Medical and Veterinary Entomology

Medical and Veterinary Entomology (2012), doi: 10.1111/j.1365-2915.2012.01012.x

Blood-feeding ecology of mosquitoes in zoos

H. C. TUTEN[1,*], W. C. BRIDGES[2], K. S. PAUL[3] and P. H. ADLER[1]

[1]Department of Entomology, Soils and Plant Sciences, Clemson University, Clemson, SC, U.S.A., [2]Department of Applied Economics and Statistics, Clemson University, Clemson, SC, U.S.A. and [3]Department of Biological Sciences, Clemson University, Clemson, SC, U.S.A.

Abstract. To determine if the unique host assemblages in zoos influence blood-feeding by mosquitoes (Diptera: Culicidae), a sampling programme was conducted in Greenville and Riverbanks Zoos, South Carolina, U.S.A., from April 2009 to October 2010. A total of 4355 female mosquitoes of 14 species were collected, of which 106 individuals of nine species were blood-fed. The most common taxa were *Aedes albopictus* (Skuse), *Aedes triseriatus* (Say), *Anopheles punctipennis* (Say), *Culex erraticus* (Dyar & Knab), *Culex pipiens* complex (L.) and *Culex restuans* (Theobald). Molecular analyses (cytochrome *b*) of bloodmeals revealed that mosquitoes fed on captive animals, humans and wildlife, and took mixed bloodmeals. Host species included one amphibian, 16 birds, 10 mammals (including humans) and two reptiles. Minimum dispersal distances after feeding on captive hosts ranged from 15.5 m to 327.0 m. Mosquito–host associations generally conformed to previous accounts, indicating that mosquito behaviour inside zoos reflects that outside zoos. However, novel variation in host use, including new, exotic host records, warrants further investigation. Zoos, thus, can be used as experiment environments in which to study mosquito behaviour, and the findings extrapolated to non-zoo areas, while providing medical and veterinary benefits to zoo animals, employees and patrons.

Key words. *Aedes*, *Anopheles*, *Culex*, bloodmeal, cytochrome *b*, mosquito, zoos.

Introduction

Zoos are unique environments in which to study mosquito foraging behaviour and to use strong hypothesis testing to elucidate host adaptations and preferences of mosquitoes. For instance, zoos can be used to investigate the role of genetic vs. environmental factors in shaping mosquito host choices, and to examine the nestedness of ectoparasite and host networks, two recently suggested goals in medical and veterinary entomology (Graham *et al.*, 2009; Chaves *et al.*, 2010). Species of captive animals represented in mosquito bloodmeals can be compared with the potential host species available in a particular zoo, and also be used to acquire information such as on flight distances from hosts (Ejiri *et al.*, 2011). Zoos are also excellent experiment environments for addressing another recently suggested research goal that involves determining the influence of environmental factors on mosquito assemblages (Beketov *et al.*, 2010).

Zoos have epidemiological consequences for captive and wild animals and humans. Mosquitoes transmit pathogens that have resulted in the deaths of captive birds and mammals, including those of endangered species (Adler *et al.*, 2011). High host heterogeneity in zoos may contribute to increases in pathogen prevalence, leading to epizootics (Kilpatrick *et al.*, 2006a), or may cause a dilution of biting rates on susceptible hosts, thereby decreasing pathogen incidence in the general population (Bradley & Altizer, 2007). Zoos, thus, are ideal environments for addressing hypotheses related not only to mosquito ecology, but also to the dynamics of pathogen transmission because: (a) mosquitoes are present; (b) the species and movements of hosts are known; (c) animals are under regular observation, and (d) wild and captive animals

Correspondence: Holly C. Tuten, Department of Entomology, University of Kentucky, S-225 Agricultural Science Center North, Lexington, KY 40546-0091, U.S.A. Tel.: +1 859 257 7450; Fax: +1 859 323 1120; E-mail: htuten@gmail.com

*Present address: Department of Entomology, University of Kentucky, Lexington, KY, U.S.A.

are in the same area. If the results of studies in zoos represent those in non-zoo environments, they provide the power to predict mosquito distributions and patterns of host usage in areas in which field studies are not feasible.

The objectives of the present study were to elucidate blood-feeding patterns, hosts and post-bloodmeal dispersal of mosquitoes in zoos. The study tested the hypotheses that mosquito bloodmeals would: (a) degrade with time; (b) represent captive animals, humans and wildlife; (c) include examples of mixed host species, and (d) conform to known mosquito–host class associations (e.g. mammals, birds).

Materials and methods

Site selection and sampling

Mosquitoes were collected from Greenville Zoo (Greenville County, 34°50′15.15″ N, 82°21′23.23″ W) and Riverbanks Zoo (Richland County, 34°00′46.39″ N, 81°04′42.93″ W), South Carolina, U.S.A.

In April 2009, 15 gravid trap sites were selected at Greenville Zoo and 19 at Riverbanks Zoo. These sites had little human traffic, partial shade and protection from wind, and artificial lighting. Gravid trap infusion water included rabbit pellets and alfalfa hay, and was based on the recipe of Jackson *et al.* (2005). Once per month, five locations at each zoo were selected randomly for gravid trap placement, with the caveat that all traps be at least 50 m apart to ensure independence (Allan *et al.*, 1987; Reiter, 2007). To encompass the period of maximum mosquito activity while minimizing exposure-induced mosquito mortality, Reiter–Cummings modified gravid traps were activated between 16.00 hours and 17.00 hours and retrieved between 08.00 hours and 09.00 hours. Two traps were run for 3 days per location, resulting in 30 trap nights per zoo per month from June to September 2009. In addition, Greenville Zoo was sampled in April 2009 and Riverbanks Zoo in May 2009. All containers were cleaned with 95% ethanol between trap catches. In 2009, two resting boxes were placed near animal habitats at both zoos, but these failed to capture mosquitoes.

In April 2010, 13 aspiration sites at Greenville Zoo and 17 aspiration sites at Riverbanks Zoo were selected; new sites were added on an ad hoc basis. Both zoos were sampled one to four times per month for periods of 1–3 days from May to September by mouth and mechanical (hand-held and backpack) aspiration. Riverbanks Zoo was also sampled in April 2010 and February 2011. During 2009 and 2010, creeping ground cover (e.g. *Hedera helix* L.) at both zoos was sampled with a modified Centers for Disease Control (CDC) backpack aspirator (model 1412; John W. Hock Co., Gainesville, FL, U.S.A.). The South Carolina Department of Health and Environmental Control sampled mosquitoes on three separate nights in the summer of 2010 using a BG-Sentinel trap baited with octenol. Voucher specimens of each mosquito species are deposited in the Clemson University Arthropod Collection, South Carolina.

