in humans develop only in individuals who are bitten by mosquitoes in close association to sylvatic habitats where *Oc. triseriatus* is present (Hanson and Hanson 1970).

Ochlerotatus triseriatus females are anautogenous, requiring a blood meal for successful development and deposition of eggs. La Crosse infected females are capable of transovarially transmitting LAC virus to subsequent progeny and produce a virus amplification effect throughout the mosquito season (Miller et al. 1977). This specific type of vertical transmission was verified by the isolation of the LAC virus from *Oc. triseriatus* larvae (Pantuwatana et al. 1974, Watts et al. 1974). Eggs deposited by *Oc. triseriatus* in the fall undergo diapause in response to shorter day lengths and over winter in the egg stage (Teng and Apperson 2000). This allows the LAC virus to maintain endemic levels between mosquito seasons. Although males do not feed on blood or transmit LAC to humans, the complexity of the LAC transmission cycle is further

compounded by the capability of transovarially infected males to infect females venereally (Thompson and Beaty 1977,1978).

Although LAC virus can persist 4 years or longer with vertical transmission to subsequent generations of *Oc. triseriatus* (Miller et al. 1977), a horizontal transmission cycle between mosquitoes and small mammals must periodically take place in order to maintain the virus at endemic levels (Moulton and Thompson 1971). In LAC endemic areas, antibody prevalence rates are highest in eastern chipmunks (Gauld et al. 1974), however the magnitude of viremias within eastern chipmunks, gray squirrels, and fox squirrels can provide sufficient virus to infect virus free *Oc. triseriatus* that feed on the infected vertebrate (Osorio et al. 1996, Pantuwatana et al. 1972). All modes of LAC transmission to *Oc. triseriatus* (transovarial, venereal, horizontal) allows for an epidemiology that complicates management efforts and increases the risk of human LAC infection.

Aedes albopictus (Skuse), the Asian tiger mosquito, is native to the Oriental and Australian regions, and a substantial breeding population was first discovered in Harris County, Texas in 1985 (Sprenger and Wuithiranyagool 1986). This species of container-inhabiting mosquito is suspected of being introduced from its natural range in Asia to the continental United States in imported tires casings (Moore and Mitchell 1997). Although *Ae. albopictus* has a short flight range of between 200 and 600 meters (Kitron et al. 1998), rapid expansions of larval populations throughout the United States were aided by extensive commercial shipments of used tires and by 1988 were abundant in discarded tires in multiple states east of the Mississippi River (Hawley 1988). *Aedes albopictus* was first collected in Knoxville, Tennessee in the summer of 1994 (R. Gerhardt pers.

comm.) and has subsequently spread to all southeastern states (Reiter 1998).

Aedes albopictus is an experimental vector of 22 arboviruses including agents within the alphavirus, flavivirus, and bunyavirus classifications and can successfully transmit the LAC, Keystone, and San Angelo viruses transovarially (Tesh and Gubler 1980, Moore and Mitchell 1997, Grimstad et al. 1989). Six viruses within the continental United States have been isolated from wild caught *Ae. albopictus* including Cache Valley and Potosi viruses (Mitchell et al. 1998), Jamestown Canyon virus (Gottfried et al. 2002), eastern equine encephalomyelitis (Mitchell et al. 1992), West Nile virus (White et al. 2001, Turell et al. 2001a), and LAC virus (Gerhardt et al. 2001). In the laboratory, *Ae. albopictus* is a more competent vector of LAC than the native vector, *Oc. triseriatus* (Grimstad et al. 1989, Cully et al. 1992).

This diurnal, container-inhabiting culicid efficiently transmits LAC transovarially, over winters in the egg stage, and utilizes the same larval habitats as the native *Oc. triseriatus* (Hawley et al. 1989, Hanson and Craig 1995). Although *Aedes albopictus* has been shown to out-compete and displace *Oc. triseriatus* in tires and artificial containers (Ho et al. 1989), the two species will coexist in natural tree-hole habitats (Livdahl and Willey 1991, Swanson et al. 2000). Adult *Ae. albopictus* opportunistically take blood meals from a wide array of vertebrates including avian and mammalian species (Niebylski et al. 1994, Savage et al. 1993). Host-feeding patterns are more diverse in comparison to *Oc. triseriatus* which may increase the exposure of LAC to humans (Hawley 1988).

Aedes albopictus is of great concern to the LAC transmission cycle of eastern Tennessee because the virus was isolated from wild caught populations in Tennessee and

North Carolina during the 1999 collecting season (Gerhardt et al. 2001). This prolific species is peridomestic in both rural and urban areas (Hawley 1988, Gerhardt et al. 2001, Erwin et al. 2002). At four eastern Tennessee LAC case sites, *Ae. albopictus* was the more dominant day time biter and exerted considerably more biting pressure than the native vector, *Oc. triseriatus* (R. Nasci pers. comm.). Thus, the potential for *Ae. albopictus* to become a primary vector in eastern Tennessee has been postulated.

Ochlerotatus japonicus (Theobald) is a container-inhabiting mosquito species that is native to and occurs throughout Japan, Korea, Taiwan, eastern China, and Russia (Tanaka et al. 1979, Falco et al. 2002). The first reported collections of *Oc. japonicus* in the United States were from New York and New Jersey in the late summer of 1998 (Peyton et al. 1999). Subsequently, *Oc. japonicus* was reported to have been collected in Connecticut in July of 1998 (Fonseca et al. 2001). This exotic species is spreading geographically, and by the end of the 2003 mosquito collection season it had been collected in 19 states including Connecticut (Fonseca et al. 2001), Delaware, Georgia, Massachusetts (Rutgers 2004), Maine (Foss and Dearborn 2001), Maryland (Sardelis and Turell 2001), North Carolina (B. Harrison pers. comm.), New Hampshire (Rutgers 2004), New Jersey, New York (Peyton et al. 1999), Ohio (Rutgers 2004), Pennsylvania (Sardelis and Turell 2001), Rhode Island, South Carolina (Rutgers 2004), Tennessee, Virginia (Harrison et al. 2002), Vermont (Graham and Turmel 2001), Washington (Falco et al. 2002), and West Virginia (Rutgers 2004). The first known collection of *Oc. japonicus* in Knox County, Tennessee occurred on June 9, 2003.

In its native range, *Oc. japonicus* has been collected as far south as 33°N and based on climatic factors alone, could expand its range in the United States as far south as

Jacksonville, Florida (Sardelis and Turell 2001). The world's northernmost collection for this species is in France at 48°N (Schaffner et al. 2003). The spread of *Oc. japonicus* may have been caused by a divergence from a single source or through separate introductions into various areas of the continental United States (Falco et al. 2002). However, the used tire trade is suspected to be the mode of introduction (Peyton et al. 1999) and movement of horses via trailers has been postulated to be the mode of transportation of larvae from state to state (Fronseca et al. 2001).

Adult *Oc. japonicus* are diurnal and inhabit forested areas similar to those of *Oc. triseriatus* and *Ae. albopictus* (Andreadis et al. 2001). The larval stages of *Oc. japonicus* develop in a wide variety of natural and artificial water-holding cavities and containers, especially in tires (Kutz et al. 2003), and are reported to prefer rock pools and earthenware containers (Scott et al. 2001a). *Ochlerotatus japonicus* over winters in the egg stage in the more northern parts of its range but can be found as larvae during the winter months as far north as Tokyo (37°N), which is at the same latitude as Norfolk, Virginia (CDC 2003).

This newly introduced invasive species is a competent vector for the West Nile virus (White et al. 2001, Turell et al. 2001b, Kutz et al. 2003) and has been shown in a laboratory setting to transmit the viruses associated with LAC encephalitis (Sardelis et al. 2002a), eastern equine encephalitis (Sardelis et al. 2002b), and St. Louis encephalitis (Sardelis et al. 2003). Its potential as a bridge vector for LAC virus is enhanced by the fact that females feed readily on avian and mammalian hosts in the laboratory (Sardelis et al. 2002a, Falco et al. 2002), as well as humans in the field (Peyton et al. 1999). The potential of *Oc. japonicus* to become an established pest and disease vector of LAC in

forested suburbs and rural areas should not be underestimated.

iv - Techniques for Monitoring Container-Inhabiting Mosquitoes

Monitoring and accurately measuring the abundance of container-inhabiting mosquitoes is essential to understanding the epidemiology of the LAC virus.

Standardized trapping techniques are critical in monitoring the changes in relative abundance and age structure of mosquito populations and for surveillance of LAC virus activity in local mosquito populations. Various trapping and collecting methods can be utilized for the surveillance of eggs, larvae, and adult container-inhabiting mosquitoes.

An ovitrap serves as a depository for container-inhabiting mosquito eggs and acts as an artificial oviposition site. It consists of a water-filled, black cup attached to a tree at approximately 0.5 meters above ground with seed germination paper affixed to the inner side (Loor and DeFoliart 1969, Yap et al. 1995, Jones et al. 1999, Nasci et al. 2000, Gerhardt et al. 2001). Continual usage of ovitraps provides an indirect measurement of container-inhabiting mosquito oviposition activity, but these egg densities cannot be directly correlated with larval and adult population frequencies (Steinly et al. 1991). This trapping technique should not be utilized as a quantitative measurement because ovitraps compete with natural oviposition sites and can increase the overall frequency of eggs deposited in sylvatic habitats. An alternative egg collection method, expanded polystyrene floats, is being investigated as a potential *Oc. japonicus* egg surveillance technique (Scott and Crans 2003). In addition to ovitraps providing a qualitative measurement, eggs collected in the field can be reared to the adult stage and tested for a broad spectrum of viruses (Clark et al. 1982). Various methods of mosquito egg collecting have a relatively low operating cost and are not as laborious in comparison to

larval and adult collections (Furlow and Young 1970).

Vectors of LAC, as well as many other mosquito species, can be further monitored through larval and adult collection techniques. Larvae of container-inhabiting mosquitoes are found in natural (tree-holes) and artificial containers and are collected by dipper or siphon. Adult vector activity, relative abundance, and age structure have been monitored through various adult trapping techniques including Center for Disease Control (CDC) light traps alone or baited with carbon dioxide (Stryker and Young 1970, Takken and Kline 1989, Reisen et al. 1999), Omni-directional Fay traps (Jensen et al. 1994), infusion-baited gravid traps (Reiter 1983, Reiter 1987, Scott et al. 2001b), and resting boxes (Crans 1989). Collection numbers and consistency of adults trapped vary and are species biased. Thus, multiple surveillance techniques should be utilized to ascertain viable information on changes in relative abundance, age structure, and virus activity.

v - Vertebrate Amplification

Vertebrates can contribute to the natural LAC transmission cycle by supplementing the numerous modes of vertical transmission with horizontal transmission (Moulton and Thompson 1971, Miller et al. 1977). The vertebrate species on which a mosquito vector feeds upon influences the dissemination potential of the LAC virus to humans. Primary amplification hosts for LAC are infected vertebrates that can provide sufficient virus to infect virus free female mosquitoes (Pantuwatana et al. 1972, Osorio et al. 1996) and include the eastern chipmunk, gray squirrel, and fox squirrel (Moulton and Thompson 1971, Cully et al. 1991, Osorio et al. 1996, Kitron et al. 1997). In LAC endemic areas, antibody prevalence rates are highest in populations of eastern chipmunk

(53%) and tree squirrels (39%) (Moulton and Thompson 1971, Gauld et al. 1974). La Crosse antibody levels have been shown to peak in eastern chipmunk and gray squirrel at approximately 21 days and are detectable at 256 days post-viremia (Ksiazek and Yuill 1977). However, free-ranging chipmunks are considered the main amplification host because of their tendency to sustain higher and longer viremias than other mammals (Thompson et al. 1967).

Secondary vertebrate hosts susceptible to infection include the red (*Vulpes vulpes*) and gray foxes (*Urocyon cinereoargenteus*), white-tailed deer (*Odocoileus virginianus*), and eastern cottontail rabbits (*S. floridanus*) (Yuill 1983). Viremic levels within secondary vertebrates may be too low to infect subsequent feeding mosquitoes; these vertebrates have been postulated to be of limited significance to horizontal transmission (Yuill 1983). Woodchucks (*M. monax*), deer mice (*Peromyscus maniculatus*), raccoons (*P. lotor*), and opossums (*D. virginiana*), have been eliminated as potential amplifiers of LAC due to low magnitude viremias or ecological incompatibilities for vector activity (Issel et al. 1972, Yuill 1983, Grimstad 1988). Caged rabbits are used as sentinel animals because they exhibit high antibody titers (Trainer 1973); wild eastern cottontail rabbits historically have shown low antibody titers (Moulton and Thompson 1971). It is suggested that lagomorphs are more abundant throughout open habitats during the late summer and fall months thus reducing the likelihood of interaction between host and vector(s) (Moulton and Thompson 1971).

Viral infection and amplification rates of LAC virus amongst vertebrates vary and are dependant upon the ecology of the species. Chipmunks are active throughout diurnal and crepuscular periods in the lower strata of the forest thus allowing for a close

association with *Oc. triseriatus* and *Ae. albopictus* (Moulton and Thompson 1971). Tree squirrels, *S. carolinensis* and *S. niger*, spend more time in the upper strata of the arboreal canopy and may not be exposed to biting mosquito vectors as often. Furthermore, the defensive behavior of the eastern chipmunk to mosquito biting activity in comparison to the gray squirrel is limited and does not significantly impact mosquito engorgement success (Edman et al. 1985).

La Crosse virus attrition can reduce the efficiency of transmission through four major factors and limits overall vertebrate amplification. The first factor effecting horizontal transmission results from mosquitoes feeding on non-amplifier species of vertebrates (DeFoliart et al. 1986). White-tailed deer do not play an important role in maintaining LAC virus because viremia levels are too low to infect susceptible *Oc. triseriatus* (Issel et al. 1972). Prior studies in Wisconsin have shown that virus attrition occurs in forested habitats where white-tailed deer are abundant. In these areas, cervids constitute 65% of *Oc. triseriatus* blood meals while only 24% were from eastern chipmunks and gray squirrels (Burkot and DeFoliart 1982). The natural host selection of *O. virginianus* by mosquito vectors decreases the viral amplification potential of LAC. The second factor effecting viral attrition involves the inefficiency of virus transfer from infective mosquito to susceptible amplifier host and back to a susceptible mosquito (DeFoliart et al. 1986). Thirdly, efficacy in small mammal amplification is limited due to LAC virus typically not being transmitted to *Oc. triseriatus* offspring in the first ovarian cycle following the engorgement of an infectious blood meal (Chandler et al. 1998). It has been calculated that there is a 90% mortality rate of orally infected mosquitoes prior to deposition of infected eggs, which limits viral amplification to succeeding progeny

(DeFoliart et al. 1986). In contradiction, a recent parity and survival study by Gottfried et al. (2002) showed a daily survival rate for *Ae. albopictus* of 96% to 99% in a high prevalence area for La Crosse. Nasci (1988) showed that longer wing lengths of *Oc. triseriatus* were related to an increase of survivability and blood-feeding success. Because the body size of an adult mosquito is determined by the quality of food as larvae (Fish and Carpenter 1982), it could be assumed that the historical variations in daily survival rates could be attributed to the quality of larval habitat. Lastly, there has been evidence provided by Thompson (1983) showing reduced oral transmission rates by the native vector, *Oc. triseriatus*, when infected venereally after engorging upon a chipmunk or squirrel with antibody to LAC. La Crosse virus neutralizing antibodies have been exhibited in sciurids at a prevalence exceeding 40-50% (Gauld et al. 1974), thus even the main vertebrate amplification hosts can reduce the efficiency of LAC virus transmission in the proven vector.

CHAPTER II

**SEASONAL DISTRIBUTION AND PARITY OF CONTAINER-INHABITING
MOSQUITOES (DIPTERA: CULICIDAE) IN A HIGH AND LOW
PREVALENCE AREA FOR LA CROSSE ENCEPHALITIS**

i – Abstract

Weekly collections of container-inhabiting mosquitoes were conducted from 19 May to 24 November, 2003, in a high (Knox County) and low (Blount County) prevalence area for La Crosse (LAC) encephalitis in eastern Tennessee, USA. *Ochlerotatus triseriatus* (Say) and *Aedes albopictus* (Skuse) were collected with 25 ovipositional traps, 2 CO₂-baited Center for Disease Control (CDC) miniature light traps, a CO₂-baited Omni-directional Fay trap, and 2 gravid traps from both sites each in Knox and Blount County. A total of 108,973 eggs of *Oc. triseriatus* (n=88,261) and *Ae. albopictus* (n=20,712) was identified from both sites combined. The season-long egg ratio in Knox County was 4.2:1 in favor of *Oc. triseriatus* to *Ae. albopictus*; a similar ratio of 4.6:1 was observed within the Blount County site. Egg densities of *Oc. triseriatus* were highest from 26 May to 23 June and are abundant during the early onset dates of LAC encephalitis cases in eastern Tennessee. In contrast, *Ae. albopictus* egg collections gradually increased in density from May to July and sustained high levels until September. Both species were abundant during July and August and likely increased the opportunities for vertical and horizontal transmission of LAC throughout the latter portion of the mosquito season. Egg numbers and adult collections fluctuated in a similar manner between sites but not between species. A total of 5,187 adult *Oc.*

triseriatus and *Ae. albopictus* was collected from all adult trapping techniques. When catches from adult host-seeking traps were combined, the adult ratio in Knox County was 1.2:1, *Ae. albopictus* to *Oc. triseriatus*; a ratio of 6.4:1, *Ae. albopictus* to *Oc. triseriatus*, existed in the Blount County site. In the Knox and Blount County sites, average monthly parity rates of dissected *Oc. triseriatus* (n = 404) and *Ae. albopictus* (n = 456) was approximately 40% and increased from 0 to 79% from May to October. The limited number of blood-engorged mosquitoes that were successfully analyzed by PCR contained mammalian blood. Each site contained sufficient populations of sciurids for LAC virus amplification. There were no differences in egg or adult collections, parity rates, or host selection preferences between high and low LAC prevalence sites.

ii - Introduction

La Crosse (LAC), a California (CAL) serogroup bunyavirus, is the most prevalent pediatric arboviral disease in the United States and accounts for virtually all encephalitis cases associated with CAL serogroup arboviruses (Kappus et al. 1982, Calisher et al. 1988, Jones 1999). Children and adolescents under 15 years of age are particularly susceptible to contracting LAC encephalitis (Estrado-Franco et al. 1995) with symptomatic cases taking place in late summer and early fall (Chun et al. 1968, Jones et al. 1999). Although LAC encephalitis has been diagnosed infrequently in southern states in comparison to midwestern states (Thompson et al. 1965, Calisher 1994), human cases have been documented in both Tennessee and North Carolina since 1964 (Kappus et al. 1982, Jones et al. 1999). In Tennessee, prior to the drastic increase of confirmed LAC cases in 1997, the sum of documented cases reported to the Tennessee Department of Health totaled 9 (Jones et al. 1999). During subsequent years in Tennessee, 10 cases

were reported in 1997, nine in 1998, six in 1999, 16 in 2000, nine in 2001, 15 in 2002, and 19 in 2003 (Jones et al. 1999, Erwin et al. 2002, K. Gottfried pers. comm.). The human seroprevalence of LAC antibodies in Tennessee (0.5%) suggests that the disease is less prevalent in the state than in western North Carolina (9.6%) (Gerhardt et al. 2001).

