

# Identification of Avian- and Mammalian-Derived Bloodmeals in *Aedes vexans* and *Culiseta melanura* (Diptera: Culicidae) and Its Implication for West Nile Virus Transmission in Connecticut, U.S.A.

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**ABSTRACT** To evaluate the host-feeding patterns of *Aedes vexans* (Meigen) and *Culiseta melanura* (Coquillett) as secondary vectors of West Nile virus (family *Flaviviridae*, genus *Flavivirus*, WNV) in Northeastern United States, we identified the source of vertebrate bloodmeals by sequencing portions of the cytochrome *b* gene of mitochondrial DNA. Analysis of polymerase chain reaction products from a total of 119 *Ae. vexans* revealed that 92.4% of individuals acquired blood solely from mammalian and 2.5% from avian hosts. Mixed bloodmeals from both avian and mammalian hosts were detected in 5% of individuals of this species. *Ae. vexans* obtained vertebrate bloodmeals most frequently from white-tailed deer (80%) followed by domestic horse, American robin, eastern cottontail, and domestic cat. In contrast, *Cs. melanura* fed predominantly on avian species (89.6%) but exhibited some inclination for mammalian blood (4.2%). Individual mosquitoes containing mixed bloodmeals were also identified in 6% of *Cs. melanura*. American robin was the most common source of vertebrate blood for *Cs. melanura* (23%), followed by wood thrush and gray catbird. American crow represented only 2% of the bloodmeals identified in *Cs. melanura*, as was similarly found with other recognized *Culex* vectors of WNV in the northeast. These findings support the view that *Ae. vexans* is likely to be a relatively important “bridge vector” to large mammals, including deer and horse, whereas *Cs. melanura* likely plays a secondary role in enzootic transmission of WNV among free-ranging birds in more rural environs.

**KEY WORDS** mosquito, host-feeding pattern, *Aedes vexans*, *Culiseta melanura*, West Nile virus

Since the discovery of West Nile virus (family *Flaviviridae*, genus *Flavivirus*, WNV) in North America in 1999 (Anderson et al. 1999, Lanciotti et al. 1999), >60 mosquito species have been found infected with this virus (CDC 2005b). In the Northeastern United States, *Culex* L. mosquitoes, i.e., *Culex pipiens* L., *Culex restuans* Theobald, and *Culex salinarius* Coquillett, are considered to be the principal vectors (Andreadis et al. 2001, 2004; Bernard et al. 2001; Kulasekera et al. 2001, Nasci et al. 2001, White et al. 2001, Anderson et al. 2004, Ebel et al. 2005, Kilpatrick et al. 2005). Other species, including *Aedes vexans* (Meigen) and *Culiseta melanura* (Coquillett), also have been implicated as potential secondary vectors because of their local abundance, demonstrated vector competence in the laboratory (Turell et al. 2005), and frequent infection with WNV in nature (Bernard et al. 2001, White et al. 2001, Andreadis et al. 2004); however, their vectorial capacity and respective role(s) in epidemic transmission to humans and/or enzootic amplification among various wild bird species have not been fully resolved.

*Ae. vexans* is an aggressive human biter that feeds almost exclusively on mammals and rarely on birds

(Tempelis et al. 1967, 1970; Edman 1971; Cupp and Stokes 1973; Magnarelli 1977a,b; Ritchie and Rowley 1981; Nasci 1984; Irby and Apperson 1988; Apperson et al. 2002, 2004; Lee et al. 2002; Hassan et al. 2003; Gingrich and Williams 2005). Because of its local abundance and biting behavior, it is considered to be a potentially important bridge vector for WNV in the region (Andreadis et al. 2004, Turell et al. 2005). However, its limited preference for feeding on birds, a presumed prerequisite for acquisition of the virus, has been suggested as a major factor that reduces its vectorial capacity (Andreadis et al. 2004). *Ae. vexans* is also fairly opportunistic in its host-seeking behavior and is known (Nasci 1984) to exhibit local variation depending on host availability. Thus, knowledge of the feeding behavior of resident populations is an essential element in assessing the vectorial capacity of this species within a given locale.

