Short Communication

Vector Competence and Capacity of *Culex erraticus* (Diptera: Culicidae) for Eastern Equine Encephalitis Virus in the Southeastern United States

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Abstract

Field studies of the ecology of eastern equine encephalitis virus (EEEV; family *Togaviridae*, genus *Alphavirus*) in the southeastern United States have demonstrated that *Culex erraticus* (Dyar and Knab) is the most common mosquito at many enzootic sites and is often infected with the virus. However, the competence of *Cx. erraticus* for EEEV has not been explored in detail. *Culex erraticus* females were collected from the field and fed upon EEEV-infected chicks. The infected mosquitoes were provided honey for nutrition and to monitor for time to infectiveness. Of the mosquitoes that survived the 14-d postfeeding period, 89% were infected and 84% had evidence of a disseminated infection, though titers were generally low. EEEV was first detected in honey 6 d postinfection and was detected in samples collected from 94% of the mosquitoes with a disseminated infection overall. These data and others were then employed to estimate the relative vectorial capacity of *Cx. erraticus* at an EEEV enzootic site in Alabama. The vectorial capacity of *Cx. erraticus* at this site was 44% of *Culiseta melanura* (Coquillett), the accepted enzootic vector, suggesting *Cx. erraticus* may play a role in transmitting EEEV in areas where it is abundant and *Cs. melanura* rare.

Key words: *Culex erraticus*, eastern equine encephalitis, arbovirus, Alabama

Eastern equine encephalitis virus (EEEV; family *Togaviridae*, genus *Alphavirus*) is the most pathogenic arbovirus endemic to the United States. Over 35% of the symptomatic cases result in fatalities, with most survivors suffering long-term neurological complications (Villari et al. 1995). In the northeastern United States, EEEV circulates primarily in a mosquito–avian cycle, usually within freshwater swamp habitats (Moncayo et al. 2000). Enzootic transmission of EEEV in this region is primarily mediated by *Culiseta melanura* (Coquillett), which feeds almost exclusively upon passerine birds (songbirds; Magnarelli 1977, Molaei et al. 2006, Burkett-Cadena et al. 2015). *Culiseta melanura*, while the most efficient enzootic vector of EEEV, is generally not considered an important bridge vector, as it rarely feeds on mammals (Edman et al. 1972, Nasci and Edman 1981, Molaei and Andreadis 2006, Estep et al. 2011). *Aedes sollicitans* (Walker), *Culex salinarius* (Coquillett), *Coquillettidia perturbans* (Williston) (Crans and Schulze 1986), and *Aedes vexans* (Meigen) have been implicated as bridge vectors in different habitats in the northeast (Chamberlain et al. 1956, Vaidyanathan et al. 1997).

The ecology of EEEV transmission in the southeastern United States may differ from that in the northeast. In the southeast, *Cs. melanura* is often rare in habitats supporting EEEV enzootic transmission (Cupp et al. 2003, 2004; Cohen et al. 2009, Mukherjee et al. 2012). Here, *Culex erraticus* (Dyar and Knab) is by far the most abundant mosquito species (Cupp et al. 2003, 2004; Cohen et al. 2009; Mukherjee et al. 2012), and is also the mosquito species most commonly infected with EEEV (Cupp et al. 2003, Mukherjee et al. 2012). *Culex erraticus* feeds primarily on avian hosts in the spring and summer months, then switches to feeding primarily upon mammalian hosts in the summer and fall (Burkett-Cadena et al. 2011, Oliveira et al. 2011). It has been hypothesized that *Cx. erraticus* may play an important role in the transmission of EEEV in the southeast (Cupp et al. 2003, Cohen et al. 2009, Mukherjee et al. 2012). However, apart from one early report listing *Cx. erraticus* as a “fair” vector for EEEV (Chamberlain et al. 1954), the competency of this species has not been explored. Here, we report studies investigating the vector competency and capacity of *Cx. erraticus* for EEEV in the southeastern United States.

