

Innate preference or opportunism: mosquitoes feeding on birds of prey at the Southeastern Raptor Center

Nathan D. Burkett-Cadena^{1,2,3✉}, Andrea M. Bingham², Christopher Porterfield^{1,4}, and Thomas R. Unnasch²

¹Department of Entomology and Plant Pathology, Auburn University, AL 36849, nburkett@health.usf.edu

²Department of Global Health, University of South Florida, Tampa, FL 33612, U.S.A.

³Department of Entomology and Nematology, University of Florida, FL 32611, U.S.A.

⁴Department of Biological Sciences, Boise State University, Boise, ID 83725, U.S.A.

Received 26 August 2013; Accepted 22 October 2013

ABSTRACT: The amplification of mosquito-borne pathogens is driven by patterns of host use by vectors. While each mosquito species is innately adapted to feed upon a particular group of hosts, this “preference” is difficult to assess in field-based studies, because factors such as host defenses and spatial and temporal overlap of mosquitoes and hosts affect which host animals actually get bitten. Here we examined patterns of host use by mosquitoes feeding on caged raptors at a rehabilitation and education center for birds of prey in Alabama, U.S.A. PCR-based techniques were used to determine the host species fed upon. Of 19 raptor species at the facility, seven were found to be fed upon by mosquitoes. Feeding indices and linear regression indicated that no species or family of raptor were significantly preferred over another ($R^2=0.46$). Relative abundance adjusted for bird size explained a statistically significant amount of the variation in relative host use ($R^2=0.71$), suggesting that bird size is an important component of host selection by mosquitoes. These findings support the hypothesis that traits of host animals drive patterns of host use by mosquitoes in nature, an interaction that leads to amplification of mosquito-borne viruses. *Journal of Vector Ecology* 39 (1): 21–31. 2014.

Keyword Index: Host preference, mosquito, raptor, bird of prey, *Culex*.

INTRODUCTION

Patterns of host use by mosquitoes govern the amplification and epidemic transmission of mosquito-borne pathogens (Weaver 2005). Innate preference for a host or group of hosts is one of the factors that drives contact rates of mosquitoes and hosts (Dekker et al. 2001) and is one of the principal factors that drives patterns of host use by mosquitoes in nature (Besansky et al. 2004). Innate patterns of host preference of mosquitoes are difficult to assess in field-based studies, because other factors that also influence patterns of host use, such as host defenses, spatial distributions of mosquitoes and vectors (Estep et al. 2012), and circadian activity patterns of the hosts and mosquitoes (Day and Edman 1984), confound the effects of innate host preference (Besansky et al. 2004). In field experiments of mosquito feeding preference conducted in California (Blackmore and Dow 1958), for example, mosquitoes were found to be more attracted to adult than to juvenile domestic pigeons. However, mosquito feeding success (percent engorgement) on juvenile birds was much greater (86.0%) than adult birds (15.3%), due to less protective plumage and quiescent behavior of the juvenile birds (Blackmore and Dow 1958). In this case, innate attraction to adult birds is not reflected in patterns of host use but is rather due to differences in host biology. The observed pattern of host use for the mosquito community therefore results from complex interactions between innate (genetic) host preference and environmental (host) factors.

Innate host preference in mosquitoes is commonly considered to be driven by olfactory attraction. Studies that attempt to determine innate host preference offer questing mosquitoes choices between host odors in experimental situations, such that

mosquitoes must choose between competing olfactory sources, such as in y-tube olfactometers (Geier and Boeckh 1999, Puri et al. 2006, Lefèvre et al. 2010). Use of y-tube olfactometers may provide insight into the groups or species of hosts that a mosquito may find more attractive. However, information derived from these methods may not correlate with observed patterns of host use in nature (Blackmore and Dow 1958).

In published reports of blood meal determinations of field-collected mosquitoes, feedings from raptors rarely constitute more than a small fraction of total avian blood meals. A study of host use by California *Culex* mosquitoes found that 3.4% of 1,320 avian blood meals were from nine species of raptors (Thiemann et al. 2012). In Tennessee, 0.3% of 755 avian blood meals were from two species of raptors (Savage et al. 2007). In Illinois, 0.6% of 756 avian blood meals were from two species of raptors (Hamer et al. 2009). In Macon County, AL (~25 km from the Southeastern Raptor Center (SRC)), 2.7% of 528 avian blood meals were from three raptor species (Estep et al. 2011). Despite the relatively low percentages of blood meals from raptors in these studies, the even lower abundances of these avian top predators, relative to other available birds, suggest that mosquitoes may actually feed upon raptors at rates much greater than their relative abundance in the bird community. Barred owl, for example, was one of only nine avian species (of 42 bird species detected in mosquito blood meals) fed upon significantly more than its relative abundance would suggest in Alabama (Estep et al. 2011). In Illinois, American kestrel (*Falco sparverius*) and Cooper's hawk (*Accipiter cooperii*) were among the top five preferred avian species (of 25 bird species detected in blood-fed mosquitoes) regardless of sampling method used (Hamer et al. 2009). The larger mass of raptors, relative to

other birds, may account for their over-representation in the blood-meal samples (Estep et al. 2012).

Here we examine patterns of host utilization by mosquitoes for a cohort of captive raptors at the SRC in Alabama, U.S.A., and test the hypothesis that mosquitoes feed on some raptor species disproportionate to their relative abundance. By investigating relative host use of these captive birds, we can reduce the effects of many of the factors that may confound studies of host preference in completely natural settings, where behavioral traits of different bird species may drive the interactions of mosquitoes and their hosts. In this behavior-limiting setting we can therefore infer the importance of innate host preference of mosquitoes in patterns of host use by mosquitoes through comparing relative abundance and relative host use of species (or families).

MATERIALS AND METHODS

Study site

Fieldwork was conducted on the grounds of the Southeastern Raptor Center, a research, education, and rehabilitation facility, supported by the College of Veterinary Medicine, Auburn University, Alabama, U.S.A. The SRC takes in between 200 and 275 birds of prey annually and normally houses 100-200 birds at any given time. Birds are kept in outdoor habitats (cages or aviaries), either singly or in small groups. The vegetation surrounding the SRC is a mixture of pastureland, pinewoods, and riparian forest. Vegetated wetlands occur within 0.5 km of the facility.