Regional host-feeding studies on local populations of *Cs. melanura* in the Northeastern United States (Jobbins et al. 1961, Crans 1964, Schober and Collins 1966, Magnarelli 1977a, Nasci and Edman 1981, Ngo and Kramer 2003, Apperson et al. 2004) clearly demonstrate that this species, in contrast to *Ae. vexans*, has a strong preference for avian hosts, especially *Passe-*

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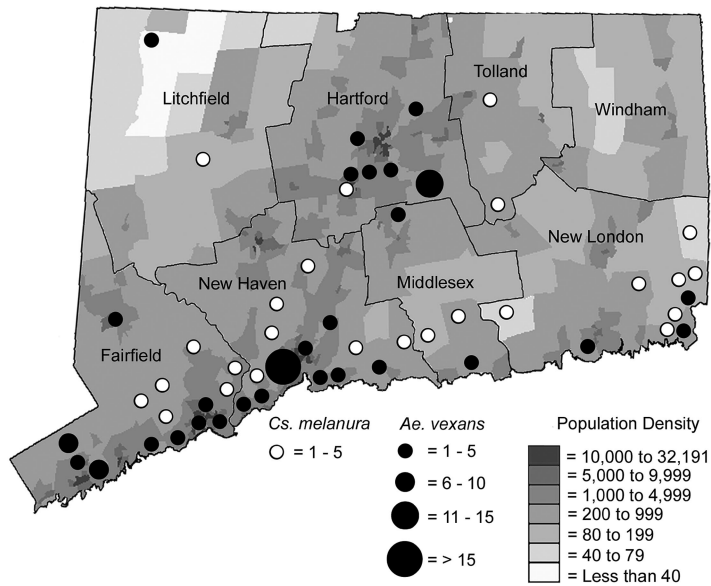


Fig. 1. Geographic distribution of mosquito collection from 54 sites within seven counties, in relation to human population density in Connecticut 2002–2004.

riformes. Occasional feeding on mammals, including humans, has been noted (Jobbins et al. 1961, Apperson et al. 2004), although this seems to be very rare and a function of local fauna and host accessibility.

With a few exceptions (Apperson et al. 2002, 2004; Lee et al. 2002; Hassan et al. 2003; Ngo and Kramer 2003), the majority of the aforementioned investigations have largely been based on serological identification of bloodmeals by using broadly reactive antisera. This has allowed for identification of mammalian hosts to the species level but for avian hosts, identification has been limited to class and at best, ordinal level only. Ngo and Kramer (2003) implemented a polymerase chain reaction (PCR)-based assay to identify host bloodmeals from mosquitoes by using primers for the cytochrome *b* gene that allowed them to distinguish between mammalian and avian bloodmeals and differentiate among four avian orders. Recently, in our analysis we have identified vertebrate bloodmeals in mosquitoes by sequencing portions of the cytochrome *b* gene of mitochondrial DNA (Molaei et al. 2006). This has allowed us to unambiguously identify both avian- and mammalian-derived bloodmeal sources to the species level.

The current research initiative was undertaken to characterize the host-feeding patterns of *Ae. vexans* and *Cs. melanura* and to evaluate their contribution as secondary vectors in enzootic maintenance of WNV among wild birds and epidemic transmission to mammalian hosts, including humans.

### Materials and Methods

**Mosquito Collection.** Mosquitoes were collected from 54 sites located within seven counties in Connecticut (Fig. 1) over a 3-yr period (June–October

2002–2004) as part of a statewide surveillance program (Andreadis et al. 2004). The majority of *Ae. vexans* was obtained from densely populated residential locales where WNV is endemic. These included parks, greenways, golf courses, undeveloped wood lots, sewage treatment plants, dumping stations, and temporary wetlands associated with waterways. Most females of *Cs. melanura* were collected from more sparsely populated rural locales that included permanent freshwater swamps (red maple/white cedar) and bogs and swamp-forest border environments. Two trap types were used: a CO<sub>2</sub>-baited Centers for Disease Control (CDC) light trap (John W. Hock Co., Gainesville, FL) and a CDC gravid mosquito trap (Reiter 1983). Traps were operated overnight and retrieved the next morning. Adult mosquitoes were transported alive to the laboratory where they were identified on chill tables with a stereomicroscope by using descriptive keys (Means 1979, Darsie and Ward 1981, Means 1987, 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  $-80^{\circ}\text{C}$ .

**DNA Isolation from Blood-Fed Mosquitoes.** Mosquito abdomens were removed and reserved for bloodmeal analysis with the aid of a dissecting microscope. Each mosquito was dissected individually on a new microscope slide by using flame-sterilized forceps to avoid cross-contamination. DNA was isolated from the abdominal contents of blood-fed mosquitoes individually using DNA-zol BD (Molecular Research Center, Cincinnati, OH) according to the manufacturer's recommendation with some modifications (Molaei et al. 2006).

