BioOne COMPLETE

# Blood Meal Identification from Florida Mosquitoes (Diptera: Culicidae)

Authors: Watts, Stacey L., Fitzpatrick, Daniel M., and Maruniak, James E.

Source: Florida Entomologist, 92(4) : 619-622

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.092.0414

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

Downloaded From: https://bioone.org/journals/Florida-Entomologist on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

# BLOOD MEAL IDENTIFICATION FROM FLORIDA MOSQUITOES (DIPTERA: CULICIDAE)

STACEY L. WATTS, DANIEL M. FITZPATRICK AND JAMES E. MARUNIAK
Department of Entomology and Nematology, University of Florida, P.O. Box 110620, Gainesville FL, 32611-0620

ABSTRACT

Mosquitoes from different species endemic to North Florida were collected over a 12-month period, and blood meal analyses were conducted on engorged females to determine the range of vertebrate hosts each species fed on. Thirty-one mosquito blood meals from 6 mosquito species were identified, and blood meal hosts identified included horse, cow, armadillo, deer, raccoon, rabbit, and owl. Several mosquitoes captured in the study should be considered potential bridge vectors for encephalitis viruses considering their ubiquity, their preference for mammalian blood meals, and their competence as viral vectors.

Key Words: host identification, host preference, Alachua County, Florida, polymerase chain reaction, viral vectors

RESUMEN

Varias especies endémicas de mosquitos de la región norte de Florida fueron colectadas durante un periodo de 12 meses y la comida de sangre de hembras fue analizada para determinar los hospederos de los cuales los mosquitos se habían alimentado. Un total de treinta y una comidas de sangre obtenidas de seis diferentes especies de mosquitos identificaron a los siguientes hospederos: caballo, vaca, armadillo, ciervos, mapache, conejo, y búho. Varios de los mosquitos capturados en este estudio pueden considerarse vectores potenciales de virus de encefalitis debido a su omnipresencia, su preferencia por sangre de mamíferos, y por ser vectores virales competentes.

Translation provided by the authors.

Mosquitoes vector a variety of pathogens of medical and veterinary importance. Growing concern over the spread of West Nile virus (WNV) and related encephalitis viruses has prompted extensive investigation into the host-feeding patterns and preferences, as well as vectoral capacities, of pests now viewed mainly as cosmopolitan nuisances (Sardelis et al. 2001; Turell et al. 2005; Rodrigues & Maruniak 2006; Molaei et al. 2008). Host-feeding patterns and preferences vary according to a number of innate, seasonal, and environmental tendencies, including host availability and abundance, flight behavior and feeding periodicity of mosquitoes (Molaei et al. 2008) as well as by region (Turell et al. 2005). The current study sought to collect an array of mosquito species native to North Florida over a 12-month period and identify the range of vertebrate hosts that those mosquitoes fed on through blood meal analysis.

## MATERIALS AND METHODS

### Mosquito Collection and Identification

Mosquitoes were collected in $CO_2$-baited Center for Disease Control (CDC) light traps (John W. Hock, Gainesville, FL) from May 2006 through Apr 2007 from 11 sites in Alachua County, Florida. The habitats chosen were sampled due to proximity either to aquatic environments or to habitats of suspected vertebrate hosts. Traps were placed on site in late afternoon and collected the next morning, approximately 18 h later. Captured mosquitoes were transferred to the University of Florida while still alive in mesh collection bags from the trap, placed in a -70°C freezer to euthanize them, and stored in the freezer until subsequent identification and processing. Mosquitoes were sorted by species as described in Darsie & Morris (2003) on a -20°C chill-table, and those without apparent blood meals were pooled in groups of up to 25 in sterile microcentrifuge tubes, while female mosquito abdomens with apparent blood meals were homogenized individually.