Mosquito collection

Mosquitoes were collected from artificial resting stations located in a wooded riparian corridor surrounding the bird housing units. Black plastic yard trash cans, placed on their sides, were used as resting stations (Burkett-Cadena et al. 2008a). Eleven resting stations were placed along a ~0.3 km footpath that ran through the riparian corridor, spaced roughly 25 m apart (Figure 1). Mosquitoes were aspirated from the resting sites using a modified Dustbuster® (Burkett-Cadena et al. 2008a). The trash can lid, with a 10-cm diameter hole cut into its center, was placed

over the mouth of the trash can at the time of collection, directing exiting mosquitoes directly into the aspirator intake (Burkett-Cadena et al. 2008a). Mosquitoes were aspirated from resting stations between 08:00 and 10:00 (Gray et al. 2011). Collections were made on 44 days between April and September, 2010, with a lapse in collecting from July 2 through August 9 (Table 1). No other mosquito collection methods were used. Mosquito samples were returned to the laboratory for identification and blood meal source determination. Females were identified to species using published keys (Darsie and Ward 2005).

Blood meal analysis

Blood-fed female mosquitoes (partially or fully engorged) were processed individually for vertebrate host blood source determination (blood meal analysis). Whole females were homogenized in 225- μ L phosphate buffered saline (PBS, pH 7.4). DNA was prepared using DNAzol BD reagent (Invitrogen, Grand Island, NY). In brief, 200 μ L of the homogenate supernatant was mixed with 400 μ L of DNAzol BD to lyse the cells. In order to precipitate the DNA, 160 μ L of isopropanol was added, the solution vortexed, and then stored for 5 min at room temperature. The sediment was precipitated by centrifugation at 8,000 RPM for 6 min. Following centrifugation, the supernatant was removed and 200 μ L of DNAzol BD was used to disperse the pellet. After another centrifugation, the pellet was washed with 1 ml of 75% ethanol and the DNA solubilized with 100 μ L of tris-EDTA buffer (Tris-EDTA, pH 8.0, Boston Bioproducts, Ashland, MA). Extracted DNA was stored at -80°C until further testing.

The identification of the blood meals from the extracted DNA required the use of three polymerase chain reaction (PCR)-based assays. A total of 2.5 μ L of DNA was used as a template in 25 μ L amplification reactions for each PCR. The initial PCR used a universal vertebrate primer set targeting 16S rRNA. Primers used in the PCR were those of Kitano and co-workers (Kitano et al. 2007) and were as follows: 5'-GCCTGTTTACCAAAAACATCAC-3' and 5'-CTCCATAGGGTCTTCTCGTCTT-3'. Reaction conditions were the same as those described previously (Burkett-Cadena et al. 2008b). Samples that did not amplify with this PCR were tested



Figure 1. Map of Southeastern Raptor Center, Auburn University, AL, U.S.A., and surrounding area. Mosquito collection locations (11) are represented by white stars.

in another PCR that used universal vertebrate primers targeting cytochrome B. The sequences of these primers were as follows: 5'-CCCCTCAGAATGATATTTGTCCTCA-3' and 5'-CCATCCAA-CATCTCAGCATGATGAAA-3' and followed the reaction conditions outlined by Hassan and co-workers (Hassan et al. 2003). Samples that did not amplify with either of the previous primer sets were then tested in a nested PCR. Nested PCR has been shown to be useful in samples with small amounts of DNA (Hassan et al. 2003). The nested PCR used the previous PCR product targeting cytochrome B as a template. The sequences of the forward primer for the second reaction were 5'-TCWRCHTGATGAACTTC-GG-3' where W = A or T, R = A or G, and H = A, C, or T. The non-coding primer used was a mixture of four primers with the following sequences: 5'-ACRAARGCRGTTGCTATTAG-3', 5'-ACRAAGGCAGTKGCTATAAG-3', 5'-ACGAARGCRGTTG-CYATGAG-3', and 5'-ACGAAGGCMGTKGCTATTAG-3', where K = G or T, Y = C or T, and M = A or C. Reaction conditions were the same as those previously described (Hassan et al. 2003). Positive samples were treated with Exosap-IT (USB, Cleveland, OH) for purification and were then sent to the GENEWIZ sequencing facility (South Plainfield, NJ) for analysis. Sequences were entered into the National Center for Biotechnology Information Basic Local Alignment Search Tool (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) database for identification, and only those sequences with a match percentage $\geq 95\%$ were accepted as belonging to the identified blood meal source.

Data analysis

We calculated forage ratios (feeding indices) for each raptor species as a metric of relative host utilization by the mosquito community (Hess and Swartz 1940, Kay et al. 1979, Manley et al. 2002). In the context of patterns of host use by mosquitoes, forage ratios represent the relationship between a host animal's relative abundance in the environment and the fraction of total blood meals that are derived from it. Forage ratios are calculated for species A as the % of total blood meals derived from A, divided by the % of the community represented by A. Forage ratios greater than 1 indicate that the proportion of total blood meals from a given host species is greater than its proportion in the community. The forage ratio was considered statistically significant if its 95% confidence interval excluded 1, i.e., when relative abundance equaled relative host use (Manley et al. 2002). Because numbers of individual raptor species housed at the Center changed throughout the study period, relative abundance of raptor species was calculated from the average abundance of bird species in June and August, the months with the greatest numbers of raptor blood meals. Blood meal data from all mosquito species were aggregated from each mosquito species for the forage ratio calculations, so as to investigate host-feeding patterns of the mosquito community (Chaves et al. 2010).

We used linear regression (SAS PROC REG) to test whether or not overall feedings on raptors was a function of their relative abundance. Two simple and one multiple regression analyses were performed, each using the proportion of total blood meals (host use) as the dependent variable. Independent variables investigated for their contribution to variation in host use were relative abundance of raptor species and relative abundance of birds in respective families (simple regressions). Relative abundances of

raptor species adjusted for bird mass (g) and bird length (m) were examined for their contribution to variation in host use using stepwise multiple regression. Relative abundances were adjusted for mass and length by multiplying the published average mass and length (beak to tail) of adults of each species by its estimated abundance (Hassan et al. 2003). All proportions were transformed using the angular transformation (arcsine of square root), prior to statistical tests. Alpha was set at 0.05 for all analyses.

