

## SCIENTIFIC NOTE

### HUMAN BLOODFEEDING BY THE RECENTLY INTRODUCED MOSQUITO, *Aedes japonicus japonicus*, AND PUBLIC HEALTH IMPLICATIONS

GOUDARZ MOLAEI,<sup>1</sup> ARY FARAJOLLAHI,<sup>2,3</sup> JAMESINA J. SCOTT,<sup>4</sup> RANDY GAUGLER,<sup>2</sup> AND THEODORE G. ANDREADIS<sup>1</sup>

**ABSTRACT.** Knowledge of the host-feeding behavior and extent of interactions with human hosts are important in evaluating the role and vector potential of invasive mosquitoes in transmission of native arboviruses. We collected blood-engorged females of the recently established exotic species *Aedes japonicus japonicus* from sites in New Jersey during 2000 to 2007 and identified the sources of vertebrate blood meals by sequencing portions of the cytochrome *b* gene of mitochondrial DNA. Over 1/3 (36%,  $n = 36$ ) of the engorged mosquitoes acquired blood meals from humans. Other mammalian hosts included white-tailed deer (53%), fallow deer (5%), horse (3%), and Virginia opossum (3%). No avian, amphibian, reptilian, or mixed blood meals were identified. Our detection of a comparatively high prevalence of human bloodfeeding in *Ae. j. japonicus* in association with its local abundance, vector competence, and repeated detection of West Nile virus from field-collected specimens illustrates the potential for this invasive mosquito to serve as a “bridge” vector in transmission of West Nile and other mosquito-borne viruses in North America.

**KEY WORDS** *Aedes japonicus japonicus*, bloodfeeding behavior, West Nile virus, arboviruses

The global spread of exotic mosquito species as nuisance pests and potential vectors of diseases poses profound impacts on human health and creates serious challenges for public health and mosquito control agencies. *Aedes japonicus japonicus* (Theobald), an invasive container habitat species native to Japan, Korea, and Eastern China (Tanaka et al. 1979), was first discovered in the northeastern USA in 1998 (Peyton et al. 1999) and has rapidly spread throughout much of eastern North America and southern Canada (Andreadis et al. 2001, Fonseca et al. 2001, Sardelis and Turell 2001, Falco et al. 2002, Harrison et al. 2002, Oliver et al. 2003, Joy 2004, Reeves and Korecki 2004, Roppo et al. 2004, Young et al. 2004, Caldwell et al. 2005, Gallitano et al. 2005, Gray et al. 2005, Joy and Sullivan 2005, Larish and Savage 2005, Sames and Pehling 2005, Holman et al. 2006, Saenz et al. 2006, Thielman and Hunter 2006, Bevins 2007, Morris et al. 2007, Hughes et al. 2008).

Laboratory studies have shown that *Ae. j. japonicus* is a highly efficient vector of West Nile (WN) and St. Louis encephalitis viruses and a moderately efficient vector of eastern equine encephalitis and La Crosse viruses (Sardelis and

Turell 2001; Sardelis et al. 2002a, 2002b; Sardelis et al. 2003). Furthermore, WN virus has been detected in field-collected *Ae. j. japonicus* from at least 9 different states in the northeastern and north central USA (New Hampshire, Massachusetts, Rhode Island, Connecticut, New York, New Jersey, Pennsylvania, Ohio, and Indiana) (CDC 2009), implicating this species as a “bridge” vector to humans.

Studies on the host-feeding behavior of *Ae. j. japonicus* are needed to define its feeding preferences in areas where it may serve as a potential vector of WN and other mosquito-borne viruses. Laboratory studies with native Asian populations indicate that this species readily feeds on chickens and mice but not on reptiles or amphibians (Miyagi 1972, Tanaka et al. 1979). However, blood meal analyses of field-collected mosquitoes in New York (Apperson et al. 2004) and Connecticut (Molaei et al. 2008) indicate that *Ae. j. japonicus* acquire blood meals exclusively from mammalian hosts, including humans. The present study examined the host-feeding habits of *Ae. j. japonicus* to extend knowledge of the behavioral ecology of this mosquito in other areas of northeastern USA and to further elucidate its role and vector potential in arbovirus transmission.