Mosquito processing

In 2009, mosquitoes were transported alive to the laboratory, killed in an ultralow freezer (−70 °C), identified to species using morphological keys (Darsie & Ward, 2004), and grouped by gonotrophic condition according to stages outlined by Sella (1920). The head plus thorax of each blood-fed mosquito was separated from the abdomen with a razor blade on a fresh KimWipe (lint-free paper cleaning wipe; Kimberly-Clark Corp., Irving, TX, U.S.A.), and each of the two sections was placed in its own autoclaved, ultraviolet (UV) light-sterilized 1.5-mL centrifuge tube. Tools were immersed in alcohol and flame-sterilized for at least 30 s after each mosquito was cut. Latex gloves were worn during sorting and processing. All collections were stored at −70 °C and later moved to −20 °C before processing. The same procedures were used in 2010, except that mosquitoes were killed on dry ice in the field.

Genomic DNA (gDNA) was extracted from the heads plus thoraxes and separately from the abdomens of blood-fed mosquitoes, using a DNAzol BD Direct Extraction Kit (Molecular Research Center, Cincinnati, OH, U.S.A.), according to the manufacturer's instructions, with slight modifications (Tuten, 2011a). The DNAzol BD is the least expensive kit on the market and has been used successfully for gDNA extractions from mosquito bloodmeals (Molaei *et al.*, 2008, 2009; Watts *et al.*, 2009). The gDNA extract was stored at 4 °C for up to 1 week, but transferred to −20 °C for longer periods. Genomic DNA was extracted from individual mosquitoes in batches of 20–30 individuals, using an initially empty, sterilized 1.5-mL tube as a negative control for contamination in each batch of extractions.

Bloodmeal analysis

Genomic DNA extracts from mosquito abdomens were amplified by polymerase chain reaction (PCR) on a Bio-Rad iCycler (Bio-Rad Inc., Hercules, CA, U.S.A.), using order-specific primers for birds and mammals and universal vertebrate primers (Table 1). All primers amplified segments of the mitochondrial cytochrome *b* gene and have been used previously in studies analysing mosquito bloodmeals. A 25-μL reaction mixture was used, containing 12.5 μL of GoTaq Colorless Master Mix (Promega Corp., Madison, WI, U.S.A.), 1 μL of forward and 1 μL of reverse (premixed) primer, 1 μL of gDNA, and 9.5 μL of nuclease-free water (provided with GoTaq). For every PCR, distilled autoclaved water was used as a negative control and domestic dog gDNA (from blood obtained at the Filariasis Research Reagent Repository, Athens, GA, U.S.A.) as a positive control for mammal-specific and universal vertebrate primers, and chicken gDNA (from blood-fed *Culex pipiens* in a colony at Ohio State University) for avian primers. The same gDNA used as positive controls was used to optimize PCR cycling conditions for each primer (Table 1). The strategy in the present study was to first attempt amplification with order-specific primers for birds and mammals. If neither of these reactions yielded a product, the sample was subjected to PCR with universal vertebrate primers. Successful amplifications

Table 1. Primers used to amplify mosquito host genomic DNA (target gene: cytochrome *b*).

Primer	Sequence (5′–3′)	Amplicon length, bp	Temperature			Cycles, *n*	Reference
			Denaturation, °C	Annealing, °C	Extension, °C		
Avian	GACTGTGACAAAA TCCCNTTCCA GGTCTTCATCTYHG GYTTACAAGAC	508	94	55	72	33	Ngo & Kramer (2003)
Mammal	CGAAGCTTGATATG AAAAACCATCGTTG TGTAGTTRTCWGGG TCHCCTA	772	94	50	72	35	Ngo & Kramer (2003)
Universal vertebrate	AAAAAGCTTCCATCCA ACATCTCAGCATGA TGAAA AAACTGCAGCCCCTCA GAATGATATTTGTC CTCA	307	94	50	72	40	Kocher *et al.* (1989)

were determined by visualizing PCR products on a 1.5% agarose gel with EDTA followed by ethidium bromide staining and UV transillumination. Gels were documented digitally on a Bio-Rad Gel Doc System (Bio-Rad, Inc.) and archived.

Products of PCR were purified out of successful reaction mixtures, using an 'Exo-AP' protocol. Briefly, a master Exo-AP mix was made by diluting (with DNA-grade H_2O) Exonuclease I at 1 : 100 and Antarctic phosphatase at 1 : 10 in the same PCR tube. Then, 1 μL of the Exo-AP mix was added to 1 μL of PCR product. The resultant mixture was placed in a PCR cycler and PCR products were purified under a thermal profile of 37 °C for 30 min and 80 °C for 15 min, and held at 4 °C. A forward or reverse primer was added to wells (depending on which primer performed better in sequencing in test trials) and purified products were sent to the Clemson University Genomics Institute for Sanger sequencing on an ABI 3130 unit (Applied Biosystems, Inc., Foster City, CA, U.S.A.). Primer sequences were removed from trace files and the remaining sequences were edited with BioEdit 7.0.5.3 freeware (Hall, 1999). The sequences were run through the GenBank nucleotide (nr) database, using the BLASTN 2.2.25+ algorithm, and vertebrate hosts identified (Altschul *et al.*, 1997). Results with the highest 'Max score' were recorded along with the 'Max ident' percentage values. For each bloodmeal, the highest percentage identity that was geographically reasonable is presented; lower percentages (<95%) indicate questionable identities.

To screen for experimenter contamination that would lead to false positive human identifications (Malmqvist *et al.*, 2004), sample sequences from successful amplifications with the mammal-specific primer set were checked against the sequence of the same amplicon from gDNA isolated from the experimenter (HCT), using the CAP contig assembly program in BioEdit (7.0.5.3) (Hall, 1999), with parameters of a one-base minimum match and 85% overlap. Other conspecific sample sequences were checked against one another similarly to ensure that non-human results did not reflect contamination.

Statistical analyses

Mosquito : host forage ratios (Hess *et al.*, 1968) were used to determine if mosquitoes exhibited host biases. Forage ratios were obtained by dividing the percentage of a particular host species represented in mosquito bloodmeals by the percentage of that host in the general population of captive animals.

Minimum flight distances were calculated, using Google Earth, for mosquitoes containing bloodmeals from captive hosts, based on the locations of mosquito captures and hosts. Because most animal enclosures were irregularly shaped, the shortest and longest distances between mosquito captures and enclosure boundaries were estimated. These distances were pooled across zoos and tested separately by host type (bird vs. mammal), Sella stage and mosquito species. If short and long distances did not differ significantly ($P < 0.05$), the average of the two distances was used. All analyses, G-tests and analyses of variance (ANOVAS) were conducted in JMP Version 9 (SAS Institute, Inc., Cary, NC, U.S.A.).

Results

Mosquito collections

Sixteen species of mosquito were collected at the zoos: 14 species were collected at Greenville Zoo and 14 species were collected at Riverbanks Zoo; 12 species were collected at both zoos (Table 2). Five species were blood-fed at Greenville and nine at Riverbanks; all of the blood-fed species found at Greenville were also found at Riverbanks. In total, 2879 individuals were collected at Greenville and 1476 at Riverbanks. Of these, 103 (2.4%) were blood-fed, 31 (1.1%) at Greenville and 72 (4.9%) at Riverbanks (Table 3). Aspiration methods yielded more blood-fed mosquitoes for effort than did mosquito-attraction traps. Approximately equal time was spent at each zoo, using either method. Aspiration methods yielded 47 (36.4%) blood-fed mosquitoes among 129 caught in total,

Table 2. Mosquito species (Diptera: Culicidae) collected with hand and backpack aspirators, light traps and (primarily) gravid traps at Greenville and Riverbanks Zoos, South Carolina, 2009–2011.