RESULTS

Eleven species of mosquitoes (Table 1) were collected from resting stations at the Southeastern Raptor Center. Species of *Anopheles* and *Culex* dominated the collections (Table 1). *Anopheles punctipennis* (Say), *Culex erraticus* (Dyar and Knab), *Culex quinquefasciatus* Say, *Culex restuans* Theobald, and *Culex territans* Walker were the most commonly collected species (Table 1), accounting for 96.4% of non-engorged and 98.3% of blood-engorged females, respectively. *Aedes albopictus* (Skuse), *Aedes triseriatus* (Say), and *Toxorhynchites rutilus* (Coquillett) were collected sporadically, although no blood-engorged specimens of these species were recovered (Table 1).

The six mosquito species examined for host blood source fed (collectively) on all four classes of vertebrates (Figure 2), belonging to 31 different species (Table 2). *Anopheles punctipennis* fed primarily upon mammals (91.2% of blood meals), yet also fed occasionally upon reptiles (2.9%) and birds (5.8%). *Culex erraticus*, *Culex quinquefasciatus*, *Culex restuans*, and *Culex salinarius* split their feedings among birds and mammals (Figure 2), with a few blood meals from amphibians (4% from amphibians for *Culex erraticus*). *Culex territans* fed heavily on amphibians (85.8%), while also taking blood meals from mammals and birds (7.1% each).

A relatively large percentage of total mosquito feedings (14.5%) were from raptors. Females of four mosquito species (all of the genus *Culex*) were found to feed upon raptors (Figure 2). Blood meals from raptors accounted for five (20%) of *Cx. erraticus* feedings (n=25), 14 (21.2%) of *Cx. quinquefasciatus* feedings (n=66), four (16%) of *Cx. restuans* feedings (n=25), and one (50%) of *Cx. salinarius* feedings (n=2). Nineteen raptor species (Latin names provided in Table 3) were housed at the center during our study (Table 3), including two individuals of species that are not native to North America (Saker falcon and Lanner falcon) and one species not native to Alabama (Harris's hawk). Native raptor species at SRC included two eagle species (Bald eagle, Golden eagle), five hawk species (Broad-winged hawk, Cooper's hawk, Red-shouldered hawk, Red-tailed hawk, Sharp-shinned hawk), one kite species (Mississippi kite), two vulture species (Black vulture, Turkey vulture), two species of falcon (American kestrel, Peregrine falcon) and four owl species (Barred owl, Eastern screech-owl, Great horned owl, Barn owl). Of the 19 available raptor species, seven (36.8%) were found to be fed upon by the mosquitoes during our study (Tables 3 and 4). Nineteen (79.2%) of twenty-four total raptor blood meals were derived from three species (combined): Barred owl (6), Great horned owl (6), and Red-tailed hawk (7). Red-tailed hawks were the most abundant birds at SRC, comprising 14.3% (May) to 25.3% (Aug) of all birds, depending on month. Barred owls made up 8.8% (Aug)

Table 1. Female mosquitoes (daily average) collected from resting stations at the Southeastern Raptor Center, Auburn University, 2010. The number of collections days for each month is provided in parentheses (n) below each month.

Mosquito	Status	Apr (n=6)	May (n=13)	Jun (n=15)	Jul (n=1)	Aug (n=7)	Sep (n=2)	Total females
<i>Aedes albopictus</i>	Not engorged	0	0	0.2	0	0.71	0	8
	Engorged	0	0	0	0	0	0	0
<i>Aedes triseriatus</i>	Not engorged	0	0	0.4	0	0	0	6
	Engorged	0	0	0	0	0	0	0
<i>Anopheles crucians</i> complex	Not engorged	0	0	0	0	0	0	0
	Engorged	0	0.08	0	0	0	0	1
<i>Anopheles punctipennis</i>	Not engorged	2.5	5.62	6.13	12	2.14	0	206
	Engorged	0.33	0.92	1.13	0	0.57	0	35
<i>Anopheles quadrimaculatus</i> group	Not engorged	0	0	0.07	0	0	0	1
	Engorged	0	0	0	0	0	0	0
<i>Culex erraticus</i>	Not engorged	0	0.15	2.2	2	3.14	0.5	60
	Engorged	0.17	0	0.93	0	2.14	0	30
<i>Culex quinquefasciatus</i>	Not engorged	0	0.23	1.93	1	15.43	3.5	148
	Engorged	0	0	0.8	1	8	0.5	70
<i>Culex restuans</i>	Not engorged	2.5	4.08	1.93	0	0	0	97
	Engorged	0.33	1.31	0.2	0	0	0	22
<i>Culex salinarius</i>	Not engorged	0	0.08	0.27	0	0	0	5
	Engorged	0.17	0.08	0	0	0	0	2
<i>Culex territans</i>	Not engorged	0.67	1.47	1.4	1	0	1	47
	Engorged	0.33	0.77	0.13	0	0	0	14
<i>Toxorhynchites rutilus</i>	Not engorged	0	0	0.07	0	0	0	1
	Engorged	0	0	0	0	0	0	0

