VECTOR-HOST INTERACTIONS OF *CULEX PIPIENS* COMPLEX IN NORTHEASTERN AND SOUTHWESTERN USA

GOUDARZ MOLAEI,[1] SHAOMING HUANG[2] AND THEODORE G. ANDREADIS[1]

ABSTRACT. Studies on the vector-host interactions of *Culex pipiens* complex mosquitoes by sequencing portions of mitochondrial cytochrome *b* gene indicate that *Cx. p. pipiens* f. pipiens predominantly feed on avian hosts (93.1%), and focus feeding activity on several key bird species, in particular the American robin, the gray catbird, and the house sparrow in Connecticut. However, *Cx. p. quinquefasciatus* indiscriminately feed on both birds and mammals. *Culex p. quinquefasciatus* in Harris County - Texas and southern California acquired 39.1% and 88.2% of bloodmeals from birds, respectively. Mammalian-derived bloodmeals constituted 52.5% and 9.6% in the two regions, respectively. The most frequent avian hosts for this mosquito species in the southwestern U.S. were the mourning dove, the white-winged dove, the house sparrow and the house finch. Humans infrequently served as the source of bloodmeals for *Cx. p. pipiens* and *Cx. p. quinquefasciatus*. Microsatellite analysis of mosquitoes from Chicago, Illinois showed that *Cx. p. pipiens* f. pipiens with mammalian- derived bloodmeals had significantly higher ancestry and proportion of hybrids from *Cx. p. pipiens* f. molestus than did those with avian-derived bloodmeals.

KEY WORDS *Culex pipiens* complex, blood-feeding patterns, mitochondrial cytochrome *b* gene, microsatellite analysis, hybrid ancestry

INTRODUCTION

The *Culex pipiens* complex in North America consists of *Cx. p. pipiens* (L.) form pipiens, *Cx. p. pipiens* f. molestus, *Cx. p. quinquefasciatus* Say, and hybrids between *Cx. p. pipiens* f. pipiens and *Cx. p. quinquefasciatus* (Barr 1957). As principal vectors of several disease-causing pathogens, including West Nile virus (WNV) and St. Louis virus (Mitchell et al. 1980, Hayes et al. 2005, Savage et al. 2007), members of the complex exhibit substantial diversity in physiology, behavior and geographic distribution.

Knowledge of the vector-host interactions and host feeding patterns of *Culex pipiens* complex in nature is essential for evaluating their vectorial capacity and for assessing the role of various vertebrates as reservoir and amplifying hosts for vector-borne zoonotic pathogens. Although earlier studies using serologic techniques have provided valuable information on the blood feeding patterns of *Cx. pipiens* complex, recent advances in molecular techniques using polymerase chain reaction-based assays have permitted the identification of hosts to the species level with a much higher degree of accuracy.

As part of ongoing efforts to better understand the vector potentials of mosquito species in transmission of arboviruses, we reviewed studies on the host feeding patterns of *Cx. pipiens* complex in the northeastern (Connecticut) and southwestern (Harris County - Texas and southern California) U.S. (Molaei et al. 2006, 2007, 2010) (Fig. 1). We also reviewed studies on the genetic structure and potential influence of introgressions on the blood feeding behavior of *Cx. p. pipiens* in northeastern and midwestern U.S. (Huang et al. 2008, 2009).

Culex. p. pipiens form pipiens

Studies on the vector-host interactions and blood feeding patterns of *Cx. p. pipiens* f. pipiens populations from north temperate regions of the U.S. have shown that these mosquitoes readily feed on avian hosts and focus blood feeding activity on several key bird species, in particular Passeriformes, that are capable of harboring arboviruses (Apperson et al. 2002, 2004; Kilpatrick et al. 2006; Molaei et al. 2006; Savage et al. 2007; Hamer et al. 2008). An analyses of engorged *Cx. p. pipiens* f. pipiens from Connecticut, for example, revealed that 93.1% of these mosquitoes acquired bloodmeals from avian hosts only, 2.5% from mammalian, 0.5% from amphibian, and 3.9% from both avian and mammalian hosts (Fig. 2) (Molaei et al. 2006). In a recent study of *Cx. p. pipiens* from Sacramento and Yolo Counties in California, an overwhelming majority (> 99%) were identified as having avian-derived bloodmeals, though the genetic identities of these mosquitoes and degrees of introgressions with other members of the *Cx. pipiens* complex were not determined (Montgomery et al. 2011). However, considerable geographic variation in the host-selection patterns of *Cx. p. pipiens* f. pipiens populations has been reported throughout their range of distribution in the U.S. Studies in more southern and midwestern regions have shown that a substantial number of individuals fed on

---

[1] Center for Vector Biology and Zoonotic Diseases, The Connecticut Agricultural Experiment Station, 123 Huntington Street, New Haven, CT 06511.

[2] San Joaquin County Mosquito and Vector Control District, 7759 South Airport Way Stockton, CA 95206-3918.