Container-inhabiting species including the native *Ochlerotatus triseriatus* (Say) and the invasive *Aedes albopictus* (Skuse) have been proven to be effective vectors of the LAC virus in the laboratory setting; in the laboratory, *Ae. albopictus* transmits LAC more efficiently than *Oc. triseriatus* (Grimstad et al. 1989, Culley et al. 1992). The LAC virus has been repeatedly isolated from field-caught larvae and adult *Oc. triseriatus* (Watts et al. 1973, Pantuwatana et al. 1974, Watts et al. 1974, Kappus et al. 1982, Szumlas et al. 1996) and was also recently isolated from *Ae. albopictus* (Gerhardt et al. 2001). The first known collection of *Ae. albopictus* in eastern Tennessee occurred during the summer of 1994 (R. Gerhardt pers. comm.) and it has been postulated that an increase in both incidence and distribution of LAC virus throughout Tennessee coincided with an expansion of the exotic *Ae. albopictus* (Gerhardt et al. 2001, Erwin et al. 2002).

The overall objective of this study was to quantify differences in seasonal patterns of *Oc. triseriatus* and *Ae. albopictus* populations between a high and low prevalence area for reported cases of LAC encephalitis. Specifically, we wanted to determine the relative rates of the following: 1) number of eggs deposited, 2) abundance of host-seeking and gravid adults, 3) monthly proportions of parous mosquitoes, 4) and mammalian host selection. Further, population estimates for the primary amplification hosts of LAC, *Tamias striatus* and *Sciurus carolinensis* (Moulton and Thompson 1971, Cully et al. 1991, Osorio et al. 1996, Kitron et al. 1997), were conducted.

iii - Materials and Methods

Collection locations: One site each was selected in Knox and Blount counties, located in the Appalachian Valley and Ridge region of eastern Tennessee, USA (Moore 1994). Collection sites were located in mixed hardwood forests approximately 30 km apart (Figure 2.1) and in close vicinity to human dwellings. Each sylvatic habitat contained numerous natural and artificial containers and was in close proximity to human interaction.

The Knox County site (35°, 69°N: 84°, 8°W) was located in the northeastern portion of the county, in the community of Karns, and was designated as the high prevalence area for LAC encephalitis. Two children with confirmed cases of LAC encephalitis in children, during 1997 and 2000, lived 3 houses apart on the same street, and were both adjacent to a 3-hectare woodlot. The forested area was bordered by 7 houses of a middle-income neighborhood atop a ridge with an apex elevation of 350.5 meters. The slope from the ridge was gradual and continuous with a southeastern aspect. Primary arboreal canopy species were white oak (*Quercus alba*), black oak (*Quercus velutina*), pignut hickory (*Carya glabra*), and tulip poplar (*Liriodendron tulipifera*). Major understory species were red maple (*Acer rubrum*), devil's walking-stick (*Aralia spinosa*), eastern redbud (*Cercis canadensis*), dogwood (*Cornus florida*), red elm (*Ulmus rubra*), Virginia creeper (*Parthenocissus quinquefolia*), poison ivy (*Rhus radicans*), and common greenbrier (*Smilax rotundifolia*).

The Blount County site (35°, 48°N: 83°, 60°W) was considered the low prevalence area for LAC encephalitis because there have been no reported cases in the county since 1999 (Morton 2002, K. Gottfried pers. comm.). The site was located in the north central

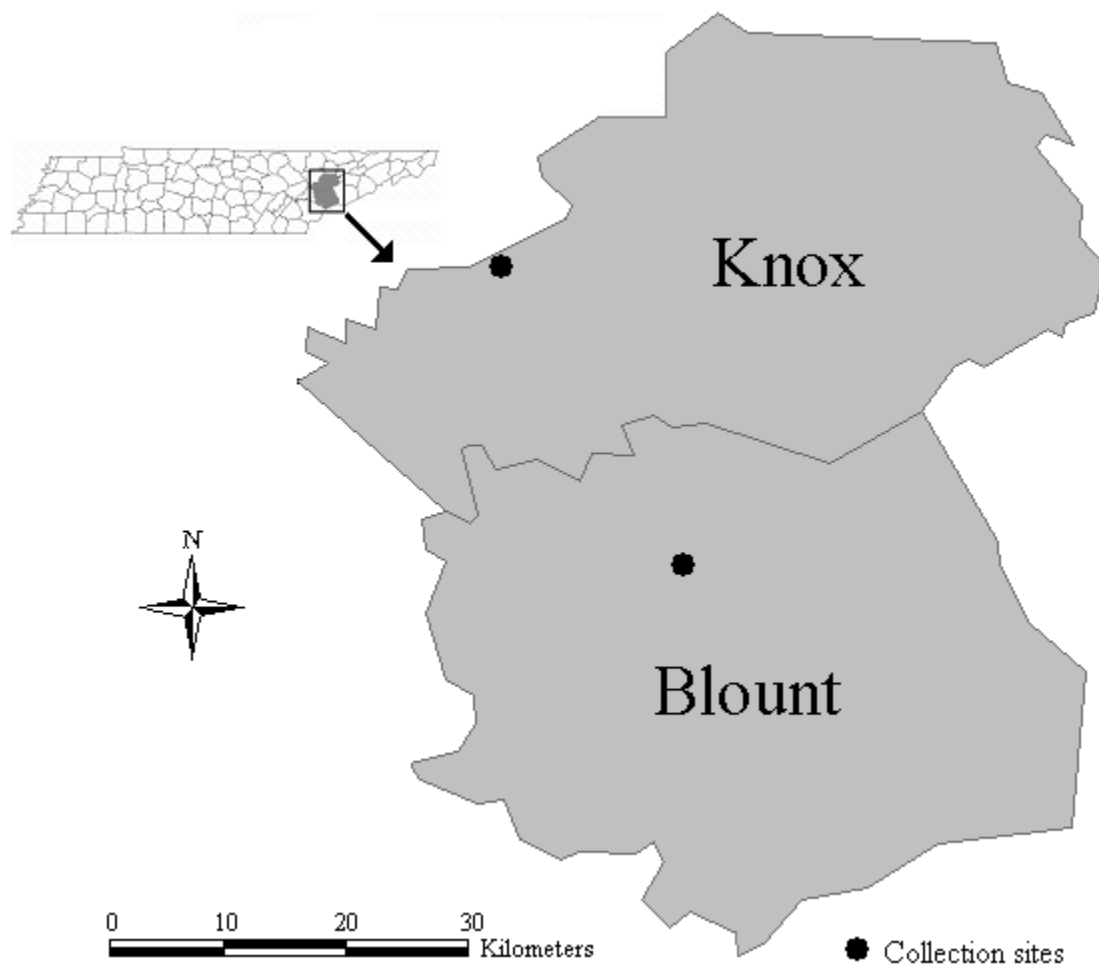


Figure 2.1. Sites in Knox and Blount County, Tennessee, USA, where mosquito population studies were conducted in 2003 and 2004.

portion of the county within the city limits of Maryville. Collections were made in the Stewardship Forest, on Maryville College campus. The forest consisted of mixed hardwoods, was approximately 121 hectares in size, and had a maximum elevation of 283.3 meters. The southwestern portion of the forest was selected as the focus of collection efforts because it contained open areas with serpentine edges and was comparable in size to the Knox County site. The southwestern edge bordered multiple middle-income residences. The overstory species were white oak (*Quercus alba*), black oak (*Quercus velutina*), pignut hickory (*Carya glabra*), and tulip poplar (*Liriodendron tulipifera*). Understory species were American beech (*Fagus grandifolia*), red maple (*Acer rubrum*), mimosa (*Albizia julibrissin*), bigleaf magnolia (*Magnolia macrophylla*), common privet (*Ligustrum vulgare*), American holly (*Ilex opaca*), English ivy (*Hedera helix*), and poison ivy (*Rhus radicans*).

Climatological Patterns: Weather data (precipitation, maximum and minimum temperature) for Knox County were gathered from the Environmental Science Division of the Oak Ridge National Laboratory (ORNL) located in Oak Ridge, TN (Anderson County). The ORNL's weather gauges were approximately 8.0 km northwest from the Knox County collection site. Daily precipitation and maximum and minimum temperatures for Blount County were supplied from a weather monitoring station located at the McGee Tyson airport, located approximately 7.0 km northwest of the Blount County collection site, and was operated by the U.S. Department of Commerce, National Weather Service (NWS).

Ovitraping and rearing: Oviposition traps (Loor and DeFoliart 1969) were set weekly in both Knox and Blount County sites to monitor egg laying by *Oc. triseriatus*

and *Ae. albopictus* from 19 May and 24 November, 2003. Twenty-five ovitraps (five sets of five) were placed between one and 10 meters inside the forest edge with an approximate spacing of 10 meters between traps. Ovitrap consisted of 473-ml black plastic cups filled with tap water and attached to trees with aluminum nails at approximately 0.5 meters above the base of the tree (Lor and DeFoliart 1969). Egg deposition substrate, 76-lb seed germination paper (Anchor Paper Company, Saint Paul, MN) was cut into 5 X 28 cm pieces, labeled, folded, and affixed to the inside of the cup via paperclips to provide an ample and attractive oviposition area (Steinly et al. 1991). A constant area for oviposition was provided by placing two holes in the rim of each cup to allow for drainage of excess water during times of high rainfall. All traps were placed on the north side of trees to avoid direct sunlight, which could cause desiccation to the eggs or drastically alter water conditions. The strips of seed germination paper from both sites were replaced weekly and deposited into plastic bags for transportation. Both clean tap water and paper strips were replenished weekly. Oviposition strips were transported back to the laboratory facility where they were partially air dried and eggs were individually counted and identified by species using a binocular dissecting scope (Pratt and Kidwell 1969). Strips were sealed in plastic bags and monitored daily. To assure adequate time for egg embryonation, the rearing process began 11 days after collection.

Strips from ovitraps were placed into clean, 4.3-liter plastic containers (2 groups of 10 and 1 group of 5) by site and submerged in 2.0 liters of tap water mixed with 4.0g of bovine liver powder (ICN Biomedicals Inc., Aurora, OH). Water temperatures were maintained at $27^{\circ}\text{C} \pm 2^{\circ}\text{C}$. After 3 days, strips were removed from the containers and newly emerged larvae were allowed to develop to 4th instar and occasionally pupal

stages; at this point, developing mosquitoes were transferred to Mosquito Breeders (BioQuip, Gardena, CA). The lower portion of the rearing chamber was covered in dark construction paper and newly emerged adults found in the upper portion of the chamber were collected and killed by freezing. Mosquitoes were identified (Darsie and Ward 1981), sexed, and sorted on a BioQuip® chill table or ice tray and placed into pools of no more than 50 individuals. All mosquitoes reared, as well as all adults collected by various trapping techniques, were placed into labeled centrifuge tubes and subsequently stored in a Baxter® Cryo-fridge at -70°C and are awaiting virus testing.

Adult mosquito collections: Weekly collections of host-seeking mosquitoes were conducted at each site from 19 May to 17 November, 2003 by suspending 2 dry ice-baited Centers for Disease Control (CDC) miniature light traps (Stryker and Young 1970) and a dry ice-baited Omni-directional Fay trap (Jensen et al. 1994) in the vicinity of ovitraps. The CDC miniature light trap (model 512) and the Omni-directional Fay trap (model 112) were constructed by the John W. Hock Company in Gainesville, FL. A 5.68-liter water cooler was loaded with dry ice was positioned adjacent to each adult trap. Traps for host-seeking mosquitoes were deployed for approximately 24-hour periods from 0800-0930 EDT and 1030-1130 EDT at the Blount and Knox County sites, respectively. Adult mosquito collections coincided with the weekly replacement of oviposition strips. The collection cup of each trap was removed and placed into a cooler with dry ice for transportation back to the laboratory. Mosquitoes were separated by species (Darsie and Ward 1981) and sex while on a chill table or ice to prevent virus degradation and placed into pools of 50 mosquitoes or less.

Two gravid traps (John W. Hock Company, Gainesville, FL), model number 1712, were placed at each site to collect gravid female mosquitoes (Reiter 1983). A concoction of tap water, fresh cow manure, and oak leaves were utilized as the ovipositional attractant. During days of high precipitation, gravid trap collection nets were covered with a plastic bag to prevent damage to scales and other essential identification markers of the mosquitoes. Gravid mosquitoes were transported and identified with all other adults.

Parity determinations: Previously identified female mosquitoes from host-seeking collections were briefly submerged in 70% ethyl alcohol and immediately placed into individual wells of saline solution (Hayes 1953). The last segment of the abdomen was torn and the ovaries and gut were removed with forceps to evaluate the ovarian tracheal skeins through a compound microscope. Individual specimens were dissected and classified as parous or nulliparous based on the condition of the ovarian trachioles (Detinova 1962). A mosquito had experienced at least one gonotrophic cycle and was thus considered parous when the skeins of the ovarian tracheal system were elongated and not tightly coiled (Detinova 1962, Nasci 1986). Conversely, mosquitoes with tightly knotted ovarian tracheal skeins were assumed to be nulliparous. The parity rate was determined for both species by calculating the monthly total of parous individuals divided by the total number of females dissected per month (Jensen et al. 1998). Power analysis using a modified version of SAS: UnifyPow (O'Brien 1998) estimated from eastern Tennessee (Gottfried et al. 2002) determined a sample size of 36 females was necessary at an $\alpha = 0.5$ (A Saxton and D Sanders pers. comm.). Therefore, we attempted to dissect 10 females each week to achieve a rounded sample of 40 females total per month.

Blood-meal identification: Attempts to aspirate blood-engorged adult mosquitoes were made near ovitraps and in surrounding areas using a modified CDC backpack aspirator (Nasci 1982), model 1412 (John W. Hock Company, Gainesville, FL). Aspiration collections were conducted bi-weekly and were performed for a minimum of 30 minutes per site. Resting boxes were placed throughout both sites to lure resting, blood-engorged females (Crans 1989). Aspirator collection samples were labeled and placed on dry ice and subsequently transported and handled in the same manner as host-seeking and gravid mosquitoes. Resting, blood-engorged specimens collected in the field were identified to species through use of a dissecting microscope, placed into a labeled centrifuge tube, and maintained at -70°C until processed. Furthermore, any females considered partially blood-engorged from host-seeking trap collections were processed for blood-meal identification.

Total genomic nucleic acids were extracted from blood-engorged specimens that exhibited signs of having a distended abdomen and/or having a red to brown colored gut. Voucher materials (DNA extractions) were stored in the medical and veterinary entomology lab at the University of Tennessee. A sodium dodecyl sulfate (SDS)/proteinase K-based extraction protocol (50mM Tris, pH 8.0; 50mM EDTA, pH 8.0; 2% SDS, 75mM NaCl; 50mM Sucrose; 100 mg proteinase K) was used for the lysis buffer (K. Moulton pers. comm.). Individual specimens were deposited into 1.7 ml eppendorf (Ep) tubes that contained 700 µl of lysis buffer. Specimens were subsequently ground with a clean blue pestle after 10 µl of 10 mg/ml proteinase K solution was added. Each Ep tube was inverted 10 times and briefly spun down with the aid of a Vortex® machine and the homogenates were incubated in a heat block at 50°C for 24 hours. The

following day, 700 μ l of phenol: chloroform: isoamyl alcohol (25:24:1) mixture (Sigma-Aldrich) was placed into each tube, mixed by inverting tubes 10 times, and spun in a microcentrifuge at 14,000 rpm for five minutes. The upper aqueous layer (600 μ l) was carefully removed via pipetting and placed into a new 1.5 ml Ep tube. Subsequently, 600 μ l of 24:1 chloroform: isoamyl alcohol solution was added, inverted 10 times, and placed into the microcentrifuge at 14,000 rpm for five minutes. Again, the upper aqueous layer (500 μ l) was removed and placed into a new 1.5 ml Ep tube. A volume of 0.1 (50 μ l) of 3M sodium acetate and 600 μ l of chilled (-20°C) isopropanol were added and gently inverted 10 times. The Ep tubes were centrifuged at 14,000 rpm for five to 10 minutes. To isolate and retain the newly formed DNA pellet inside of the Ep tube, the supernatant was removed via pipette and was subsequently washed twice, once with 1 ml of 70% ethanol and then with 95% ethanol. At the end of each washing, the supernatant was removed. The DNA pellet was left to air dry inside of the Ep tube for no less than four hours and was subsequently resuspended in 50 to 200 μ l (depending upon the size of the pellet) of 1X TE solution. Templates were placed inside a -20°C freezer until further processed.

Universal forward and reverse avian and mammalian-specific oligonucleotide primers (Table 2.1) were designed from conserved regions of the large mitochondrial ribosomal subunit (16S) and ordered from Sigma-Genosys (The Woodlands, TX). These primers were designed using a nucleotide alignment of 16S containing numerous avian and mammalian species. The primers and polymerase chain reaction (PCR) protocols published by Ngo and Kramer (2003) were utilized in the early portion of this study; however, searches conducted in BLAST (NCBI 2004) found primers to be unreliable

Table 2.1. Oligonucleotide primers used in the PCR-based identification of mosquito blood-meal sources collected in eastern Tennessee between 19 May and 24 November, 2003.

Name	Sequence (5' to 3') ¹	Length	Position ²	Amplicon Size
Avian 16S 5'	MMCAAGTATTGAAGGTGA	18	2,020	--
Avian 16S 3'	AGGATTTGTTCTCCTCCA	18	2,311	326 bp
Mammalian 16S 5'	CCTGTTTACCAAAAACATCAC	21	2,513	--
Mammalian 16S 3'	AYTGTCGATAKGRACCTWRARTAG	25	2,949	481 bp

¹ Redundancy codes: Y = C or T, R = A or G, K = G or T, M = A or C, and W = A or T.

² Positions of the 3' nucleotides of primers are given in reference to the complete mitochondrial genome sequences of *Anser albifrons* (Slack et al. 2003) and *Homo sapiens* (Moilanen and Majamaa 2003), respectively.

due to instances of mismatches with published sequences.

DNA amplifications were performed in a 50 µl solution comprised of 36 µl ddH₂O, five µl of 10X PCR buffer (Takara/Panvera Corp., WI), 1.5 µl of 50 mM MgCl₂, two µl of each primer (10pmol/µl), four µl of 10mM dNTPs, one unit of Taq polymerase (ExTaq Hot Start, Takara/Panvera Corp., WI), and one µl of template DNA. Successful amplification of avian and mammalian 16S fragments were processed by a three-step touchdown PCR. The program included a two minute denaturation at 94°C for 30 seconds, 62°C for 30 seconds, 72°C for one minute followed by 5 cycles of 94°C for 30 seconds and 72°C for one minute. The program finished with 33 cycles of 94°C for 30 seconds, 54°C for 30 seconds, and 72°C for one minute. Qualification of PCR reactions was conducted by way of horizontal electrophoresis in 1% agarose.

Population assessment of amplification hosts: Gray squirrel (*S. carolinensis*) and eastern chipmunk (*T. striatus*) trapping occurred at each site on alternating dates between 27 May to 31 May, 20 October to 22 October, and 17 November to 21 November, 2003. At each site, fifty collapsible, single door, Tomahawk® traps (25 traps = 4 x 4 x 16 inches, 25 traps = 6 x 6 x 19 inches) were wired open and pre-baited with sunflower seeds for three consecutive days prior to the initial trapping date. In both Knox and Blount County sites, all trap-lines were positioned in the vicinity of adult mosquito collection devices and were spaced at 10 meter intervals. During collection dates, cages were baited with a mixture of sunflower seed, apple, and peanut butter and set at dawn. Traps were monitored at 2 hour intervals with collections continuing throughout the day light hours. At dusk, cages were wired open to avoid the trapping of nocturnal vertebrates.