**Bloodmeal Analysis.** Isolated DNA from the mosquito bloodmeals served as DNA templates in subse-

**Table 1. Sequences of primers, length of amplification products, and thermal cycling conditions used in PCR for bloodmeal analysis**

Primer sequence	Product (bp)	Denaturation	Cycling condition	
			Annealing	Extension
GAC TGT GAC AAA ATC CCN TTC CA (f) <sup>a</sup>	508	94°C (30 s)	60°C (50 s)	72°C (40 s)
GGT CTT CAT CTY HGG YTT ACA AGA C (r)				
CCC TCA GAA TGA TAT TTG TCC TCA (f) <sup>b</sup>	515	95°C (1 min)	58°C (1 min)	72°C (1 min)
CCT CAG AAK GAT ATY TGN CCT CAK GG (r)				
CGA AGC TTG ATA TGA AAA ACC ATC GTT G (f) <sup>c</sup>	772	94°C (30 s)	55°C (45 s)	72°C (1:30 min)
TGT AGT TRT CWG GGT CHC CTA (r)				
GCG TAC GCA ATC TTA CGA TCA A (f)	195	95°C (1 min)	54°C (1 min)	72°C (1 min)
CTG GCC TCC AAT TCA TGT GAG (r)				
CCA TCC AAC ATC TCA GCA TGA TGA AA (f)	395	95°C (1 min)	55°C (1 min)	72°C (1 min)
GCC CCT CAG AAT GAT ATT TGT CCT CA (r)				

<sup>a</sup> Cicero and Johnson (2001).

<sup>b</sup> Sorenson et al. (1999).

<sup>c</sup> See Ngo and Kramer (2003).

quent PCR reactions. PCR primers were either based upon a multiple alignment of cytochrome *b* sequences of avian and mammalian species obtained from GenBank or previously published primer sequences (Table 1). A *Taq*PCR core kit (QIAGEN, Valencia, CA) was used for all PCR reactions according to the manufacturer's recommendation. A 50- $\mu$ l reaction volume was prepared with 3  $\mu$ l of template DNA, 4  $\mu$ l of each primer (0.1–0.5  $\mu$ M), 5  $\mu$ l of 10 $\times$  QIAGEN PCR buffer (containing 15 mM MgCl<sub>2</sub>), 1  $\mu$ l of dNTP mix (10 mM each), 0.25  $\mu$ l of *Taq*DNA Polymerase (1.25 U/reaction), and 32.75  $\mu$ l of water. All PCR reactions were performed using the GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA). PCR-amplified products were purified using QIAquick PCR purification kit (QIAGEN) and sequenced directly in cycle sequencing reactions at the Keck Sequencing Facility (Yale University, New Haven, CT) by using the sequencer 3730xl DNA Analyzer (Applied Biosystems). Sequences were annotated using ChromasPro, version 1.22 (Technelysium Pty Ltd., Tewantin, Australia) and identified by comparison with the GenBank DNA sequence database (National Center for Biotechnology Information, Bethesda, MD; <http://www.ncbi.nlm.nih.gov/Genbank/index.html>). The performance of the molecular-based assay was previously validated (Molaei et al. 2006).

## Results

Bloodmeal sources were successfully identified by DNA sequencing from 119 of 120 *Ae. vexans* and 48 of 49 *Cs. melanura*. Of 119 *Ae. vexans* analyzed, 110 (92.4%) contained mammalian blood only, three (2.5%) avian blood only, and six (5%) both avian and mammalian blood. Of 48 *Cs. melanura* analyzed, 43 (89.6%) contained avian blood only, two (4.2%) mammalian blood only, and three (6.2%) both avian and mammalian blood.

An analysis of the mammalian and avian bloodmeal sources for *Ae. vexans* is shown in Table 2. In total, seven mammalian host species were identified for *Ae. vexans*. White-tailed deer, *Odocoileus virginianus* (Zimmermann), was the most frequently identified host (79.8% of total), followed by horse, *Equus caball-*

*lus* (L.) (9.2%), eastern cottontail, *Sylvilagus floridanus* (Allen) (3.4%), and domestic cat, *Felis catus* (L.) (2.5%). All (including mixed bloodmeals) of the avian-derived bloodmeals found in *Ae. vexans* were identified as American robin, *Turdus migratorius* (L.).