Bloodfed mosquitoes were collected from a variety of localities in rural and suburban New Jersey including farms, wood lots, waste tire and automobile disposal sites, residential backyards, and an animal park during 2000 to 2007 using New Jersey light trap collections (JW Hock Company, Gainesville, FL), grass infusion-baited

<sup>1</sup> The Connecticut Agricultural Experiment Station, 123 Huntington Street, P.O. Box 1106, New Haven, CT 06511.

<sup>2</sup> Center for Vector Biology, Rutgers University, 180 Jones Avenue, New Brunswick, NJ 08901.

<sup>3</sup> Mercer County Mosquito Control, 300 Scotch Road, West Trenton, NJ 08628.

<sup>4</sup> Lake County Vector Control District, P.O. Box 310, 410 Esplanade, Lakeport, CA 95453.

Table 1. Number and percentage of mammalian-derived blood meals identified from *Aedes japonicus japonicus* collected in New Jersey, 2000–07.

Vertebrate host species	No.	% total	Trap type			
			GT <sup>1</sup>	NJ LT	CO <sub>2</sub> LT	ASP
White-tailed deer, <i>Odocoileus virginianus</i>	19	52.8	8	3	3	5
Human, <i>Homo sapiens</i>	13	36.1	1	—	—	12
Fallow deer, <i>Dama dama</i>	2	5.5	—	2	—	—
Horse, <i>Equus caballus</i>	1	2.8	1	—	—	—
Virginia opossum, <i>Didelphis virginiana</i>	1	2.8	—	—	1	—
Total	36	100.0	10	5	4	17

<sup>1</sup> GT, gravid trap; NJ LT, New Jersey light trap; CO<sub>2</sub> LT, CO<sub>2</sub>-baited light trap; ASP, aspirator.

gravid traps (BioQuip Products, Inc., Rancho Dominguez, CA), carbon dioxide-baited Centers for Disease Control and Prevention (CDC) and encephalitis virus surveillance light traps (Bio-Quip), and aspiration from resting sites by using a backpack aspirator (BioQuip). Engorged mosquitoes were identified to species level on a chill table with the aid of a stereomicroscope using descriptive keys (Tanaka et al. 1979). Individual mosquitoes were dissected on microscope slides using flame-sterilized scalpels and forceps or disposable razor blades, and DNA was isolated from the abdominal contents of engorged mosquitoes using DNA-zol BD (Molecular Research Center, Cincinnati, OH) according to the manufacturer's recommendation with some modifications as described elsewhere (Scott 2003; Molaei et al. 2006, 2008). Isolated DNA from the mosquito blood meals served as DNA templates in subsequent polymerase chain reaction (PCR) with primers based on cytochrome *b* sequences of avian and mammalian species using previously described thermal-cycling conditions (Scott 2003; Molaei et al. 2006, 2008). The GeneAmp PCR system 9700 (Applied Biosystems, Foster City, CA) was used to perform PCR reactions and sequenced directly in cycle sequencing reactions using the sequencer, 3730xl DNA analyzer (Applied Biosystems) at the Keck Sequencing Facility, Yale University, New Haven, CT, or using ABI Prism® 3100 automated capillary genetic analyzer (Applied Biosystems) at the Biotechnology Center for Agriculture and the Environment, Rutgers University. Sequences were analyzed and annotated by using Chroma-sPro version 1.22 or 2.2.6 (Technelysium Pty Ltd., Tewantin, Australia) and identified by comparison to the GenBank DNA sequence database (NCBI 2008).

Analysis of the vertebrate blood meal sources for *Ae. j. japonicus* identified 5 mammalian hosts (Table 1). White-tailed deer, *Odocoileus virginianus* (Zimmermann), was the most frequently identified host (52.8% of total), followed by human, *Homo sapiens* L. (36.1%). Specimens with human-derived blood meals were collected by aspiration (92.3%,  $n = 12$ ) and a gravid trap

(7.7%), from woodlots (69.2%), waste tire disposal sites (15.4%), and residential backyards (15.4%). Other mammalian species identified included horse, *Equus caballus* (L.), and Virginia opossum, *Didelphis virginiana* (Kerr). Two mosquitoes collected within a captive wildlife facility had acquired blood meals from fallow deer, *Dama dama* L. This small facility (ca. 5 acres) houses a group of 15 fallow deer that are allowed to roam freely within the enclosure. No avian, amphibian, reptilian, or mixed blood meals were identified.