### PCR Amplification and Sequencing of Mosquito Blood Meals

DNA was extracted with the DNAzol® Direct Extraction Kit (MRC, Cincinnati, OH). Polymerase chain reactions (PCR) were conducted on extracted DNA with vertebrate-specific primer sets and cycling conditions described in Cupp et al. (2004) that preferentially amplified a 290-bp region from the cytochrome *b* gene within the mitochondrial DNA of vertebrates. Briefly, the PCR

Downloaded From: https://bioone.org/journals/Florida-Entomologist on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

reactions were conducted in 50 µL reactions with the following reagents: 5.0 µL of 10X reaction buffer (600 mM Tris-HCl pH 8.5, 150 mM $(NH_4)_2SO_4$, 35 mM $MgCl_2$), 0.2 mM dNTPs, 25 pmol of each primer, 2.5 units of *Taq* Polymerase (Invitrogen, Carlsbad, CA) and 2.5 µL of DNA template. If 290 bp amplicons were not produced or were not able to be identified, a second PCR was conducted with mammalian-specific primer sets and cycling conditions described in Ngo & Kramer (2003) to preferentially amplify a 772-bp region from the cytochrome b gene. Briefly, the PCR reactions were conducted in 25 µL reactions with the following reagents: 2.5 µL of 10X reaction buffer (200 mM Tris-HCl pH 8.4, 500 mM KCl), 3.0 mM $MgCl_2$, 0.5 mM dNTPs, 5.0 pmol of each primer, 1.25 units of *Taq* Polymerase, and 2.5 µL of blood meal DNA template. Agarose gel electrophoresis was used to separate amplicons, and DNA was purified from excised bands with the expected size with QIAquick columns (QIAGEN, Valencia, CA). Fragments were sequenced with BigDye Terminator Sequencing Ready Reaction Kits (Applied Biosystems, Foster City, CA) at the Interdisciplinary Center for Biotechnology Research at the University of Florida. Sequences were analyzed and edited with Sequencher™ software (Gene Codes Co., Ann Arbor, MI), and were compared with the GenBank database entries by the BLAST program from NCBI (http://blast.ncbi.nlm.nih.gov) to identify the vertebrate host on which each mosquito had fed.

## Results

Sixty-one of the 45,692 mosquitoes collected showed apparent blood meals. Upon amplification, 57 of those 61 samples (93%) showed amplicons of the expected size. Of those, vertebrate hosts were identified in 33 individuals (58%) (Table 1). Blood meals were most commonly identified from hosts fed on by *Coquillettidia perturbans* Walker, a particularly opportunistic feeder (Molaei et al. 2008). *Coquillettidia perturbans* blood meals represented 5 mammalian species (horse, armadillo, deer, rabbit, and raccoon) and 1 avian species (owl). Blood meals were identified from 6 horses and 4 cows in the 10 *Mansonia titillans* Walker, from 2 horses and 1 cow in 3 *Aedes vexans* Meigen, from 1 cow and 1 deer in 2 *Culex salinarius* Coquillett, and from 1 rabbit in *Culex erraticus* Dyer & Knab, while all 3 *Anopheles crucians* Wiedemann fed upon horses. The identity of the blood meal match with GenBank sequences ranged from 89-100%, and the best match was shown in Table 1.

## Discussion

Of the 6 species of blood-fed mosquitoes captured in the study, 4 species fed from horses,

Table 1. Vertebrate-derived bloodmeals identified from mosquito species captured in Alachua Co. (Florida) from 2006-2007.

| Mosquito species | Associated with Arbovirus[1] | Total Hosts Identified | Number of Confirmed Hosts (Range of % Nucleotide Identity with GenBank database)[2] | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Horse | Cow | Armadillo | Deer | Rabbit | Raccoon | Owl |
| *Ae. vexans* | EEE[3],SLE[3],WEE[3],WNV[4] | 3 | 2 (91-97) | 1 (100) | | | | | |
| *An. cruicans* | EEE[5],WNV[6] | 3 | 3 (95-99) | | | | | | |
| *Cq. perturbans* | EEE[7],WNV[8] | 14 | 5 (89-97) | | 3 (97) | 2 (97-98) | 2 (96-97) | 1 (91) | 1 (92) |
| *Cx. erraticus* | EEE[5],SLE[5] | 1 | | | | | 1 (96) | | |
| *Cx. salinarius* | EEE[3],SLE[3] | 2 | | 1 (99) | | 1 (96) | | | |
| *Ma. titillans* | WNV[5] | 10 | 6 (91-97) | 4 (99-100) | | | | | |

1 - Known association with other viruses with a similar transmission cycle. EEE, eastern equine encephalomyelitis virus; SLE, St. Louis encephalitis virus; WEE, western equine encephalomyelitis virus, WNV, West Nile virus. Based on 3 (Turell et al. 2005), 4 (Goddard et al. 2002), 5 (Cupp et al. 2004), 6 (CDC 2007), 7 (Vaidyanathan et al. 1997), 8 (Sardelis et al. 2001).

