

Host-Feeding Patterns of Potential Mosquito Vectors in Connecticut, USA: Molecular Analysis of Bloodmeals from 23 Species of *Aedes*, *Anopheles*, *Culex*, *Coquillettidia*, *Psorophora*, and *Uranotaenia*

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ABSTRACT We evaluated the blood-feeding patterns in several mosquito species that may serve as vectors of disease agents in the northeastern United States. Blood-fed mosquitoes were collected from 91 different sites throughout Connecticut over a 6-yr period (June–October 2002–2007), and the host-feeding patterns of 23 mosquito species representing six genera were examined by using a polymerase chain reaction-based assay and sequencing portions of the *cytochrome b* gene of mitochondrial DNA. This study was part of a statewide surveillance program and for some of the mosquito species a limited number of specimens were examined [e.g., *Aedes communis* (De Geer) (1), *Anopheles barberi* Coquillett (1), *Uranotaenia sapphirina* (Osten Sacken) (5)]. With the exception of *Culex territans* Walker that acquired bloodmeals from all four classes of vertebrates—birds, reptiles, amphibians, and mammals—all species of *Aedes*, *Anopheles*, *Coquillettidia*, *Psorophora*, and to a lesser degree, *Uranotaenia*, were found to feed predominately upon mammalian hosts. Fourteen mammalian species were identified as sources of blood, but the majority of feedings were taken from the white-tailed deer, *Odocoileus virginianus*. Human-derived bloodmeals were identified from 13 of the 23 mosquito species. Limited avian-derived bloodmeals were detected in *Aedes canadensis* (Theobald), *Aedes cantator* (Coquillett), *Aedes cinereus* Meigen, *Aedes triseriatus* (Coquillett), *Aedes trivittatus* (Coquillett), *Coquillettidia perturbans* (Walker) *Cx. territans*, *Psorophora ferox* (von Humboldt), and *Ur. sapphirina*. American robin, *Turdus migratorius*, was the most common source of avian blood, followed by a few other mostly Passeriformes birds. We conclude that the white-tailed deer serve as the main vertebrate host for these mammalophilic mosquitoes in this region of the United States. This feeding pattern supports enzootic amplification of arboviruses, including Jamestown Canyon, Cache Valley, and Potosi viruses that perpetuate in cervid hosts. Occasional feeding on avian hosts suggests that some of these mosquito species, such as *Cq. perturbans*, also could facilitate transmission of West Nile and eastern equine encephalitis viruses from viremic birds to mammalian hosts.

KEY WORDS Blood-feeding behavior, mosquitoes, *cytochrome b*, vector, arboviruses

Detailed knowledge of the blood-feeding behavior of mosquito populations in nature is an essential component for evaluating their vectorial capacity and for assessing the role of individual vertebrates as potential reservoir hosts involved in maintenance and amplification of zoonotic agents of human diseases. The host-feeding patterns of mosquitoes are governed by a number of factors such as innate tendencies, host availability and abundance, defensive behavior of hosts, as well as flight behavior and feeding periodicity of mosquitoes (Clements 1999).

The feeding patterns and the degree of contact of mosquito vectors with potential hosts may be directly estimated by analyzing the bloodmeal contents of field-derived females. Advances in molecular techniques for bloodmeal analyses by using polymerase chain reaction (PCR)-based assays and direct sequencing of the *cytochrome b* gene, have permitted the identification of hosts to the species level with a much higher degree of accuracy than could be achieved with previous serologic techniques. These molecular techniques have recently been used to examine the feeding patterns of the primary and secondary mosquito vectors of West Nile (family *Flaviviridae*, genus *Flavivirus*, WN) and eastern equine encephalitis (family *Togaviridae*, genus *Alphavirus*, EEE) viruses in the northeastern United States (primarily *Culex* and *Culiseta* spp., respectively) (Apperson et al. 2002, 2004; Molaei et al. 2006a, 2006b; Molaei and Andreadis