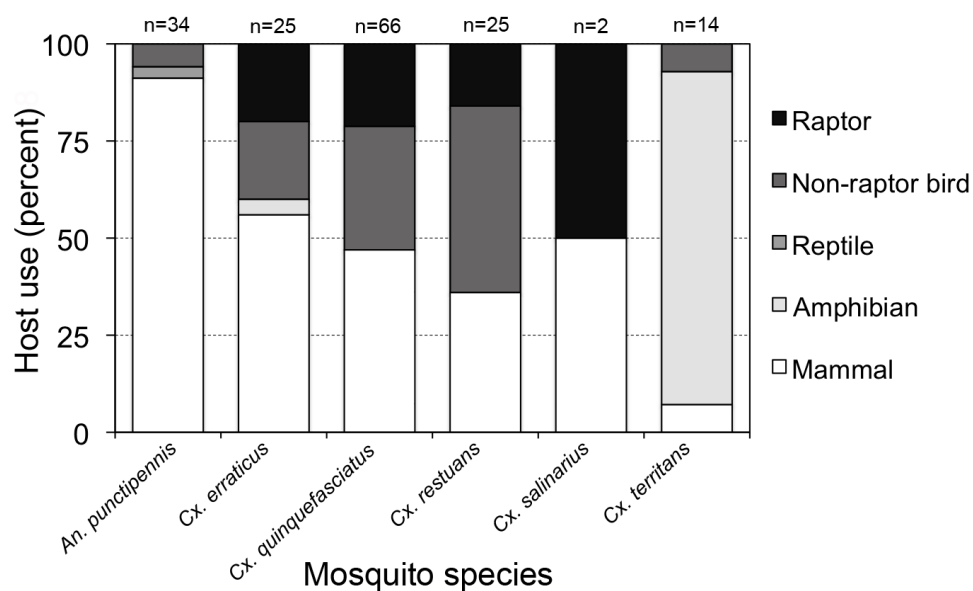


Figure 2. Patterns of host use from mosquitoes collected from the grounds of the Southeastern Raptor Center. Numbers over bars indicate total number of blood meals identified for each mosquito species.

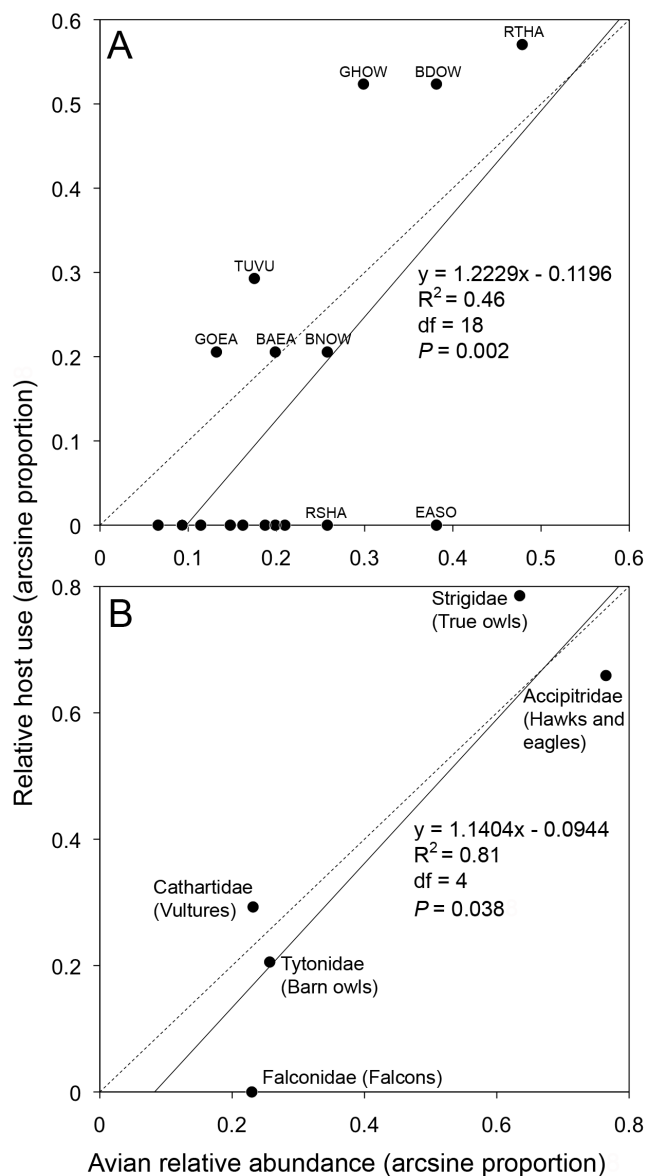


Figure 3. Relationship between abundance and mosquito feedings on birds of prey at the Southeastern Raptor Center, 2010. Circles represent 19 individual bird species (A) or 5 bird families (B) housed at the center during the study period. The dotted lines represent hypothetical one-to-one relationships between relative abundance and relative host use. See Table 3 for AOU abbreviations.

Table 2. Vertebrate blood meals from field-collected female mosquitoes from the Southeastern Raptor Center, Auburn University, 2010.

Host (common name)	Class	<i>Anopheles punctipennis</i>	<i>Culex erraticus</i>	<i>Culex quinquefasciatus</i>	<i>Culex restuans</i>	<i>Culex salinarius</i>	<i>Culex territans</i>
Bullfrog	Amphibian	0	1	0	0	0	7
Green frog	Amphibian	0	0	0	0	0	3
Green tree frog	Amphibian	0	0	0	0	0	2
Bald eagle	Bird	0	1	0	0	0	0
Barn owl	Bird	0	0	1	0	0	0
Barred owl	Bird	0	1	4	1	0	0
Black-crowned night heron	Bird	0	1	0	0	0	0
Blue jay	Bird	0	0	1	0	0	0
Brown thrasher	Bird	0	0	1	0	0	0
Carolina chickadee	Bird	0	0	2	0	0	0
Carolina wren	Bird	0	1	2	1	0	0
Common grackle	Bird	0	0	1	0	0	0
Eastern phoebe	Bird	0	0	2	0	0	0
Golden eagle	Bird	0	0	1	0	0	0
Great horned owl	Bird	0	1	5	0	0	0
House finch	Bird	0	0	0	1	0	0
Mourning dove	Bird	2	0	5	3	0	0
Northern cardinal	Bird	0	2	5	7	0	1
Red-tailed hawk	Bird	0	1	3	2	1	0
Tufted titmouse	Bird	0	0	1	0	0	0
Turkey vulture	Bird	0	1	0	1	0	0
Wild turkey	Bird	0	1	1	0	0	0
Brown rat	Mammal	0	1	6	0	0	0
Cow	Mammal	17	3	0	0	1	0
Horse	Mammal	2	0	0	0	0	0
Human	Mammal	1	5	23	8	0	1
Raccoon	Mammal	0	2	0	1	0	0
Virginia opossum	Mammal	0	1	0	0	0	0
White tailed deer	Mammal	11	2	2	0	0	0
Green anole	Reptile	1	0	0	0	0	0
Total		34	25	66	25	2	14

to 18.8% (May) of all birds. Eastern screech owls made up 11.0% (Aug) to 15.7% (June) of all birds. Combined, Barn owls and Red-shouldered hawks comprised between 5.5% and 15.0% of the bird community, depending on month. All other species comprised 5.5% or less of the raptors at SRC, regardless of month. The most common raptor species at SRC (owls and hawks) declined in absolute abundance during the study period (Table 3), with the exception of Red-tailed hawks, which were more abundant in August (23 birds) than in May (19 birds). Overall, blood meals from raptors constituted a greater percentage of total blood meals in August (21.2%) than in May (7.0%) or June (10.4%).