2 - Best Match GenBank Accession Numbers: Horse (AY819737.1, EU308069.1, or EU433683.1), Cow (EU365345.1), Armadillo (Y11832.1), Deer (AF535866.1), Rabbit (AY292722.1), Raccoon (AB291073.1), Owl (DQ190851.1).

Downloaded From: https://bioone.org/journals/Florida-Entomologist on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

which are particularly susceptible to diseases caused by encephalitis viruses. Preferential feeding on horses by *Ae. vexans* is a major concern, as *Ae. vexans* is considered a competent to highly-efficient vector for Saint Louis encephalitis (SLE), western equine encephalomyelitis (WEE), eastern equine encephalomyelitis (EEE), and WNV (Goddard et al. 2002; Turell et al. 2005).

*Coquillettidia perturbans* is considered a moderately competent vector of EEE (Vaidyanathan et al. 1997) and an inefficient but competent vector for WNV (Sardelis et al. 2001) in lab settings. EEE and WNV have been isolated from mosquitoes in nature throughout the Eastern and Southeastern United States (Srihongse et al. 1980; Crans & Schulze 1986; Edman et al. 1993; Godsey et al. 2005; Lukacik et al. 2006). *Anopheles crucians* and *Ma. titillans* showed a considerable affinity for horse hosts in a previous study by Cupp et al. (1986) as well, and have been positive for WNV isolations in the field (CDC 2007; Cupp et al. 2007). The paucity of research on *An. crucians* and *Ma. titillans* vectoral capacities for encephalitis viruses indicates that neither have historically been considered important disease vectors. However, considering their pervasiveness and their preference for mammalian blood meals in our study, as well as their moderate competence as viral vectors (Molaei et al. 2008), the potential for *Cq. perturbans* and *Ma. titillans* to be bridge vectors in the transfer of EEE and WNV from viremic primary hosts to mammals should still be considered.

$CO_2$-baited light traps, used in a series of studies conducted on the mosquitoes captured, are not considered particularly attractive to engorged mosquitoes, thus explaining the low percentage (61 of 45,692, or > 0.002%) of blood-fed mosquitoes captured. Future studies in mosquito blood meal analysis will supplement $CO_2$-baited light traps with mosquito resting boxes.

Of particular interest is the low yield of positive blood meal identifications in *An. crucians* (3 positive identifications in 12 blood meals). Townzen et al. (2008) had similar complications, reporting that co-amplification of *Anopheles* genes occurred often with cyt b primer sets, which consequently prevented positive host identification.

## Acknowledgments

We thank Dr. Alejandra Garcia-Maruniak for invaluable contributions and for help with editing the manuscript. This research was funded by USDA-CSREES, T-STAR grant # 00057913.

## References Cited

CDC—Centers For Disease Control and Prevention. 2007. West Nile Virus. http://www.cdc.gov/ncidod/dvbid/westnile/surv&controlCaseCount06_detailed.htm.

Crans, W. J., and Schulze, T. L. 1986. Evidence incriminating *Coquillettidia perturbans* (Diptera: Culicidae) as an epizootic vector of eastern equine encephalitis. Isolation of EEE virus from *Cq. perturbans* during an epizootic among horses in New Jersey. Bull. Soc. Vector Ecol. 11: 178-184.

Cupp, E. W., Hassan, H. K., Yue, X., Oldland, W. K., Lilley, B. M., and Unnasch, T. R. 2007. West Nile Virus infection in mosquitoes in the Mid-South U.S.A., 2002–2005. J. Med. Entomol. 44: 117-125.

Cupp, E. W., Scherer, F., Lok J. B., Brenner R. J., Dziem, G. M., and Ordonez, J. V. 1986. Entomological studies at an enzootic Venezuelan equine encephalitis virus focus in Guatemala, 1977-1980. American J. Trop. Med. Hyg. 35: 851-859.