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2006). Further identification of the avian reservoir and mammalian hosts to the species level has significantly advanced our epidemiologic knowledge of these two mosquito-borne viruses in this region. Analyses of the feeding patterns of local populations of species of *Aedes* Meigen, *Anopheles* Meigen, *Coquillettidia* Dyar, *Psorophora* Robineau-Desvoidy, and *Uranotaenia* Lynch Arribalzaga have been more limited in scope (Edman 1971; Cupp and Stokes 1973; Tempelis 1975; Magnarelli 1977; Nasci and Edman 1981; Ritchie and Rowley 1981; Nasci 1982, 1984; Irby and Apperson 1988; Apperson et al. 2002, 2004; Gingrich and Williams 2005; Savage et al. 2007), and their respective roles in transmitting these and other enzootic arboviruses in the region are relatively unknown. The latter include four viruses that have been associated with human diseases: Cache Valley (family *Bunyaviridae*, genus *Orthobunyavirus*, CV), Jamestown Canyon (family *Bunyaviridae*, genus *Orthobunyavirus*, JC), La Crosse (family *Bunyaviridae*, genus *Orthobunyavirus*, LAC), and Trivittatus, as well as Flanders, Highlands J (family *Togaviridae*, genus *Alphavirus*, HJ), Keystone, and Potosi (family *Bunyaviridae*, genus *Orthobunyavirus*, POT) viruses (Grimstad 1988, Sexton et al. 1997).

The current study was thus designed to examine the host-feeding patterns of 23 species in the aforementioned genera in an attempt to extend information on their behavioral ecology. This work is a part of our continued efforts to evaluate the role of various mosquito species and to elucidate their vector potentials for arboviruses that currently circulate in Connecticut. Blood-fed mosquitoes were collected during the period 2002 through 2007 from a variety of locales throughout the state, and the sources of bloodmeals were determined by sequencing PCR products of the *cytochrome b* gene of mitochondrial DNA.

Materials and Methods

Collection of Mosquitoes. Mosquitoes were collected from 91 different sites located within seven counties in Connecticut during June through October 2002–2007 as part of a statewide surveillance program (Andreadis et al. 2004). Trap sites included parks, greenways, golf courses, undeveloped wood lots, sewage treatment plants, dumping stations, and temporary wetlands associated with waterways. Two trap types were used: a CO₂ (dry ice)-baited CDC miniature light trap (John W. Hock Co., Gainesville, FL) and a CDC gravid mosquito trap containing a hay/lactalbumin/yeast or sod-grass infusion (Reiter 1983). Traps were operated overnight and retrieved the following morning. Supplementary collections were made from natural resting sites at four locations in 2005 and 2006. These were conducted by sweeping low-lying vegetation with a battery-powered modified CDC backpack aspirator (John W. Hock Co.) during the early morning.

Mosquitoes were transported alive on dry ice to the laboratory where they were promptly identified on chill tables with the aid of a stereomicroscope by using

descriptive keys (Darsie and Ward 1981, Andreadis et al. 2005). All mosquitoes with fresh or visible blood remnants were transferred into individual 2-ml tubes labeled according to species, date of collection, and locale and stored at -80°C .

DNA Isolation from Blood-Fed Mosquitoes. DNA was isolated from the abdominal contents of the blood-fed mosquitoes individually by using DNA-zol BD (Molecular Research Center, Cincinnati, OH) according to the manufacturer's recommendation, with some modifications as described previously (Molaei et al. 2006a, 2006b, 2007; Molaei and Andreadis 2006).