Forage ratios and associated confidence intervals suggest that none of the raptor species were significantly preferred (Table 4), although the lower confidence interval of Great horned owls (0.89) approaches statistical significance (>1.0). Golden eagles and Turkey vultures, two of the least common birds at SRC,

had relatively high forage ratios of 2.406 and 2.750, respectively. However, the lower confidence intervals for these birds were quite low (-2.211 and -0.899, respectively). In general, the most abundant raptor species were also those that were most commonly fed upon (Figure 3A). The relationship between relative abundance and relative host use of raptor species was highly significant ($R^2=0.445$; $df=18$; $P=0.0015$). When raptor species were aggregated by family, a strong, statistically significant positive association ($R^2=0.807$; $df=4$; $P=0.038$) was found between relative abundance and relative host use (Figure 3B). Multiple regression of size-adjusted abundance found that length-adjusted abundance explained more variation in host use ($R^2=0.71$; $df=6$; $P=0.0178$) than did mass-adjusted abundance.

Table 3. Bird of prey abundance and mosquito feedings, by month, at the Southeastern Raptor Center, Auburn University, 2010. Two blood meals from Red-tailed hawk (Apr. 26 and Sept. 15) not shown. In July, one round of collection was made (July 1), but yielded no raptor-fed mosquitoes. *No AOU Abbreviation.

Common name (AOU abbreviation) Latin name	May		June		July	
	Birds available	Blood meals	Birds available	Blood meals	Birds available	Blood meals
American kestrel (AMKE) <i>Falco sparverius</i>	2		3		2	
Bald eagle (BAEA) <i>Haliaeetus leucocephalus</i>	7		5	1	4	
Barn owl (BNOW) <i>Tyto alba</i>	11		10		5	1
Barred owl (BDOW) <i>Strix varia</i>	25	1	24		8	5
Black vulture (BLVU) <i>Coragyps atratus</i>	3		3		3	
Broad-winged hawk (BWHA) <i>Buteo platypterus</i>	4		7		3	
Cooper's hawk (COHA) <i>Accipiter cooperii</i>	4		4		5	
Eastern screech owl (EASO) <i>Megascops asio</i>	20		22		10	
Golden eagle (GOEA) <i>Aquila chrysaetos</i>	2		2		2	1
Great horned Owl (GHOW) <i>Bubo virginianus</i>	12		11	2	9	4
Harris's hawk (HAHA) <i>Parabuteo unicinctus</i>	1		1		1	
Lanner falcon (*) <i>Falco biarmicus</i>	1		1		1	
Mississippi kite (MIKI) <i>Ictinia mississippiensis</i>	2		2		6	
Peregrine falcon (PEFA) <i>Falco peregrinus</i>	2		2		1	
Red-shouldered hawk (RSHA) <i>Buteo lineatus</i>	15		10		5	
Red-tailed hawk (RTHA) <i>Buteo jamaicensis</i>	19	1	26	2	23	2
Saker falcon (*) <i>Falco cherrug</i>	1		1		1	
Sharp-shinned hawk (SSHA) <i>Accipiter striatus</i>	0		1		0	
Turkey vulture (TUVU) <i>Cathartes aura</i>	2	1	5		2	1
Total	133	3	140	5	91	14

Table 4. Mosquito utilization (host preference) of birds of prey at the Southeastern Raptor Center, 2010.

Species	Relative abundance*	Blood meal proportion	Forage ratio	Standard error	95% CI
American kestrel	0.022	0	0	0	
Bald eagle	0.039	0.042	1.069	1.047	-0.983-3.121
Barn owl	0.065	0.042	0.642	0.628	-0.590-1.873
Barred owl	0.139	0.250	1.805	0.638	0.554-3.055
Black vulture	0.026	0	0	0	
Broad-winged hawk	0.043	0	0	0	
Cooper's hawk	0.039	0	0	0	
Eastern screech-owl	0.139	0	0	0	
Golden eagle	0.017	0.042	2.406	2.356	-2.211-7.023
Great horned owl	0.087	0.250	2.888	1.021	0.887-4.888
Harris' hawk	0.009	0	0	0	
Lanner falcon	0.009	0	0	0	
Mississippi kite	0.035	0	0	0	
Peregrine falcon	0.013	0	0	0	
Red-shouldered hawk	0.065	0	0	0	
Red-tailed hawk	0.212	0.292	1.375	0.437	0.518-2.232
Saker falcon	0.009	0	0	0	
Sharp-shinned hawk	0.004	0	0	0	
Turkey vulture	0.030	0.083	2.750	1.862	-0.899-6.399

*Relative abundance calculated from average abundance in June and August (months with greatest numbers of raptor blood meals).

DISCUSSION

Given the importance of mosquito host use in the transmission of pathogens, it is important to delineate the relative contribution of preference vs opportunism in mosquito feeding behavior. The positive linear relationship between relative abundance and relative mosquito feedings on raptors (species and family) indicates a lack of innate preference for any species or family of raptors over another (Figures 3A,3B). This semi-field experiment nearly eliminates many of the variable behavioral traits of the different avian species housed at SRC that might normally drive feeding success (Edman et al. 1974), and by extension, overall patterns of host use by mosquitoes (Estep et al. 2012). All birds at SRC were housed in cages that restrict their aerial movements, making them fairly equally available to foraging mosquitoes. Differences in temporal activity patterns were also minimized (Day and Edman 1984), so birds, such as owls, that would normally be on the wing at dusk (therefore unavailable to foraging mosquitoes) were likewise available during the mosquito activity period. The effects of other traits (Estep et al. 2012) that have been hypothesized to affect patterns of host use by mosquitoes (spatial availability, habitat-edge affiliation, nesting and roosting habits) were also minimized under the circumstances at SRC. Differences in body size (mass, surface area) and behavioral defenses (stomping, shaking, flapping) among species, traits that have been shown to affect the feeding success (Edman et al. 1974) and patterns of host use by mosquitoes (Hassan et al. 2003), were not controlled in the current study. Adjusting abundance data for bird length increased the accuracy of the regression equation for estimating host use, suggesting that bird size, in the broad sense, is indeed an important determinant of host use by mosquitoes. That length-adjusted abundance was a better predictor than mass-adjusted abundance may indicate that visual cues (a larger silhouette) are of greater importance than metabolic cues (CO_2 and heat) for host-seeking mosquitoes, at least over short distances.