Fig. 1. Collection sites of blood-fed (A) *Cx. p. pipiens* (n = 15 sites) in Chicago, Illinois, 2005–2006, (B) *Cx. p. pipiens* (n = 31 sites) in Connecticut, 2002–2004, (C) *Cx. p. quinquefasciatus* (n = 69 sites) in Orange, Riverside, and San Bernardino Counties, southern California, 2006–2008, (D) *Cx. p. quinquefasciatus* (n = 268 sites) in Harris County, Texas, 2005. (Panel A courtesy of Dr. Gabriel L. Hamer.)

mammals including humans: Washington DC and Maryland - 13% (Kilpatrick et al. 2006), Chicago, Illinois - 22.4% (Hamer et al. 2008), Tennessee - 24% (Apperson et al. 2004), and New Jersey - 38% (Apperson et al. 2004).

Avian hosts of Cx. p. pipiens f. pipiens: Several studies have reported American robins as the most frequent hosts for *Cx. p. pipiens* f. pipiens from various regions in the U.S. (Apperson et al. 2002, 2004; Kilpatrick et al. 2006; Molaei et al. 2006; Hamer et al. 2008; Montgomery et al. 2011). Among 27 avian species, the American robin, *Turdus migratorius* Linnaeus (37.7% of all vertebrate-derived bloodmeals), the gray catbird, *Dumetella carolinensis* (L.) (10.4%), the house sparrow, *Passer domesticus* (L.) (9.9%), the European starling, *Sturnus vulgaris* L. (6.6%), the mourning dove, *Zenaida macroura* (L.) (6.1%), and a few other mostly Passeriformes birds were identified as the most frequent hosts for *Cx. p. pipiens* f. pipiens in Connecticut (Molaei et al. 2006) (Fig. 3).

Populations of American robins use a wide variety of open and forested habitats in urban/suburban and rural settings throughout most of North America (Martin 1973, Hutto 1995). These birds are competent amplifying hosts for arboviruses including WNV and eastern equine encephalitis virus (EEEV), based on the intensity of infection and duration of viremias (Komar et al. 1999, 2003). Furthermore, WNV and EEEV have been isolated from wild American robins and antibody prevalence studies indicate that these birds are frequently exposed to the aforementioned

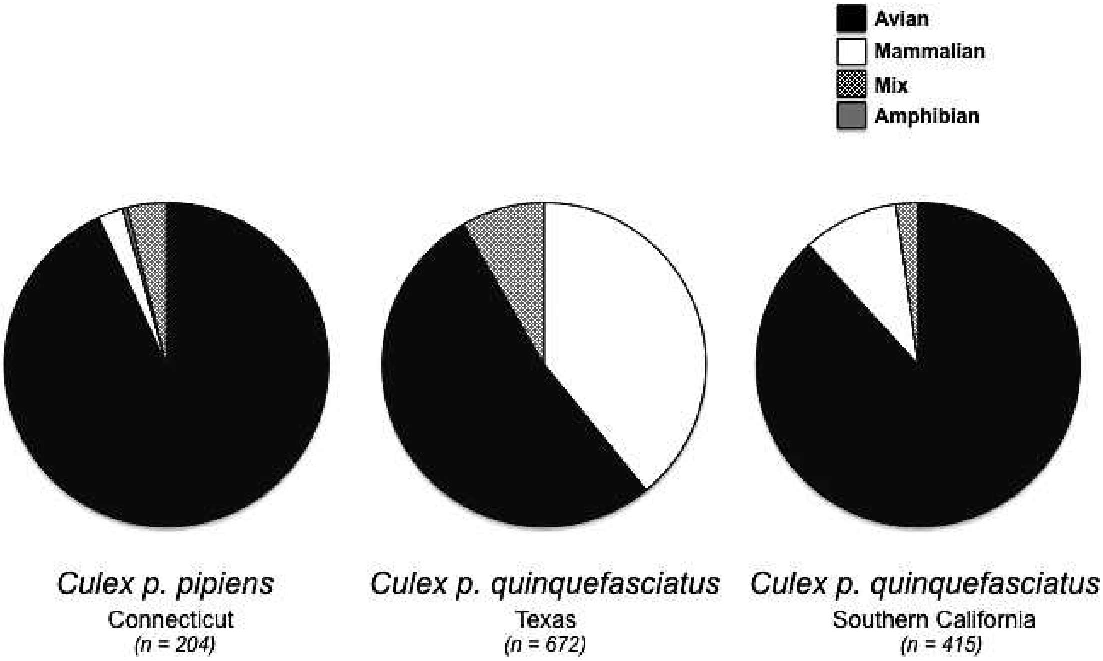

Fig. 2. Proportion of vertebrate blood meals for *Cx. p. pipiens* from Connecticut, and *Cx. p. quinquefasciatus* from Harris County in Texas, and from Orange, Riverside, and San Bernardino Counties in southern California.

viruses (Dalrymple et al. 1972, Bast et al. 1973, Morris et al. 1975, Main et al. 1988, Crans et al. 1994, Komar et al. 2001, Hamer et al. 2009, Kent et al. 2009). These findings highlight the importance of American robins in the amplification of arboviruses throughout their range of distribution in the U.S.