Species	GZ, total *n*	GZ, %	RZ, total *n*	RZ, %
Aedes albopictus	281	9.8	261	17.7
Aedes canadensis canadensis	0	0	1	0.1
Aedes japonicus	7	0.7	0	0
Aedes triseriatus	22	0.8	11	0.8
Aedes vexans	2	0.1	12	0.8
Anopheles punctipennis	6	0.2	54	3.7
Anopheles quadrimaculatus complex	1	0.1	5	0.3
Culex erraticus	1	0.1	64	4.3
Culex pipiens complex	1707	59.3	860	58.3
Culex pipiens/restuans	132	4.6	11	0.8
Culex restuans	700	24.3	108	7.3
Culex spp.	1	0.1	22	1.5
Culex territans	17	0.6	64	4.3
Orthopodomyia signifera	1	0.1	0	0
Psorophora ferox	0	0	1	0.1
Uranotaenia sapphirina	1	0.1	2	0.1
Total	2879		1476	

GZ, Greenville Zoo; RZ, Riverbanks Zoo.

whereas mosquito-attraction traps yielded 56 (1.3%) blood-fed mosquitoes among 4226 captures.

Genomic DNA amplifications and identifications

Hosts were identified successfully from 65.1% of bloodmeals (35.5% at Greenville, 77.8% at Riverbanks). Although the magnitude of unsuccessful identifications was greater for the Greenville samples, the failure trend across species did not differ between zoos. Two multiple bloodmeals were detected in mosquitoes at Riverbanks: one wild bird plus captive mammal in *Anopheles punctipennis* and one wild bird plus reptile (wild/captive status undetermined) in the *Cx. pipiens* complex. One of the human results, which was removed for reasons of possible experimenter contamination, may have been part of a mixed human and wild bird bloodmeal in a female of the *Cx. pipiens* complex from Greenville. Four human sequences had high homology with the experimenter sample (one *Culex erraticus* from Riverbanks, three *Cx. pipiens* complex from Greenville). Because the primer set was universally mammal-specific, the four excluded sequences may not have come from the experimenter but, rather, were possibly legitimate; nonetheless, the four data points were not included in statistical analyses or in tables, although their inclusion did not alter the significance of statistical tests.

The likelihood of bloodmeal identification success was significantly lower for *Culex restuans* than for *Aedes albopictus*, *An. punctipennis*, *Cx. erraticus*, *Cx. pipiens* complex and *Culex territans*, and for the *Cx. pipiens* complex and *Cx. territans* than for *An. punctipennis* and *Cx. erraticus* (G-test, G = 30.442, d.f. = 5, $P < 0.0001$). Overall success rates in bloodmeal identification by Sella stage were 81.0% (30/37) for Sella stage II, 81.0% (17/21) for Sella stage III, 81.8% (9/11) for Sella stage IV, 33.3% (1/3) for Sella stage V, and 26.3% (5/19) for Sella stage VI (Table 3). Bloodmeal identification success declined significantly with increasing Sella stage; identification in stages II, III and IV differed significantly from that in stage VI [G-test, G = 21.414, d.f. = 4, $P < 0.0003$; (Sella stage V excluded because of low sample size)]. Sella stage was independent of host type (i.e. bird or mammal) [G-test, G = 4.338, d.f. = 6, $P = 0.6310$ (Sella stage V excluded)]. Of 31 blood-fed mosquitoes captured at Greenville Zoo, 10 were in Sella stage II, III or IV, two were in Sella stage V, and 18 were in Sella stage VI; one was of unknown stage. Of 72 blood-fed mosquitoes captured at Riverbanks Zoo, 59 were in Sella stage II, III or IV, one was in Sella stage V, one was in Sella stage VI, and 11 were of unknown stage.

Flight distances

Minimum flight distances (dispersal) between mosquito captures and host locations ranged from 15.5 m to 327.0 m (mean ± standard error: 94.1 ± 13.4 m). Although flight distances differed by host type (bird vs. mammal), this difference was not significant (Welch's ANOVA, WF = 4.2395, d.f. 1 = 1, d.f. 2 = 17.82, $P = 0.0527$) (Table 3; three *Cx. erraticus* reptile bloodmeals were not included because flight distances of 26 m were identical). Flight distances did not differ significantly among *An. punctipennis* ($n = 9$), *Cx. erraticus* ($n = 10$) and the *Cx. pipiens* complex ($n = 10$) ($F = 2.2438$, d.f. 1 = 2, d.f. 2 = 26, $P = 0.1262$) (*Aedes triseriatus* and *Anopheles quadrimaculatus* were not included because sample numbers were $n = 2$ for each). The average flight distance of mosquitoes in Sella stage III was significantly greater than for those in Sella II ($F = 3.8099$, d.f. 1 = 2, d.f. 2 = 28, $P < 0.0344$), but neither differed from that of mosquitoes in Sella stage IV.

Mosquito hosts

Of the four species with more than five bloodmeals across both zoos, the *Cx. pipiens* complex and *Cx. erraticus* fed on a significantly different ratio of avian to mammalian to reptilian hosts in captivity than did *An. punctipennis* (G-test, G = 14.848, d.f. = 4, $P = 0.005$) (Fig. 1, Table 4). No significant differences were found among these four species feeding in the wild category (G-test, G = 5.296, $P = 0.5065$). Bloodmeals from humans were included in the wild category (removing them did not change the significance). The *Cx. pipiens* complex and *Cx. erraticus* showed a slight bias for birds, and *An. punctipennis* showed a strong bias for mammals (Table 5). *Aedes albopictus* fed on only wild animals, including three birds and two mammals (including one human).

Collections with more than four identified bloodmeals at Riverbanks Zoo did not show a trend toward increased use of captive animals in the zoo interior vs. the periphery (Fig. 2); however, the only human bloodmeals were from a more interior location (i.e. within the perimeter of the outer customer

Table 3. Average GenBank BLAST percentage identity, percentage of host genomic DNA amplification success, and average flight distances from hosts in zoos in South Carolina, 2009–2011, by mosquito species and Sella stage.