An attempt was made to collect and tag 25 sciurids at each site during both the spring and fall months. All trapped animals were anesthetized with isoflurane to ease handling and reduce stress levels (Kreeger et al. 1998, Imai et al. 1997, D. O'Rourke pers. comm.). Captured sciurids were released at the identical trap site after being sexed, checked for ectoparasites, and tagged on both ears. Furthermore, a small amount of blood was collected on Nobuto® strips (Advantec Manufacturing Inc., CA) to be tested for LAC antibodies at a later date.

Statistical analysis: To test for differences in the abundance of adults and eggs collected at each site, all data were analyzed with PROC MIXED of SAS 9.1, 2004. The density of adult collections was log transformed to normalize the distribution before analyses. Raw means and standard deviations of adults and eggs were determined with PROC MEANS of SAS 9.1, 2004. Monthly comparisons in the proportion of parous females between sites and species were tested using univariate analysis of variance (ANOVA) (SPSS for Windows, version 12). Parity data was tested for normality with the Shapiro-Wilk test (EXPLORE of SPSS), and Pearson correlation was utilized to determine the relationship between parity and date. Population estimates for the potential amplification hosts were conducted using the program "CAPTURE" and allowed for population estimates of capture-recapture data on closed populations (Otis et al. 1978, White et al. 1982).

iv - Results

Climatological patterns: Between 19 May and 24 November, 2003 average daily temperatures ranged from lows of 3.1°C and 3.9°C (both on 14 November) to highs of

25.3°C (15 August) and 28.3°C (27 August) in Knox and Blount County sites, respectively (Fig. 2.2). Throughout the entire collecting season, the daily mean temperature at the Knox County (18.6°C) site averaged 2°C lower than the Blount County site (20.9°C). An average weekly accumulation of 2.58 cm at the Knox County site and 2.55 cm of rain at the Blount County site were recorded by the ORNL and NWS, respectively (Fig. 2.2). Rainfall accumulations for the 27 weeks were 68.93 cm in Knox County and 69.73 cm in Blount County. Precipitation was unseasonably high at both the high and low prevalence areas for LAC during the 2003 mosquito season in comparison to the average accumulation of rainfall for the same time period, between 1971 and 2000, which was reported to be 62.61 cm and 53.99 cm in Knox and Blount County, respectively.

Ovitraping and rearing success: Over a collection period of 9,331 ovitrap days (derived from the sum of all ovitraps utilized each week multiplied by seven days) a total of 108,973 eggs were collected from the Knox and Blount County site (Tables 2.2, 2.3, 2.4 and 2.5). The mean number of *Oc. triseriatus* eggs collected in each ovitrap per trap day over the entire 27 week study was 9.1 in the Knox County site and 9.7 in the Blount County site. The daily mean density of *Ae. albopictus* egg deposition was 2.2 eggs per trap in both Knox and Blount County sites. There was a large and significant difference between the total egg densities of *Oc. triseriatus* and *Ae. albopictus* at both sites ($P < 0.0001$).

Egg collections for *Oc. triseriatus* was bi-modal peaking at both sites during the week of 23 June followed by a decrease and another peak in mid to late August

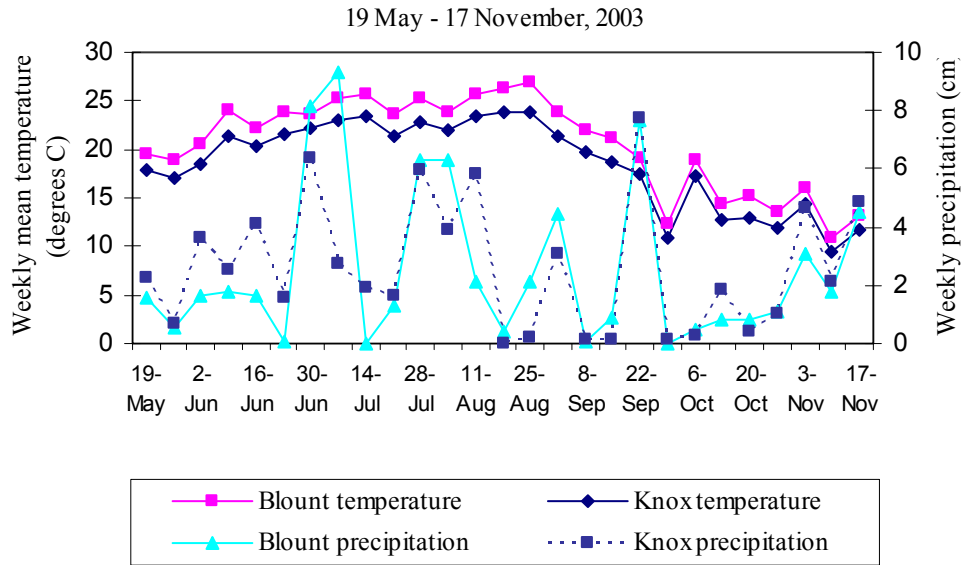


Figure 2.2. Temperature ($^{\circ}\text{C}$) and precipitation (cm) provided by the National Weather Service and the Oak Ridge National Laboratory from 19 May to 24 November, 2003, for the sites located in Blount and Knox County, TN, respectively.

Table 2.2 Number of *Ochlerotatus triseriatus* eggs (collected in a 7 day period beginning with the dates listed) and rearing success of eggs from Knox County, TN, between 19 May and 24 November, 2003.

<i>Ochlerotatus triseriatus</i>							
Date	No. ovitraps	Ovitrap days ¹	No. eggs	Mean No. eggs/trap day	Adults reared		Rearing Success ²
					Female	Male	
19-May-03	25	175	645	3.69	141	137	43.10
26-May-03	25	175	3400	19.43	89	88	5.21
2-Jun-03	25	175	2808	16.05	550	413	34.29
9-Jun-03	25	175	3578	20.45	96	95	5.34
16-Jun-03	25	175	3203	18.30	0	0	0.00
23-Jun-03	25	175	3757	21.47	542	285	22.01
30-Jun-03	25	175	1856	10.61	265	229	26.62
7-Jul-03	25	175	1477	8.44	72	127	13.47
14-Jul-03	25	175	1894	10.82	314	249	29.73
21-Jul-03	24	168	1907	11.35	194	108	15.84
28-Jul-03	25	175	908	5.19	47	32	8.70
4-Aug-03	25	175	1589	9.08	210	193	25.36
11-Aug-03	25	175	2220	12.69	133	112	11.04
18-Aug-03	24	168	3548	21.12	366	366	20.63
25-Aug-03	24	168	3056	18.19	88	74	5.30
1-Sep-03	25	175	1770	10.11	142	105	13.95
8-Sep-03	25	175	736	4.21	50	33	11.28
15-Sep-03	25	175	801	4.58	140	109	31.09
22-Sep-03	25	175	1598	9.13	84	74	9.89
29-Sep-03	25	175	322	1.84	34	47	25.16
6-Oct-03	25	175	1002	5.73	40	59	9.88
13-Oct-03	25	175	104	0.59	2	2	3.85
20-Oct-03	23	161	168	1.04	4	3	4.17
27-Oct-03	24	168	12	0.07	0	0	0.00
3-Nov-03	23	161	87	0.54	4	4	9.20
10-Nov-03	25	175	0	0.00	0	0	0.00
17-Nov-03	23	161	1	0.01	0	0	0.00
Average				9.06			14.26
Total	665	4655	42447		3607	2944	

¹Ovitrap days = number of ovitraps x 7 days

² Rearing Success = (number of females and males reared/number of eggs) x 100

Table 2.3 Number of *Aedes albopictus* eggs (collected in a 7 day period beginning with the dates listed) and rearing success of eggs from Knox County, TN, between 19 May and 24 November, 2003.

Date	No. ovitraps	Ovitrap days ¹	<i>Aedes albopictus</i>				
			No. eggs	Mean No. eggs/trap day	Adults reared		Rearing Success ²
					Female	Male	
19-May-03	25	175	260	1.49	2	0	0.77
26-May-03	25	175	157	0.90	8	5	8.28
2-Jun-03	25	175	94	0.54	9	8	18.09
9-Jun-03	25	175	215	1.23	2	4	2.79
16-Jun-03	25	175	278	1.59	3	5	2.88
23-Jun-03	25	175	221	1.26	24	20	19.91
30-Jun-03	25	175	954	5.45	35	25	6.29
7-Jul-03	25	175	918	5.25	20	29	5.34
14-Jul-03	25	175	705	4.03	86	89	24.82
21-Jul-03	24	168	1225	7.29	49	38	7.10
28-Jul-03	25	175	738	4.22	76	73	20.19
4-Aug-03	25	175	445	2.54	72	80	34.16
11-Aug-03	25	175	490	2.80	108	169	56.53
18-Aug-03	24	168	467	2.78	143	86	49.04
25-Aug-03	24	168	1209	7.20	198	226	35.07
1-Sep-03	25	175	543	3.10	80	83	30.02
8-Sep-03	25	175	124	0.71	26	24	40.32
15-Sep-03	25	175	111	0.63	24	25	44.14
22-Sep-03	25	175	249	1.42	70	48	47.39
29-Sep-03	25	175	101	0.58	19	11	29.70
6-Oct-03	25	175	443	2.53	28	24	11.74
13-Oct-03	25	175	33	0.19	1	1	6.06
20-Oct-03	23	161	61	0.38	0	0	0.00
27-Oct-03	24	168	28	0.17	0	0	0.00
3-Nov-03	23	161	38	0.24	0	0	0.00
10-Nov-03	25	175	4	0.02	0	0	0.00
17-Nov-03	23	161	1	0.01	0	0	0.00
Average				2.17			18.54
Total	665	4655	10112		1083	1073	

¹Ovitrap days = number of ovitraps x 7 days

² Rearing Success = (number of females and males reared/number of eggs) x 100

Table 2.4 Number of *Ochlerotatus triseriatus* eggs (collected in a 7 day period beginning with the dates listed) and rearing success of eggs from Blount County, TN, between 19 May and 24 November, 2003.

Date	No. ovitraps	Ovitraps days ¹	<i>Ochlerotatus triseriatus</i>				
			No. eggs	Mean No. eggs/trap day	Adults reared		Rearing Success ²
					Male	Female	
19-May-03	25	175	1951	11.15	397	452	43.52
26-May-03	25	175	3957	22.61	115	129	6.17
2-Jun-03	25	175	2188	12.50	439	521	43.88
9-Jun-03	25	175	3975	22.71	116	222	8.50
16-Jun-03	25	175	3057	17.47	0	0	0.00
23-Jun-03	25	175	4004	22.88	302	482	19.58
30-Jun-03	25	175	1612	9.21	162	167	20.41
7-Jul-03	25	175	1087	6.21	23	107	11.96
14-Jul-03	25	175	1613	9.22	177	222	24.74
21-Jul-03	25	175	1110	6.34	160	196	32.07
28-Jul-03	25	175	852	4.87	110	189	35.09
4-Aug-03	25	175	1161	6.63	177	208	33.16
11-Aug-03	25	175	1800	10.29	110	174	15.78
18-Aug-03	25	175	2476	14.15	341	488	33.48
25-Aug-03	25	175	3729	21.31	0	20	0.54
1-Sep-03	25	175	2192	12.53	62	99	7.34
8-Sep-03	25	175	3076	17.58	142	216	11.64
15-Sep-03	25	175	2033	11.62	257	396	32.12
22-Sep-03	25	175	1241	7.09	93	133	18.21
29-Sep-03	25	175	787	4.50	77	73	19.06
6-Oct-03	25	175	902	5.15	180	156	37.25
13-Oct-03	25	175	644	3.68	141	137	43.17
20-Oct-03	24	168	83	0.49	15	13	33.73
27-Oct-03	23	161	173	1.07	44	46	52.02
3-Nov-03	23	161	25	0.16	0	0	0.00
10-Nov-03	25	175	79	0.45	18	19	46.84
17-Nov-03	23	161	7	0.04	1	1	28.57
Average				9.70			24.40
Total	668	4676	45814		3659	4866	

¹Ovitraps days = number of ovitraps x 7 days

² Rearing Success = (number of females and males reared/number of eggs) x 100

Table 2.5 Number of *Aedes albopictus* eggs (collected in a 7 day period beginning with the dates listed) and rearing success of eggs from Blount County, TN, between 19 May and 24 November, 2003.

Date	No. ovitraps	Ovitrap days ¹	<i>Aedes albopictus</i>				
			No. eggs	Mean No. eggs/trap day	Adults reared		Rearing Success ²
					Male	Female	
19-May-03	25	175	373	2.13	3	2	1.34
26-May-03	25	175	223	1.27	6	6	5.38
2-Jun-03	25	175	87	0.50	13	17	34.48
9-Jun-03	25	175	186	1.06	4	2	3.23
16-Jun-03	25	175	196	1.12	0	0	0.00
23-Jun-03	25	175	340	1.94	18	22	11.76
30-Jun-03	25	175	1002	5.73	51	51	10.18
7-Jul-03	25	175	999	5.71	42	45	8.71
14-Jul-03	25	175	864	4.94	71	96	19.33
21-Jul-03	25	175	831	4.75	40	40	9.63
28-Jul-03	25	175	475	2.71	30	42	15.16
4-Aug-03	25	175	96	0.55	15	29	45.83
11-Aug-03	25	175	969	5.54	104	98	20.85
18-Aug-03	25	175	773	4.42	168	177	44.63
25-Aug-03	25	175	1232	7.04	0	0	0.00
1-Sep-03	25	175	253	1.45	79	74	60.47
8-Sep-03	25	175	432	2.47	91	86	40.97
15-Sep-03	25	175	235	1.34	62	63	53.19
22-Sep-03	25	175	235	1.34	62	62	52.77
29-Sep-03	25	175	117	0.67	25	23	41.03
6-Oct-03	25	175	166	0.95	24	20	26.51
13-Oct-03	25	175	264	1.51	36	41	29.17
20-Oct-03	24	168	82	0.49	14	7	25.61
27-Oct-03	23	161	89	0.55	6	9	16.85
3-Nov-03	23	161	13	0.08	2	1	23.08
10-Nov-03	25	175	63	0.36	4	1	7.94
17-Nov-03	23	161	5	0.03	0	0	0.00
Average				2.25			22.52
Total	668	4676	10600		970	1014	

¹Ovitrap days = number of ovitraps x 7 days

² Rearing Success = (number of females and males reared/number of eggs) x 100

(Figure 2.3). An increase in egg deposition during late June and early July and a subsequent rise throughout August and early September were recorded for *Ae. albopictus* (Figure 2.3). There was a high correlation between the numbers of eggs collected for both *Oc. triseriatus* (n = 42,447 and 45,814) and *Ae. albopictus* (n = 10,112 and 10,600) between the Knox and Blount County sites, respectively. There was no significant differences between sites when comparing egg counts of both species over the season ($F = 0.18$, $df = 1$, $P = 0.6728$).

Egg prevalence in oviposition traps was high for both species during the majority of the collection season (Table 2.6); however, *Oc. triseriatus* accounted for 81.99% of the eggs deposited on oviposition strips and were consistently more abundant on individual strips. The season long egg ratio in Knox County was 4.2:1 in favor of *Oc. triseriatus* to *Ae. albopictus*; a similar ratio of 4.6:1 was observed within the Blount County site. Monthly egg ratios at each site fluctuated in a similar manner (Table 2.7).

A total of 19,216 adults (17.63%) were successfully reared from eggs collected from the high and low prevalence areas for LAC encephalitis (Tables 2.2, 2.3, 2.4 and 2.5). Weekly rearing success of all eggs collected by ovitraps ranged from 0-60.47% and was calculated by dividing the sum of eggs reared to the adult stage by the total number of eggs counted. Mean rearing success of eggs collected in Knox County was highest for *Ae. albopictus* (18.54%) while in Blount County it was highest for *Oc. triseriatus* (24.40%).

Adult mosquito collections: Between 19 May and 17 November a total of 2,602 adult mosquitoes was collected (*Oc. triseriatus* (n= 719) and *Ae. albopictus* (n=1,883)) from 4 CO₂-baited CDC miniature light traps and 2 CO₂-baited Omni-directional Fay

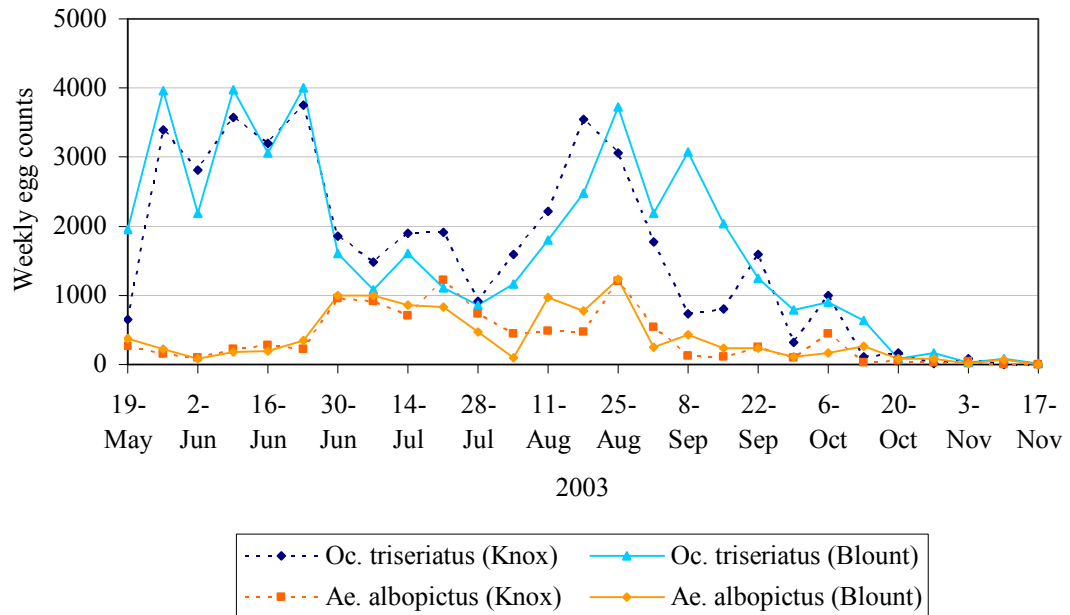


Figure 2.3. Number of eggs collected weekly for *Ochlerotatus triseriatus* and *Aedes albopictus* at each site in Knox and Blount County, TN, between 19 May and 24 November, 2003. Ovitrap collected eggs at 7 day increments beginning with the dates listed.

Table 2.6. Egg prevalence in ovposition traps for *Ochlerotatus triseriatus*, *Aedes albopictus*, or both species at each site in Knox and Blount County, TN, between 19 May and 24 November, 2003.

Month	<i>Oc. triseriatus</i>		<i>Ae. albopictus</i>		Both species	
	Knox	Blount	Knox	Blount	Knox	Blount
May	92.0	94.0	90.0	94.0	82.0	88.0
June	100.0	99.2	89.6	95.2	89.6	94.4
July	100.0	95.0	97.0	96.0	97.0	92.0
August	100.0	95.0	93.9	88.0	93.9	85.0
September	96.8	98.4	83.2	85.6	81.6	85.6
October	69.8	68.0	55.2	78.4	45.8	60.8
November	18.3	35.2	19.7	18.3	09.9	08.5

Table 2.7. Monthly egg ratios for *Ochlerotatus triseriatus* and *Aedes albopictus* collected by ovitraps in Knox and Blount County sites between 19 May and 24 November, 2003.