Bloodmeal Identification. Isolated DNA from the mosquito bloodmeals served as DNA templates in subsequent PCR reactions. PCR primers were based on *cytochrome b* sequences of avian and mammalian species. DNA templates were initially screened with avian- and mammalian-specific primer pairs, by using previously described protocols (Molaei et al. 2006a, 2006b, 2007; Molaei and Andreadis 2006). Avian-specific primer pairs were 5'-GAC TGT GAC AAA ATC CCN TTC CA-3' (forward) and 5'-GGT CTT CAT CTY HGG YTT ACA AGA C-3' (reverse), with amplified product size of 508 bp. Mammalian-specific primer pairs were 5'-CGA AGC TTG ATA TGA AAA ACC ATC GTT G-3' (forward) and 5'-TGT AGT TRT CWG GGT CHC CTA-3' (reverse), with amplified product size of 772 bp. Mammalian primer set successfully amplified the reptilian and amphibian DNA. PCR reaction and cycling conditions were as described previously (Molaei et al. 2006a, Molaei and Andreadis 2006). A *Taq*PCR core kit (QIAGEN, Valencia, CA) was used for all PCR reactions according to the manufacturer's recommendation. PCR reactions were performed with the GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA). PCR-amplified products were purified by using QIAquick PCR purification kit (QIAGEN) and sequenced by using the sequencer, 3730xl DNA Analyzer (Applied Biosystems) at the Keck Sequencing Facility, Yale University, New Haven, CT. Sequences of both DNA strands were analyzed and annotated by using ChromasPro version 1.22 (Technelysium Pty Ltd., Tewantin, Australia) and identified by comparison to the GenBank DNA sequence database (National Center for Biotechnology Information available online: www.ncbi.nlm.nih.gov/blast/Blast.cgi). Positive identification and host species assignment were made when exact or nearly exact match (>95%) were obtained. Sequences that did not meet the criteria were assumed unknown. These could be due to the quality of the sequences or the possibility that the bloodmeals were derived from vertebrates for which *cytochrome b* sequences are not yet available.

Results

Bloodmeal sources were successfully identified from 747 (90.6%) of 824 field-collected mosquitoes with visible bloodmeals, representing 23 species in six genera (Table 1). Of the remaining (9.4%) of the blood-fed mosquitoes either we did not obtain visible

Table 4. Number of amphibian- and reptilian-derived bloodmeals identified from four species of mosquitoes collected in Connecticut, 2002–2007

Mosquito species	Total no.	Turtle	Frog
<i>Ae. canadensis</i>	2	2	
<i>Ae. cantator</i>	1	1	
<i>Cx. territans</i>	7	4	3
<i>Ur. sapphirina</i>	1	1	

strongest association (seven of 19 total) with these two classes of vertebrate hosts.

Discussion

Our study on the blood-feeding behavior of the mosquitoes in the genera *Aedes*, *Anopheles*, *Culex*, *Coquillettidia*, *Psorophora*, and *Uranotaenia* identified white-tailed deer as the most common vertebrate host. Deer-derived bloodmeals accounted for >83% of all vertebrate feedings ($n = 747$) and >88% of all mammal feedings ($n = 699$). Although care should be exercised in interpreting findings for those species where a limited number of specimens were examined (i.e., *Ur. sapphirina*, *An. barberi*, and *An. communis*). Our results with local populations in Connecticut are in agreement with a recent analysis of mammalian-derived bloodmeals from a similar composition of mammalophilic species (16 species in six genera) from neighboring New York and New Jersey, where nearly 90% ($n = 747$) of mammalian-derived bloodmeals were from white-tailed deer (Apperson et al. 2004). This differs from a study conducted in Connecticut >30 yr ago by Magnarelli (1977), who identified comparatively little feeding on deer (3.8%; $n = 399$) among 11 of the same mammalophilic species examined in our investigation. The apparent prevalence of the white-tailed deer as hosts for mosquitoes in these most recent studies is likely a function of deer abundance and availability in this region of the northeastern United States. Deer populations have grown exponentially during the past three decades throughout eastern North America (Conover et al. 1995) concomitant with reforestation and the decline in traditional agriculture (UMass-Center for Agriculture Census Analysis 2008). In Connecticut for example, white-tailed deer populations have increased from an estimated 18,000 in 1975–80,000 at present (Williams et al. 2008). White-tailed deer and humans represent the two dominant large mammals in the region.