Blood-fed species	Sella stage*	GenBank BLAST identity, mean ± SE, % (*n*)		Amp. success, % (in successful/total tested)	Flight distance, mean ± SE, m (*n*)	
		GZ	RZ	Total	Avian	Mammalian
Aedes albopictus	Unknown	96.0 (1)	93.0 ± 7.0 (3)	80.0 (4/5)	NA	NA
	II	99.0 (1)	NA	100 (1/1)	NA	NA
Aedes triseriatus	II	NA	99.0 ± 0.0 (2)	100 (2/2)	59.0 (1)	23.0 (1)
	Unknown	NA	99.0 (1)	100 (1/1)	NA	79.5 (1)
Anopheles punctipennis	II	99.0 (1)	99.4 ± 0.2 (8)	90.0 (9/10)	75.5 (1)	53.9 ± 31.0 (7)
	VI	NA	95.0 (1)	50.0 (1/2)	NA	NA
Anopheles quadrimaculatus complex	III	NA	100 (1)	100 (1/1)	55.0 (1)	NA
	IV	NA	99.0 (1)	100 (1/1)	NA	68.0 (1)
Culex erraticus	II	NA	95.0 ± 2.0 (10)	90.9 (10/11)	253.0 (1)	112.0 ± 83.4 (2)
	III	NA	95.0 ± 2.0 (11)	91.7 (11/12)	248.5 ± 111.0 (2)	NA
	IV	NA	99.0 ± 1.0 (2)	66.7 (2/3)	193.0 (1)	NA
	V	NA	95.0 (1)	100 (1/1)	92.5 (1)	NA
	Unknown	NA	NA	0 (0/5)	NA	NA
Culex pipiens complex	II	100 (1)	98.0 ± 2.0 (6)	100 (7/7)	61.8 ± 44.0 (3)	NA
	III	99.0 (1)	93.0 ± 7.0 (4)	62.5 (5/8)	235.0 (1)	98.5 ± 97.6 (2)
	IV	99.5 ± 0.5 (2)	99.0 (3)	83.3 (5/6)	97.3 ± 59.8 (2)	110.0 ± 81.3 (2)
	V	NA	NA	0 (0/1)	NA	NA
	VI	92.0 ± 5.0 (4)	NA	28.6 (4/14)	NA	NA
Culex restuans	II	NA	NA	0 (0/1)	NA	NA
	IV	NA	93.0 (1)	100 (1/1)	NA	NA
	V	NA	NA	0 (0/1)	NA	NA
	VI	NA	NA	0 (0/3)	NA	NA
Culex territans	II	NA	99.0 (1)	20.0 (1/5)	NA	NA
Psorophora columbiae	Unknown	NA	NA	0 (0/1)	NA	NA

*Abdominal appearance by Sella stage: stage I, empty; stage II, with red blood and ovaries occupying up to three ventral and up to four dorsal segments; stage III, with dark red blood and ovaries occupying up to three ventral and five dorsal segments; stage IV, with dark red blood and ovaries occupying four to five ventral and six dorsal segments; stage V, with black blood and blood volume greatly reduced (some red clot) and ovaries occupying most of ventral and dorsal segments; stages VI and VII, abdomen with only trace of black blood or none, and ovaries occupying all ventral and dorsal segments (Sella, 1920).

GZ, Greenville Zoo; RZ, Riverbanks Zoo; SE, standard error; NA, not applicable.

walkway) in the zoo. Of the four reptile hosts identified, three came from mosquitoes captured within 37 m of their hosts: captive giant tortoises. A bloodmeal from an amphibian host (American green treefrog) came from a *Cx. territans* captured near an alligator pond. For the two species with the most hosts, the *Cx. pipiens* complex and *Cx. erraticus*, host identities were summed across zoos and years and a seasonal change in host use became evident (Fig. 3).

Discussion

The success rate (65.1%) of bloodmeal identification and the percentage of multiple bloodmeals (3.0%) are within the ranges of those reported in previous studies using similar methods and species (Molaei *et al.*, 2008, 2009; Ejiri *et al.*, 2011). The success rates of bloodmeal identification for Sella stages were similar to those found by Ejiri *et al.* (2011), who used their own abdominal grading scheme in which 'full-fed' = Sella stage II, 'partially fed' = Sella stage III, 'half-gravid' = Sella stages IV and V, and 'gravid' = Sella stages VI and VII. The decline in the rate of successful bloodmeal identification is likely to reflect the decreasing bloodmeal volume and, hence, decreasing amounts of DNA (Ejiri *et al.*, 2011) and DNA degradation caused by the digestive action of bloodmeal nucleases. Although the likelihood of obtaining a host identification decreased with increasing Sella stage, if gDNA was amplified, the average GenBank BLAST percentage identity between the purified amplicon sequences and GenBank sequences was similar across stages, suggesting that regardless of bloodmeal age, a successful identification is likely if host gDNA is recovered. Host DNA identifications from mosquitoes collected at Riverbanks Zoo were more successful than those from samples collected at Greenville Zoo, possibly because a higher percentage of mosquitoes were captured in late (V and VI) Sella stages at Greenville Zoo than at Riverbanks Zoo. In addition, the Greenville Zoo samples were subjected to an additional thaw–freeze cycle during transportation.

The relative proportion of captive hosts represented in mosquito bloodmeals in the current study was lower than that in a study conducted by Nelder (2007) at Riverbanks Zoo:

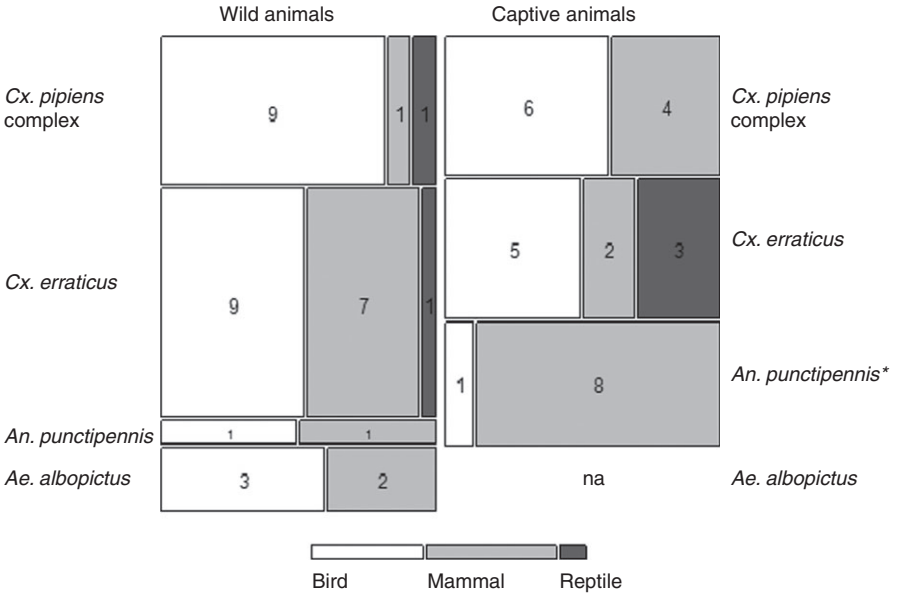

Fig. 1. Identity of mosquito bloodmeals during 2009–2011, across two South Carolina zoos, by captive vs. wild status. Numbers in boxes refer to number of hosts; sizes of boxes represent different host numbers. Wild category includes human bloodmeals for *Anopheles punctipennis* ($n = 1$), *Culex erraticus* ($n = 1$) and *Culex pipiens* complex ($n = 3$). **An. punctipennis* differs significantly from the two other species in the captive category (G-test, $P \leq 0.05$). NA, not applicable.