County	Month	ovitraps ¹	Total egg numbers		Ratio (T:A)
			<i>Oc.</i> <i>triseriatus</i>	<i>Ae.</i> <i>albopictus</i>	
Knox	May	50	4045	417	9.7:1
	June	125	15202	1762	8.6:1
	July	99	6186	3586	1.7:1
	August	98	10413	2611	4.0:1
	September	125	5227	1128	4.6:1
	October	97	1286	565	2.3:1
	November	71	88	43	2.0:1
	Total	665	42447	10112	
Blount	May	50	5908	596	9.9:1
	June	125	14836	1811	8.2:1
	July	100	4662	3169	1.5:1
	August	100	9166	3070	3.0:1
	September	125	9329	1272	7.3:1
	October	97	1802	601	3.0:1
	November	71	111	81	1.4:1
	Total	668	45814	10600	

¹ Total number of ovitraps at each site multiplied by the number of weeks in each month

traps. At each established site, host-seeking *Oc. triseriatus* appeared to be bi-modal and peaked in early to mid June followed by a smaller increase in August (**Figure 2.4**).

Aedes albopictus adult collections steadily increased between May and August and peaked on 25 August and 1 September (**Figure 2.5**).

When collection data from all adult host-seeking traps were combined, the adult ratio in Knox County was 1.2:1 in favor of *Ae. albopictus* to *Oc. triseriatus*. A ratio of 6.4:1 in favor of *Ae. albopictus* was observed at the Blount County site. However, species abundance varied according to the adult trapping technique utilized. In general, weekly collection abundance for both container-inhabiting species was greatest in CO²-baited CDC miniature light traps. All CO²-baited CDC miniature light traps collected more *Ae. albopictus* in comparison to *Oc. triseriatus* over the course of the entire collection period. The mean number of collected *Ae. albopictus* per CO²-baited CDC miniature light trap was 7.15 (SD = 8.67) and 23.43 (SD = 49.52) in Knox and Blount County, respectively. In contrast, the mean number of *Oc. triseriatus* per trap in the Knox and Blount County sites was 6.02 (SD = 7.94) and 3.69 (SD = 4.58).

Fewer numbers of both species were collected from the Omni-directional Fay traps; however, the CO²-baited Omni-directional Fay trap in Knox County had a larger quantity of both *Oc. triseriatus* and *Ae. albopictus* in comparison to Blount County (**Table 2.8**). The trap located in the Knox County site had an average of 2 more *Ae. albopictus* per collection period in comparison to *Oc. triseriatus*; there were no differences among the mean weekly collections of *Oc. triseriatus* and *Ae. albopictus* in the Blount County site.

Of the 168 gravid females collected by 2 gravid traps in the Knox County site, the

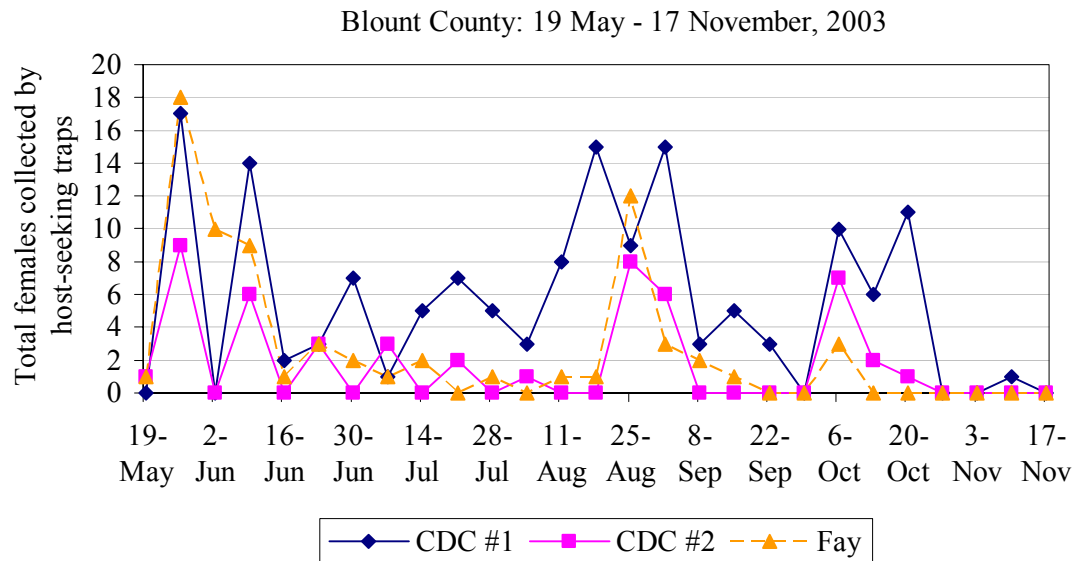
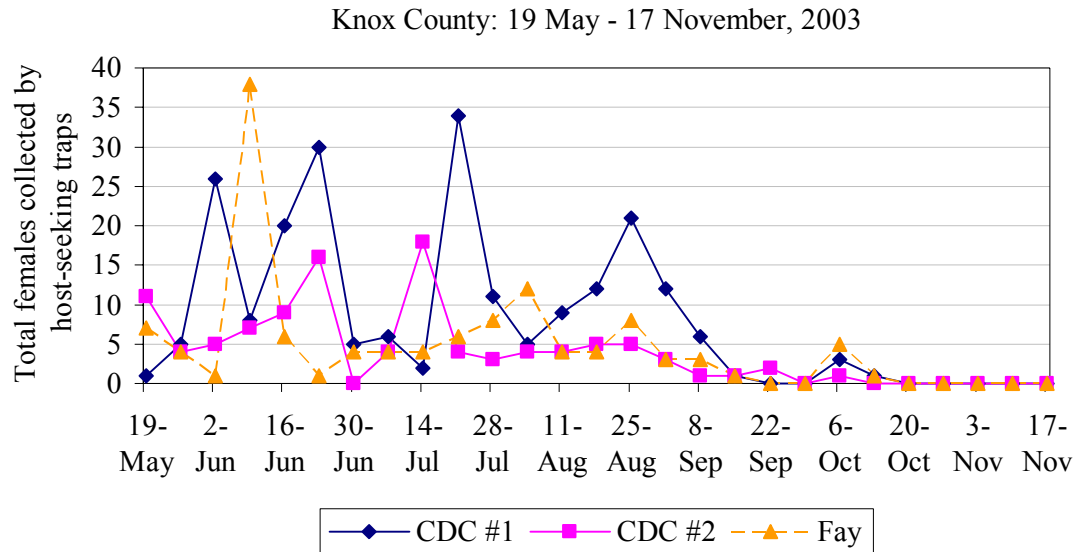


Figure 2.4. Total number of *Ochlerotatus triseriatus* females collected by 2 CO₂-baited CDC miniature light traps (labeled CDC #1 and CDC #2) and a single CO₂-baited Omni-directional Fay trap in Knox and Blount County, TN, from 19 May to 17 November, 2003. Each trap collected host-seeking females once a week, for a period of 24 hours beginning with the dates listed.

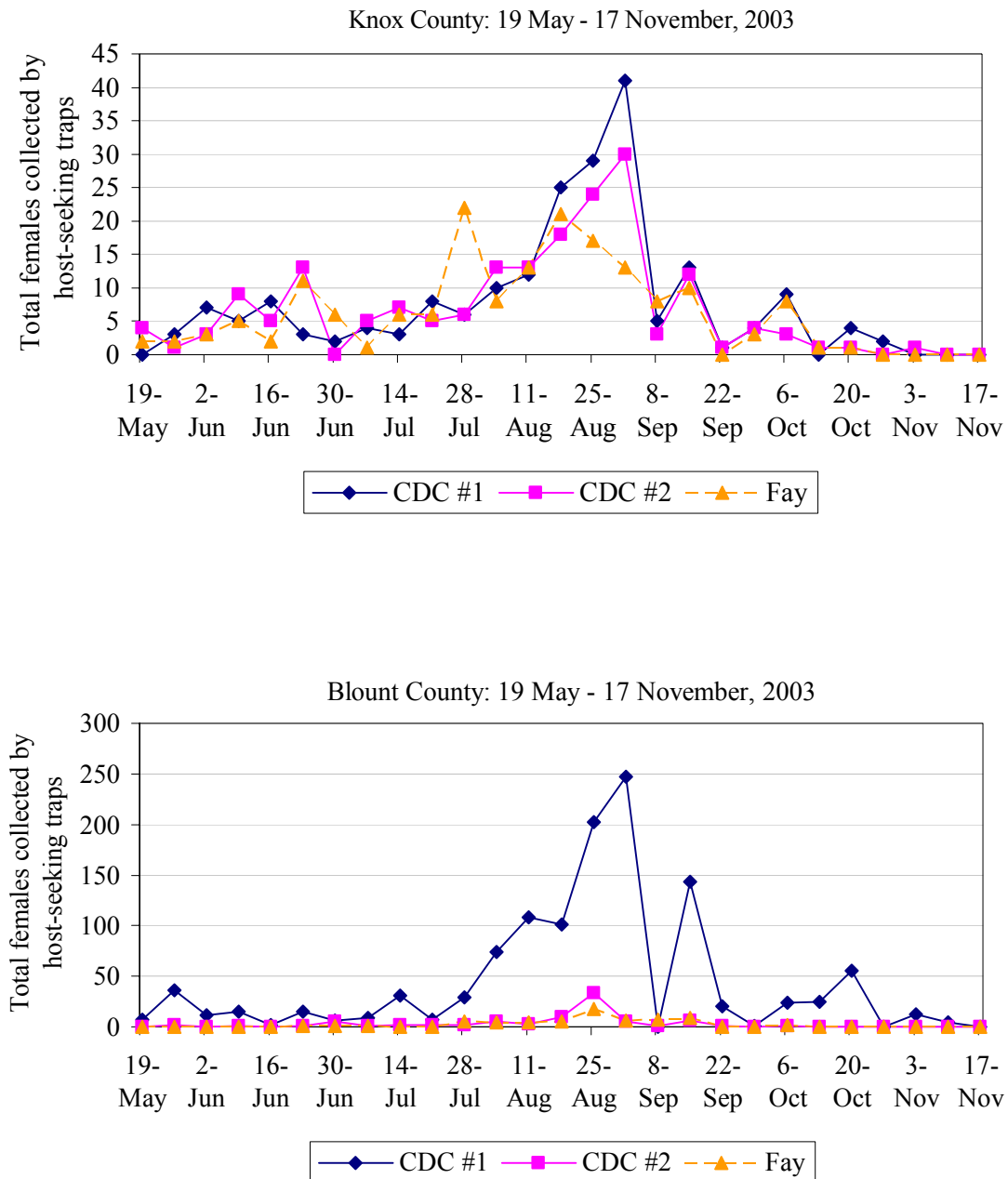


Figure 2.5. Total number of *Aedes albopictus* females collected from 2 CO₂-baited CDC miniature light traps (labeled CDC #1 and CDC #2) and a single CO₂-baited Omni-directional Fay trap from Knox and Blount County, TN, from 19 May to 17 November, 2003. Each trap collected host-seeking females once a week, for a period of 24 hours beginning with the dates listed.

Table 2.8. Seasonal distribution and abundance of female *Ochlerotatus triseriatus* and *Aedes albopictus* collected by date with a CO₂-baited Omni-directional Fay trap in Knox and Blount County, TN, between 19 May and 17 November, 2003. Traps collected host-seeking mosquitoes for a 24 hour period beginning with the dates listed.

		Date																												Total
		May		June					July				August				September					October				November				
County	Species	19	26	2	9	16	23	30	7	14	21	28	4	11	18	25	1	8	15	22	29	6	13	20	27	3	10	17		
Knox	<i>Oc. triseriatus</i>	7	4	1	38	6	1	4	4	4	6	8	12	4	4	8	3	3	1			5	1						124	
	<i>Ae. albopictus</i>	2	2	3	5	2	11	6	1	6	6	22	8	13	21	17	13	8	10	3		8	1	1					169	
Blount	<i>Oc. triseriatus</i>	1	18	10	9	1	3	2	1	2		1		1	1	12	3	2	1			3							71	
	<i>Ae. albopictus</i>						1	1	1			5	4	4	5	18	6	7	9			2							63	

majority were *Ae. albopictus* (n = 89) and *Oc. triseriatus* (n = 51). In the Blount County site, *Oc. triseriatus* (n = 31) were collected in greater quantity in comparison to *Ae. albopictus* (n = 22). The seasonal distribution of gravid *Oc. triseriatus* peaked in June and July while *Ae. albopictus* gravid collections peaked in August (Figure 2.6).

Parity: Host-seeking adults of *Oc. triseriatus* (n = 404) and *Ae. albopictus* (n = 456) were dissected to determine parity at the high and low prevalence areas for LAC encephalitis. Females that were determined to be gravid at the time of dissection were excluded from the parity study. In all months that had at least 40 females dissected per species and site, there were no significant differences in the monthly parity rates of *Oc. triseriatus* (Table 2.9) and *Ae. albopictus* (Table 2.10) (ANOVA, $P < 0.05$). Parity was normally distributed (Shapiro-Wilk = 0.939), and there was a strong Pearson correlation which depicted a very strong, positive, linear correlation for parity and date of both species ($r = 0.910$, $P < 0.001$). Thus, a significant portion of the variance in parity can be explained by its linear relationship with date ($r^2 = 0.799$, $P < 0.001$). The monthly percentage of parous *Oc. triseriatus* in Knox County ranged from 0.10 to 0.64 and Blount County proportions ranged from 0.05 to 0.79. The percentage of parous *Ae. albopictus* in Knox County by month varied from 0.08 to 0.77 and Blount County parity rates varied from 0.00 to 0.75. The total average monthly parity at each established site was over 40% parous for both container-inhabiting species over the entire year.

Blood-meal identification: Resting, blood engorged *Oc. triseriatus* and *Ae. albopictus* were collected with backpack aspirator and carbon dioxide traps with limited success. A total of 29 blood engorged females including *Ae. vexans* Meigen (n = 20), *Oc. triseriatus* (n = 7), and *Ae. albopictus* (n = 2) were collected. Approximately 52% of all

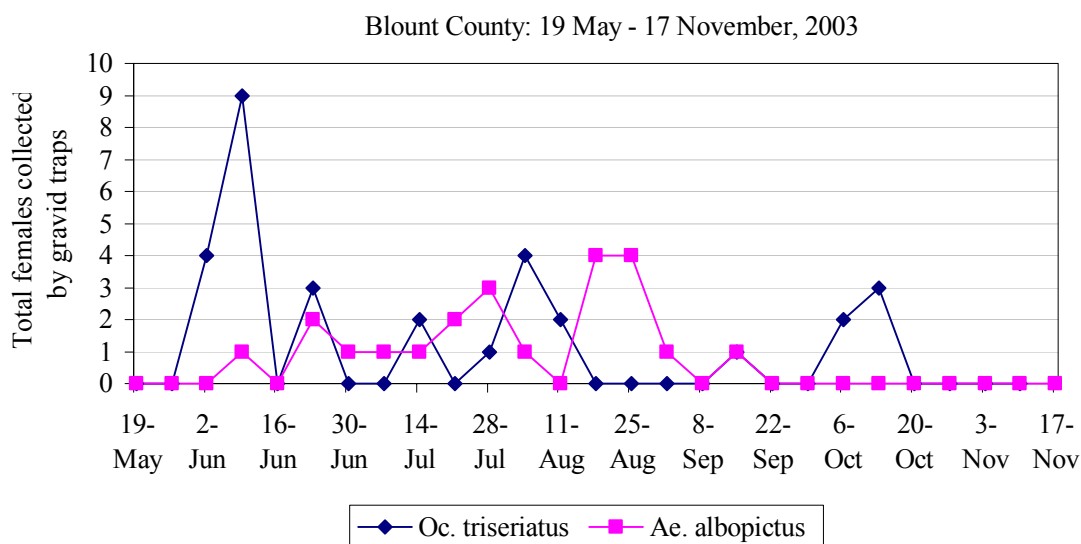
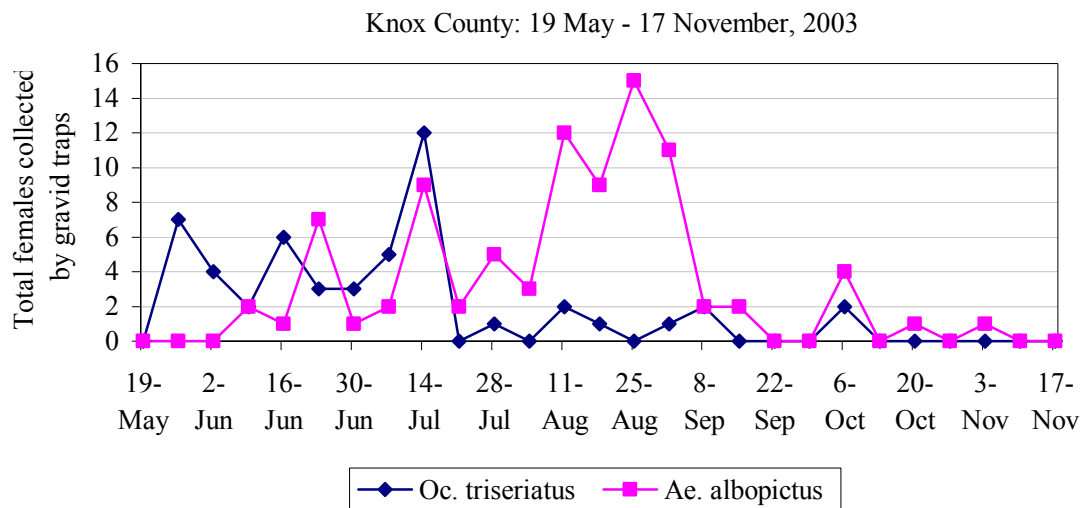


Figure 2.6. Seasonal distribution of all *Ochlerotatus triseriatus* and *Aedes albopictus* females collected from 2 gravid traps from Knox and Blount County, TN, between 19 May and 17 November, 2003. Each trap collected gravid females once a week, for a period of 24 hours beginning with the dates listed.

Table 2.9. Monthly proportion of parous *Ochlerotatus triseriatus* collected in Knox and Blount County, TN, between 19 May and 31 October, 2003.

Month	Knox County				Blount County			
	Total dissected	Parous	Nulliparous	Parity rate	Total dissected	Parous	Nulliparous	Parity rate
May	30	3	27	0.10	19	1	18	0.05
June	52	8	44	0.15	42	14	28	0.33
July	40	19	21	0.48	25	16	9	0.64
August	41	19	22	0.46	40	15	25	0.38
September	31	19	12	0.61	38	30	8	0.79
October	11	7	4	0.64	35	24	11	0.69
Total	205	75	130	0.41	199	100	99	0.48

Table 2.10. Monthly proportion of parous *Aedes albopictus* collected in Knox and Blount County, TN, between 19 May and 31 October, 2003.

Month	Knox County				Blount County			
	Total dissected	Parous	Nulliparous	Parity rate	Total dissected	Parous	Nulliparous	Parity rate
May	12	1	11	0.08	20	0	20	0.00
June	48	6	42	0.13	43	10	33	0.23
July	43	18	25	0.42	44	21	23	0.48
August	43	15	28	0.35	42	16	26	0.38
September	44	28	16	0.64	47	29	18	0.62
October	30	23	7	0.77	40	30	10	0.75
Total	220	91	129	0.40	236	106	130	0.41

blood engorged females (n=15) were collected from the Knox County site by aspiration. Sixteen blood-engorged mosquitoes (*Ae. vexans* = 13, *Oc. triseriatus* = 2, *Ae. albopictus* = 2) were successfully analyzed by PCR. All contained mammalian blood.