Mammalian hosts of Cx. p. pipiens f. pipiens: Although infrequent, 7 mammalian species were identified as the source of bloodmeals for this

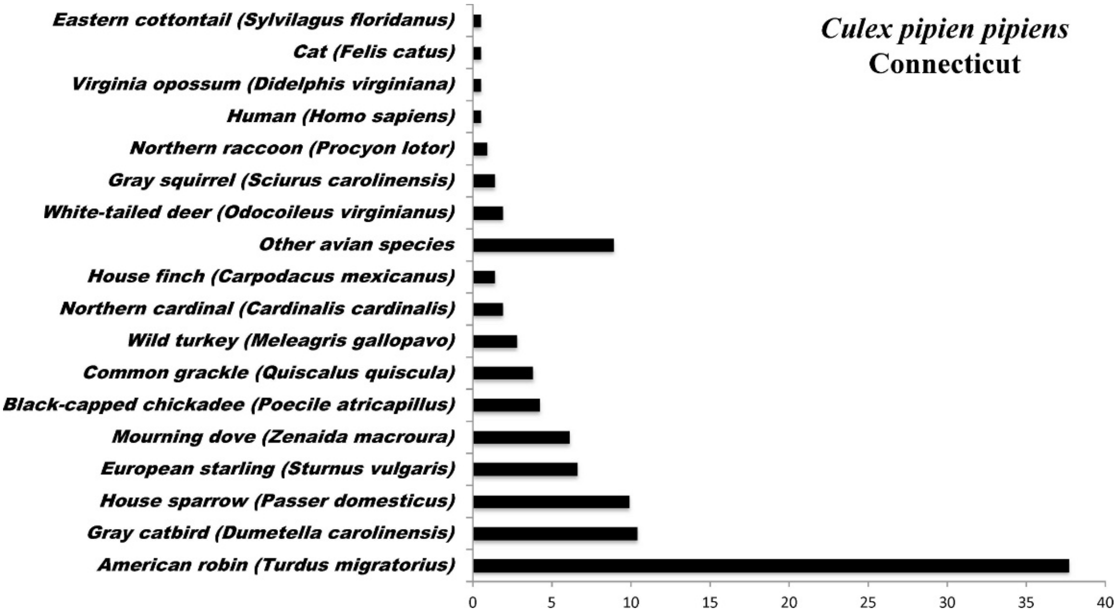

Fig. 3. Percentage of avian- and mammalian-derived blood meals identified from *Cx. p. pipiens* (n= 204) in Connecticut, 2002–2004.

mosquito species in Connecticut (Molaei et al. 2006). Human-derived bloodmeals were infrequently detected (0.5%), whereas white-tailed deer, *Odocoileus virginianus* Zimmermann was the most frequent mammalian host (1.9% of all vertebrate-derived bloodmeals) (Fig. 3). White-tailed deer are the most abundant large mammals in the region besides humans. The role of deer in the ecology and transmission dynamics of arboviruses, including WNV and EEEV, is unknown. Neutralizing antibodies for WNV have been detected from free-ranging white-tailed deer in Iowa and New Jersey (Farajollahi et al. 2004, Santaella et al. 2005). Seroprevalence of WNV antibodies was 0%–6% among hunter-killed deer from New Jersey in 2001 (Farajollahi et al. 2004), which suggests infrequent exposure to WNV relative to avian hosts, but frequency of exposure was still greater than that in humans (Centers for Disease Control and Prevention 2001). Serological evidence of EEEV exposure in white-tailed deer has also been reported from Florida, Iowa, Nebraska, New York, North Dakota, Texas, Wisconsin, Wyoming, and Georgia in the U.S., and Quebec in Canada (Trainer and Hanson, 1969, Whitney et al. 1969, Hoff et al. 1973, Whitney 1973, Bigler et al. 1975, Forrester 1992, Tate et al. 2005). Although white-tailed deer are infected by WNV and EEEV, it is not known whether they develop sufficient viremias to infect mosquitoes and contribute to local transmission cycles.

Culex p. quinquefasciatus

Studies indicate an opportunistic blood feeding behavior of *Cx. p. quinquefasciatus* from the southern U.S. (Reisen et al. 1990; Zinser et al. 2004; Molaei et al. 2007, 2010; Savage et al. 2007). This behavioral characteristic highlights the importance of *Cx. p. quinquefasciatus* in both enzootic and epidemic transmission of arboviruses. Bloodmeal analyses have demonstrated that this species indiscriminately acquires bloodmeals from a wide range of vertebrate species including birds and mammals in Harris County, Texas (Molaei et al. 2007). Of 672 blood-fed *Cx. p. quinquefasciatus*, 39.1% obtained bloodmeals from avian hosts, 52.5% from mammalian, and 8.3% had mixed-blood of avian and mammalian origins (Fig. 2). Results of bloodmeal analysis in Harris County, Texas were consistent with previous studies from other geographic locales that have examined the host feeding patterns of *Cx. p. quinquefasciatus*: Tucson, Arizona - 32% avian and nearly 65% from mammalian hosts including humans (Zinser et al. 2004), Sao Paulo, Brazil - 22% avian and 70% mammalian (Gomes et al. 2003), Northern Queensland, Australia - 29.7% avian and 62.9% mammalian (Kay et al. 1979).