The composition of avian- and mammalian-derived bloodmeals for *Cs. melanura* is shown in Table 3. In total, 18 species of birds were identified. The most frequently identified hosts were American robin (23.0% of total); wood thrush, *Hylocichla mustelina* (Gmelin) (12.5%); gray catbird, *Dumetella carolinensis* (L.) (10.4%); tufted titmouse, *Baeolophus bicolor* (L.) (6.3%); veery, *Catharus fuscescens* (Stephens) (6.3%); song sparrow, *Melospiza melodia* (Wilson) (6.3%); and common grackle, *Quiscalus quiscula* (L.) (6.3%). Only one (2.1%) American crow-derived bloodmeal was identified. The most common mammalian species was white-tailed deer (6.3% of total) followed by domestic cat (2.1%) and northern raccoon, *Procyon lotor* (L.) (2.1%).

## Discussion

Our investigations of the host-feeding patterns of local populations of *Ae. vexans* and *Cs. melanura*, as determined by bloodmeal analysis, provide meaningful information relative to their respective roles as

**Table 2. Number and percentage of mammalian and avian bloodmeals identified from *Ae. vexans* collected in Connecticut, 2002–2004**

Host	No. <sup>a</sup>	% total
Mammalian		
White-tailed deer	95	79.8
Horse	11	9.2
Eastern cottontail	4	3.4
Domestic cat	3	2.5
Domestic dog, <i>Canis familiaris</i> (L.)	1	0.8
Virginia opossum, <i>Didelphis virginiana</i> (Kerr)	1	0.8
Red fox, <i>Vulpes vulpes</i> (L.)	1	0.8
Avian		
American robin	9	7.6

<sup>a</sup> Includes six specimens from which double bloodmeals were identified.

**Table 3.** Number and percentage of avian and mammalian bloodmeals identified from *Cs. melanura* collected in Connecticut, 2002–2004

Host	No. <sup>a</sup>	% total
<b>Avian</b>		
American robin	11	22.9
Wood thrush, <i>Hylocichla mustelina</i> (Gmelin)	6	12.5
Gray catbird	5	10.4
Tufted titmouse	3	6.3
Veery, <i>Catarus fuscescens</i>	3	6.3
Song sparrow	3	6.3
Common grackle	3	6.3
Common yellowthroat, <i>Geothlypis trichas</i> (L.)	2	4.2
Cedar waxwing, <i>Bombycilla cedrorum</i> (Vieillot)	1	2.1
Northern cardinal, <i>Cardinalis cardinals</i> (L.)	1	2.1
American crow, <i>Corvus brachyrhynchos</i> (Brehm)	1	2.1
Brown-headed cowbird, <i>Molothrus ater</i> (Boddaert)	1	2.1
Northern parula, <i>Parula americana</i> (L.)	1	2.1
Black-capped chickadee, <i>Parus atricapillus</i> (L.)	1	2.1
House sparrow, <i>Passer domesticus</i> (L.)	1	2.1
Scarlet tanager, <i>Piranga olivacea</i> (Gmelin)	1	2.1
Ovenbird, <i>Seiurus aurocapillus</i> (L.)	1	2.1
Mourning dove, <i>Zenaidura macroura</i> (L.)	1	2.1
<b>Mammalian</b>		
White-tailed deer	3	6.3
Domestic cat	1	2.1
Northern raccoon	1	2.1

<sup>a</sup> Includes 3 specimens from which double-blood meals were identified.

potential secondary vectors of WNV in this region of the Northeastern United States.

We find that *Ae. vexans* feeds mainly on mammalian species, white-tailed deer and horse. This finding is in agreement with previous studies conducted in northeastern and other regions of the United States where the species has similarly been shown to have a decided preference for large mammals (Tempelis et al. 1967; Edman 1971; Suyemoto et al. 1973; Magnarelli 1977a,b; Ritchie and Rowley 1981; Nasci 1984; Irby and Apperson 1988, Ngo and Kramer 2003, Apperson et al. 2004). The identification of moderate numbers (7.6%) of avian-derived bloodmeals from a competent reservoir (American robins) (Komar et al. 2003) as well as mixed bloodmeals (5.0%) from both avian and mammalian hosts are particularly significant as these findings demonstrate a minimally necessary condition for acquisition of the virus from an avian host and subsequent transmission to mammals.