Materials and Methods

Mosquito collections were carried out at John B. Sergeant Park and Lettuce Lake Park in Hillsborough County, FL. Details of these sites may be found in a previous publication (Bingham et al. 2014). Resting adult mosquitoes were collected from natural and artificial resting sites (Burkett-Cadena 2011) using a handheld aspirator (Burkett-Cadena et al. 2008a). Mosquitoes were transported to the laboratory and identified to species using standard morphological keys for mosquitoes of the southeastern United States (Burkett-Cadena 2013).

Culex erraticus females were divided evenly among host exposure chambers. The chambers were constructed of polypropylene light trap collection containers (Model 2801B, BioQuip Products, Rancho Dominguez, CA). The top of the chamber was fitted with netting to allow mosquito blood feeding, but prevent escape. The bottom of the chamber consisted of 40 by 40 aluminum mesh. A 10-mm-diameter hole was drilled into the side of each mosquito chamber to allow the insertion and removal of a 1.5-ml microcentrifuge tube with the bottom portion cut away. Mosquitoes were given access to carbohydrates via honey placed inside the microcentrifuge tube. In this way, honey could be replaced and tested for presence of EEEV. The honey drops present in each of the containers were tested for the presence of EEEV by reverse transcription-polymerase chain reaction (RT-PCR) as previously described (Bingham et al. 2014), to ensure that none of the mosquitoes included in the study were previously infected with EEEV. Mosquitoes were maintained at 28°C under a photoperiod of 13:11 (L:D) h. Prior to transmission experiments, mosquitoes were fed honey for 4 d, then starved of honey for 48 h before being allowed to feed upon an infected chicken.

Five 8-d-old chickens were infected via subcutaneous injection with 1×10^4 plaque forming units (PFU) of EEEV strain M05-316, which was originally isolated from a pool of *Culex nigripalpus* Theobald collected in Volusia County, FL, in May 2005 and passaged twice in Vero cells (White et al. 2011). The infected birds were returned to isolation cages (Tecniplast, Buguggiate, Italy) and maintained at 28°C for 24 h to permit them to develop an infectious viremia. The infected birds were then immobilized on the containers containing the adult *Cx. erraticus* described above. The mosquitoes were permitted to feed ad libitum on the infected chickens. The infected birds were euthanized, and the concentration of EEEV in their blood was determined by RT-PCR and plaque assay as previously described (Beatty et al. 1989, Lambert et al. 2003). The mean titer of the five infected birds was $1.01 \times 10^5 \pm 4 \times 10^4$ PFU/ml, a titer similar to that seen in wild avian reservoirs of the virus (Arrigo et al. 2010). Blood-engorged mosquitoes were identified by visual inspection of their abdomens, transferred to individual containers and incubated for 14 d postinfection. Fresh honey was supplied every 2 d, and used honey tested for the presence of EEEV by RT-PCR. Following the 14-d experimental period, mosquitoes were sacrificed, separated into legs and bodies, and tested for the presence of EEEV RNA by quantitative RT-PCR as previously described (Bingham et al. 2014). Samples were scored positive only if they gave a positive result (Ct value <40) in both initial and confirmatory assays. Viral titers in positive samples were calculated by reference to Ct values derived from a standard curve consisting of RNA extracted from dilutions of a standard culture whose titer was determined by plaque assay (Beatty et al. 1989). Vectorial capacity was calculated using the formula

$$V = \frac{ma^2 p^n b}{-lmp}$$

following Garret-Jones (Garret-Jones 1964). The 95% confidence intervals (95% CI) surrounding the percent estimates were calculated as previously described (Apperson et al. 2002).

Animal experiments were performed under Protocol R4158 approved by the Institutional Animal Care and Use Committee and Protocol number 0972 approved by the Institutional Biosafety Committee of the University of South Florida. Experiments were performed in an ABSL3 facility approved for select agent use (EEEV) by the Centers for Disease Control (registration number C20112014-1298).