41.7% vs. 71.4% for *Cx. erraticus*, and 47.6% vs. 62.5% for the *Cx. pipiens* complex. However, the sample size in the current study was more than twice that of Nelder (2007), which may account for the discrepancy. The 16 bird and 10 mammal species identified in the present study are comparable with the 17 bird and seven mammal species identified in the study by Ejiri *et al.* (2011), and the two studies show one mammalian host (*Bos taurus*) and one avian genus (*Parus*) in common. Two of the most prevalent mammals (horse, *Equus caballus*, and human, *Homo sapiens*) and two of the most common birds (grey catbird, *Dumetella carolinensis*, and northern cardinal, *Cardinalis cardinalis*) in the current study were also reported among the most common mammals and birds in a previous meta-analysis of 12 bloodmeal studies conducted primarily in the eastern U.S.A. (Chaves *et al.*, 2010).

Of the species with more than five bloodmeals from captive animals across both zoos, the *Cx. pipiens* complex and *An. punctipennis* showed host-class usage similar to that reported previously from wild animals (Molaei *et al.*, 2008, 2009; Hamer *et al.*, 2009), whereas *Cx. erraticus* showed an avian bias by contrast with the opportunistic host usage and mammal bias reported previously (Irby & Apperson, 1988; Robertson *et al.*, 1993; Savage *et al.*, 2007). An avian bias was seen previously for *Cx. erraticus* at Riverbanks Zoo (Nelder, 2007). *Aedes albopictus* also showed avian associations to a greater degree than previously reported (Savage *et al.*, 1993; Richards *et al.*, 2006). For *Cx. erraticus* and the *Cx. pipiens* complex, a seasonal shift was detected in the use of birds vs. mammals (e.g. early-season bird feeding); this shift has been previously noted for the *Cx. pipiens* complex (Kilpatrick *et al.*, 2006b), but not for *Cx. erraticus* (Irby & Apperson, 1988). The seasonal change is unlikely to be related to shifts in the availability of zoo animals, which are accessible throughout the year.

Host usage by *Ae. albopictus* and *Cx. erraticus* in zoos merits further investigation. *Aedes albopictus* is reported to use avian hosts infrequently, but, as it is opportunistic and ground-associated, it may take bloodmeals from any hosts it encounters during appetitive flights (Dennett *et al.*, 2007). Zoos possibly represent predator-limited areas for wild birds, especially urban-associated passerines, which may forage more often on the ground, where *Ae. albopictus* would encounter them. Alternatively, they may forage more often on the ground or spend more time foraging because there is less competition in zoos from ground-dwelling mammals that are subject to pest control programmes. The northern cardinal has been reported once as a host of *Ae. albopictus* (Richards *et al.*, 2006), but, to the authors' knowledge, the other two avian hosts of *Ae. albopictus* in the current study are novel records. *Culex erraticus* has been described as opportunistic (Irby & Apperson, 1988), although it may feed preferentially on large birds with lowered defences (e.g. nesting birds) that occur in high abundance (Hassan *et al.*, 2003; Unnasch *et al.*, 2006; Mackay, 2007). Five of the 14 avian hosts of *Cx. erraticus* in the present study were captive and large (e.g. flamingo). Of the previous studies reporting host usage in zoo mosquitoes, Nelder (2007) found a similar avian association in *Cx. erraticus*, and Ejiri *et al.* (2011) reported *Ae. albopictus* feeding on four humans, one rat and one black-necked swan.

Bird-feeding in zoos implies the potential transmission of malarial agents to wild and captive birds (Ejiri *et al.*, 2011), and the transmission of West Nile virus (WNV) from birds,

Table 4. Hosts of blood-fed mosquitoes collected at Greenville (GZ) and Riverbanks (RZ) Zoos, South Carolina, 2009–2011. If more than one mosquito was positive for a given host, the separate mosquitoes are indicated in parentheses, with zoo and GenBank BLAST percentage identities. Ratios of host types are presented.

Blood-fed spp.	Hosts ID/total GZ, *n* (%)	Hosts ID/total RZ, *n* (%)	Hosts ID/total, *n* (%)	Host species* (zoo, GenBank BLAST % identity†)	C : H : W, GZ	C : H : W, RZ	A : M : Re, GZ	A : M : Re, RZ
Aedes albopictus	2/2 (100)	3/5 (60)	5/7 (71)	Avian: northern cardinal (*Cardinalis cardinalis*) (RZ, 79), Carolina chickadee‡ (*Poecile carolinensis*) (RZ, 100), mourning dove‡ (*Zenaida macroura*) (RZ, 100) Mammal: Virginia opossum (*Didelphis virginiana*) (GZ, 96), human (*Homo sapiens*) (GZ, 99)	0 : 1 : 1	0 : 0 : 4	0 : 2 : 0	3 : 0 : 0
Aedes triseriatus	0/0 (NA)	2/2 (100)	2/2 (100)	Avian: common ostrich‡ (*Struthio camelus*) (RZ, 99) Mammal: brown bear‡ (*Ursus arctos*) (RZ, 99)	NA	2 : 0 : 0	NA	1 : 1 : 0
Anopheles punctipennis	1/2 (50)	10/10§ (100)	11/12 (92)	Avian: summer tanager‡ (*Piranga rubra*) (RZ, 99¶), Common ostrich‡ (RZ, 99) Mammal: auroch (i.e. cow) (*Bos taurus*) (RZ, 99; RZ, 99¶), goat (*Capra hircus*) (GZ, 99), spotted hyena‡ (*Crocuta crocuta*) (RZ, 100), horse (*Equus caballus*) (RZ, 100; RZ, 100; RZ, 99; RZ, 99), human (RZ, 95)	1 : 0 : 0	8 : 1 : 1	0 : 1 : 0	2 : 8 : 0
Anopheles quadrimaculatus complex	0/0 (NA)	2/2 (100)	2/2 (100)	Avian: common ostrich‡ (RZ, 100) Mammal: brown bear‡ (RZ, 99)	NA	2 : 0 : 0	NA	1 : 1 : 0
Culex erraticus	0/0 (NA)	24/28 (86)	24/28 (86)	Avian: grey crowned crane‡,** (*Balearica regulorum*) (RZ, 97), northern cardinal (RZ, 99; RZ, 99), grey catbird (*Dumetella carolinensis*) (RZ, 88), Carolina chickadee (RZ, 89), American flamingo‡ (*Phoenicopterus ruber*) (RZ, 99; RZ, 95), keel-billed toucan‡ (*Ramphastos sulfuratus*) (RZ, 100), common ostrich‡ (RZ, 100), common starling (*Sturnus vulgaris*) (RZ, 89; RZ, 89), mourning dove (RZ, 100; RZ, 99; RZ, 99) Mammal: horse (RZ, 100), human (RZ, 95; RZ, 88; RZ, 82), raccoon (*Procyon lotor*) (RZ, 99; RZ, 90), ring-tailed lemur‡ (*Lemur catta*) (RZ, 95) Reptile: Galapagos tortoise‡,** (*Chelonoidis nigra*) (RZ, 98; RZ, 98, RZ, 98)	NA	10 : 3 : 11	NA	14 : 7 : 3
Culex pipiens complex	8/21 (38)	13/19§ (68)	21/40 (50)	Avian: wreathed hornbill‡ (*Rhyticeros undulatus*) (GZ, 100), northern cardinal (GZ, 100; RZ, 99), yellow-throated warbler (*Dendroica dominica*) (RZ, 72¶), grey catbird (RZ, 89), tufted titmouse (*Baeolophus bicolor*) (GZ, 77), American flamingo‡ (RZ, 100; RZ, 100), Carolina chickadee (GZ, 99), Toco toucan‡ (*Ramphastos toco*) (RZ, 100), common ostrich‡ (RZ, 99), Carolina wren (*Thryothorus ludovicianus*) (RZ, 99), northern red-billed hornbill‡ (*Tockus erythrorhynchus*) (RZ, 99), mourning dove (RZ, 100; RZ, 99) Mammal: auroch (RZ, 99), spotted hyena‡ (*Crocuta crocuta*) (RZ, 99), human (GZ, 100; RZ, 95), ring-tailed lemur‡ (GZ, 99), siamang (*Symphalangus syndactylus*)‡,†† (GZ, 99) Reptile: American box turtle (*Terrapene carolina*)** (RZ, 99¶)	3 : 1 : 4	7 : 0 : 6	5 : 3 : 0	10 : 2 : 1
Culex restuans	0/4 (0)	1/2 (50)	1/6 (17)	Avian: northern cardinal (RZ, 93)	NA	0 : 0 : 1	NA	1 : 0 : 0
Culex territans	0/2 (0)	1/3 (33)	1/5 (20)	Amphibian: green treefrog (*Hyla cinerea*) (RZ, 99)	NA	0 : 0 : 1	NA	0 : 0 : 0
Psorophora columbiae	0/0 (NA)	0/1 (0)	0/1 (0)	NA	NA	NA	NA	NA
Total	11/31 (36)	56/72 (78)	67/103 (65)	—	4 : 2 : 5	29 : 4 : 24	5 : 6 : 0	32 : 19 : 4