In the Blount County site, 26 potentially blood engorged females were collected including *Ae. vexans* (n = 9), *Ae. albopictus* (n = 9), *Oc. triseriatus* (n = 6), *Culex pipiens quinquefasciatus* Edwards (n = 1), and *Anopheles punctipennis* Say (n = 1). Only seven of the Blount County specimens were collected by the backpack aspirator. All other blood engorged females were collected by either CO²-baited CDC miniature light traps or CO²-baited Omni-directional Fay trap. A total of 11 of the 26 specimens were completely processed. Of these, *Ae. vexans* (n = 1) and *Ae. albopictus* (n = 4) tested positive for mammalian blood.

Sciurid population assessment: The number of potential amplification hosts (capture-recapture) collected at both sites is shown in **Tables 2.11, 2.12, and 2.13**. The null (Mo) model in the program “CAPTURE” was continuously selected as the most appropriate model to determine population sizes for the capture-recapture data at both Knox and Blount County sites. Sources of variation including time, behavior, and heterogeneity did not appear to affect capture probabilities; however, sample sizes for captures were low and may have affected population estimates (White et al. 1982). Due to limited numbers of collected sciurids in October, a population estimate was not plausible. Furthermore, since populations assessments are based on a closed population, animals that were tagged in May and recaptured in November were not considered recaptures.

Table 2.11. Capture-recapture data for grey squirrel (*Sciurus carolinensis*) collections made during three separate periods (May, October, and November) in 2003, at a site in Knox County, TN.

Collection Date	<i>Sciurus carolinensis</i>		
	Animals Caught	Newly Caught	Accumulated # Tagged
27-May	8	8	8
29-May	5	3	11
31-May	10	8	18
20-Oct	0	0	0
22-Oct	1	1	1
18-Nov	5	5	5
20-Nov	7	3	8
21-Nov	5	3	11

Table 2.12. Capture-recapture data for chipmunks (*Tamias striatus*) collections made during three separate periods (May, October, and November) in 2003, at a site in Knox County, TN.

Collection Date	<i>Tamias striatus</i>		
	Animals Caught	Newly Caught	Accumulated # Tagged
27-May	3	3	3
29-May	4	3	6
31-May	2	1	7
20-Oct	3	3	3
22-Oct	5	2	5
18-Nov	4	4	4
20-Nov	4	4	8
21-Nov	6	4	12

Table 2.13. Capture-recapture data for grey squirrel (*Sciurus carolinensis*) collections made during three separate periods (May, October, and November) in 2003, at a site in Blount County, TN.

Collection Date	<i>Sciurus carolinensis</i>		
	Animals Caught	Newly Caught	Accumulated # Tagged
28-May	11	11	11
30-May	16	14	25
21-Oct	3	3	3
17-Nov	13	13	13
19-Nov	18	8	21

In Knox County, 18 of the 23 *S. carolinensis* were collected once during the three trapping occasions in May (Table 2.10). The population was estimated to be 31 individuals (SE = 9.02). The approximate 95% confidence interval (CI) was 22 to 68 total individuals. In November, a total 14 *S. carolinensis* were collected, with two recaptures. The November squirrel population was estimated at 11 individuals (SE = 1.82) with a 95 % CI ranging from 11 to 20. Nine *T. striatus* were collected in the Knox County site during May with 4 recaptures. The population was estimated to be five individuals (SE = 0.96) with a range from 5 to 5 (95% CI). In contrast, November collections of *T. striatus* depicted the population to be at 28 individuals (SE = 14.67) with the 95% CI ranging from 16 to 86.

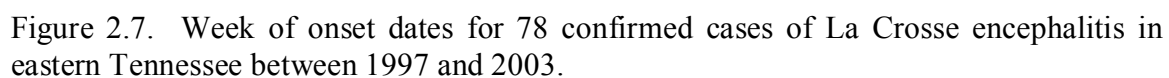
The Blount County site contained a larger number of *S. carolinensis* in comparison to the Knox County site. Of the 27 squirrels collected in May, only 2 were recaptures. The population was estimated to be 85 individuals (SE = 48.73). The approximate 95% CI was 40 to 267 total individuals. In November, during 2 trapping occasions, 21 of the 31 *S. carolinensis* were collected once. The population was estimated to be at 27 individuals (SE = 4.6) with a range from 23-43 individuals (95% CI). No *T. striatus* were collected during all trapping occasions in the Blount County site.

v - Discussion

Climatological patterns: The total accumulation of precipitation in 2003 was consistent with the annual averages between 1961 and 1990 that recorded sums of 134.6 cm and 119.4 cm in Knox and Blount County sites, respectively (U.S. Department of Agriculture 1999). However, rainfall accumulation in April and May of 2003

exceeded the recorded averages between 1971 and 2000 by 21.78 cm and 13.64 cm in Knox and Blount County, respectively. Furthermore, all months from July through September had monthly rainfall levels above average. Precipitation and temperature patterns varied only slightly between the two mixed hardwood forest sites.

Ovitraping and rearing success: The week of onset for confirmed cases of LAC encephalitis in eastern Tennessee, between 1997 and 2003, has ranged from 26 May to 13 October (**Figure 2.7**) (Jones et al. 1999, K. Gottfried pers. comm.). Approximately 62% of these reported cases have occurred in the months of July and August. Although collection sites were approximately 30 km apart, egg densities for each species fluctuated in a similar manner (**Figure 2.3**) and may suggest that all comparable habitats throughout eastern Tennessee have container-inhabiting egg production fluctuating in a similar fashion. When eggs found in ovitraps were utilized as an index of population density (Craig 1983), the data showed that *Oc. triseriatus* populations were highest from 26 May to 23 June and are likely associated with the early onset dates of LAC encephalitis cases in eastern Tennessee. Collections of *Oc. triseriatus* eggs declined throughout July with a smaller peak in August. In contrast, *Ae. albopictus* egg numbers gradually increased in density from May to July and sustained high levels until September. Egg densities of both *Oc. triseriatus* and *Ae. albopictus* were present during July and August and increased the opportunities for vertical and horizontal transmission throughout the mosquito season. However, the number of LAC encephalitis cases is generally highest in August (**Figure 2.7**); therefore, increased populations of *Ae. albopictus* from July to September may associate this container-inhabiting mosquito with the transmission of LAC encephalitis.



Eggs of both container-inhabiting mosquitoes were identified from the first week of collection and suggest that the onset of ovipositional activity began prior to 19 May, 2003 (Gottfried 2000, Chapman 2001). Variation in total ovitrap days between sites was attributed to missing oviposition strips particularly during the months of October and November and appeared to be a result of sciurid activity.

A comparison of egg densities between 1998 (Gottfried et al. 2002) and 2003 for *Oc. triseriatus* and *Ae. albopictus* was conducted from ovitrap collections located within the identical site in Knox County. When *Ae. albopictus* egg numbers were used as a fixed regression factor, there was no effect on the season long egg totals of *Oc. triseriatus* when taking into consideration daily mean temperature and precipitation ($t = 0.83$; $df = 23$; $P = 0.4139$). From this data, a stable and sympatric relationship appears to be occurring for both container-inhabiting species in the high prevalence area for LAC encephalitis (**Figure 2.8**).

There were no significant differences in the numbers of adults reared from eggs between the high and low prevalence areas for LAC encephalitis. Nearly all eggs from the week of 16 June failed to hatch or be reared to the adult stage and was likely due to contamination of rearing containers. Eggs that were successfully reared to the adult stage for *Oc. triseriatus* (17.08%) and *Ae. albopictus* (19.99%) will be tested for an assortment of viruses at a later date.

Adult mosquito collections: The seasonal distribution of adult *Oc. triseriatus* and *Ae. albopictus* fluctuated in a similar manner in comparison to egg collections. However, weekly adult collection densities were extremely variable between established sites as well as by the adult trapping technique utilized. Host-seeking collection ratios were

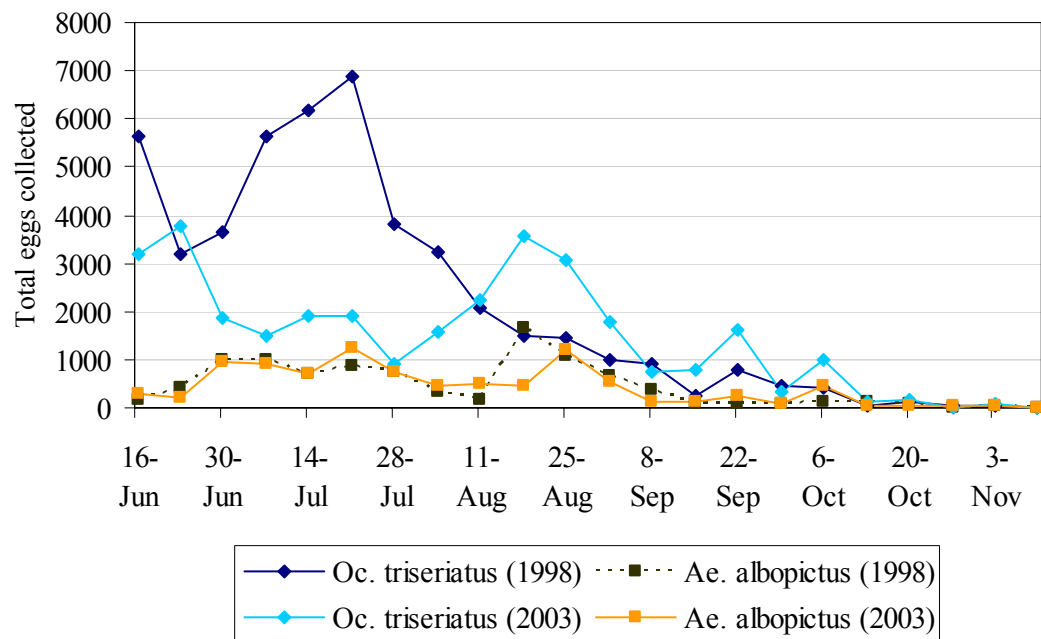


Figure 2.8. Seasonal distribution of *Ochlerotatus triseriatus* and *Aedes albopictus* eggs collected in ovitraps from the identical site in Knox County, TN during 1998 (Gottfried et al. 2002) and 2003.

similar at the high prevalence area for LAC encephalitis with *Ae. albopictus* being slightly more abundant in all traps utilized. Within the low prevalence area for LAC encephalitis, *Ae. albopictus* abundance was greater than that of *Oc. triseriatus*. Approximately 88% of all *Ae. albopictus* adults (n=1184) collected in Blount County were from a single CDC miniature light trap (CDC #1), located on the forest edge, directly behind human residences, and in the vicinity of a large number of artificial containers (tires, oil pans, abandoned car, washer and dryer, and other discarded containers). The adult ratio of collected females within CDC trap #1 was 7.9:1 in favor of *Ae. albopictus* to *Oc. triseriatus* (**Figures 2.4 and 2.5**) and drastically influenced the variation of adult collection ratios between sites.

However, a set of five ovitraps was located in the vicinity of the CDC #1 trap and had an average egg ratio of 3.7:1 in favor of *Oc. triseriatus* (n=13,409) to *Ae. albopictus* (n=3,614). From this data one can infer that adult *Ae. albopictus* are more readily attracted to CO²-baited CDC miniature light traps. However, based upon previous studies by Livdahl and Willey (1991) and Swanson et al. (2000) the adult collections at CDC #1 could imply that *Ae. albopictus* outcompetes *Oc. triseriatus* in artificial larval habitats within the low prevalence area for LAC encephalitis. Furthermore, larvae of *Toxorhynchites rutilus septentrionalis* were identified from artificial containers within 50 meters of the CDC #1 trap (Caldwell 2004, Chapter 3) and could imply that immature stages of *Ae. albopictus* are better adapted to avoid predation by *Tx. rutilus septentrionalis* in comparison to *Oc. triseriatus*.

Parity: Few container-inhabiting mosquitoes were collected in May and October thus preventing an accurate determination of parity for these particular months. Due to

the overall limited abundance of collected *Oc. triseriatus* adults, statistical comparisons in Knox County could only be made in the months of June, July, and August.

Furthermore, statistical comparisons of *Oc. triseriatus* in Blount County could only be made in June and August. However, data for all months were placed into **Tables 2.10 and 2.11** in order to provide general fluctuations patterns throughout the entire collection season. A decrease in the proportion of parous *Oc. triseriatus* and *Ae. albopictus* females during August was postulated to be affected by a large recruitment of emerging adults during periods of high rainfall in late July and early August. This may have increased the proportion of potentially infected females. Based upon the general increase in parity from May to October, opportunities for vertical and horizontal transmission by *Oc. triseriatus* and *Ae. albopictus* should have increased throughout the mosquito season.

Blood meal identification: The PCR test for mosquito blood-meal identification was in the early stages of development and only differentiated between mammalian and avian primers. However, since all blood from *Oc. triseriatus* and *Ae. albopictus* was identified as mammalian, the opportunities for horizontal transmission of the LAC virus from amplification host to vector(s) is increased. The low number of successfully analyzed blood-meals for all species and sites could be attributed to the low number of blood-engorged females collected or DNA degradation. Furthermore, the aspiration technique collected gravid females which may have contained older blood and likely reduced the efficiency and success of blood-meal identification.

Population assessment of amplification hosts:

Assessments of *S. carolinensis* populations varied significantly between sites with larger populations occurring in the Blount County site. However, estimates of population

size at each site were larger in May in comparison to November and may be attributed to breeding cycles and gestation periods. In Tennessee, *S. carolinensis* breeds from January to February and again in May to early July (Whitaker and Hamilton 1998). The increased number of captures in May could have resulted from increased ground activity and foraging by arboreal squirrels.

Chipmunks, *T. striatus*, were only collected at the high prevalence area for LAC encephalitis. This particular ground squirrel mates in late February to early April in eastern Tennessee, with a gestation of 31 to 32 days (Whitaker and Hamilton 1998), and could have impacted low collection numbers in May. The population estimate drastically increased in November and was likely closer to actual densities of *T. striatus*.

Based upon the mosquito research conducted in the high (Knox) and low (Blount) prevalence areas for LAC encephalitis, there does not appear to be any significant differences in *Oc. triseriatus* and *Ae. albopictus* egg production, adult collections, parity rates, or host feeding preferences. The fact that *T. striatus* was not detected in the low prevalence area for LAC encephalitis may impact the incidence of horizontal amplification of LAC to mosquito vector(s). However, with the large population of *S. carolinensis* in Blount County, there appears to be sufficient numbers of potential amplification hosts. Furthermore, the low number of LAC encephalitis cases in Blount County may be due to reduced levels of the circulating bunyavirus in the area. Future efforts dealing with the blood samples collected from all live-trapped sciurids may give insight into the prevalence of LAC antibodies and therefore the LAC virus at both sites in eastern Tennessee.

CHAPTER III
SEASONAL ABUNDANCE, OCCURENCE, AND DIVERSITY OF
MOSQUITOES AT TWO SITES IN EASTERN TENNESSEE

i - Abstract

Weekly collections of mosquito species were conducted between 19 May and 17 November, 2003, and 24 May and 5 July, 2004, at two forested sites (Knox and Blount County) in eastern Tennessee, USA. Seasonal abundance, occurrence, and diversity of mosquito species was evaluated through the utilization of two CO₂-baited Center for Disease Control (CDC) miniature light traps, a CO₂-baited Omni-directional Fay trap, two gravid traps, and larval collections from both sites. The overall abundance of adult mosquitoes collected and identified to species was similar between Knox (n = 2590) and Blount (n = 2667) counties. Nineteen species representing seven genera were identified during the two collection seasons with an accumulation of 4,961 host-seeking and 296 gravid females. Diversity of collected species was highest in June. The proven and potential La Crosse virus (LACV) vectors, *Ochlerotatus triseriatus* (Say) and *Aedes albopictus* (Skuse), constituted 59.3% and 73.8% of all adult species collected in the 2003 and 2004 season, respectively. *Aedes albopictus* individuals were prevalent in both collection years; however, density levels in 2004 were reduced for *Oc. triseriatus*. In general, peak adult densities occurred in June for *Oc. triseriatus* and August for *Ae. albopictus*. *Aedes vexans* (Meigen), a potential bridge vector of West Nile virus (WNV), accounted for approximately 28% of all host-seeking and gravid females collected. In 2004, *Ae. vexans* was extremely low at both sites and was likely effected by lower amounts of precipitation in the early portion of the mosquito season. Gravid traps did not

collect a large abundance or diversity of *Culex* species in either year. In Knox County, *Oc. japonicus* was collected by all trapping techniques utilized and appeared to have over-wintered successfully in the northeastern portion of Knox County.

ii - Introduction

Evaluating seasonal abundance, occurrence, and diversity of adult mosquitoes is critical to understanding the biology and epidemiology of current and potential mosquito vectors in eastern Tennessee. The introduction of the West Nile virus (WNV) led to increased reports of La Crosse (LAC) encephalitis cases (Jones et al. 1999, Gerhardt et al. 2001). The recent isolation of the Jamestown Canyon (JC) virus (Gottfried et al. 2002) in eastern Tennessee increases the necessity to determine the seasonal abundance and distribution of proven and potential mosquito vectors. However, climatological patterns influence the density of mosquito populations and their importance should not be underestimated. The effects of temperature and precipitation on populations could provide abatement and educational programs valuable information on where to place the emphasis of mosquito control efforts.

Culex species are suspected to be of primary importance to the natural transmission cycle of the WNV in the United States (Turell et al. 2001a, Turell et al. 2001b, Goddard et al. 2002, Blackmore et al. 2003). However, WNV has been isolated from 43 field-caught mosquito species (CDC 2004) including *Aedes vexans* Meigen (Bernard et al. 2001), *Ae. albopictus* Skuse, *Ochlerotatus canadensis* Theobald, *Oc. triseriatus* Say, *Oc. trivittatus* Coquillett, and *Psorophora ferox* Von Humboldt and could potentially play a role as bridge vectors.

Container-inhabiting species including *Oc. triseriatus* and *Ae. albopictus* are competent vectors of multiple California serogroup arboviruses. Each species has sufficiently proven their transmission efficiency of the LAC virus in the laboratory setting (Grimstad et al. 1989, Culley et al. 1992); furthermore, the LAC virus has been isolated from field-caught individuals of *Oc. triseriatus* (Watts et al. 1973, Pantuwatana et al. 1974, Watts et al. 1974) and *Ae. albopictus* (Gerhardt et al. 2001). Although LAC has been diagnosed infrequently in southern states, human cases of LAC encephalitis have been documented in both Tennessee and North Carolina since 1964 (Kappus et al. 1982, Jones et al. 1999). In Tennessee, prior to the drastic increase of confirmed LAC cases in 1997, the sum of documented cases reported to the Tennessee Department of Health totaled 9 (Jones et al. 1999). During subsequent years in Tennessee, 10 cases were reported in 1997, 9 in 1998, 6 in 1999, 16 in 2000, 9 in 2001, 15 in 2002, and 19 in 2003 (Jones et al. 1999, Erwin et al. 2002, K. Gottfried pers. comm.).

Ochlerotatus japonicus japonicus Theobald, an exotic container-inhabiting mosquito species, was first collected from New York and New Jersey in the late summer of 1998 (Peyton et al. 1999). Subsequently, *Oc. japonicus* was reported in Connecticut in July of 1998 (Fonseca et al. 2001). This newly introduced invasive species is spreading geographically, is a competent vector of the West Nile virus (White et al. 2001, Turell et al. 2001b, Kutz et al. 2003), and has been shown in a laboratory setting to transmit the viruses associated with LAC encephalitis (Sardelis et al. 2002a), eastern equine encephalitis (Sardelis et al. 2002b), and St. Louis encephalitis (Sardelis et al. 2003). Its potential as a bridge vector is enhanced by the fact that females are catholic feeders and

readily feed on avian and mammalian hosts in the laboratory (Sardelis et al. 2002a, Falco et al. 2002), as well as humans in the field (Peyton et al. 1999).