Analysis of 415 engorged *Cx. p. quinquefasciatus* from southern California (Molaei et al. 2010) has shown that 88.2%, 9.6%, and 2.2% of mosquitoes acquired bloodmeals from birds, mammals, and from both bird and mammals in mixed-bloodmeals, respectively (Fig. 2).

Similarly, 79% of *Cx. p. quinquefasciatus* from southwestern Queensland, Australia, had avian- and 16% had mammalian-derived bloodmeals (Kay et al. 1985). Nevertheless, studies also have shown considerable differences in the actual ratio of avian and mammalian feedings in various geographic regions. A large study of blood-fed *Cx. p. quinquefasciatus* (n = 10,769) on Oahu Island, Hawaii, similarly found that 69% had acquired blood from birds, and 31% had fed on mammals (Tempelis et al. 1970). These widely divergent results in the actual ratio of avian and mammalian feedings in various geographic regions indicate that populations of *Cx. p. quinquefasciatus* are much more opportunistic in blood-feeding than *Cx. p. pipiens* f. pipiens, which, in North America, is predominantly ornithophilic.

Avian hosts of Cx. p. quinquefasciatus: Of 30 avian species, the mourning dove (18.3% of all vertebrate-derived bloodmeals), the white-winged dove, *Zenaida asiatica* (L.) (4.3%), the house sparrow (3.2%), the house finch, *Carpodacus mexicanus* (Müller) (3.0%), the gray catbird (3.0%), and the American robin (2.5%) were the most frequent hosts in Harris County, Texas (Molaei et al. 2007) (Fig. 4). Columbiformes comprised > 52% of all avian-derived bloodmeals, and the mourning dove and the white-winged dove represented 41.7% and 9.7% of all avian-derived bloodmeals, respectively. The predominance of bloodmeals from mourning doves and white-winged doves suggests an opportunistic feeding behavior for *Cx. p. quinquefasciatus* in Harris County, Texas. In addition, nearly 44% of the avian-derived bloodmeals from *Cx. p. quinquefasciatus* were determined to be from Passeriformes including house sparrows, house finches, gray catbirds and American robins.

Of 25 avian host species for *Cx. p. quinquefasciatus* in southern California, the house finch was the most frequent source of bloodmeals (34.2% of all vertebrate-derived bloodmeals), followed by the house sparrow (19.3%), the mourning dove (18.9%), the American robin (4.5%) and the American crow, *Corvus brachyrhynchos* Brehm (2.8%) (Molaei et al. 2010) (Fig. 5). The two Passeriformes, the house finch and the house sparrow, are abundant and widely distributed in peridomestic habitats in southern California, and together they represented 53.5% of the bloodmeals acquired by *Cx. p. quinquefasciatus* (Molaei et al. 2010). The house finch and the house sparrow are competent bird species and contribute substantially to the maintenance and amplification of arboviruses, including WNV, in the