Although no bird of prey species was found to be fed upon significantly more than their relative abundance, the largest forage ratios were from Barred owl (1.81), Great horned owl (2.89), Golden eagle (2.41), and Turkey vulture (2.75). Two of these species, Barred owl and Great horned owl, were among 25 bird species (including wading birds, song birds, raptors, and domestic fowl) examined for mosquito feeding tolerance in cage experiments by Edman et al. (1974). Barred owl and Great horned owl were considered to be the most tolerant of all bird species tested, with 82% and 90%, respectively, of recovered mosquitoes being fully blood fed (Edman et al. 1974). Eastern screech-owl and Red-shouldered hawk comprised 20% (combined) of the raptor community in the current study, yet no blood meals from these hosts were detected. Edman et al. (1974) concluded that Eastern screech-owl and Red-shouldered hawk were far less tolerant than the large owls, with 38% of mosquitoes engorging on captive Eastern screech-owl and just 11% of mosquitoes engorging on Red-shouldered hawk (Edman et al. 1974). The results of Edman et al. (1974) and the current study suggest that a host's tolerance of biting mosquitoes is an important driver of patterns of host use by mosquitoes.

Overall patterns of host use by various mosquito species in the current study generally support findings of other published

studies. *Anopheles punctipennis*, as expected, fed predominantly upon mammals, but also fed upon birds (mourning dove), as well as one reptile species (green anole). In studies from North Carolina and New Jersey, *An. punctipennis* took 81% and 70% of blood meals, respectively, from mammals, with the remaining meals from birds, reptiles, and amphibians (Irby and Apperson 1988; Apperson et al. 2004). *Culex territans* fed predominantly on frogs, as expected, taking half of its blood meals from a single species, the Bullfrog. At a study site in neighboring Macon County, Bullfrog was found to be a preferred host of *Cx. territans* (Burkett-Cadena et al. 2008b). *Culex erraticus*, *Cx. quinquefasciatus*, and *Cx. salinarius* fed roughly equally on birds and mammals (Figure 2). Other studies generally agree with this finding. The proportion of blood meals taken from these two host groups varies considerably depending on location and season for these mosquito species. The degree of bird and mammal host use by *Cx. erraticus* shifts with season (Burkett-Cadena et al. 2011) with up to 100% of blood meals derived from avian hosts during the bird breeding season (Burkett-Cadena et al. 2012) and as little as 2% during other parts of the year (Burkett-Cadena et al. 2012). *Culex quinquefasciatus* s.s., *Culex pipiens* s.s., and their hybrids occur at the latitude of Auburn, AL (Kothera et al. 2009). *Culex quinquefasciatus* s.s. and hybrids normally take approximately 70% of blood meals from birds (Edman 1974; Savage et al. 2007). Our finding that *Culex quinquefasciatus* s.l. took only 53% of blood meals from avian hosts, when abundant, confined, relatively large birds were available, was unexpected. Humans were the major mammalian host of *Cx. quinquefasciatus* in this study, constituting 74.2% of mammalian blood meals. It is possible that an abundance of breeding sites and/or human hosts in the neighboring mobile home park (Figure 1) contributed to the relatively large number of blood meals from humans. Only two blood-fed females of *Cx. salinarius* were recovered, one feeding upon a Red-tailed hawk, the other feeding upon a cow. In Delaware, *Cx. salinarius* females were found to be attracted to and successfully feed upon a wide variety of bird and mammal species (Murphey et al. 1967) in host-baited traps. Studies in Florida (Edman 1974), Louisiana (Schaefer and Steelman 1969), Maryland (LeDuc et al. 1972), New Jersey (Apperson et al. 2004), North Carolina (Irby and Apperson 1988), and New York (Apperson et al. 2002) found that *Cx. salinarius* females feed predominantly upon mammals, although their selection of hosts is apparently opportunistic (Cupp and Stokes 1976). *Culex restuans* females fed primarily upon birds (64% of total), as expected, yet also took 32% of blood meals from humans. This finding was unexpected, since *Cx. restuans* is rarely found to take >1% of blood meals from humans in the southern U.S. (Savage et al. 2007). That *Cx. restuans* fed upon humans in this setting is significant, because this mosquito is an important enzootic vector of West Nile virus (WNV) in the eastern U.S.A. (Savage et al. 2007). Our data suggest that *Cx. restuans* may also serve as a bridge vector of WNV to humans in the southern U.S.A.

Raptor blood meals from *Cx. erraticus*, *Cx. quinquefasciatus*, *Cx. restuans* and *Cx. salinarius* were expected since these mosquito species are known to feed upon a wide variety of avian hosts in the southern U.S.A., including raptors (Savage et al. 2007, Hamer et al. 2009, Estep et al. 2011). Interestingly, the artificially high density of raptors at SRC was reflected in an increased proportion of blood meals from raptors in the mosquito community, compared

to studies of unmanipulated host populations in more natural settings (Thiemann et al. 2012, Savage et al. 2007, Hamer et al. 2009, Estep et al. 2011). For example, analysis of mosquito blood meals from Tuskegee National Forest, in neighboring Macon Co., AL, found that just 2.7% of avian blood meals were from raptors (Estep et al. 2011). In the current study, 58.5% of avian blood meals were from raptors. This suggests that the low numbers of raptor blood meals from field studies is related to their low relative abundance in the avian community. The lower percentage of blood meals taken from raptors in the early season (May and June) compared to later in the season (August) may reflect a seasonal shift in the mosquito community composition. In May and June the two most common blood-fed mosquitoes were *Cx. territans* and *An. punctipennis*. Combined, these species comprised 53.5% and 37.5% of the total bloodfed females collected in May and June (Table 1), respectively, yet were not found to feed upon raptors (Table 2).