Examination of the host-feeding behavior of mosquitoes is vital to understanding their vectorial capacity in a new distribution range, particularly in instances where humans may be the preferred host and the mosquito species are capable of transmitting viruses that circulate annually and cause human disease. Our blood meal analysis of *Ae. j. japonicus* identified exclusively mammalian-derived blood meals with a high prevalence of human feeding that was notably greater than that reported for other mammalophilic mosquito species in the northeastern USA (Apperson et al. 2002, 2004; Molaei et al. 2009). This finding, in concert with the detection of WN virus from field-collected females throughout New Jersey in 8 of the last 9 years ( $n = 24$  WN virus positive pools) (CDC 2008), supports a likely bridge vector role for this species in transmission of WN virus to humans in the region that has not been previously recognized. Our results also corroborate the reported attraction of *Ae. j. japonicus* to human bait stations and collection of indoor biting females in suburban and rural environs in Connecticut and New Jersey (Andreadis et al. 2001, Scott 2003).

Our results with populations in New Jersey are further consistent with the report of exclusive mammalian-derived blood meals from *Ae. j. japonicus* in New York and Connecticut, where 98% and 67% of mammalian-derived blood meals were from white-tailed deer, respectively (Apperson et al. 2004, Molaei et al. 2008). The prevalence of white-tailed deer as hosts for *Ae. j. japonicus* and other mammalophilic mosquitoes in the present and the most recent studies is likely

a function of deer abundance in the northeastern USA (Molaei et al. 2006, 2008).

The absence of avian-derived blood meals from *Ae. j. japonicus* in this and other studies from the region is enigmatic. Laboratory observations indicate that *Ae. j. japonicus* readily feeds on birds in addition to mammalian species (Miyagi 1972, Scott 2003), and vector competency studies in the USA use chickens as the blood meal source for this mosquito (Sardelis and Turell 2001; Sardelis et al. 2002a, 2002b; Sardelis et al. 2003). Furthermore, a free mating colony of *Ae. j. japonicus* established since 2000 at Rutgers University has been exclusively maintained by feeding on bobwhite quail, *Colinus virginianus* (L.) (Williges et al. 2008). The absence of avian-derived blood meals from field-collected mosquitoes could be due to the relatively small numbers and inherent experimental bias in collecting engorged mosquitoes. Samples analyzed in our study were collected at ground level, which may not harbor a large number of *Ae. j. japonicus* that feed on birds in canopies and rest near hosts (Scott 2003). Efforts in collecting this mosquito species in the canopy in New Jersey have been unsuccessful; however, Andreadis and Armstrong (2007) reported the collection of a relatively small number (16.1%,  $n = 535$ ) of *Ae. j. japonicus* in CO<sub>2</sub>-baited CDC light traps in tree canopies (7.6 m) when compared with light traps placed at ground level (1.5 m) in Connecticut.

Lack of avian feeding is also problematic with regard to the acquisition of WN virus by this species, since birds are viewed as the principal reservoir and amplifying hosts. Serological evidence of WN virus infection has been noted in white-tailed deer populations in New Jersey (Farajollahi et al. 2004) and Iowa (Santaella et al. 2005); however, it is not known whether they develop a sufficient viremia to infect mosquitoes and contribute to local transmission cycles. Alternatively, WN virus could be acquired from other mammals, such as eastern chipmunk, *Tamias striatus* (L.) (Platt et al. 2007), and fox squirrels, *Sciurus niger* L. (Root et al. 2006, Platt et al. 2008), for example, which have been shown to develop serum titers sufficient to infect mosquitoes in the laboratory.