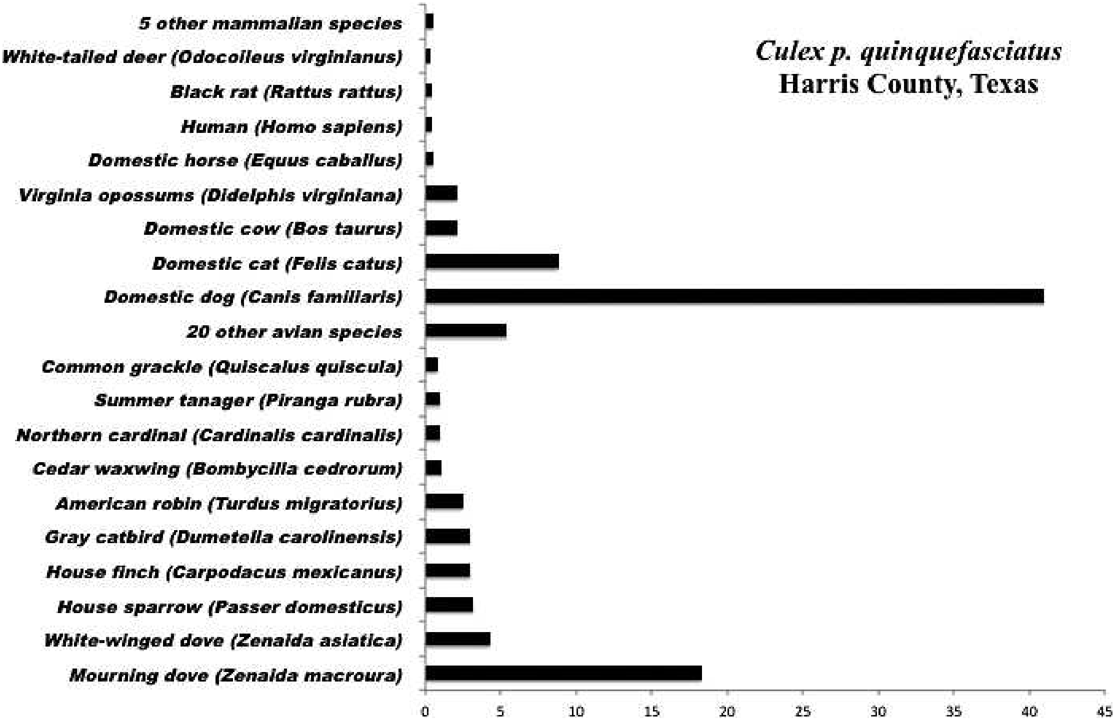

Fig. 4. Percentage of avian- and mammalian-derived blood meals identified from *Cx. p. quinquefasciatus* (n = 672) in Harris County, Texas, 2005.

region. However, the role that Colombiformes such as the mourning dove and the white-winged dove may play as amplification hosts is unclear, because of their relatively low reservoir competence (Komar et al. 2003).

A comparison of the proportion of *Cx. p. quinquefasciatus* bloodmeals from a specific avian species and the frequency of species in Harris County, Texas and Orange County, California was performed. The proportion of this mosquito species that fed on birds such as house finches, mourning doves, house sparrows and a few other birds was as expected, based on their estimated frequencies. However, for certain other birds, particularly the American crow and the blue jay, it was substantially lower than expected. American crows and blue jays are closely associated with human populations in urban/suburban habitats and are considered important amplification hosts for arboviruses. These corvids have shown elevated infections and high viremias, and have suffered substantial mortalities since the outbreak of WNV in the U.S. (Komar et al. 2001, 2003). Therefore, it was of considerable interest to determine the proportion of bloodmeals acquired by the members of *Cx. pipiens* complex from these birds. In an analysis of bloodmeals from *Cx. p. pipiens* f. pipiens in Connecticut, a limited number of mosquitoes were identified with American crow-derived bloodmeals despite their abundance in the region (Hanisek 2005). Similar findings with regard to underrepresentation of American crows as the source of bloodmeals for *Cx. p. pipiens* f. pipiens, have been reported from New York (Apperson et al. 2002) and New Jersey (Apperson et al. 2004). In the study of the host feeding patterns of *Cx. p. quinquefasciatus* from mostly urban/suburban area in Harris County, Texas (Molaei et al. 2007), no crow feedings were observed, and a limited number of mosquitoes acquired bloodmeals from blue jays (0.1% of total). Similarly, in an analysis of *Cx. p. quinquefasciatus* from southern California (Molaei et al. 2010), American crows were represented in only 2.8% of blood feedings. These results indicated that the proportion of bloodmeals from American crows and blue jays was much lower than would be expected based on the frequency data and abundance of these birds locally. Nonetheless, because of the inherent biases in mosquito collection methods from various sites, and heterogeneity in spatial and temporal distribution of American crows and blue jays in urban/suburban areas, caution should be exercised in interpretation of the bloodmeal data and underrepresentation of these birds as hosts for mosquitoes.

Mammalian hosts of Cx. p. quinquefasciatus: Mammalian hosts for *Cx. p. quinquefasciatus* constituted nearly 60% (including the mixed