The overall objective of this research was to compare the abundance of selected suspected vector species at two sites in eastern Tennessee throughout the 2003 and early portion of the 2004 mosquito season while examining differences in seasonal patterns between years.

iii - Materials and Methods

Collection locations: A study area was established each in Knox and Blount counties in order to assess the diversity, abundance and seasonal distribution of mosquito species in eastern Tennessee. Both collection sites were located within mixed hardwood forests approximately 30 km apart and were in close vicinity to human dwellings. The location, topography, and vegetation for each county are described elsewhere (Caldwell 2004, Chapter 2).

Climatological Patterns: Weather data (precipitation, maximum and minimum temperature) for Knox County were gathered from the Environmental Science Division of the Oak Ridge National Laboratory (ORNL) located in Oak Ridge, TN (Anderson County). The ORNL's weather gauges were approximately 8.0 km northwest from the Knox County collection site. Daily precipitation and maximum and minimum temperatures for Blount County were supplied from a weather monitoring station located at the McGee Tyson airport, located approximately 7.0 km northwest of the Blount County collection site, and was operated by the U.S. Department of Commerce, National Weather Service (NWS).

Mosquito collections: Mosquitoes were collected from the 2 sites between May 19 and November 17, 2003 and 24 May and 5 July, 2004. Weekly host-seeking mosquito collections were obtained from each site by suspending two dry ice-baited Centers for Disease Control (CDC) miniature light traps (Stryker and Young 1970, Takken and Kline 1989, Reisen et al. 1999) and a dry ice-baited Omni-directional Prince-Fay trap (Jensen et al. 1994). These host-seeking traps were deployed for approximately 24-hour periods from 0800-0930 EDT at the Blount County site and 1030-1130 EDT at the Knox County site. The collection cup was removed from each and placed into a cooler with dry ice for transportation back to the laboratory where mosquitoes were identified (Darsie and Ward 1981, Darsie 2002) and sexed.

Two gravid traps, model number 1712 (John W. Hock Company, Gainesville, FL), were placed at each site to collect gravid female mosquitoes (Reiter 1983). A concoction of tap water, fresh cow manure, and oak leaves were utilized as the ovipositional attractant with an attempted resemblance to hay infused water (Gjullin et al. 1965). During days of high precipitation, gravid trap collection nets were covered with a plastic bag to prevent damage to scales and other essential identification markers of the mosquitoes. Gravid females were transported, identified, and stored with all other adult mosquitoes.

Larval collections: Larval sampling was conducted in 2003 from stagnant water found in tree holes, vugs, or man made containers (Belkin 1954, Waters and Slaff 1987). A siphon or small pipette was utilized as the sampling tool and subsequently collected larvae and pupae were transported back to the laboratory in sealed plastic bags for rearing purposes. Immature stages from field collected samples were placed into Mosquito

Breeders (BioQuip, Gardena, CA) and given sufficient amounts of bovine liver powder (ICN Biomedicals Inc., Aurora, OH) or live mosquito larvae, in the case of *Toxorhynchites* species, to successfully develop to the adult stage. Emerged adults were identified to species.

iv - Results

Climatological patterns:

2003: Over the 196 day collection period from 19 May through 24 November 2003, mean daily temperatures ranged from a low of 3.1°C and 3.9°C (both on 14 November) to a high of 25.3°C (15 August) and 28.3°C (27 August) in Knox and Blount County sites, respectively (Fig. 3.1). The daily mean temperature at the Knox County site (18.6°C) was on average of 2°C lower than the Blount County site (20.9°C). An average weekly precipitation accumulation of 2.58 cm at the Knox County site and 2.55 cm at the Blount County site was recorded by the ORNL and NWS, respectively (Fig. 3.1). Accumulations for the 27 weeks were 68.93 cm in Knox County and 69.73 cm in Blount County. Precipitation was unseasonably high at both sites during the 2003 mosquito season in comparison to the average accumulation of rainfall between 1971 and 2000 (62.61 cm and 53.99 cm in Knox and Blount County, respectively).

2004: Between 24 May and 5 July 2004, temperatures were similar in range and overall pattern between Knox and Blount County sites (Fig. 3.1). Average daily temperatures ranged from 19.44°C to 27.22°C in each site. Precipitation was extremely low in the spring of the 2004 collecting season with a drastic increase in rainfall occurring during the later portion of June (Fig 3.1). Weekly accumulations of rainfall for Knox and

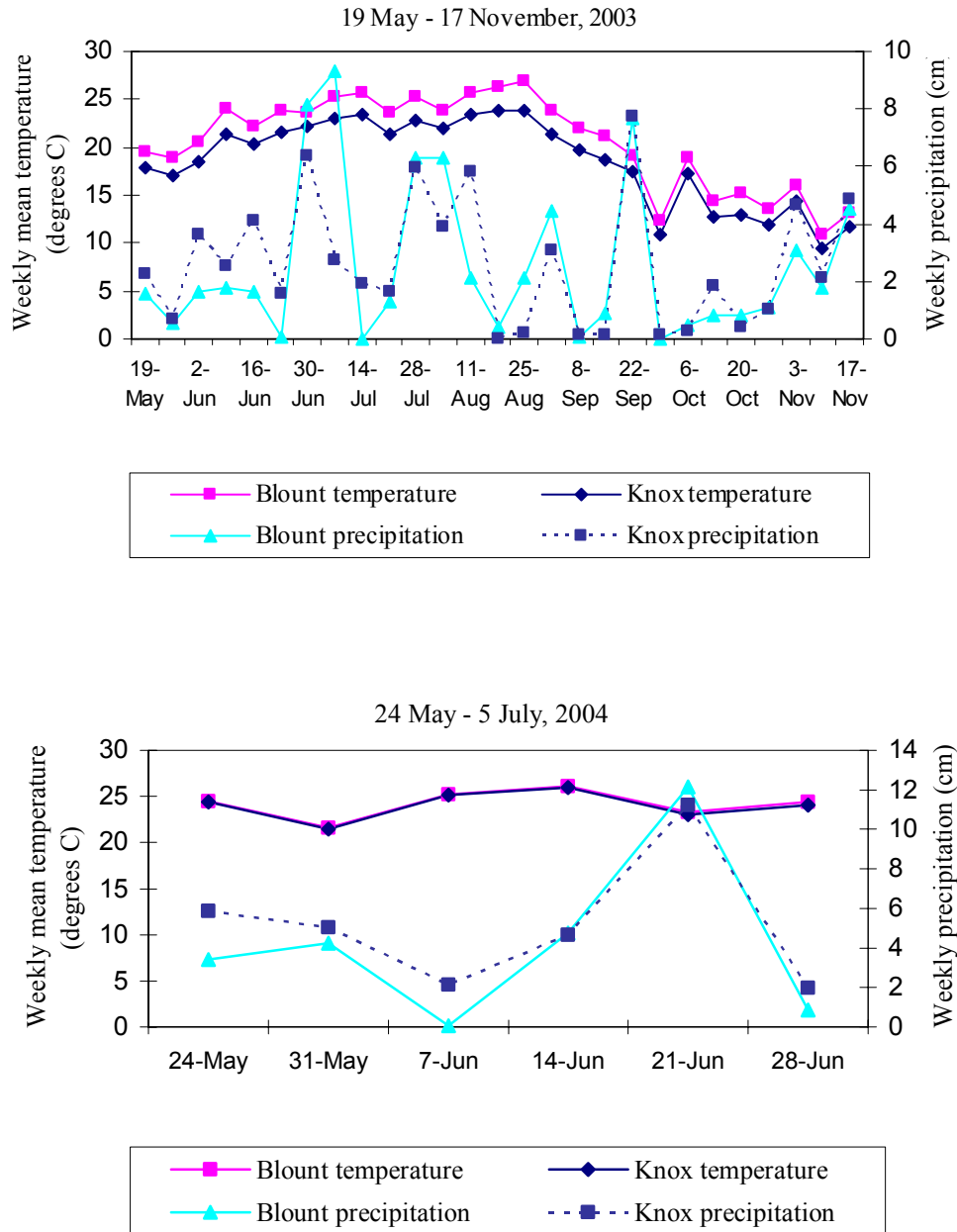


Figure 3.1. Temperature ($^{\circ}\text{C}$) and precipitation (cm) provided by the National Weather Service and the Oak Ridge National Laboratory from 19 May to 24 November, 2003, and 24 May to 5 July, 2004, for the sites located in Blount and Knox County, TN, respectively.

Blount counties averaged 5.16 cm and 4.24 cm, respectively; the total accumulation for the 7 week study was 30.94 cm and 25.45 cm for Knox and Blount counties.

Mosquito collections: During the 2003 collection period, 19 species representing 7 genera were collected at two sites with 12 of the species collected at both the Knox and Blount County sites. A total of 4,811 mosquitoes were collected from adult host-seeking traps of which 92.3% were females and could be identified to species. Another 296 adult mosquitoes were collected from gravid traps of which 271 were identified to species and determined to be females.

In 2004, a total of 12 species in six genera was collected from the Knox and Blount County sites. Half of the mosquito species collected were identified from both established sites. Of the 538 mosquitoes collected from host-seeking traps, 87.7% were females and identifiable to species. Approximately 93% of the total number of females collected by gravid trap (n = 27) were identified to species.

Knox County (2003):

Host-seeking collections: Eleven species in 5 genera were collected in 2 CO₂-baited CDC miniature light traps with an accumulation of 1,828 female mosquitoes identified. *Aedes vexans* (n = 824) was the most abundant species and comprised 47.1% of all mosquitoes collected by CO₂-baited CDC miniature light traps (Table 3.1). Over half of the *Ae. vexans* females (n = 415) were collected on the night of 2 - 3 June. The three other species that were collected in high numbers were *Ae. albopictus*, *Oc. triseriatus*, and *Anopheles punctipennis* Say. Both *Oc. triseriatus* and *An. punctipennis* collections were highest throughout the month of June; in contrast, the numbers of *Ae. albopictus* steadily increased during the season and was the most common species during

Table 3.1. Combined seasonal distribution and abundance of female mosquito species collected from 2 CO₂-baited CDC miniature light traps in Knox County, TN, from 19 May to 17 November, 2003. Traps collected host-seeking mosquitoes for a 24 hour period.

Species	Date																								Total			
	May		June				July				August				September				October				November					
	19	26	2	9	16	23	30	7	14	21	28	4	11	18	25	1	8	15	22	29	6	13	20	27		3	10	17
<i>Ae. vexans</i>	151	43	415	45	41	10	4	5	4	14	14	6	16	3	17	16	5	5	3		1	4	2					824
<i>Ae. albopictus</i>	4	4	10	14	13	16	2	9	10	13	12	23	25	43	53	71	8	25	2	8	12	1	5	2	1			386
<i>Oc. triseriatus</i>	12	9	31	15	29	46	5	10	20	38	14	9	13	17	26	15	7	2	2		4	1						325
<i>An. punctipennis</i>	2	27	80	35	4	12	1	5	12	2	3		8	1	13	6	4	1										216
<i>Cx. restuans</i>	6	12	6	2	1						3	2																32
<i>Oc. trivittatus</i>		2	5	1	1	5	1		1					1														17
<i>Cx. pipiens</i>			1						3				2		1	2			1									10
<i>Oc. japonicus</i>					1				1					2	3	1	2											10
<i>Ps. ferox</i>					2	1							1															4
<i>Cx. tarsalis</i>		1	2																									3
<i>Ps. cyanescens</i>																					1							1
Total	175	98	550	115	90	89	13	29	51	70	45	38	65	67	113	111	26	33	8	8	18	6	7	2	1	0	0	1828

the month of August (Table 3.1). *Aedes albopictus* females were collected in all weeks between 19 May and 3 November, 2003 and peaked on 1 September.

In general, more individual species were caught in CO₂-baited CDC miniature light traps than the CO₂-baited Omni-directional Fay trap. The single CO₂-baited Omni-directional Fay trap collected 417 female of which 9 species in 5 genera were identified (Table 3.2). *Aedes albopictus* accounted for 40.5% of all individuals identified and was the most abundantly collected species between the months of July and October. Seasonal patterns for *Ae. albopictus* fluctuated similarly between the two types of host-seeking traps (Figure 3.2).

Gravid collections: In 2003, gravid collections had the largest diversity of mosquito genera (n = 6) sampled amongst all trap types. Of the 168 gravid female mosquitoes collected by gravid traps the majority were *Ae. albopictus* (n = 89) followed by *Oc. triseriatus* (n = 51), *Culex restuans* Theobald (n = 7), *Ae. vexans* (n = 6), *Oc. japonicus* (n = 5), *An. punctipennis* (n = 4), *Orthopodomyia signifera* Coquillett (n = 3), *Psorophora ferox* (n = 1), *Oc. canadensis* (n = 1) and *Cx. tarsalis* Coquillett (n = 1). Peak gravid collections for *Oc. triseriatus* occurred in June and July while *Ae. albopictus* collections peaked in August (Fig. 3.3 and 3.4). The majority of all other gravid female species were collected in June and July with the exception of a single gravid *Oc. japonicus* collected on 11 August and 3 November, 2003.

Larval collections: Larvae of *Oc. triseriatus* (n=98) and *Ae. albopictus* (n=28) were collected from five natural and eight artificial containers and reared successfully to the adult stage during the 2003 collection season. Two larvae of *Oc. japonicus* were positively identified (Peyton et al. 1999) on 22 July; attempts to rear this species to the

Table 3.2. Seasonal distribution and abundance of female mosquito species collected from a single CO₂-baited Omni-directional Fay trap in Knox County, TN, from 19 May to 17 November, 2003. The trap collected host-seeking mosquitoes for a 24 hour period.

Species	Date																												Total
	May		June				July				August				September				October				November						
	19	26	2	9	16	23	30	7	14	21	28	4	11	18	25	1	8	15	22	29	6	13	20	27	3	10	17		
<i>Ae. albopictus</i>	2	2	3	5	2	11	6	1	6	6	22	8	13	21	17	13	8	10		3	8	1	1					169	
<i>Oc. triseriatus</i>	7	4	1	38	6	1	4	4	4	6	8	12	4	4	8	3	3	1			5	1						124	
<i>Ae. vexans</i>	16	8	8	14	5	2	2	2	6	4	13			3		1		1		2		3	8					98	
<i>An. punctipennis</i>		1	1	3					2		1		1		1	1												11	
<i>Cx. restuans</i>	1			1	3						1																	6	
<i>Oc. trivittatus</i>				1	4																							5	
<i>Ps. ferox</i>				2																								2	
<i>An. barberi</i>																		1										1	
<i>Oc. japonicus</i>						1																						1	
Total	26	15	14	67	16	15	12	7	18	16	45	20	18	28	26	18	11	13	0	5	13	5	9	0	0	0	0	417	

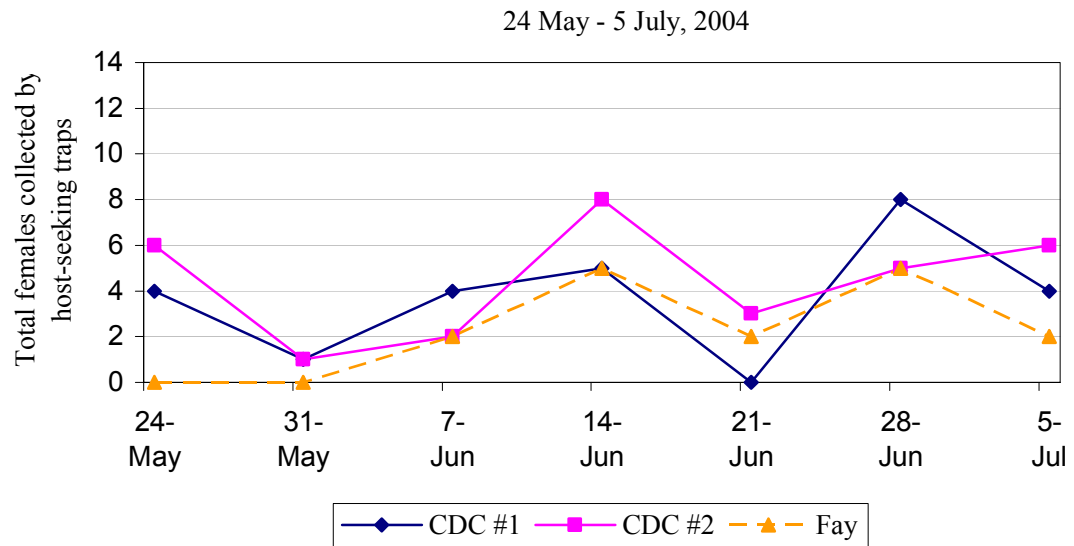
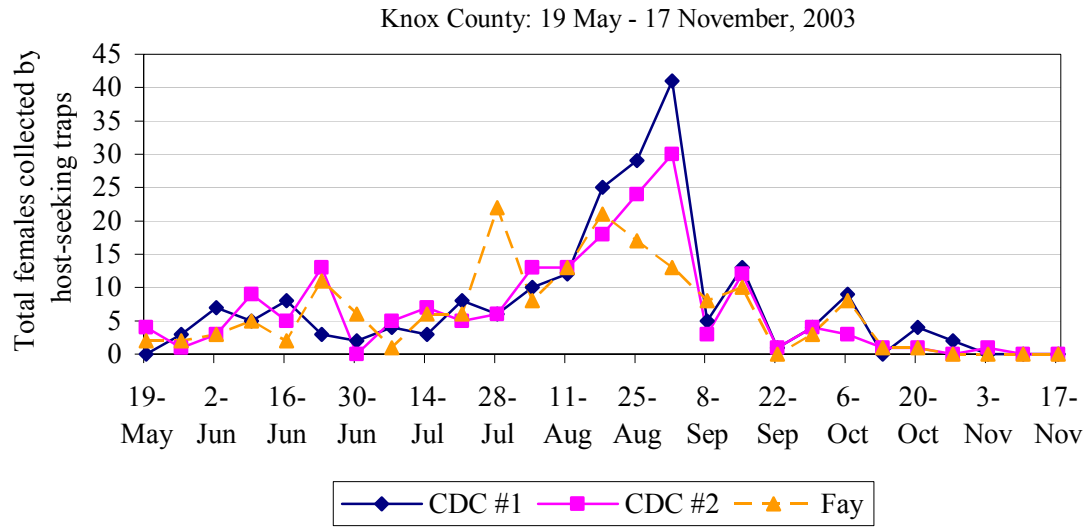


Figure 3.2. Seasonal patterns of abundance for *Aedes albopictus* females collected by 2 CO₂-baited CDC miniature light traps (labeled CDC #1 and CDC #2) and a single CO₂-baited Omni-directional Fay trap in Knox County, TN, from 19 May to 17 November, 2003, and 24 May to 5 July, 2004.