The apparent affinity of *Ae. vexans* for deer over other mammalian species in the current study is likely the function of their local abundance and availability. Although no formal census of host density was taken, white-tailed deer are the most abundant large mammals in the region after humans and were likewise found to be the predominant mammalian host for *Cx. salinarius* collected from similar locales within the state (Molaei et al. 2006). Nasci (1984) also has suggested that large mammals such as deer may have a greater tolerance for mosquito biting, resulting in more successful feeding on these hosts. The identi-

cation of a moderate number of horse-derived bloodmeals (9.2%) is significant and is consistent with the view that *Ae. vexans* is likely to be a relatively important "bridge vector" to horses in this region, given its abundance, prevalence of WNV infection (Andreadis et al. 2004), and moderate vector competence (Turell et al. 2001, 2005). However, in the absence of any identified human-derived bloodmeals, we cannot reliably comment on the role this mosquito may play in epidemic transmission of WNV to humans. Our analysis of bloodmeals from wild-caught female *Cs. melanura* revealed a very strong tendency for avian hosts (95.8% of total including avian/mammalian mixed meals) in agreement with previous investigations of populations from other regions of the Northeastern United States and elsewhere (Jobbins et al. 1961, Crans 1964, Schober and Collins 1966, Edman et al. 1972, Muul et al. 1975, Magnarelli 1977a, Nasci and Edman 1981, Irby et al. 1988, Ngo and Kramer 2003, Apperson et al. 2004). However, >10% of the bloodmeals identified from *Cs. melanura* were mammalian-derived. This finding was significant and nearly identical to results obtained in nearby New Jersey (Apperson et al. 2004), where 10.3% of the bloodmeals identified in *Cs. melanura* (seven of 68) collected from dense pine plantations in the vicinity of permanent freshwater swamps (red maple–white cedar) were of mammalian origin as well (white-tailed deer, raccoon, and human). It is not unreasonable to thus infer that although *Cs. melanura* is largely ornithophilic in its feeding patterns, it could occasionally function as a bridge vector of WNV to mammals, especially white-tailed deer. White-tailed deer are among the most abundant large mammals in the Northeastern United States and exposure to WNV has been documented previously (Farajollahi et al. 2004). The identification of mixed avian- and mammalian-derived bloodmeals in 6% of the analyzed bloodmeals for this species is also consistent with this conclusion.

Seventeen species of Passeriformes and one species of Columbiformes (mourning dove) were identified as avian hosts for *Cs. melanura* in our study. This was double the number of bird species identified by Apperson et al. (2004) from a similar sample size ( $n = 44$ ) of *Cs. melanura* collected in New Jersey. This difference was most likely due to our collections from a more diverse array of sampling sites. American robin, wood thrush, and gray catbird were among the most common, in contrast to northern cardinal, tufted titmouse, and American robin in the Apperson et al. (2004) study. In both instances, American crow-derived bloodmeals were either not detected (Apperson et al. 2004) or extremely rare ( $n = 1$ ) as was similarly reported with other established mosquito vectors (*Cx. pipiens*, *Cx. restuans*, and *Cx. salinarius*) of WNV in the northeast (Apperson et al. 2002, 2004; Molaei et al. 2006). Mortality attributed to WNV infection has been reported in all 18 of the avian species identified in our study (CDC 2005a), four of which (American crow, American robin, house sparrow, and mourning dove) have been evaluated and determined to be reservoir competent (Komar et al. 2003). Seroprevalence stud-



ies of local populations from several metropolitan areas in the vicinity of New York City (Komar et al. 2001, Nasci et al. 2002) have further detected neutralizing antibodies in American robin, gray catbird, northern cardinal, and house sparrow. American robin has been correspondingly shown to be the most common source of avian blood for local northeastern populations of other ornithophilic mosquitoes, *Cx. pipiens* and *Cx. restuans* (Apperson et al. 2004, Molaei et al. 2006), further implicating the potential role of this bird as an important amplifying host in the WNV transmission cycle in this region. Although the vector competence of *Cs. melanura* for WNV has not been fully evaluated, the species is susceptible and develops disseminated infections after feeding on viremic birds, a necessary condition for transmission (Turell et al. 2005). Its abundance in sylvan environments and frequent isolation of WNV in nature (Andreadis et al. 2004), in concert with the present findings on its host feeding preferences for reservoir competent birds, are consistent with the view that this species plays a secondary role in enzootic transmission of WNV among free-ranging birds in more rural environments.

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