Cupp, E. W., Zhang, D., Yue, X., Cupp, M. S., Guyer, C., Sprenger, T. R., and Unnasch, T. R. 2004. Identification of reptilian and amphibian blood meals from mosquitoes in an eastern equine encephalomyelitis virus focus in central Alabama. American J. Trop. Med. Hyg. 71: 272-276.

Darsie, Jr., R. F., and Morris, C. D. 2003. Keys to the Adult Females and Fourth Instar Larvae of the Mosquitoes of Florida. Vol. 1 (revised). Fort Myers, Florida: Florida Mosquito Control Assoc. 159 pp.

Edman, J. D., Timpari, R., and Werner, B. 1993. Epidemiology of eastern equine encephalitis in Massachusetts. J. Florida Mosq. Control Assoc. 864: 84-96.

Goddard, L. B., Roth, A. E., Reisen, W. K., and Scott, T. W. 2002. Vector competence of California mosquitoes for West Nile Virus. Emerg. Infect. Dis. 8: 1385-1391.

Godsey, Jr., M. S., Nasci, R., Savage, H. M., Aspen, S., King, R., Powers, A. M., Burkhatler, K., Colton, L., Charnetzky, D., Lasater, S., Taylor, V., and Palimisano, C. T. 2005. West Nile virus-infected mosquitoes, Louisiana, 2002. Emerg. Infect. Dis. 11: 1399-1404.

Lukacik, G., Anand, M., Shusas, E. J., Howard, J. J., Oliver, J., Chen, H., Backenson, P. B., Kauffman, E. B., Bernard, K. A., Kramer, L. D., and White, D. J. 2006. West Nile virus surveillance in mosquitoes in New York State, 2000-2004. J. American Mosq. Control Assoc. 22: 264-271.

Molaei, G., Andreadis, T. G., Armstrong, P. M., and Diuk-Wasser, M. 2008. Host-feeding patterns of potential mosquito vectors in Connecticut, U.S.A.: Molecular analysis of bloodmeals from 23 species of *Aedes*, *Anopheles*, *Culex*, *Coquillettidia*, *Psorophora*, and *Uranotaenia*. J. Med. Entomol. 45: 1143-1151.

Ngo, K. A., and Kramer, L. D. 2003. Identification of mosquito blood meals using polymerase chain reaction (PCR) with order-specific primers. J. Med. Entomol. 40: 215-222.

Rodrigues, S. C. G., and Maruniak, J. E. 2006. Blood meal identification from mosquitoes collected at a commercial alligator farm. J. American Mosq. Control Assoc. 22: 557-660.

Sardelis, M. R., Turell, M. J., Dohm, D. J., and O'Guinn, M. L. 2001. Vector competence of selected North American *Culex* and *Coquillettidia* mosquitoes for West Nile virus. Emerg. Infect. Dis. 7: 1018-1022.

Srihongse, S. J., Woodall, P., Grayson, M. A., and Deibel, R. 1980. Arboviruses in New York state: surveillance in arthropods and nonhuman vertebrates, 1972-1977. Mosq. News 40: 269-276.

Downloaded From: https://bioone.org/journals/Florida-Entomologist on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

TOWNZEN, J. S., BROWER, A. V., AND JUDD, D. D. 2008. Identification of mosquito bloodmeals using mitochondrial cytochrome oxidase subunit I and cytochrome b gene sequences. Med. Vet. Entomol. 22: 386-93.

TURELL, M. J., DOHM, D. J., SARDELIS, M. R., O' GUINN, M. L., ANDREADIS, T. G., AND BLOW, J. A. 2005. An update on the potential of North American mosquitoes (Diptera: Culicidae) to transmit West Nile Virus. J. Med. Entomol. 42: 57-62.

VAIDYANATHAN, R., EDMAN, J. D., COOPE, L. A., AND SCOTT, T. W. 1997. Vector competence of mosquitoes (Diptera: Culicidae) from Massachusetts for a sympatric isolate of eastern equine encephalomyelitis virus. J. Med. Entomol. 34: 346-52.

Downloaded From: https://bioone.org/journals/Florida-Entomologist on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use