*Common and Latin names follow those of the International Ornithologist's Union for birds (www.worldbirdnames.org), Wilson and Reeder's Mammal Species of the World, 3rd edn., online searchable database for mammals (www.bucknell.edu/msw3), and the International Union for Conservation of Nature and Natural Resources (IUCN) Red List of Threatened Species for reptiles and amphibians (www.iucnredlist.org).

†Three bloodmeal identifications of wild bird hosts of *Culex erraticus* (two common starlings, one grey catbird) and one of *Culex pipiens* complex (grey catbird) were not the highest percentage hits returned by GenBank, but were the highest hits that made sense geographically.

‡Novel host record (results with <95% identity not evaluated).

§One mosquito with mixed bloodmeal.

¶Mixed bloodmeal.

**IUCN 2.3 'Vulnerable'.

††IUCN 2.3 'Endangered'.

A, avian; C, captive; H, human; M, mammalian; Re, reptilian; W, wild; NA, not applicable.

Medical and Veterinary Entomology © 2012 The Royal Entomological Society, *Medical and Veterinary Entomology*, doi: 10.1111/j.1365-2915.2012.01012.x

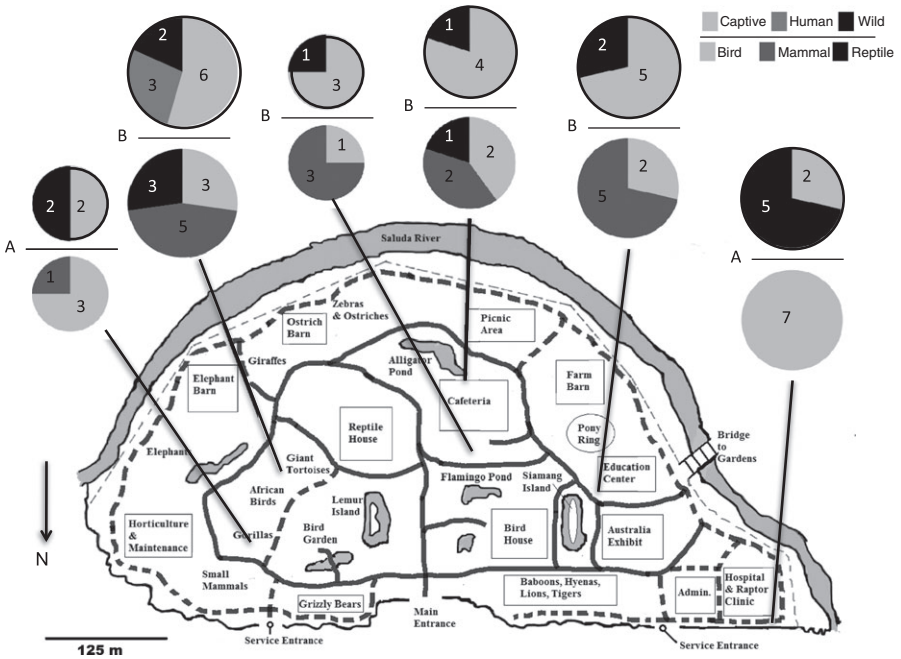

Fig. 2. Mosquito hosts (captive, human, wild above line; avian, mammal, reptile below line) in Riverbanks Zoo, South Carolina, 2009–2011. 'A' indicates a gravid trap site and 'B' a mechanical (hand-held) aspiration site. Dashed lines indicate zoo boundaries and employee access roads. Solid lines are visitor walkways.

Table 5. Forage ratios of mosquitoes on captive animals in South Carolina zoos, 2009–2011.

Species	Current study, bloodmeals, *n*	
Culex pipiens complex	Avian (6)	Mammal (4)
In bloodmeals, %	60.0	40.0
In zoo population, %*	52.0	48.0
Forage ratio	1.2	0.8
Culex erraticus	Avian (5)	Mammal (2)
In bloodmeals, %	71.0	29.0
In zoo population, %*	52.0	48.0
Forage ratio	1.4	0.6
Anopheles punctipennis	Avian (1)	Mammal (8)
In bloodmeals, %	12.5	87.5
In zoo population, %*	52.0	48.0
Forage ratio	0.2	1.8

*Estimate of percentage of individuals in each class exposed to mosquitoes at zoos, summed across both zoos; standing population numbers in zoos were obtained from zoo registers.
A forage ratio of >1 indicates a preference for that host type because the mosquito is taking more bloodmeals from that host type than expected based on the standing population.