Results

In total, 35/37 *Cx. erraticus* successfully fed upon infected chicks. Of these, 19/35 (54%; 95% CI 38–70%) survived through the 14-d postinfection study period (Fig. 1). Of the mosquitoes that survived, 17/19 (89%; 95% CI 76–100%) had detectable EEEV in their bodies, indicating that they had become infected. Of the survivors, 16/19 (84%; 95% CI 68–100%) had detectable EEEV RNA in their legs, indicative of a disseminated infection. Of the mosquitoes that did not survive, 5/16 were recovered in a decomposed state and could not be tested. Of those that did not survive and could be assayed, 10/11 (91%; 95% CI 74–100%) contained detectable EEEV in their bodies and 2/11 (18%; 95% CI 0–41%) contained EEEV in both bodies and legs. Viral titers in surviving positive mosquitoes ranged from $1-7 \times 10^6$ PFU/ml in the positive bodies and $1-7 \times 10^5$ PFU/ml in the legs (Fig. 2). Two of the 19 survivors (10.5%; 95% CI 0–24%) exhibited titers in the legs of $>1 \times 10^5$ /ml, the mean titer seen in laboratory-infected *Cs. melanura* (Scott et al. 1984).

The honey drops provided to the individual mosquitoes were also collected and assayed for the presence of EEEV. EEEV RNA first appeared in the collected honey 6 d postinfection. Overall, 15/16 (94%; 95% CI 82–100%) of the mosquitoes with a disseminated infection expectorated detectable RNA into the honey drops at least once in days 6–14 postinfection. None of the honey drops collected from the mosquitoes that tested negative in both the body or leg assays were positive, nor were positive honey drops detected from the single mosquito found to have detectable virus in the body but not the legs.

These data were combined with data from previously published laboratory and field studies to estimate the relative vectorial capacity of *Cx. erraticus* and *Cs. melanura* at an EEEV enzootic site in the Tuskegee National Forest of Alabama (TNF), where *Cx. erraticus* is common and *Cs. melanura* is relatively rare (Cupp et al. 2003). Assuming that only individuals with titers $>1 \times 10^5$ /ml were

Fig. 1. Daily survival of *Cx. erraticus* infected with EEEV.

Fig. 2. Titer of EEEV in bodies and legs of EEEV-infected *Cx. erraticus*. Titers were determined using quantitative RT-PCR with reference to a standard curve derived from serial dilutions of a viral culture.

infectious, the relative vectorial capacity of *Cx. erraticus* was calculated to be 44% of *Cs. melanura* at the TNF site (Table 1).

Discussion

We were unable to colonize *Cx. erraticus* in the laboratory, and thus had to rely upon wild-caught specimens to conduct these experiments, resulting in a relatively small number of mosquitoes examined. However, as >80% of the *Cx. erraticus* that survived were found to have developed a disseminated infection, we could conclude that viral development in this species occurs frequently. Despite this, it appears that overall replication of EEEV in *Cx. erraticus* was inefficient, with just 10.5% of the surviving mosquitoes exhibiting titers that were comparable with those seen in *Cs. melanura* (Scott et al. 1984). This proportion agrees with the findings of Chamberlain, who reported that 14% of experimentally infected *Cx. erraticus* became infectious with EEEV; Chamberlain rated *Cx. erraticus* as a “fair” EEEV vector based upon this finding (Chamberlain et al. 1954). However, 94% of the females with disseminated infections in this study deposited viral RNA in the honey. It is likely that this was a result of the presence of EEEV RNA in their saliva and not a result of direct passage of virus present in the bloodmeal through the digestive tract, as only the honey samples from mosquitoes with a disseminated infection were found to be positive, and positive honey samples were not detected until 6 d postinfection. As we were not able to induce these females to oviposit and take another bloodmeal in the laboratory, it was not possible for us to determine if these mosquitoes were actually capable of infecting another host. If these low-titer individuals were capable of transmission in some cases, *Cx. erraticus* would be a more competent vector than the viral titers may indicate, though still not as efficient as *Cs. melanura*.