Successful establishment, rapid range expansion, abundance, repeated isolation of WN virus in nature, and vector competency at rates comparable to other susceptible bridge vectors in concert with pronounced bloodfeedings on human hosts are consistent with the view that *Ae. j. japonicus* likely plays a role as a bridge vector in transmission of this virus and conceivably a number of other mosquito-borne viruses to humans and other mammals in northeastern and other regions of the USA.

We are grateful to Wayne Crans for his insights and encouragement and Michael Thomas, John

Shepard, and Melanie J. Raubeson for technical assistance. We thank Linda McCuiston for identification assistance and pooling of mosquito specimens, along with Maureen Musarra, Thomas L. Scott, Jennifer Gruener, and John Phelps. We also thank the superintendents and staff of Bergen County Mosquito Control (Peter Pluchino, Jimmy Bartlett, and Warren Staudinger), Burlington County Mosquito Control (Dominic Chappine and Tom Verna), Cumberland County Mosquito Control (Heather Lomberk and Doug Abdil), Sussex County Division of Mosquito Control (John Holick and Marta Iwaseczko), and the Warren County Mosquito Extermination Commission (Christine Musa, Abie Musa, Bob Duryea, Sara May, Teresa Duckworth, Katy Parise, Heather Buckley, and Veronica Ronnie Galbraith), and Jessie Sebbo for assistance in collecting specimens. Funding for this research was provided in part by Laboratory Capacity for Infectious Diseases Cooperative Agreement Number U50/CCU6806-01-1 from the Centers for Disease Control and Prevention, US Department of Agriculture (USDA) Specific Cooperative Agreement Number 58-6615-1-218, USDA-administered Hatch funds CONH00768 to the Connecticut Agricultural Experiment Station.

#### REFERENCES CITED

- Andreadis TG, Anderson JF, Munstermann LE, Wolfe RJ, Florin DA. 2001. Discovery, distribution and abundance of a newly introduced mosquito, *Ochlerotatus japonicus* (Diptera: Culicidae) in Connecticut, USA. *J Med Entomol* 38:774-779.
- Andreadis TG, Armstrong PM. 2007. A two-year evaluation of elevated canopy trapping for *Culex* mosquitoes and West Nile virus in an operational surveillance program in the northeastern United States. *J Am Mosq Control Assoc* 23:137-148.
- Apperson CS, Harrison BA, Unnasch TR, Hassan HK, Irby WS, Savage HM, Aspen SE, Watson DW, Rueda LM, Engber BR, Nasci RS. 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.
- Apperson CS, Hassan HK, Harrison BA, Savage HM, Aspen SE, Farajollahi A, Crans W, Daniels TJ, Falco RC, Benedict M, Anderson M, McMillen L, Unnasch TR. 2004. Host-feeding patterns of established and potential mosquito vectors of West Nile virus in the eastern United States. *Vector Borne Zoonotic Dis* 4:71-82.
- Bevins SN. 2007. Establishment and abundance of a recently introduced mosquito species *Ochlerotatus japonicus* (Diptera: Culicidae) in the Southern Appalachians, USA. *J Med Entomol* 44:945-952.
- Caldwell ND, Gerhardt RR, Jones CJ. 2005. First collection of *Ochlerotatus japonicus japonicus* in the state of Tennessee. *J Am Mosq Control Assoc* 21:322-324.