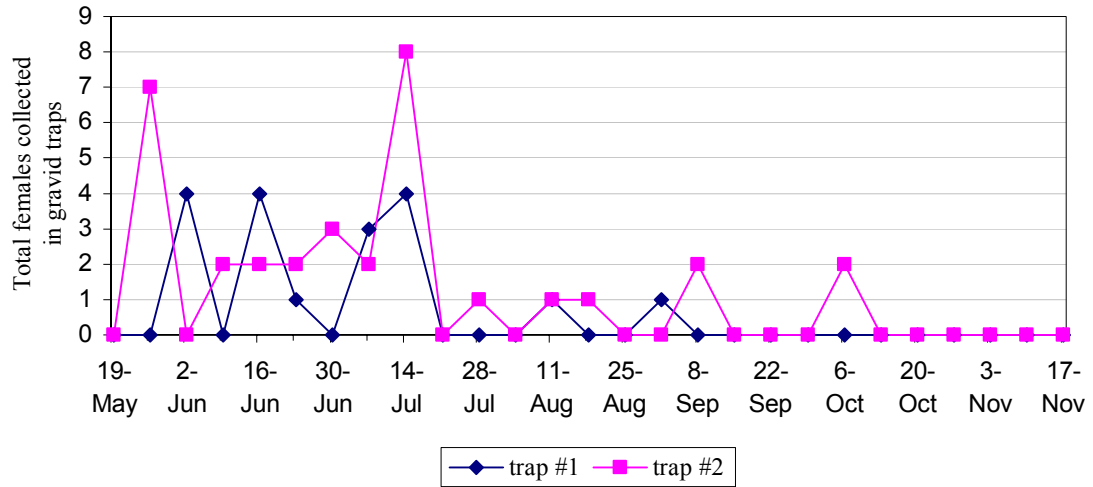


Figure 3.3. Total number of *Ochlerotatus triseriatus* females collected in 2 gravid traps (labeled trap #1 and trap #2) from Knox County, TN, from 19 May to 17 November, 2003. Each trap collected gravid females once a week, for a period of 24 hours.

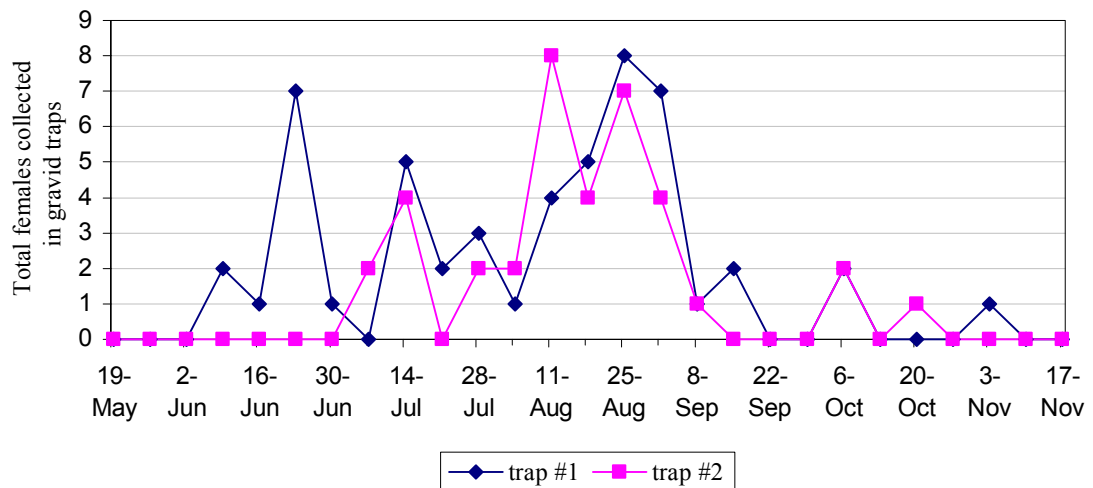


Figure 3.4. Total number of *Aedes albopictus* females collected in 2 gravid traps (labeled trap #1 and trap #2) from Knox County, TN, from 19 May to 17 November, 2003. Each trap collected gravid females once a week, for a period of 24 hours.

adult stage was not successful.

Knox County (2004):

Host-seeking collections: Only 9 species representing 5 genera were identified from 2 CO₂-baited CDC miniature light traps in the shorter 2004 season (**Table 3.3**). These traps collected the most specimens (n = 124) with the highest diversity of female mosquito species in the Knox County site. *Aedes albopictus* (n = 57) was the most frequently collected species and accounted for approximately 46% of all mosquitoes collected.

The Omni-directional Fay trap, with dry ice as the only attractant, had the lowest diversity of mosquito species (n = 3) and genera (n=2) (**Table 3.4**). Over 50% of all mosquitoes identified were *Ae. albopictus* (n = 16). The quantity of *Ae. albopictus* in the CO₂-baited Omni-directional Fay trap collections was consistently equal to or less dense than each CO₂-baited CDC miniature light trap (**Figure 3.2**); weekly collections of *Ae. albopictus* in 2004 were consistent with early season 2003 levels. All other species were collected in fewer numbers in 2004.

Gravid collections: Five species representing 3 genera were collected with the 2 gravid traps. Overall abundance of mosquitoes collected by gravid traps was extremely low. Of the 17 females, the majority were *Ae. albopictus* (n = 11) followed by *Oc. triseriatus* (n = 2), *Oc. japonicus* (n = 2), *Ae. vexans* (n = 1), and *An. punctipennis* (n = 1). The collection dates of gravid *Ae. albopictus* ranged from 24 May to 5 July. All other species were collected on either 31 May or 7 June, 2004.

Table 3.3. Female mosquito species collected from 2 CO₂-baited CDC miniature light traps in Knox County, TN, from 24 May to 5 July, 2004. Traps collected host-seeking mosquitoes for a 24 hour period.

Species	Date							
	May		June				July	Total
	24	31	7	14	21	28	5	
<i>Ae. albopictus</i>	10	2	6	13	3	13	10	57
<i>Oc. triseriatus</i>	9	2	3	5		10	5	34
<i>Ae. vexans</i>		3	12		2	2	3	22
<i>Oc. japonicus</i>	2	2	3					7
<i>An. punctipennis</i>	2	1	1					4
<i>Oc. trivittatus</i>						2		2
<i>Cx. erraticus</i>					1			1
<i>Cx. tarsalis</i>	1							1
<i>Ps. ferox</i>							1	1
Total	24	10	25	18	6	27	19	129

Table 3.4. Female mosquito species collected from a CO₂-baited Omni-directional Fay traps in Knox County, TN, from 24 May to 5 July, 2004. The trap collected host-seeking mosquitoes for a 24 hour period.

Species	Date							
	May		June				July	Total
	24	31	7	14	21	28	5	
<i>Ae. albopictus</i>			2	5	2	5	2	16
<i>Oc. triseriatus</i>		3	4	1		3		11
<i>Ae. vexans</i>						3	1	4
Total	0	3	6	6	2	11	3	31

Blount County (2003):

Host-seeking collections: During the 27 week collection period, 15 species in six genera were collected in two CO₂-baited CDC miniature light traps with an accumulation of 1,915 female mosquitoes identified. The CO₂-baited CDC miniature light trap had the broadest diversity of mosquito species for any trap type in Blount County. *Aedes albopictus* (n = 1265) was the most abundant species comprising 66.1% of all mosquitoes collected (Table 3.5). During the entire 2003 mosquito season, approximately 94% of *Ae. albopictus* females (n = 1184) were trapped in one of the two CO₂-baited CDC miniature light traps (labeled CDC #1) (Figure 3.5). *Aedes albopictus* females were most abundant during the month of August with a peak in collected individuals on 1 September. Three other species were collected in high frequency and includes *Ae. vexans*, *Oc. triseriatus*, and *An. punctipennis*. The seasonal distribution of *An. punctipennis* peaked in early collections while *Ae. vexans* populations fluctuated throughout the 2003 season; collections of *Oc. triseriatus* were bi-modal and peaked during late May and August (Table 3.5).

The Omni-directional Fay trap baited with dry-ice caught 282 females over the course of the 2003 collection period (Table 3.6). Of the 12 species and 6 genera represented, *Ae. vexans* (n = 86) was collected in the highest numbers. Captures of *Ae. vexans*, *Oc. triseriatus* and *An. punctipennis* peaked on 26 May. *Aedes albopictus* females were the most abundantly collected species during the month of August with the peak collections occurring on 28 July.

Gravid collections: A total of 103 gravid female mosquitoes were collected in 2003. The most abundant species was *Oc. triseriatus* (n = 31) followed by *Cx. restuans*

Table 3.5. Combined seasonal distribution and abundance of female mosquito species collected from 2 CO₂-baited CDC miniature light traps in Blount County, TN, from 19 May to 17 November, 2003. Traps collected host-seeking mosquitoes for a 24 hour period.

	May		June				July				August				September					October				November				Total
	19	26	2	9	16	23	30	7	14	21	28	4	11	18	25	1	8	15	22	29	6	13	20	27	3	10	17	
Species																												
<i>Ae. albopictus</i>	7	38	11	16	2	16	11	10	33	9	31	79	111	111	235	252	1	149	21	1	25	25	55		12	4		1265
<i>Ae. vexans</i>	23	9	13	18	4	1	8	4	5	25	7	6	25	2	2	15	2	2	7	2		9	35	1	3			228
<i>Oc. triseriatus</i>	1	26		20	2	6	7	4	5	9	5	4	8	15	17	21	3	5	3		17	8	12			1		199
<i>An. punctipennis</i>	6	46	8	7	6	11	3	6	1	2	1	1	1	7	0	4	1	1	1	1	1		1					116
<i>Cx. restuans</i>		27	8	2	3	2	1	1		5	1																	50
<i>Oc. trivittatus</i>	1	23	8	2					4	1				3	1													43
<i>Oc. canadensis</i>	4	10	8	2	1																							25
<i>Ps. ferox</i>									1	2					1													4
<i>Cx. pipiens</i>									1						1						1							3
<i>Ae. dupreei</i>												1																1
<i>An. quadrimaculatus</i>																1												1
<i>Cx. erraticus</i>																1												1
<i>Or. siginifera</i>	1																											1
<i>Ps. columbiae</i>																		1										1
<i>Ps. cyanescens</i>																							1					1
Total	43	179	56	67	18	36	30	25	50	53	45	91	148	136	256	294	7	158	32	4	44	42	103	1	15	5	0	1938

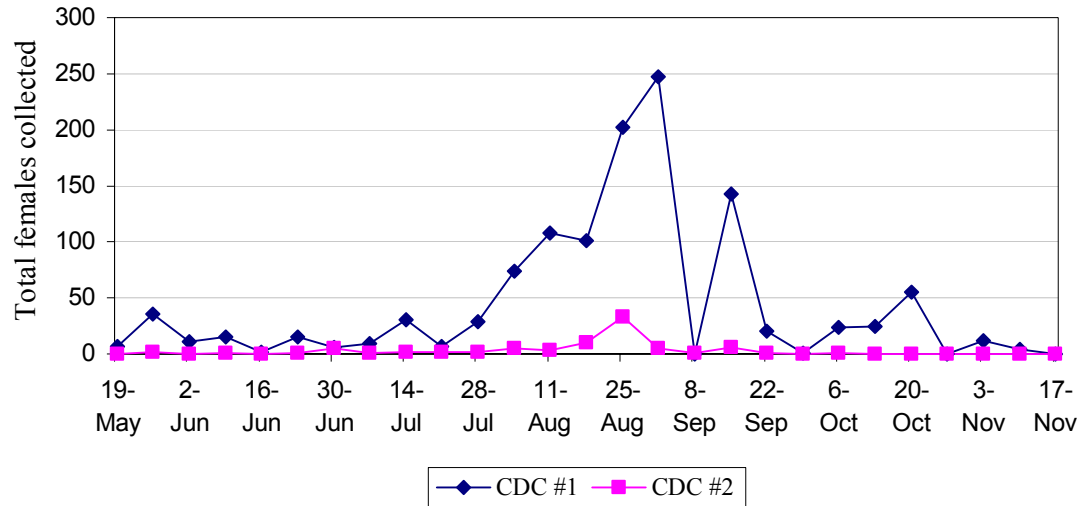


Figure 3.5. Total number of *Aedes albopictus* females collected from 2 CO₂-baited CDC miniature light traps (labeled CDC #1 and CDC #2) from Blount County, TN, from 19 May to 17 November, 2003. Each trap collected gravid females once a week, for a period of 24 hours.

Table 3.6. Seasonal distribution and abundance of female mosquito species collected from a single CO₂-baited Omni-directional Fay trap in Blount County, TN, from 19 May to 17 November, 2003. The trap collected host-seeking mosquitoes for a 24 hour period.

Species	Date																								Total			
	May		June				July				August				September					October				November				
	19	26	2	9	16	23	30	7	14	21	28	4	11	18	25	1	8	15	22	29	6	13	20	27		3	10	17
<i>Ae. vexans</i>	17	32			5	2	3		4			7	1	5	2		2	3		1		1				1		86
<i>Oc. triseriatus</i>	1	18	10	9	1	3	2	1	2			1		1	1	12	3	2	1		3							71
<i>Ae. albopictus</i>						1	1	1				5	4	4	5	18	6	7	9		2							63
<i>An. punctipennis</i>	1	16			3		2							1					1					1	1			26
<i>Oc. trivittatus</i>	6	10			2		2					1		1														22
<i>Cx. restuans</i>	1	1			1	1	1				1																	6
<i>Oc. canadensis</i>		2			1																							3
<i>An. quadrimaculatus</i>		1																										1
<i>Cx. erraticus</i>															1													1
<i>Cx. pipiens</i>																		1										1
<i>Ps. ferox</i>																			1									1
<i>Ur. sapphirina</i>																		1										1
Total	26	80	10	21	4	12	3	6	2	0	14	6	11	9	31	11	14	12	1	0	6	0	0	1	2	0	0	282

(n = 29), *Ae. albopictus* (n = 22) *Ae. vexans* (n = 18), *Cx. pipiens quinquefasciatus* (n = 2) and *An. punctipennis* (n = 1). All gravid *Cx. restuans* were trapped in June with peak collections in the early portion of the month. All but one *Ae. vexans* were collected on 30 June, 2003. Collections of *Oc. triseriatus* and *Ae. albopictus* (Figures 3.6 and 3.7) fluctuated in a similar manner relative to adult host-seeking traps as well as to the gravid traps located in the Knox County site (Figures 3.3 and 3.4).

Larval collections: Larvae of *Oc. triseriatus* (n = 66), *Ae. albopictus* (n = 35), *Toxorhynchites rutilus septentrionalis* Coquillett (n = 6) and *Or. signifera* (n = 5) were reared successfully to the adult stage from collections made from three natural and 13 artificial containers.

Blount County (2004):

Host-seeking collections: The two CO₂-baited CDC miniature light traps had the largest quantity of mosquitoes (n = 266) with a total of six species representing four genera (Table 3.7). *Aedes albopictus* (n = 138) was the most abundant species and accounted for approximately 52% of all mosquitoes collected. Over 86% of all *Ae. albopictus* (n = 119) collected from CO₂-baited CDC miniature light traps during the shortened season were from one trap (labeled CDC #1). The two container-inhabiting species (*Ae. albopictus* and *Oc. triseriatus*) were collected on all trapping dates; in contrast, all *Oc. trivittatus* females (n = 27) were collected on 5 July, 2004.

The single CO₂-baited Omni-directional Fay trap captured the same number of species (n = 6) as the light traps baited with dry ice; however, it collected more genera (n = 5) (Table 3.8). Of the 70 females collected, *Ochlerotatus triseriatus* (n = 23) was the most abundant species with a peak in collection density on 14 June. Peak collections for

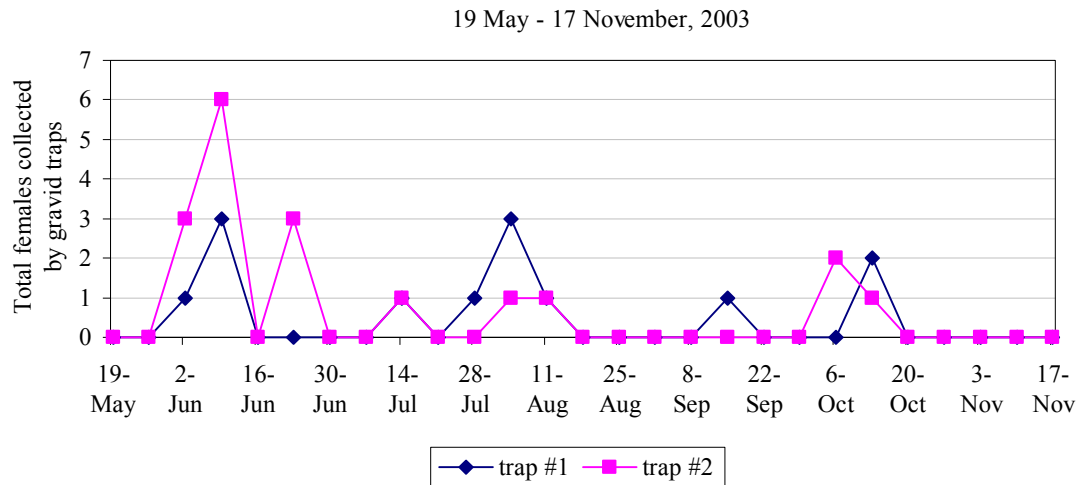


Figure 3.6. Total number of *Ochlerotatus triseriatus* females collected in 2 gravid traps (labeled trap #1 and trap #2) from Blount County, TN, from 19 May to 17 November, 2003. Each trap collected gravid females once a week, for a period of 24 hours.

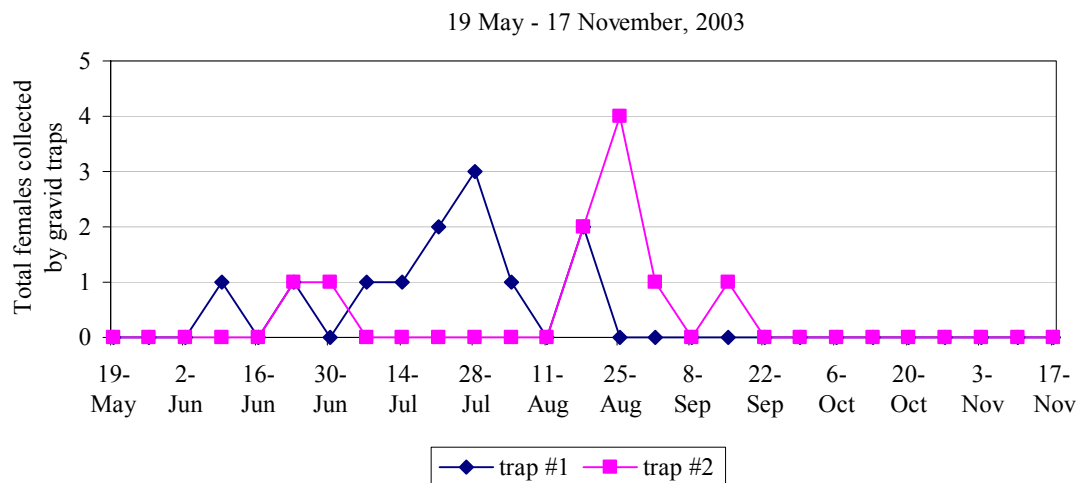


Figure 3.7. Total number of *Aedes albopictus* females collected in 2 gravid traps (labeled trap #1 and trap #2) from Blount County, TN, from 19 May to 17 November, 2003. Each trap collected gravid females once a week, for a period of 24 hours.

most abundant species with a peak in collection density on 14 June. Peak collections for

Table 3.7. Female mosquito species collected from 2 CO₂-baited CDC miniature light traps in Blount County, TN, from 24 May to 5 July, 2004. Traps collected host-seeking mosquitoes for a 24 hour period.

Species	Date							Total
	May		June				July	
	24	31	7	14	21	28	5	
<i>Ae. albopictus</i>	23	3	1	42	20	19	30	138
<i>Oc. triseriatus</i>	23	10	11	3	5	14	14	80
<i>Ae. vexans</i>	1	1	8	1	1		2	14
<i>Oc. trivittatus</i>							27	27
<i>An. punctipennis</i>			4	1				5
<i>Oc. canadensis</i>						1		1
<i>Or. signifera</i>						1		1
Total	47	14	24	47	26	35	73	266

Table 3.8. Female mosquito species collected from a CO₂-baited Omni-directional Fay traps in Blount County, TN, from 24 May to 5 July, 2004. The trap collected host-seeking mosquitoes for a 24 hour period.