White-tailed deer are generally regarded as the principal amplification hosts for JC, CV, and POT viruses (Issel et al. 1972, Watts et al. 1979, Grimstad 1983, Blackmore and Grimstad 1998). Serologic evidence of JC virus in white-tailed deer populations in Connecticut has been reported to be relatively high; 21% of the hunter-killed deer were antibody positive as determined by an enzyme-linked immunosorbent assay and confirmed by plaque reduction neutralization testing (Zamparo et al. 1997). It has been suggested (Boromisa and Grimstad 1986, Grimstad et al.

1987) that the transmission of JC virus involves a single seasonal peak of virus amplification in primarily white-tailed deer, with vertical transmission by *Aedes* mosquitoes early in the season, horizontal amplification in deer population in June and July, followed by early autumn amplification cycle involving Anopheline mosquitoes, including *An. punctipennis* and *An. quadrimaculatus* (Grimstad 1988, 2001). In Connecticut, JC virus has been consistently isolated from a diverse array of mosquitoes, especially *Ae. abserratus*, *Ae. canadensis*, *Ae. cantator*, *An. punctipennis*, and *Cq. perturbans* (Andreadis et al. 2008). The feeding association of these mosquitoes with white-tailed deer as hosts in conjunction with high rates of JC virus infection in nature, suggest that they may serve as potential vectors of JC virus in the state.

Human-derived bloodmeals were identified from 13 (56.5%) of the mosquito species that were examined in our study, but made up <5% of the total bloodmeals analyzed. This overall proportion was lower than the 8.3% reported from eight of the same mammalophilic mosquitoes analyzed by Magnarelli (1977) in 1975, but greater than the 2.2% observed recently by Apperson (2004) in nine of 15 species analyzed from similar locales in New York and New Jersey. It is evident that the majority of these mammalophilic mosquitoes will feed on humans in addition to other species. However, we suspect the rather low overall frequency of feeding on humans is most likely a function of less host accessibility during the peak mosquito feeding activity in comparison with other large mammals, particularly white-tailed deer.

It is noteworthy that we identified human-derived blood for the first time from *Ae. japonicus*, a species recently introduced from Asia (Peyton et al. 1999, Andreadis et al. 2001) that has been shown to be a competent vector of EEE, LAC, and WN viruses (Turell et al. 2001; Sardelis et al. 2002a, 2002b) and from which WN virus has been occasionally isolated in nature (Bernard and Kramer 2001, Lukacik et al. 2006). It is also notable that *Ae. japonicus* was one of only two mosquitoes (in addition to *Ae. cinereus*) that fed on eastern chipmunk, *Tamias striatus*, the natural vertebrate host for LAC virus (Moulton and Thompson 1971, Pantuwatana et al. 1972). Antibodies to LAC virus have been detected in these rodents captured in Massachusetts (Walker et al. 1993), and recently the virus was isolated from *Ae. triseriatus* collected in Connecticut (Armstrong and Andreadis 2006).

Our analysis of blood-fed *Cq. perturbans* revealed that although this mosquito species fed predominately on mammals (86.8%), it also acquired a moderate number of bloodmeals from several different avian hosts (11.8%). This pattern of mixed feeding behavior is consistent with findings reported before in Connecticut (61.9% mammalian and 38.1% avian, $n = 21$; Magnarelli 1977), New York (96 and 3.4%, $n = 29$) (Apperson et al. 2004), and Florida (91% and 9%, $n = 673$) (Edman 1971). Conversely, Crans (1964) found *Cq. perturbans* populations in New Jersey fed almost exclusively on mammals (97.6%, $n = 41$).