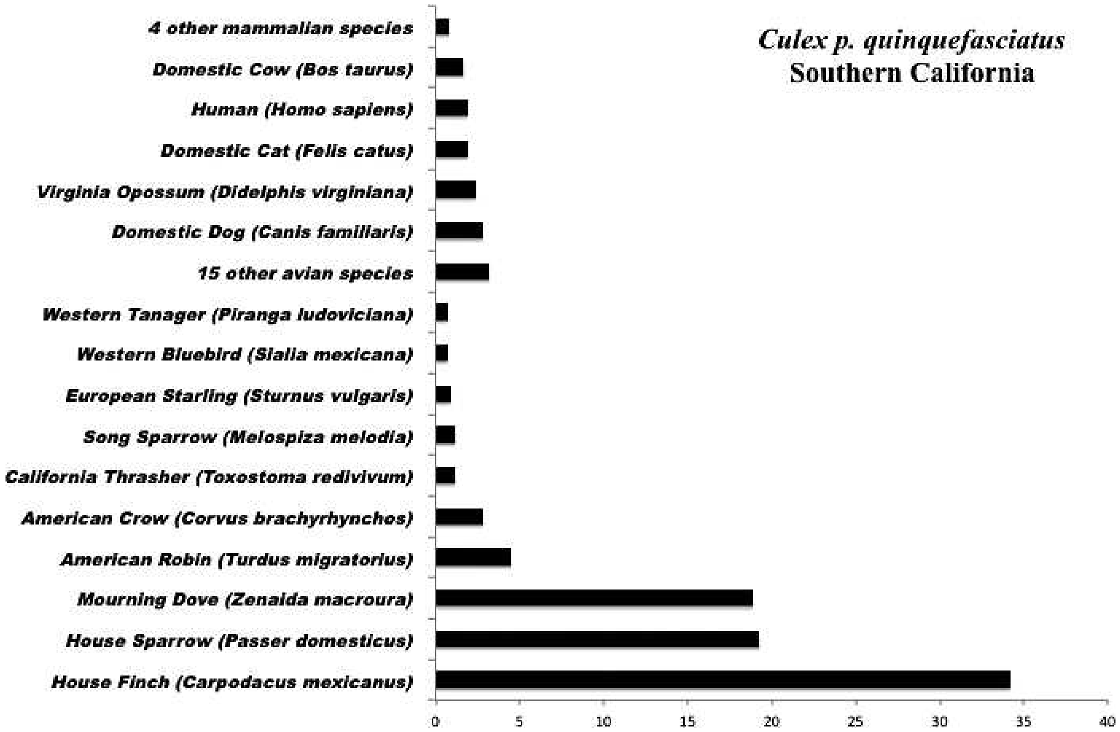

Fig. 5. Percentage of avian- and mammalian-derived blood meals identified from *Cx. p. quinquefasciatus* (n = 415) in Orange, Riverside, and San Bernardino Counties, southern California, 2006–2008.

feedings, representing 13 species) and 12% (representing 9 species) of all identified bloodmeals in Harris County - Texas and southern California, respectively (Molaei et al. 2007, 2010) (Figs. 4 and 5). Yet of the total, only 0.4% and 1.9% contained human-derived bloodmeals in the two regions, respectively, despite the fact that humans are the most abundant large mammal in the area. The underrepresentation of humans as the source of bloodmeals for *Cx. p. quinquefasciatus* is more likely due to the fact that people in the region are less exposed to mosquitoes during the summer and the period of peak activity, as many people stay indoors after dusk, when these mosquitoes are actively feeding (Molaei et al. 2007). Studies in other regions of the U.S. and the world indicate that *Cx. p. quinquefasciatus* readily feeds on humans when accessible (Beier et al. 1990, Niebylski and Meek 1992, Gomes et al. 2003, Samuel et al. 2004, Zinser et al. 2004).

The domestic dog, *Canis familiaris* L. and the domestic cat, *Felis catus* L., frequently served as hosts for *Cx. p. quinquefasciatus* in Harris County - Texas and southern California (Molaei et al. 2007, 2010). Because of the relatively mild climate and open spaces in the region, many pet dogs and cats spend a considerable amount of time outdoors, particularly in residential areas, and therefore are probably much more accessible to blood-seeking mosquitoes than humans (Molaei et al. 2007). Higher WNV antibody prevalence in dogs has been reported from Harris County, Texas (Molaei et al. 2007), indicating that many dogs are infected by the virus presumably from the bite of infected mosquitoes. A retrospective serologic survey of dogs in New York City after the 1999 outbreak of WNV revealed that 10% of local dogs were infected with WNV (Komar et al. 2001). Relatively little is known about the pathogenesis of WNV in dogs and cats or their potential role in the ecology of WNV. In an experimental infection of domestic dogs and cats with WNV by mosquito bites, viremia of low magnitude and short duration developed in four dogs, but they did not display signs of disease (Austgen et al. 2004). Among eight cats, four animals became viremic with peak titers ranging from $10^{3.0}$ to $10^{4.0}$ plaque-forming units/ml, and three of them developed neurologic signs of disease (Austgen et al. 2004).

Seasonal variations in blood-feeding patterns of the *Cx. pipiens* complex

Bridging transmission of arboviruses by the *Cx. pipiens* complex would require flexibility in the phenotype, such that an earlier feeding on birds (virus amplifying hosts) follows a later feeding on mammalian hosts particularly humans. Overall, availability and abundance of various vertebrate hosts and introgressions among members of the *Cx. pipiens* complex may influence temporal heterogeneity in the host-feeding patterns and seasonal shifts from avian to mammalian hosts or from a number of avian species to different birds (Molaei et al. 2006, Kilpatrick et al. 2007, Hamer et al. 2008). Other factors, such as increased mosquito abundance, physiological changes in mosquito host preference, and defensive behavior in birds, also have been postulated as underlying causes for seasonal shifts in blood feedings of *Culex* mosquitoes (Tempelis et al. 1965, Edman et al. 1974, Nelson et al. 1976, Thiemann et al. 2011). In the study of *Cx. p. pipiens* f. pipiens from Connecticut (Molaei et al. 2006), no seasonal shift in blood feeding from avian to mammalian hosts was noticed. Nevertheless, the proportion of American robin–derived bloodmeals decreased from June to October, where by September greater percentages of avian-derived bloodmeals were obtained from gray catbirds and mourning doves, while none was identified as being from American robins. Analyses of engorged *Cx. p. quinquefasciatus* from Harris County, Texas (Molaei et al. 2007) revealed that with one exception (in May), the proportions of avian- and mammalian-derived bloodmeals during the spring and summer months (March to August) were nearly equal. However, a pronounced seasonal shift from avian to mammalian hosts was detected during the late summer and fall (September to November). Analyses of the seasonal proportions of bloodmeals acquired by *Cx. p. quinquefasciatus* from various vertebrate hosts in southern California (Molaei et al. 2010) showed no significant temporal changes in the host-feeding pattern of this mosquito species.