Species	Date							Total
	May		June				July	
	24	31	7	14	21	28	5	
<i>Oc. triseriatus</i>			2	11	1	2	7	23
<i>Ae. albopictus</i>			2	2	1	3	10	18
<i>Oc. trivittatus</i>							17	17
<i>Ae. vexans</i>			2		1		3	6
<i>Ps. ferox</i>						2	1	3
<i>Cx. restuans</i>							2	2
<i>An. punctipennis</i>							1	1
Total	0	0	6	13	3	7	41	70

all other species occurred on 5 July.

Gravid collections: From two gravid traps placed within the Blount County site, 8 females were collected; *Ae. albopictus* (n = 7) were caught in all three months. A single *Oc. triseriatus* was collected on 24 May.

v - Discussion

The total rainfall in 2003 was consistent with the annual averages between 1961 and 1990 that recorded sums of 134.6 cm and 119.4 cm in Knox and Blount County sites, respectively (U.S. Department of Agriculture 1999). However, for the entire collecting season between 19 May and 17 November, 2003, monthly rainfall levels were above average with precipitation patterns varying slightly between the two mixed hardwood forest sites. Mosquito activity declined during October and was likely affected by declining temperatures and shortening day length. In 2004, there was less precipitation in April and May than in comparison to 2003 with a difference of 27.43 cm and 15.41 cm in Knox and Blount County, respectively. During the shortened collecting season, nearly half of the rainfall at each site occurred between 21 June and 27 June. Mean daily temperature did not vary drastically between Knox and Blount County years. Reduced mosquito collections and species diversity in 2004 was likely the result of differing environmental conditions between the two years and shorter time of collection.

Aedes vexans, a flood plain mosquito, is primarily active during crepuscular hours (Gingrich and Casillas 2004) and constituted approximately 28% of all mosquitoes collected in 2003. In contrast, *Ae vexans* individuals were scarce in collections conducted in 2004. During the spring of the 2003 collecting season, large amounts of rainfall caused an over saturation of clay and silt-loam soils. Depression areas proceeded

to fill with water and created an abundance of flooded larval habitat at or near both research sites. Adult collection densities were extremely high on the first date of collection and are postulated to have been a prevalent species in the weeks preceding. The Blount County site contained many low-lying areas and continued to produce substantial numbers of *Ae. vexans* from May to October, 2003 (**Table 3.5**). *Aedes vexans* adult populations were extremely low at both sites, from 24 May to 5 July of 2004, and were likely effected by lower amounts of precipitation in the early portion of the mosquito season. The density of collected females did not significantly increase after the large amounts of precipitation that occurred between 21 June and 27 June, 2004. West Nile virus has been isolated from field-caught *Ae. vexans* on multiple occasions in other areas of the country (Bernard et al. 2001) and could potentially increase the risk of WNV transmission during periods of high precipitation in eastern Tennessee.

Anopheles and *Culex* species are permanent-pool mosquitoes which comprised approximately 8% and 3% of all adults collected, respectively. *Culex* species are considered major bridge vectors of WNV which has had the virus repeatedly isolated from field-caught specimens (Bernard et al. 2001, Blackmore et al. 2003). However, gravid traps did not collect a large abundance or diversity of *Culex* species and could have been impacted by large amounts of precipitation that increased oviposition sites for gravid females. Furthermore, the attractiveness of water or trap locations may have altered trapping success. Lastly, *Culex* species may simply not be as abundant in the two established collection sites in comparison to other forested regions of Tennessee. In 2003, two comparable research sites in Memphis, TN, collected approximately 8.5 times the number of *Culex pipiens* (sensu lato) (D. Sanders pers. comm.) in comparison to all of

the collected *Culex* species (n = 251) identified from the Knox and Blount County research areas.

The proven and potential LAC virus vectors, *Oc. triseriatus* (Watts et al. 1973, Pantuwatana et al. 1974, Grimstad and Haramis 1984, Gerhardt et al. 2001) and *Ae. albopictus* (Grimstad et al. 1989, Gerhardt et al. 2001) collected in the 2003 and 2004 season, constituted 59.3% and 73.8% of all adult species collected, respectively. Although collection sites were approximately 30 km apart, the seasonal abundance of adult container-inhabiting species fluctuated in a similar manner between Knox and Blount counties (**Figure 3.8**). These data suggest that comparable habitats throughout eastern Tennessee have *Oc. triseriatus* and *Ae. albopictus* adult populations which fluctuate similarly but not always at the same densities. In 2004, the weekly abundance of host-seeking *Ae. albopictus* individuals were similar in comparison to 2003 figures; however, density levels in 2004 were reduced for *Oc. triseriatus*. In general, peak adult densities occurred in June for *Oc. triseriatus* and August for *Ae. albopictus*. The season long prevalence of each species may increase the incidence and expand the range of onset dates for LAC encephalitis cases within endemic areas of eastern Tennessee.

The overall abundance of adult mosquitoes identified to species was similar between Knox (n = 2590) and Blount (n = 2667) counties. Twelve of the 19 mosquito species collected were found at both sites. A review of mosquito species collected in Knox County, TN, reported that 32 species were collected by all various trapping techniques (Hribar and Gerhardt 1986). However, in 1994, *Ae. albopictus* was discovered for the first time in Knox County, TN (R. Gerhardt pers. comm.) thus

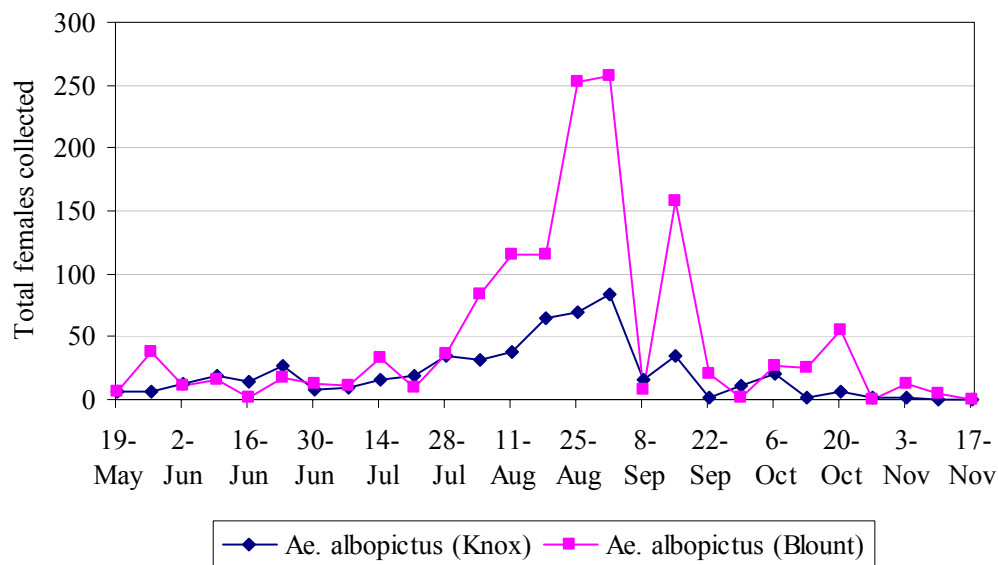
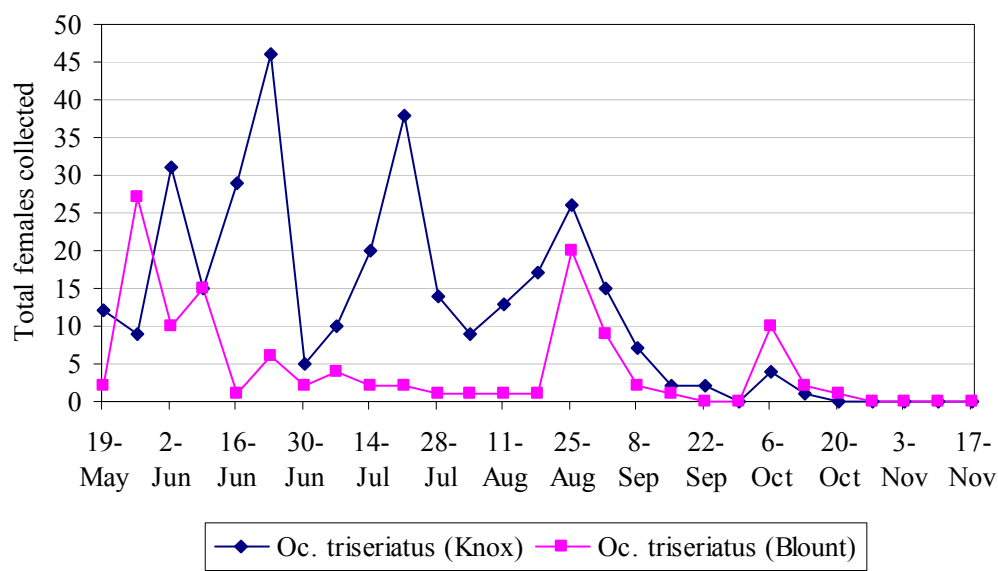


Figure 3.8. Comparison of *Ochlerotatus triseriatus* and *Aedes albopictus* collected by all host-seeking traps combined (2 CO₂-baited CDC miniature light traps and 1 CO₂-baited Omni-directional Fay trap) at Knox and Blount County, TN, from 19 May to 17 November, 2003.

increasing the total species count to 33. The diversity of mosquito species collected in 2003 and 2004 was comparable to other mosquito studies conducted in sylvatic habitats of eastern Tennessee (Ogg 1975, Hribar et al. 1984, Gottfried et al. 2002). Species diversity could have been impacted by the low number of collection sites, the adult trapping techniques utilized, or by the similarities between established sites.

Furthermore, adult mosquito collection techniques are species biased because not all species are uniformly attracted to host-seeking and gravid traps. Thus, overall species diversity and abundance may have been negatively impacted.

The Blount County site had more mosquito species ($n = 17$) with *Ae. albopictus* being the most abundant adult species collected in both years. However, approximately 87% and 74% of all *Ae. albopictus* adults collected, from all trapping techniques combined, were from a single CDC miniature light trap (CDC #1) in 2003 ($n = 1,324$) and 2004 ($n = 133$), respectively. The trap was located on the forest edge, directly behind two homes and in the vicinity of a large quantity of artificial containers (tires, oil pans, abandoned car, washer and dryer, and other discarded containers). *Ochlerotatus triseriatus* were collected in CDC #1 less frequently during the 2003 ($n = 150$) and 2004 ($n = 80$) collection periods. In 2003, an oviposition survey in the vicinity of the CDC #1 trap had a season long egg ratio of 3.7:1 in favor of *Oc. triseriatus* ($n = 13,409$) to *Ae. albopictus* ($n = 3,614$) (Caldwell 2004, Chapter 2) thus suggesting the actual *Oc. triseriatus* adult population may be higher than can be inferred from adult trapping techniques. However, the differences could be attributed to *Ae. albopictus* out competing and displacing *Oc. triseriatus* in larval habitats of artificial containers (Ho et al. 1989);

furthermore, *Ae. albopictus* may be better adapted to avoid predation by *Toxorynchites* larvae, which were all collected in the vicinity of CDC trap #1.

Diversity and abundance of larval species collected in 2003 was low and can be attributed to high precipitation which increased sites for egg deposition and larval development. This, in turn, reduced the sensitivity and efficiency of collection efforts. Larval sampling was confined to natural and artificial containers and severely reduced the diversity of collected species. However, two *Oc. japonicus* larvae were found at the Knox County site. Other known container-inhabiting mosquito species collected as adults including *An. barberi* and *Cx. pipiens quinquefasciatus* were not collected as larvae.

An adult female *Oc. japonicus*, was collected and identified (Tanaka et al. 1979, Darsie 2002) for the first time in Tennessee on June 9, 2003 from a CO₂-baited CDC miniature light trap located within the established site in Knox County. *Ochlerotatus japonicus* adults were repeatedly collected throughout the 2003 (n = 17) and 2004 (n = 9) mosquito surveillance season. Thus, this exotic and invasive species appears to have over-wintered successfully and is hypothesized to be established in at least the northeastern portion of the county. Thus, the total mosquito species checklist in Knox County, Tennessee should now include *Oc. japonicus* and total 34. The potential of *Oc. japonicus* to become an established pest and disease vector in forested suburbs and rural areas should not be underestimated.

CHAPTER IV

SCIENTIFIC NOTE

FIRST COLLECTION OF *OCHLEROTATUS JAPONICUS JAPONICUS* (THEOBALD) (DIPTERA: CULICIDAE) IN THE STATE OF TENNESSEE

Abstract. This is the first reported occurrence of *Ochlerotatus j. japonicus* in the state of Tennessee, USA. Adults were collected at 5 sites in northwestern Knox County from 9 June through 3 November, 2003 and from one site in both 2003 and 2004. The implications of these findings are discussed.

Ochlerotatus japonicus japonicus (Theobald) is a container-inhabiting mosquito species that is native to and occurs throughout Japan, Korea, Taiwan, eastern China, and Russia (Tanaka et al. 1979, Falco et al. 2002). The first reported collections of *Oc. japonicus* in the United States were from New York and New Jersey in the late summer of 1998 (Peyton et al. 1999) and subsequently it was reported from Connecticut in July of 1998 (Fonseca et al. 2001). By the end of 2003, published reports had it occurring in 13 states including Connecticut (Fonseca et al. 2001), Georgia (Reeves and Korecki 2004, Gray et al. 2004), Maine (Foss and Dearborn 2001), Maryland (Sardelis and Turell 2001), North Carolina (Gray et al. 2004), New Jersey and New York (Peyton et al. 1999), Ohio (Fonesca et al. 2001), Pennsylvania (Sardelis and Turell 2001), South Carolina (Reeves and Korecki 2004), Virginia (Harrison et al. 2002), Vermont (Graham and Turmel 2001), and Washington (Falco et al. 2002, Roppo et al. 2004). In its native range, *Oc. japonicus* has been collected as far south as 33°N, and based on climatic factors alone, could expand its range in the United States as far south as Jacksonville, Florida (Sardelis and Turell 2001). The world's northernmost collection occurred in France at 48°N (Schaffner

et al. 2003).

This newly introduced invasive species is a competent vector for West Nile (White et al. 2001, Turell et al. 2001b, Kutz et al. 2003) and has been shown to be a laboratory vector of La Crosse (LAC) encephalitis (Sardelis et al. 2002a), eastern equine encephalitis (Sardelis et al. 2002b), and St. Louis encephalitis viruses (Sardelis et al. 2003). Its potential as a bridge vector is enhanced by the fact that females are catholic feeders and feed readily on avian and mammalian hosts in the laboratory (Sardelis et al. 2002a, Falco et al. 2002), as well as humans in the field (Peyton et al. 1999).

During weekly surveillance at a research site in proximity to 2 previous cases of LAC encephalitis, a single female *Oc. japonicus* was collected from a CO₂-baited CDC miniature light trap on June 9, 2003 in northeastern Knox County, Tennessee, USA. The coordinates of this index site was (35°, 69'N: 84°, 8'W). Subsequently, from 16 June to 3 November, 2003, a total of 16 adults were collected by host seeking (CO₂-baited CDC miniature light trap and CO₂-baited Omni-directional Fay trap) and gravid traps. Two larvae of *Oc. japonicus* were collected from an abandoned tire on 22 July and positively identified (Peyton et al. 1999); but attempts to rear these specimens to the adult stage failed. Oviposition traps (Loor and DeFoliart 1969) were positioned throughout the index site and successfully collected 38 *Oc. japonicus* eggs which were reared to the adult stage. Each week, from 19 June to 9 October, 2003, 3 gravid traps (baited with a concoction of tap water, manure, and oak leaves) were operated for a 24 hour period in other areas of Knox County, with an emphasis put towards collecting near major interstates, highways, and railroads. *Ochlerotatus japonicus* were collected in 4 additional sites all within an approximate 8 km radius of the index site (**Figure 4.1**).

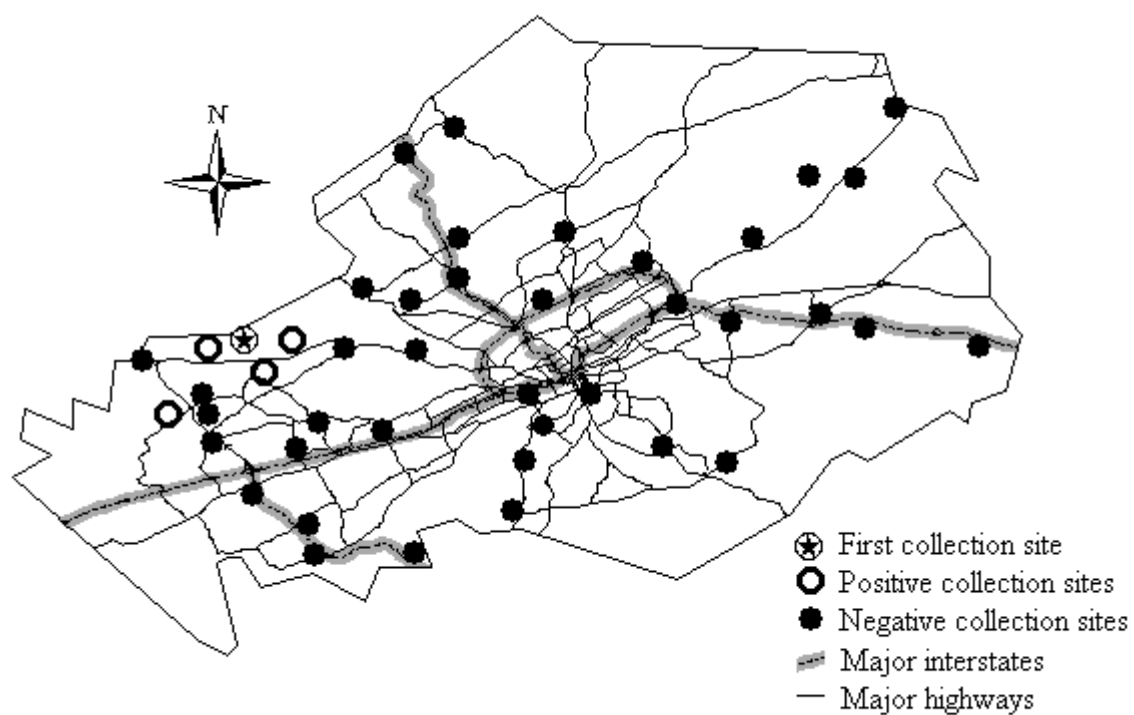


Figure 4.1. All gravid trap collection sites established in Knox County, Tennessee, USA, for the surveillance of *Ochlerotatus japonicus* between 9 June and 3 November, 2003.

However, all gravid trap collections outside of the northeastern portion of the county were negative for *Oc. japonicus*. Adult collections at the same index site in 2004, between 24 May and 5 July, resulted in 7 more *Oc. japonicus* indicating that it overwintered successfully in Tennessee. Furthermore, in June of 2004, adult *Oc. japonicus* were collected in northern Knox County approximately 20 km from the index site (A. Haddow pers. comm.).

Ochlerotatus japonicus has the potential to become an established pest and disease vector in forested suburbs and rural areas of eastern Tennessee; thus, continued surveillance for *Oc. japonicus* is essential to the determination of distribution, abundance, and potential vector capacity.

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