- CDC [Centers for Disease Control and Prevention]. 2009. *Epi-X Forum. National WNV surveillance data* [Internet]. Atlanta, GA: Centers for Disease Control and Prevention [accessed April 09, 2009]. Available from: <https://epixforum.cdc.gov/forum/wbpx.dll/~epix/login>.
- Falco RC, Daniels TJ, Slamecka MC. 2002. Prevalence and distribution of *Ochlerotatus japonicus* (Diptera: Culicidae) in two counties in southern New York State. *J Med Entomol* 39:920–925.
- Farajollahi A, Gates R, Crans W, Komar N. 2004. Serologic evidence of West Nile virus and St. Louis encephalitis virus infections in white-tailed deer (*Odocoileus virginianus*) from New Jersey, 2001. *Vector Borne Zoonotic Dis* 4:379–383.
- Fonseca DM, Campbell S, Crans WJ, Mogi M, Miyagi I, Toma T, Bullians M, Andreadis TG, Berry RL, Pagac B, Sardelis MR, Wilkerson RC. 2001. *Aedes (Finlaya) japonicus* (Diptera: Culicidae), a newly recognized mosquito in the United States: analyses of genetic variation in the United States and putative source populations. *J Med Entomol* 38:135–146.
- Gallitano S, Blaustein L, Vonesh J. 2005. First occurrence of *Ochlerotatus japonicus* in Missouri. *J Vector Ecol* 30:347–348.
- Gray EW, Harrison BA, Womack ML, Kerce J, Neely CJ, Noblet R. 2005. *Ochlerotatus japonicus japonicus* (Theobald) in Georgia and North Carolina. *J Am Mosq Control Assoc* 21:144–146.
- Harrison BA, Whitt PB, Cope SE, Payne GR, Rankin SE, Bohn LJ, Stell FM, Neely CJ. 2002. Mosquitoes (Diptera: Culicidae) collected near the Great Dismal Swamp: new state records, notes on certain species, and a revised checklist for Virginia. *Proc Entomol Soc Wash* 104:655–662.
- Holman MS, Darsie RF Jr., Foss KA. 2006. A checklist of the mosquitoes of Maine with new state records. *J Am Mosq Control Assoc* 22:327–329.
- Hughes TH, Irwin PM, Kaufman A, Sage H, Pagac Jr. BB, Paskewitz SM. 2008. First records of *Aedes japonicus japonicus* in Wisconsin. *J Am Mosq Control Assoc* 24:583–584.
- Joy JE. 2004. Larval mosquitoes in abandoned tire pile sites from West Virginia. *J Am Mosq Control Assoc* 20:12–17.
- Joy JE, Sullivan SN. 2005. Occurrence of tire inhabiting larvae in different geographic regions of West Virginia. *J Am Mosq Control Assoc* 21:380–386.
- Larish LB, Savage HM. 2005. Introduction and establishment of *Aedes (Finlaya) japonicus japonicus* (Theobald) on the island of Hawaii: implications for arbovirus transmission. *J Am Mosq Control Assoc* 21:318–321.
- Miyagi I. 1972. Feeding Habits of some Japanese mosquitoes on cold-blooded animals in the laboratory. *Trop Med* 14:203–217.
- Molaei G, Andreadis TG, Armstrong PM, Anderson JF, Vossbrinck CR. 2006. Host feeding patterns of *Culex* mosquitoes and West Nile virus transmission, Northeastern United States. *Emerg Infect Dis* 12:468–474.
- Molaei G, Andreadis TG, Armstrong PM, Diuk-Wasser M. 2008. 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*. *J Med Entomol* 45:1143–1151.
- Morris JA, Lampman RL, Ballmes G, Funes J, Halvorsen J, Novak RJ. 2007. First record of *Aedes japonicus japonicus* in Illinois: defining its spatial distribution and associated mosquito species. *J Am Mosq Control Assoc* 23:243–251.
- NCBI [National Center for Biotechnology Information]. 2008. *GenBank DNA sequence database* [Internet]. Bethesda, MD: National Center for Biotechnology Information [accessed Month 00, 2009]. Available from: <http://www.ncbi.nlm.nih.gov/>.
- Oliver J, Means RG, Howard JJ. 2003. Geographic distribution of *Ochlerotatus japonicus* in New York State. *J Am Mosq Control Assoc* 19:121–124.
- Peyton EL, Campbell SR, Candeletti TM, Romanowski M, Crans WJ. 1999. *Aedes (Finlaya) japonicus japonicus* (Theobald), a new introduction into the United States. *J Am Mosq Control Assoc* 15:238–241.
- Platt KB, Tucker BJ, Halbur PG, Blitvich BJ, Fabiosa FG, Mullin K, Parikh GR, Kitikoon P, Bartholomay LC, Rowley WA. 2008. Fox squirrels (*Sciurus niger*) develop West Nile virus viremias sufficient for infecting select mosquito species. *Vector Borne Zoonotic Dis* 8:225–233.
- Platt KB, Tucker BJ, Halbur PG, Tiawsirisup S, Blitvich BJ, Fabiosa FG, Bartholomay LC, Rowley WA. 2007. West Nile virus viremia in eastern chipmunks (*Tamias striatus*) sufficient for infecting different mosquitoes. *Emerg Infect Dis* 13:831–837.
- Reeves WK, Korecki JA. 2004. *Ochlerotatus japonicus japonicus* (Theobald) (Diptera: Culicidae), a new invasive mosquito for Georgia and South Carolina. *Proc Entomol Soc Wash* 106:233–234.
- Root JJ, Oesterle PT, Nemeth NM, Klenk K, Gould DH, McLean RG, Clark L, Hall JS. 2006. Experimental infection of fox squirrels (*Sciurus niger*) with West Nile virus. *Am J Trop Med Hyg* 75:697–701.
- Roppo MR, Lilja JL, Maloney FR, Sames WJ. 2004. First occurrence of *Ochlerotatus japonicus* in the state of Washington. *J Am Mosq Control Assoc* 20:83–84.
- Saenz VL, Townsend LH, Vanderpool RM, Schardein MJ, Trout RT, Brown GC. 2006. *Ochlerotatus japonicus* in the state of Kentucky. *J Am Mosq Control Assoc* 22:754–755.
- Sames WJ, Pehling D. 2005. Update on *Ochlerotatus japonicus* in the state of Washington. *J Am Mosq Control Assoc* 21:98–99.
- Santaella J, McLean R, Hall JS, Gill JS, Bowen RA, Hadow HH, Clark L. 2005. West Nile virus serosurveillance in Iowa white-tailed deer (1999–2003). *Am J Trop Med Hyg* 73:1038–1042.
- Sardelis MR, Turell MJ. 2001. *Ochlerotatus j. japonicus* in Frederick County, Maryland: discovery, distribution, and vector competence for West Nile virus. *J Am Mosq Control Assoc* 17:137–141.
- Sardelis MR, Dohm JD, Pagac B, Andre RG, Turell MJ. 2002a. Experimental transmission of eastern equine encephalitis virus by *Ochlerotatus j. japonicus* (Diptera: Culicidae). *J Med Entomol* 39:480–484.
- Sardelis MR, Turell MJ, Andre RG. 2002b. Laboratory transmission of La Crosse virus by *Ochlerotatus j. japonicus* (Diptera: Culicidae). *J Med Entomol* 39:635–639.
- Sardelis MR, Turell MJ, Andre RG. 2003. Experimental transmission of St. Louis encephalitis virus by *Ochlerotatus j. japonicus*. *J Am Mosq Control Assoc* 19:159–162.