Cq. perturbans is regarded as a moderately competent vector for EEE (Vaidyanathan et al. 1997) and WN (Turell et al. 2005) viruses. EEE virus has occasionally been isolated from field-collected females in several states in the northeastern United States, including Connecticut (Andreadis et al. 1998), Massachusetts (Edman et al. 1993), New Jersey (Crans and Schulze 1986), and New York (Srihongse et al. 1980). WN virus also has been isolated from *Cq. perturbans*, but prevalence seems to be less frequent (Godsey et al. 2005, Lukacik et al. 2006). The association of *Cq. perturbans* with both avian and mammalian hosts identified in our investigation is consistent with the view that this species could serve as a potential bridge vector in transferring EEE and WN from viremic birds to mammals if other required conditions, such as abundance, vector competence, and behavioral adaptations exist.

Our analysis of bloodmeals from female *Ae. canadensis* revealed an almost exclusive feeding on mammalian hosts (98%). Earlier analysis of bloodmeals from this mosquito species collected in Connecticut had shown pronounced feeding on mammals (86.5%, $n = 37$), including humans, and a moderate tendency for avian (8.1%) and amphibian (5.4%) hosts (Magnarelli 1977). More recently, Apperson et al. (2004) similarly reported an exclusive mammalian feedings for a limited number ($n = 5$) of *Ae. canadensis* collected from Westchester County, NY. *Ae. canadensis* is among the most widely distributed and commonly trapped mosquito species in Connecticut (Andreadis et al. 2004, 2008). Vector competence for WN (Turell et al. 2005) and JC (Heard et al. 1991) viruses has been confirmed in the laboratory, and several arboviruses, including CV (Ngo et al. 2006), EEE (Edman et al. 1993), HJ (Andreadis et al. 1998), JC (Grayson et al. 1983, Howard et al. 1988, Heard et al. 1990, Andreadis et al. 2008), POT (Armstrong et al. 2005), and WN (Andreadis et al. 2004) viruses, have been isolated from field populations in the northeastern United States. *Ae. canadensis* is widely distributed in Connecticut, and JC virus has been frequently isolated from this mosquito species (Andreadis et al. 2008).

We identified almost exclusively (>96%) mammalian-derived bloodmeals from *Ae. cantator*, a largely coastal salt marsh-inhabiting species. Earlier analysis of the bloodmeals from this mosquito collected in Connecticut also indicated strong blood-feeding on mammals (85.7%, $n = 140$), with moderate tendency for birds (12.9%), particularly Passeriformes, as well as amphibians (1.4%) (Magnarelli 1977). *Ae. cantator* is capable of experimentally transmitting EEE virus (Merrill et al. 1934, Ten Broeck and Merrill 1935, Davis 1940) and several arboviruses, including EEE (Srihongse et al. 1980, Andreadis et al. 1998), HJ (Andreadis et al. 1998), JC (Main et al. 1979, Grayson et al. 1983, Takeda et al. 2003, Andreadis et al. 2008), POT (Armstrong et al. 2005), and WN virus (Bernard et al. 2001, Andreadis et al. 2004, Anderson et al. 2006) have been isolated from local populations of this mosquito in the northeast.

Nine species of mosquitoes analyzed during the current study acquired bloodmeals from many avian as well as mammalian hosts. American robin was the most commonly identified avian host along with mostly other Passeriformes birds, including common grackle, gray catbird, wood thrush, and house finch, many of which have been shown to be capable of maintaining and amplifying a variety of arboviruses in the region (Main et al. 1988; Crans et al. 1994; Komar et al. 1999, 2003; Howard et al. 2004). Close association of the mosquitoes with the American robin as a common host in the current study parallels the results of previous examinations of the host preferences of largely ornithophilic *Culex* and *Culiseta* species (Apperson et al. 2004; Molaei et al. 2006a, 2006b; Molaei and Andreadis 2006; Kilpatrick et al. 2006; Savage et al. 2007; Hamer et al. 2008), and merits further investigations to determine the contribution that this avian species makes as a reservoir or amplifying host to the transmission of WNV in northeastern and other regions of the United States.

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