Cx. erraticus appears to be inferior to *Cs. melanura* as a vector in two additional respects. First, the extrinsic incubation period for *Cx. erraticus* (6 d) appears to be twice that of *Cs. melanura* (3 d; Scott and Burrage 1984). Second, while *Cs. melanura* feeds almost exclusively upon avian hosts, *Cx. erraticus* is a much more catholic feeder (Oliveira et al. 2011). However, the longer incubation period and lower feeding rates on the enzootic reservoirs (birds) are

Table. 1. Calculation of relative vectorial capacity of *Cx. erraticus* and *Cs. melanura* at TNF

Variable	Definition	<i>Cx. erraticus</i> (source)	<i>Cs. melanura</i> (source)
M	Relative population density	46 (Cupp et al. 2003)	1 (Cupp et al. 2003)
A	Proportion of avian bloodmeals	0.33 (Oliveira et al. 2011)	0.94 (Molaei et al. 2006)
P	Daily survival probability	0.954 (this study)	0.961 (Cooper et al. 2000)
b	Vector competence	0.105 (this study)	1.0 (Scott et al. 1984)
n	Extrinsic incubation period	6 (this study)	3 (Scott and Burrage 1984)
V	Vectorial capacity	8.4 (this study)	19.2 (this study)

balanced by the greater relative abundance of *Cx. erraticus* in the southeastern United States (Cupp et al. 2004, Mukherjee et al. 2012, Bingham et al. 2014). *Culex erraticus* is by far the most abundant mosquito at most of the EEEV enzootic sites in the southeastern United States examined, while *Cs. melanura* is often rare (Cupp et al. 2003, 2004; Mukherjee et al. 2012), especially during the peak epizootic transmission months of June, July, and August (Burkett-Cadena et al. 2008a). Furthermore, due to the catholic feeding behavior, utilizing avian, mammalian, and ectothermic hosts (Burkett-Cadena et al. 2008b), *Cx. erraticus* may act both as the enzootic amplification and bridge vector for EEEV. *Culex erraticus* exhibits a shift in feeding behavior, frequently feeding upon avian species in the spring and summer and then shifting almost exclusively to feeding upon mammals in midsummer through the autumn (Burkett-Cadena et al. 2011, Oliveira et al. 2011). This pattern corresponds to patterns of EEEV infection observed in mammals (particularly horses) throughout most of the virus’ range (Scott and Weaver 1989). Interestingly, the favored avian hosts of *Cx. erraticus* are wading birds (Hassan et al. 2003, Bingham et al. 2014), a group that has been shown to be quite susceptible to EEEV infection and may thus represent important enzootic amplification hosts for the virus (McLean et al. 1995). Taken together, these studies suggest, given its high abundance at many sites in the southeastern United States, *Cx. erraticus* is likely to play a role in EEEV transmission in this region.

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References Cited

Apperson, C. S., B. A. Harrison, T. R. Unnasch, H. K. Hassan, W. S. Irby, H. M. Savage, S. E. Aspen, D. W. Watson, L. M. Rueda, B. R. Engber, et al. 2002. Host-feeding habits of *Culex* and other mosquitoes (Diptera: Culicidae) in the borough of Queens in New York City, with characters and techniques for identification of *Culex* mosquitoes. *J. Med. Entomol.* 39: 777–785.

Arrigo, N. C., A. P. Adams, D. M. Watts, P. C. Newman, and S. C. Weaver. 2010. Cotton rats and house sparrows as hosts for North and South American strains of eastern equine encephalitis virus. *Emerg. Infect. Dis.* 16: 1373–1380.

Beatty, B. J., C. H. Calisher, and R. E. Shope. 1989. Arboviruses, pp. 797–855. In N. Schmidt and R. Emmons (eds.), *Diagnostic procedures for viral*,