- Scott JJ. 2003. *The ecology of the exotic mosquito Ochlerotatus (Finlaya) japonicus japonicus (Theobald 1901) (Diptera: Culicidae) and an examination of its role in the West Nile virus cycle in New Jersey* [Ph.D. dissertation]. Rutgers, New Brunswick, NJ.
- Tanaka K, Mizusawa K, Saugstad ES. 1979. A revision of the adult and larval mosquitoes of Japan (including Ryukyu Archipelago and the Ogasawara Islands) and Korea (Diptera: Culicidae). *Contrib Am Entomol Inst (Ann Arbor)* 16:1–987.
- Thielman A, Hunter FF. 2006. Establishment of *Ochlerotatus japonicus* (Diptera: Culicidae) in Ontario, Canada. *J Med Entomol* 43:138–142.
- Williges E, Farajollahi A, Scott JJ, McCuiston LJ, Crans WJ, Gaugler R. 2008. Laboratory colonization of *Aedes japonicus japonicus*. *J Am Mosq Control Assoc* 24:591–593.
- Young CLE, Beery JA, Sheffer RE, Rand KM. 2004. First record of *Ochlerotatus japonicus* (Diptera: Culicidae) in St. Joseph County, Indiana. *Gt Lakes Entomol* 37:196–197.