The low sample sizes (24 raptor blood meals of 166 total identified blood meals) and reliance upon a single mosquito sampling strategy (resting stations) are limitations of the current study that likely reduce the power of the analysis. A larger blood meal dataset may have tightened the confidence intervals around the forage ratios, for example, indicating mosquito “preference” for one or more raptor species, such as Great horned owl or Red-tailed hawk. Nonetheless, our dataset was sufficiently robust to demonstrate statistically significant relationships between relative abundance of raptor species and their utilization by mosquitoes (Figures 3A, 3B). Additionally, it is possible that some of the raptor blood meals in our study were not from captive birds but from wild individuals in the environment. However, detailed studies of the avian community in neighboring Macon Co., AL, found that birds of prey constituted less than 1% of the total avian species (McClure et al. 2011), suggesting that wild raptors likely contribute little to the mosquito blood meals in the current work. The dataset and resulting analysis were derived from a single sampling method. Use of additional sampling mosquito methods, such as CO₂-baited light traps, would have added to the information on other mosquito species present at the site. However, light traps capture relatively few blood-engorged females (Komar et al. 1995), so little additional information on mosquitoes feeding upon raptors would have been gained by employing other techniques. In addition, calculating forage ratios for multiple (combined) mosquito species may seem counterintuitive, since innate host preference is often considered a largely genetic trait and host preferences of individual mosquito species may vary. However, since the mosquito species analyzed here all fed upon birds of prey, they are physiologically adapted to locate and feed upon members of that group and thus demonstrate common host associations. Therefore, investigating patterns of host use that focus on the host group, rather than the mosquito species, is probably appropriate.

This study found no support for the hypothesis that certain bird of prey species are preferred over others, when their behavioral characteristics are minimized through confinement. Instead, the results presented here support the idea that traits of host animals, more-so than innate preference, drive patterns of host use by mosquitoes, at least among birds of prey. Day and Edman (1984) remark that although mosquitoes are unlikely to seek out specific host species in nature, blood meal analyses indicate that some

mosquito species appear to be selective in their choice of hosts. Each mosquito species is, in fact, likely adapted to locating and successfully feeding upon a group of hosts (innate preference). Within that host group, however, there also likely exists a considerable amount of plasticity, so that behavior of the host (tolerance, activity patterns) drives which species of the group are successfully fed upon, and therefore overall patterns of host use. To state that mosquitoes do not seek out specific hosts in nature is probably not completely accurate. *Culex territans*, for example, feeds heavily upon frogs even when other hosts are available. *Culex territans* is even attracted to frog calls (Bartlett-Healy et al. 2008) and therefore has adaptations for locating frogs in nature. However, the species of frogs fed upon by *Cx. territans* shifts with season, as this mosquito feeds predominantly on the frog species that are breeding at a given time (Burkett-Cadena et al. 2012). It is surmised that *Cx. territans* feeds primarily upon calling male frogs, which are so distracted by their mate attraction that they reduce their vigilance to predators and parasites (Burkett-Cadena et al. 2012). *Culex territans* is therefore physiologically adapted to locating and successfully feeding upon frogs more so than other animals and frogs normally comprise the bulk of total feedings. However, *Cx. territans* also feeds occasionally upon other animals, suggesting that given the opportunity, this mosquito will feed upon a wide range of hosts. This interplay of innate host preference and opportunism likely produces the observed patterns of host use by *Cx. territans* and other mosquitoes.

Acknowledgments

The authors thank Roy Crowe, Marianne Murphy, and Jamie Bellah DVM (director) of the SRC, for permission to access and collect from SRC grounds. This research was supported by grants from the National Institute of Allergy and Infectious Diseases, Project # R01AI049724 and R56AI101072.

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, and R.S. Nasci. 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 C.S., H.K. Hassan, B.A. Harrison, H.M. Savage, S.E. Aspen, A. Farajollahi, W. Crans, T.J. Daniels, R.C. Falco, M. Benedict, M. Anderson, L. McMillen, and T.R. Unnasch. 2004. Host feeding patterns of established and potential mosquito vectors of West Nile virus in the eastern United States. *Vector-Borne Zoonot. Dis.* 4: 71–82.
- Bartlett-Healy K., W. Crans, and R. Gaugler. 2008. Phonotaxis to amphibian vocalizations in *Culex territans* (Diptera: Culicidae). *Ann. Entomol. Soc. Am.* 101: 95–103.
- Besansky N.J., C.A. Hill, and C. Costantini. 2004. No accounting for taste: host preference in malaria vectors. *Trends Parasitol.* 20: 249–251.
- Blackmore, J.S. and R.P. Dow. 1958. Differential feeding of *Culex tarsalis* on nestling and adult birds. *Mosq. News.* 18: 15–17.