- rickettsial and chlamydial infections, 6th ed. American Public Health Association, Washington, DC.
- Bingham, A., N. Burkett-Cadena, H. K. Hassan, C.J.W. McClure, and T. R. Unnasch. 2014. Field investigations of winter transmission of Eastern Equine Encephalitis virus in Florida. *Am. J. Trop. Med. Hyg.* 91: 685–693.
- Burkett-Cadena, N. D. 2011. A wire-frame shelter for collecting resting mosquitoes. *J. Am. Mosq. Control Assoc.* 27: 152–155.
- Burkett-Cadena, N. D. 2013. Mosquitoes of the Southeastern United States, University of Alabama Press, Tuscaloosa, AL.
- Burkett-Cadena, N. D., M. D. Eubanks, and T. R. Unnasch. 2008a. Preference of female mosquitoes for natural and artificial resting sites. *J. Am. Mosq. Control Assoc.* 24: 228–235.
- Burkett-Cadena, N. D., A. M. Bingham, B. Hunt, G. Morse, and T. R. Unnasch. 2015. Ecology of *Culiseta melanura* and other mosquitoes (Diptera: Culicidae) from Walton county, Florida during winter period 2013–2014. *J. Med. Entomol.* 54: 1074–1082.
- Burkett-Cadena, N. D., S. P. Graham, H. K. Hassan, C. Guyer, M. D. Eubanks, C. R. Katholi, and T. R. Unnasch. 2008b. Blood feeding patterns of potential arbovirus vectors of the genus *Culex* targeting ectothermic hosts. *Am. J. Trop. Med. Hyg.* 79: 809–815.
- Burkett-Cadena, N. D., C. J. McClure, R. A. Ligon, S. P. Graham, C. G. Guyer, G. E. Hill, S. S. Ditchkoff, M. D. Eubanks, H. K. Hassan, and T. R. Unnasch. 2011. Host reproductive phenology drives seasonal patterns of host use in mosquitoes. *PLOS ONE* 7: e17681.
- Chamberlain, R. W., R. K. Sikes, D. B. Nelson, and W. D. Sudia. 1954. Studies on the North American arthropod-borne encephalitis. VI. Quantitative determinations of virus-vector relationships. *Am. J. Hyg.* 60: 278–285.
- Chamberlain, R. W., R. E. Kissling, D. D. Stamm, and W. D. Sudia. 1956. Transmission of eastern equine encephalitis to horses by *Aedes sollicitans* mosquitoes. *Am. J. Trop. Med. Hyg.* 5: 802–808.
- Cohen, S. B., K. Lewoczko, D. B. Huddleston, E. Moody, S. Mukherjee, J. R. Dunn, T. F. Jones, R. Wilson, and A. C. Moncayo. 2009. Host feeding patterns of potential vectors of eastern equine encephalitis virus at an enzootic focus in Tennessee. *Am. J. Trop. Med. Hyg.* 81: 452–456.
- Cooper, L. A., B. J. Sina, M. J. Turell, and T. W. Scott. 2000. Effects of initial dose on eastern equine encephalomyelitis virus dependent mortality in intrathoracically inoculated *Culiseta melanura* (Diptera: Culicidae). *J. Med. Entomol.* 37: 815–819.
- Crans, W. J., and T. L. Schulze. 1986. Evidence incriminating *Coquillettidia perturbans* (Diptera: Culicidae) as an epizootic vector of eastern equine encephalitis. I. Isolation of EEE virus from *C. perturbans* during an epizootic among horses in New Jersey. *Bull. Soc. Vector Ecol.* 11: 178–184.
- Cupp, E. W., K. Klingler, H. K. Hassan, L. M. Viguers, and T. R. Unnasch. 2003. Transmission of eastern equine encephalomyelitis virus in central Alabama. *Am. J. Trop. Med. Hyg.* 68: 495–500.
- Cupp, E. W., K. J. Tennessen, W. K. Oldland, H. K. Hassan, G. E. Hill, C. R. Katholi, and T. R. Unnasch. 2004. Mosquito and arbovirus activity during 1997–2002 in a wetland in northeastern Mississippi. *J. Med. Entomol.* 41: 495–501.
- Edman, J. D., L. A. Webber, and H. W. Kale. 1972. Host-feeding patterns of Florida mosquitoes. II. *Culiseta*. *J. Med. Entomol.* 9: 429–434.
- Estep, L. K., C.J.W. McClure, N. Burkett-Cadena, H. K., Hassan, T. L. Hicks, T. R. Unnasch, and G. E., Hill. 2011. A multi-year study of mosquito feeding patterns on avian hosts in a southeastern focus of eastern equine encephalitis virus. *Am. J. Trop. Med. Hyg.* 84: 718–726.