Population genetic structure and blood-feeding patterns of the *Cx. pipiens* complex

Limited studies have been conducted on the potential mechanisms that may influence feeding preference and seasonal variations in members of the *Cx. pipiens* complex. Varying degrees of introgression between the aboveground *Cx. p. pipiens* f. pipiens and the underground *Cx. p. pipiens* f. molestus have been suggested as an important contributing factor to blood feeding preference. Similarly, introgressions between the two distinct genetic entities, *Cx. p. pipiens* f. pipiens and *Cx. p. quinquefasciatus* in the stable hybrid zone in the U.S., have also been postulated as a determining factor for variations in the host-feeding patterns of these mosquitoes, among other factors such as mosquito abundance and host availability.

Attempts to associate genetic composition of *Cx. p. pipiens* f. pipiens with relatively greater prevalence of blood feedings on mammalian hosts (Kilpatrick et al. 2007) have been largely based on the occurrence of possible hybridization between the ornithophilic *Cx. p. pipiens* f. pipiens and the mammalophilic *Cx. p. pipiens* f. molestus (Spielman 2001, Fonseca et al. 2004). Although geographic distribution and feeding preference of *Cx. p. pipiens* f. molestus have not been thoroughly investigated in the U.S., this species has been shown to be an aggressive human biter in Europe (Harbach et al. 1984). The feeding pattern of hybrid populations has only been studied in a mixed population containing both forms in Boston, Massachusetts (Spielman 1964, 2001), where 6 of 353 mosquitoes (1.7%) were reportedly heterozygotes for autogeny (Spielman 1964), consistent with low degrees of hybridization demonstrated in other *Cx. p. pipiens* f. pipiens populations in northeastern U.S. (Huang et al. 2008). However, 3 of 9 and 6 of 13 human-biting mosquitoes were identified as heterozygotes in the aforementioned studies (Spielman 1964, 2001).

Microsatellite analyses of engorged *Cx. p. pipiens* f. pipiens mosquitoes from Chicago, Illinois, using 10 polymorphic markers (Huang et al. 2009) revealed a significantly higher proportion of ancestry from *Cx. p. pipiens* f. molestus, suggesting an underlying genetic basis for mammalian versus avian host selection. These mosquitoes had been earlier identified with a relatively greater percentage of mammalian-derived bloodmeals (Hamer et al. 2008). No association between *Cx. p. quinquefasciatus* ancestry and mammalian blood feeding by *Cx. p. pipiens* f. pipiens was identified (Huang et al. 2009). In a recent population study of mosquitoes from Champaign, Illinois, by using DV/D ratio, 7.5% hybrids were detected (Sanogo et al. 2008). This finding suggests that the hybridization zone may be wider than previously thought. The collection site for the latter study was approximately 160 km south of the area where mosquitoes were collected for bloodmeal identification and subsequent population genetic analyses using microsatellite markers in Chicago, Illinois (Hamer et al. 2008, Huang et al. 2009). Blood-meal analysis of *Cx. pipiens* complex from Memphis, Tennessee, did not reveal greater mammalian blood feeding among hybrids of *Cx. p. pipiens* f. pipiens and *Cx. p. quinquefasciatus* (Savage et al. 2007), and the impact of hybridization was not assessed.

In a bloodmeal analysis of engorged *Cx. p. pipiens* mosquitoes collected from Washington DC and Maryland, a substantially greater percentage of mosquitoes were identified with bloodmeals from mammalian hosts, particularly humans

(Kilpatrick et al. 2006). A subsequent genetic study showed higher fractions of this population with ancestry from *Cx. p. pipiens* f. molestus (Kilpatrick et al. 2007). This research finding reinforced propositions that host selection by *Cx. p. pipiens* f. pipiens mosquitoes may be influenced by genetic predisposition. Although host availability and abundance play significant roles in seasonal variations in blood-feedings by mosquitoes, the impact of extensive gene flow on the population structure, and potential influence of genetic factors on the blood-feeding preference are relatively unknown. It would be of value to determine if a selectable phenotype exists, albeit likely a complex one.