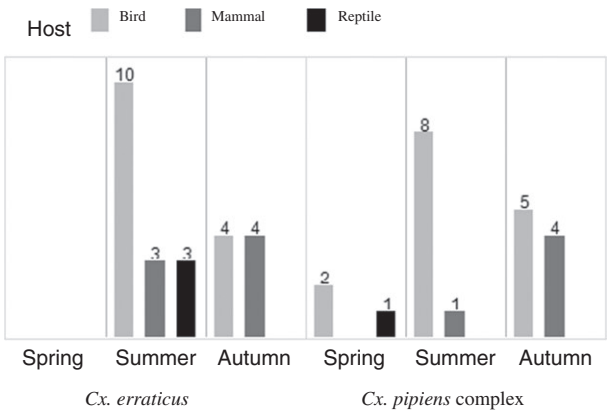

Fig. 3. Host types for *Culex erraticus* and *Culex pipiens* complex summed across 2009–2011 and Greenville and Riverbanks Zoos, South Carolina. The total number of hosts is given at the top of the bar.

which act as natural amplification reservoirs, to humans (Kilpatrick *et al.*, 2006b). The first fully sequenced strain of WNV from North America was isolated from a flamingo at the Bronx Zoo (Lanciotti *et al.*, 1999). *Aedes albopictus*, *Ae. triseriatus*, *Cx. erraticus*, *Cx. pipiens* complex and *Cx. restuans* have been implicated as vectors of eastern equine encephalitis virus, La Crosse encephalitis virus and WNV (Wozniak *et al.*, 2001; Dennett *et al.*, 2007; Kilpatrick *et al.*, 2007), and the current study showed some species of bridge vectors (e.g. *Cx. pipiens* complex) feeding on WNV-susceptible birds, such as the northern cardinal (Hamer *et al.*, 2009).

Mammals in zoos are also at risk for infection by mosquito-borne pathogens. Eight mammal hosts (brown bear, cow, goat, horse, human, lemur, opossum, raccoon) in the present study are susceptible to WNV (Blitvich, 2008). *Anopheles punctipennis*, a common mosquito species in zoos, has been implicated as a vector of *Dirofilaria immitis* (Nematoda: Filarioidea) in Georgia, U.S.A. (Licitra *et al.*, 2010). *Dirofilaria immitis* can infect large carnivores and has been implicated in the deaths of zoo animals (Adler *et al.*, 2011). The incidence of *D. immitis*

in wild-caught mosquitoes in the southeastern U.S.A. (e.g. Florida, Georgia, North Carolina) is 0.07–2.3% (Parker, 1993; Watts *et al.*, 2001; Licitra *et al.*, 2010). None of the tested mosquitoes ($n = 45$) in the present study were positive for *D. immitis* (Tuten, 2011a). Additionally, many zoos house rare and exotic reptiles and amphibians that may be vulnerable to mosquito-borne pathogens. *Culex territans*, for example, is a vector of reptile and amphibian trypanosomes (Bartlett-Healy *et al.*, 2009).

Mosquito dispersal following a bloodmeal from a captive animal represents an average net movement of <100 m, suggesting that selection favours limited dispersal after blood-feeding. The implication is that zoos are likely to maintain mosquito populations as long as sufficient habitats are available for larval development (Tuten, 2011b). Although Ejiri *et al.* (2011) found significantly longer flight distances for gravid females than for full-fed, partially fed and half-gravid females, no differences were noted in flight distances among the 33 mosquitoes with known Sella stages in the present study. This difference between studies may reflect differential availabilities of oviposition sites (Tuten, 2011b).

Blood-feeding behaviour of mosquitoes in zoos conforms to the behaviour previously recorded outside zoos, but differs sufficiently to merit further investigation. The study of blood-feeding ecology in zoos also carries medical and veterinary benefits. The present work demonstrates that by engaging zoos in experiments on mosquito behaviour, for example through the elucidation of host–mosquito–pathogen dynamics, insights can be gained into similar interactions in the more inclusive natural environment.

Acknowledgements

We thank C. Climer and the laboratory of A. Lawton-Rauh for sharing their 'Exo-AP' protocol; the South Carolina Department of Health and Environmental Control and especially C. Evans for confirmation of identifications, help with field collections and insight, and K. Benson DVM, J. Bullock, B. Foster, K. Gilchrist, H. Miller DVM, J. Lineberger, A. Norris, and S. Reno at the Greenville and Riverbanks Zoos for encouraging and facilitating this research. This is Technical Contribution No. 5982 of the Clemson University Experiment Station and is based on work supported in part by the National Institute of Food and Agriculture, U.S. Department of Agriculture, under project number SC-1700276.

References

Adler, P.H., Tuten, H.C. & Nelder, M.P. (2011) Arthropods of medicoveterinary importance in zoos. *Annual Review of Entomology*, **56**, 123–142.

Allan, S.A., Day, J.F. & Edman, J.D. (1987) Visual ecology of biting flies. *Annual Review of Entomology*, **32**, 297–316.

Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D.J. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research*, **25**, 3389–3402.

Bartlett-Healy, K., Crans, W. & Gaugler, R. (2009) Vertebrate hosts and phylogenetic relationships of amphibian trypanosomes from a potential invertebrate vector, *Culex territans* Walker (Diptera: Culicidae). *Journal of Parasitology*, **95**, 381–387.

Beketov, M.A., Yurchenko, Y.A., Belevich, O.E. & Liess, M. (2010) What environmental factors are important determinants of structure, species richness, and abundance of mosquito assemblages. *Journal of Medical Entomology*, **47**, 129–139.

Blitvich, B.J. (2008) Transmission dynamics and changing epidemiology of West Nile virus. *Animal Health Research Reviews*, **9**, 71–86.

Bradley, C.A. & Altizer, S. (2007) Urbanization and the ecology of wildlife diseases. *Trends in Ecology and Evolution*, **22**, 95–102.

Chaves, L.F., Harrington, L.C., Keogh, C.L., Nguyen, A.M. & Kitron, U.D. (2010) Blood-feeding patterns of mosquitoes: random or structured? *Frontiers in Zoology*, **7**, 3.

Darsie, R.F. & Ward, R.A. (2004) *Identification and Geographical Distribution of the Mosquitoes of North America, North of Mexico.* University Press of Florida, Gainesville, FL.

Dennett, J.A., Bala, A., Wuithiranyagool, T. *et al.* (2007) Associations between two mosquito populations and West Nile virus in Harris County, Texas, 2003–2006. *Journal of the American Mosquito Control Association*, **23**, 264–275.

Ejiri, H., Sato, Y., Kim, K.S. *et al.* (2011) Entomological study on transmission of avian malaria parasites in a zoological garden in Japan: bloodmeal identification and detection of avian malaria parasite DNA from blood-fed mosquitoes. *Journal of Medical Entomology*, **48**, 600–607.

Graham, S.P., Hassan, H.K., Burkett-Cadena, N.D., Guyer, C. & Unnasch, T.R. (2009) Nestedness of ectoparasite–vertebrate host networks. *PLoS ONE*, **4**, e7873.

Hall, T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, **41**, 95–98.

Hamer, G.L., Kitron, U.D., Goldberg, T.L. *et al.* (2009) Host selection by *Culex pipiens* mosquitoes and West Nile virus amplification. *American Journal of Tropical Medicine and Hygiene*, **80**, 268–278.

Hassan, H.K., Cupp, E.W., Hill, G.E., Katholi, C.R., Klingler, K. & Unnasch, T.R. (2003) Avian host preference by vectors of eastern equine encephalomyelitis virus. *American Journal of Tropical Medicine and Hygiene*, **69**, 641–647.