- Garret-Jones, C. 1964. Prognosis for the interruption of malaria transmission through assessment of a mosquito's vectorial capacity. *Nature* 204: 1173–1175.
- Hassan, H. K., E. W. Cupp, G. E. Hill, C. R. Katholi, K. Klingler, and T. R. Unnasch. 2003. Avian host preference by vectors of Eastern Equine Encephalomyelitis virus. *Am. J. Trop. Med. Hyg.* 69: 641–647.
- Lambert, A. J., D. A. Martin, and R. S. Lanciotti. 2003. Detection of North American eastern and western equine encephalitis viruses by nucleic acid amplification assays. *J. Clin. Microbiol.* 41: 379–385.
- Magnarelli, L. A. 1977. Host feeding patterns of Connecticut mosquitoes (Diptera: Culicidae). *Am. J. Trop. Med. Hyg.* 26: 547–552.
- McLean, R. G., W. J. Crans, D. F. Caccamise, J. McNelly, L. J. Kirk, C. J. Mitchell, and C. H. Calisher. 1995. Experimental infection of wading birds with eastern equine encephalitis virus. *J. Wildl. Dis.* 31: 502–508.
- Molaei, G., and T. G. Andreadis. 2006. 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. *J. Med. Entomol.* 43: 1088–1093.
- Molaei, G., J. Oliver, T. G. Andreadis, P. M. Armstrong, and J. J. Howard. 2006. Molecular identification of blood-meal sources in *Culiseta melanura* and *Culiseta morsitans* from an endemic focus of Eastern Equine Encephalitis virus in New York. *Am. J. Trop. Med. Hyg.* 75: 1140–1147.
- Moncayo, A. C., J. D. Edman, and J. T. Finn. 2000. Application of geographic information technology in determining risk of eastern equine encephalomyelitis virus transmission. *J. Am. Mosq. Control Assoc.* 16: 28–35.
- Mukherjee, S., E. E. Moody, K. Lewoczko, D. B. Huddleston, J. Huang, M. E. Rowland, R. Wilson, J. R. Dunn, T. F. Jones, and A. C. Moncayo. 2012. Eastern equine encephalitis in Tennessee: 2002–2008. *J. Med. Entomol.* 49: 731–738.
- Nasci, R. S., and J. D. Edman. 1981. Blood-feeding patterns of *Culiseta melanura* (Diptera: Culicidae) and associated sylvan mosquitoes in southeastern Massachusetts eastern equine encephalitis enzootic foci. *J. Med. Entomol.* 18: 493–500.
- Oliveira, A., C. R. Katholi, N. D. Burkett-Cadena, H. K. Hassan, S. Kristensen, and T. R. Unnasch. 2011. Temporal analysis of feeding patterns of *Culex erraticus* in Central Alabama. *Vector Borne Zoon. Dis.* 11: 413–421.
- Scott, T. W., and T. G. Burrage. 1984. Rapid infection of salivary glands in *Culiseta melanura* with eastern equine encephalomyelitis virus: An electron microscopy study. *Am. J. Trop. Med. Hyg.* 33: 961–964.
- Scott, T. W., and S. C. Weaver. 1989. Eastern equine encephalomyelitis virus: Epidemiology and evolution of mosquito transmission. *Adv. Virus Res.* 37: 277–328.
- Scott, T. W., S. W. Hildreth, and B. J. Beaty. 1984. The distribution and development of Eastern Equine Encephalitis virus in its enzootic mosquito vector, *Culiseta melanura*. *Am. J. Trop. Med. Hyg.* 33: 300–310.
- Vaidyanathan, R., J. D. Edman, L. A. Cooper, and T. W. Scott. 1997. Vector competence of mosquitoes (Diptera: Culicidae) from Massachusetts for a sympatric isolate of eastern equine encephalomyelitis virus. *J. Med. Entomol.* 34: 346–352.
- Villari, P., A. Spielman, N. Komar, M. McDowell, and R. J. Timperi. 1995. The economic burden imposed by a residual case of eastern encephalitis. *Am. J. Trop. Med. Hyg.* 52: 8–13.
- White, G. S., B. E. Pickett, E. J. Lefkowitz, C. L. Ottendorfer, L. M. Stark, and T. R. Unnasch. 2011. Phylogenetic analysis of eastern equine encephalitis virus isolates from Florida. *Am. J. Trop. Med. Hyg.* 84: 709–717.