- 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. Contr. Assoc.* 24: 228-235.
- 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.W. McClure, R.A. Ligon, S.P. Graham, C. 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* 6, e17681. doi:10.1371/journal.pone.0017681
- Burkett-Cadena, N.D., H.K. Hassan, M.D. Eubanks, E.W. Cupp, and T.R. Unnasch. 2012. Winter severity predicts the timing of host shifts in the mosquito *Culex erraticus*. *Biol. Lett.* published online 7 March 2012. doi:10.1098/rsbl.2012.0075.
- Chaves, L.F., L.C. Harrington, C.L. Keogh, A.M. Nguyen, and U.D. Kitron. 2010. Blood feeding patterns of mosquitoes: random or structured? *Front. Zool.* 7: 3.
- 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 epizootic focus in Tennessee. *Am. J. Trop. Med. Hyg.* 81: 452-456.
- Crans, W.J. 1970. The blood feeding habits of *Culex territans* Walker. *Mosq. News* 30: 445-447.
- Cupp, E.W. and G.M. Stokes. 1976. Feeding patterns of *Culex salinarius* Coquillett in Jefferson Parish, Louisiana. *Mosq. News* 36: 332-335.
- Darsie, R.F. and R.A. Ward. 2005. *Mosquitoes of North America, North of Mexico*. University of Florida Press, Gainesville, FL. 383 pp.
- Day, J.F. and J.D. Edman. 1984. Mosquito engorgement on normally defensive hosts depends on host activity patterns. *J. Med. Entomol.* 21: 732-740.
- Dekker, T., W. Takken, and M.A.H. Braks. 2001. Innate preference for host-odor blends modulates degree of anthropophagy of *Anopheles gambiae* sensu lato (Diptera: Culicidae). *J. Med. Entomol.* 38: 868-871.
- Edman, J.D. 1974. Host-feeding patterns of Florida mosquitoes III. *Culex (Culex)* and *Culex (Neoculex)*. *J. Med. Entomol.* 11: 95-104.
- Edman, J.D., L.A. Webber, and A.A. Schmid. 1974. Effect of host defenses on the feeding pattern of *Culex nigripalpus* when offered a choice of blood sources. *J. Parasitol.* 60: 874-883.
- Edman, J.D., J.F. Day, and E.D. Walker. 1984. Field confirmation of laboratory observations on the differential antimosquito behavior of herons. *Condor* 86: 91-92.
- Estep, L.K., C.J.W. McClure, N.D. 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.
- Estep, L.K., C.J.W. McClure, N.D. Burkett-Cadena, H.K. Hassan, T.R. Unnasch, and G.E. Hill. 2012. Developing models for the forage ratios of *Culiseta melanura* and *Culex erraticus* using species characteristics for avian hosts. *J. Med. Entomol.* 49: 378-387.
- Geier, M. and J. Boeckh. 1999. A new Y-tube olfactometer for mosquitoes to measure the attractiveness of host odours. *Entomol. Exp. Appl.* 92:9-19.
- Gray, K.M., N.D. Burkett-Cadena, M.D. Eubanks, and T.R. Unnasch. 2011. Crepuscular Flight Activity of *Culex erraticus* (Diptera: Culicidae). *J. Med. Entomol.* 48:167-172.
- Hamer, G.L., U.D. Kitron, T.L. Goldberg, J.D. Brawn, S.R. Loss, M.O. Ruiz, D.B. Hayes, and E.D. Walker. 2009. Host selection by *Culex pipiens* mosquitoes and West Nile Virus amplification. *Am. J. Trop. Med. Hyg.* 80: 268-278.
- 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.
- Hess, A.D. and A. Swartz. 1940. The forage ratio and its use in determining the food grade of streams. *Progr. Fish Culturist.* 7: 22-23.
- Irby, W.S. and C.S. Apperson. 1988. Hosts of mosquitoes in the Coastal Plain of North Carolina. *J. Med. Entomol.* 25: 85-93.
- Kay, B.H., P.F.L. Boreham, and J.D. Edman. 1979. Application of the "feeding index" concept to studies of mosquito host-feeding patterns. *Mosq. News* 39: 68-72.
- Kitano, T., K. Umetsu, W. Tian, and M. Osawa. 2007. Two universal primer sets for species identification among vertebrates. *Intl. J. Legal Med.* 121: 423-427.
- Komar, N., R.J. Pollack, and A. Spielman. 1995. A nestable fiber pot for sampling resting mosquitoes. *J. Am. Mosq. Contr. Assoc.* 11: 463-467.
- Kothera, L., E.M. Zimmerman, C.M. Richards, and H.M. Savage. 2009. Microsatellite characterization of subspecies and their hybrids in *Culex pipiens* complex (Diptera: Culicidae) mosquitoes along a north-south transect in the central United States. *J. Med. Entomol.* 46: 236-248.
- LeDuc, J.W., W. Suyemoto, B.F. Eldridge, and S. Saugstad. 1972. Ecology of arboviruses in a Maryland freshwater swamp. II. Blood feeding patterns of potential mosquito vectors. *Am. J. Epidemiol.* 96: 123-128.
- Lefèvre, T., L.-C. Gouagna, K.R. Dabiré, E. Elguero, D. Fontenille, F. Renaud, C. Costantini, and F. Thomas. 2010. Beer consumption increases human attractiveness to malaria mosquitoes. *PLoS ONE* 5: e9546. doi:10.1371/journal.pone.0009546.
- Manly, B.F., L. McDonald, D.L. Thomas, T.L. McDonald, and W. P. Erickson. 2002. *Resource selection by animals: statistical design and analysis for field studies*. Dordrecht, The Netherlands: Kluwer Academic Publishers.
- McClure, C.J.W., L.K. Estep, and G.E. Hill. 2011. Using public land cover data to determine habitat associations of breeding birds in Tuskegee National Forest, Alabama. *South. J. Appl. For.* 35: 199-209.
- Murphey, F.J., P.P. Burbutis, and D.F. Bray. 1967. Bionomics of *Culex salinarius* Coquillett. II. Host acceptance and feeding by adult females of *C. salinarius* and other mosquito species. 27: 366-374.
- Puri, S.N., M.J. Mendki, D. Sukumaran, K. Ganesan, S. Prakash, and K. Sekhar. 2006. Electroantennogram and behavioral responses of *Culex quinquefasciatus* (Diptera: Culicidae)

- females to chemicals found in human skin emanations. J. Med. Entomol. 43: 207-213.
- Savage, H.M., D. Aggarwal, C.S. Apperson, C.R. Katholi, E. Gordon, H.K. Hassan, M. Anderson, D. Charnetzky, L. Mcmillen, E.A. Unnasch, and T.R. Unnasch. 2007. Host choice and West Nile Virus infection rates in blood-fed mosquitoes, including members of the *Culex pipiens* complex, from Memphis and Shelby County, Tennessee, 2002-2003. Vector-Borne Zoonotic Dis. 7: 365-386.
- Schaefer, R.E. and C.D. Steelman. 1969. Determination of mosquito hosts in salt marsh areas of Louisiana. J. Med. Entomol. 6: 131-134.
- Vuilleumier, F. (Editor). 2011. *Birds of North America: Eastern region*. American Museum of Natural History. 480 pp.
- Thiemann, T.C., D.A. Lemenager, S. Kluh, B.D. Carroll, H.D. Lothrop, and W.K. Reisen. 2012. Spatial variation in host feeding patterns of *Culex tarsalis* and the *Culex pipiens* complex (Diptera: Culicidae) in California. J. Med. Entomol. 49: 903-916.
- Weaver, S.C. 2005. Host range, amplification and arboviral disease emergence. Arch. Virol. Suppl. 19: 33-44.