Hess, A.D., Hayes, R.O. & Tempelis, C.H. (1968) The use of the forage ratio technique in mosquito host preference studies. *Mosquito News*, **28**, 386–389.

Irby, W.S. & Apperson, C.S. (1988) Hosts of mosquitoes in the coastal plain of North Carolina. *Journal of Medical Entomology*, **25**, 85–93.

Jackson, B.T., Paulson, S.L., Youngman, R.R., Scheffel, S.L. & Hawkins, B. (2005) Oviposition preferences of *Culex restuans* and *Culex pipiens* (Diptera: Culicidae) for selected infusions in oviposition traps and gravid traps. *Journal of the American Mosquito Control Association*, **21**, 360–365.

Kilpatrick, A.M., Daszak, P., Jones, M.J., Marra, P.P. & Kramer, L.D. (2006a) Host heterogeneity dominates West Nile virus transmission. *Proceedings of the Royal Society B: Biology*, **273**, 2327–2333.

Kilpatrick, A.M., Kramer, L.D., Jones, M.J., Marra, P.P. & Daszak, P. (2006b) West Nile virus epidemics in North America are driven by shifts in mosquito feeding behaviour. *PLoS Biology*, **4**, e82.

Kilpatrick, A.M., LaDeau, S.L. & Marra, P.P. (2007) Ecology of West Nile virus transmission and its impact on birds in the western hemisphere. *The Auk*, **124**, 1121–1136.

Kocher, T.D., Thomas, W.K., Meyer, A., Edwards, S.V., Paabo, S., Villablanca, F.X. & Wilson, A.C. (1989) Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proceedings of the National Academy of Sciences of the United States of America*, **86**, 6196–6200.

Lanciotti, R.S., Roehrig, J.T., Deubel, V. *et al.* (1999) Origin of the West Nile virus responsible for an outbreak of encephalitis in the northeastern United States. *Science*, **286**, 2333–2337.

Licitra, B., Chambers, E.W., Kelly, R. & Burkot, T.R. (2010) Detection of *Dirofilaria immitis* (Nematoda: Filarioidea) by polymerase chain reaction in *Aedes albopictus*, *Anopheles punctipennis*, and *Anopheles crucians* (Diptera: Culicidae) from Georgia, U.S.A. *Journal of Medical Entomology*, **47**, 634–638.

Mackay, A.J. (2007) Detection of West Nile virus activity in male and female mosquitoes, and evaluation of host-utilization patterns of mosquitoes, in East Baton Rouge parish, Louisiana. PhD Dissertation. Louisiana State University, Baton Rouge, LA.

Malmqvist, B., Strasevicius, D., Hellgren, O., Adler, P.H. & Bensch, S. (2004) Vertebrate host specificity of wild-caught blackflies revealed by mitochondrial DNA in blood. *Proceedings of the Royal Society: Biology*, **271** (Suppl.), 152–155.

Molaei, G., Andreadis, T.G., Armstrong, P.M. & 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*, *Coquilletidia*, *Psorophora*, and *Uranotaenia*. *Journal of Medical Entomology*, **45**, 1143–1151.

Molaei, G., Farajallohi, A., Armstrong, P.M., Oliver, J., Howard, J.J. & Andreadis, T.G. (2009) Identification of bloodmeals in *An. quadrimaculatus* and *An. punctipennis* from eastern equine encephalitis virus foci in northeastern U.S.A. *Medical and Veterinary Entomology*, **23**, 350–356.

Nelder, M. (2007) Arthropods at the interface of exotic and native wildlife: a multifaceted approach to medical and veterinary entomology in the zoos of South Carolina. PhD Dissertation. Clemson University, Clemson, SC.

Ngo, K.A. & Kramer, L.D. (2003) Identification of mosquito bloodmeals using polymerase chain reaction (PCR) with order-specific primers. *Journal of Medical Entomology*, **40**, 215–222.

Parker, B.M. (1993) Variation of mosquito (Diptera: Culicidae) relative abundance and *Dirofilaria immitis* (Nematoda: Filarioidea) vector potential in coastal North Carolina. *Journal of Medical Entomology*, **30**, 436–442.

Reiter, P. (2007) Oviposition, dispersal, and survival in *Aedes aegypti*: implications for the efficacy of control strategies. *Vector-Borne and Zoonotic Diseases*, **7**, 261–273.

Richards, S.L., Ponnusamy, L., Unnasch, T.R., Hassan, H.K. & Apperson, C.S. (2006) Host-feeding patterns of *Aedes albopictus* (Diptera: Culicidae) in relation to availability of human and domestic animals in suburban landscapes of central North Carolina. *Journal of Medical Entomology*, **43**, 543–551.

Robertson, L.C., Prior, S., Apperson, C.S. & Irby, W.S. (1993) Bionomics of *Anopheles quadrimaculatus* and *Culex erraticus* (Diptera: Culicidae) in the Falls Lake Basin, North Carolina: seasonal changes in abundance and gonotrophic status, and host-feeding patterns. *Journal of Medical Entomology*, **30**, 689–698.

Savage, H.M., Niebylski, M.L., Smith, G.C., Mitchell, C.J. & Craig, G.B. (1993) Host-feeding patterns of *Aedes albopictus* (Diptera: Culicidae) at a temperate North American site. *Journal of Medical Entomology*, **30**, 27–34.

Savage, H.M., Aggarwal, D., Apperson, C.S. et al. (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 and Zoonotic Diseases*, **7**, 365–386.

Sella, M. (1920) *Lotta Antimalarica a Fiumicino (Roma): Seconda Relazione, Parte II* (ed. by B. Grassi). Ministero dell'Interno, Rome.

Tuten, H.C. (2011a) Zoos as experiment environments: biology of larval and adult mosquitoes (Diptera: Culicidae). PhD Dissertation. Clemson University, Clemson, SC.

Tuten, H.C. (2011b) Habitat characteristics of larval mosquitoes in zoos of South Carolina, U.S.A. *Journal of the American Mosquito Control Association*, **27**, 111–119.

Unnasch, R.S., Cupp, E.W. & Unnasch, T.R. (2006) Host selection and its role in transmission of arboviral encephalitides. *Disease Ecology* (ed. by S. K. Collinge & C. Ray), pp. 73–89. Oxford University Press, New York, NY.

Watts, K.J., Reddy, G.R., Holmes, R.A., Lok, J.B., Knight, D.H., Smith, G. & Courtney, C.H. (2001) Seasonal prevalence of third-stage larvae of *Dirofilaria immitis* in mosquitoes from Florida and Louisiana. *Journal of Parasitology*, **87**, 322–329.

Watts, S.L., Fitzpatrick, D.M. & Maruniak, J.E. (2009) Bloodmeal identification from Florida mosquitoes (Diptera: Culicidae). *Florida Entomologist*, **92**, 619–622.

Wozniak, A., Dowda, H.E., Tolson, M.W. *et al.* (2001) Arbovirus surveillance in South Carolina, 1996–1998. *Journal of the American Mosquito Control Association*, **17**, 73–78.

Accepted 16 December 2011