In conclusion, *Cx. pipiens* complex mosquitoes exhibit heterogeneity in vector-host interactions and blood feeding patterns in various regions that cannot be explained only by host availability and abundance. *Culex p. pipiens* f. pipiens is principally an ornitophilic mosquito with regional differences among populations. However, the probability of genetic ancestry from *Cx. p. pipiens* f. molestus might predispose these mosquitoes to feed more readily on mammals. Because genetic mechanisms that may control these divergent feeding habits in mosquitoes are not known, caution should be exercised in using hybrid ancestry as a basis to interpret the differences in host feeding patterns of *Cx. p. pipiens* f. pipiens populations. *Culex p. quinquefasciatus* is an opportunistic mosquito that readily feeds on a variety of birds and mammals, including humans.

ACKNOWLEDGMENTS

We would like to thank the authors of the original articles who made this publication possible as well as to the technical support staff from the following institutions: The Connecticut Agricultural Experiment Station, New Haven, CT; Mosquito Control Division, Harris County Public Health and Environmental Services, Houston, TX; Department of Pathology and Center for Biodefense and Emerging Infectious Diseases, University of Texas Medical Branch, Galveston, TX; Orange County Vector Control District, Garden Grove, CA; West Valley Mosquito and Vector Control District, Ontario, CA; Northwest Mosquito and Vector Control District, Corona, CA; Department of Fisheries and Wildlife, Michigan State University, East Lansing, MI; and Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI. We also would like to express our gratitude to the members of the organizing committee of the symposium on "Global perspective on the *Culex pipiens* complex in the 21st Century: The interrelationship of *Culex pipiens, quinquefasciatus, molestus* and others", for the AMCA Annual Meeting 2011, for making the symposium a successful scientific event, and this series of publications feasible.

REFERENCES CITED

Apperson CS, Harrison BA, Unnasch TR, Hassan HK, Irby WS, Savage HM, Aspen SE, Watson DW, Rueda LM, Engber BR, Nasci RS. 2002. Host-feeding habits of *Culex* and other mosquitoes (Diptera: Culicidae) in the Borough of Queens in New York City, with characters and techniques for identification of *Culex* mosquitoes. *J Med Entomol* 39:777–785.

Apperson CS, Hassan HK, Harrison BA, Savage HM, Aspen SE, Farajollahi A, Crans W, Daniels TJ, Falco RC, Benedict M, Anderson M, McMillen L, Unnasch TR. 2004. Host feeding patterns of established and potential mosquito vectors of West Nile virus in the eastern United States. *Vector-Borne Zoonotic Dis* 4:71–82.

Austgen LE, Bowen RA, Bunning ML, Davis BS, Mitchell CJ, Chang GJ. 2004. Experimental infection of cats and dogs with West Nile virus. *Emerg Infect Dis* 10:82–86.

Barr AR. 1957. The distribution of *Culex p. pipiens* and C. p. quinquefasciatus in North America. *Am J Trop Med Hyg* 6:153–165.

Bast TF, Whitney E, Benach JL. 1973. Considerations on the ecology of several arboviruses in eastern Long Island. *Am J Trop Med Hyg* 22:109–115.

Beier JC, Odago WO, Onyango FK, Asiago CM, Koech DK, Roberts CR. 1990. Relative abundance and blood feeding behavior of nocturnally active culicine mosquitoes in western Kenya. *J Am Mosq Control Assoc* 6:207–212.

Bigler WJ, Lassing E, Buff E, Lewis AL, Hoff GL. 1975. Arbovirus surveillance in Florida: wild vertebrate studies 1965–1974. *J Wildl Dis* 11:348–356.

Centers for Disease Control and Prevention. 2001, Serosurveys for West Nile virus infection—New York and Connecticut counties, 2000. *MMWR Morb Mortal Wkly Rep* 50:37–39.

Crans WJ, Caccamise DF, McNelly JR. 1994. Eastern equine encephalomyelitis virus in relation to the avian community of a coastal cedar swamp. *J Med Entomol* 31:711–728.

Dalrymple JM, Young OP, Eldridge BF, Russell PK. 1972. Ecology of arboviruses in a Maryland freshwater swamp. III. Vertebrate hosts. *Am J Epidemiol* 96:129–140.

Edman JD, Webber LA, Schmid AA. 1974. Effect of host defenses on the feeding pattern of *Culex nigripalpus* when offered a choice of blood sources. *J Parasitol* 60:874–883.

Farajollahi A, Gates R, Crans W, Komar N. 2004. Serologic evidence of West Nile virus and St. Louis encephalitis virus infections in white-tailed deer (*Odocoileus virginianus*) from New Jersey, 2001. *Vector-Borne Zoonotic Dis* 4:379–83.

Fonseca DM, Keyghobadi N, Malcolm CA, Mehmet C, Schaffner F, Mogi M, Fleischer RC, Wilkerson RC. 2004. Emerging vectors in the *Culex pipiens* complex. *Science* 303:1535–1538.

Forrester DJ. 1992. White-tailed deer. In: Forrester DJ, ed. *Parasites and diseases of wild mammals in Florida.*

Gainesville, Florida: University Press of Florida. p 275–353.

Gomes AC, Silva NN, Marques GR, Brito M. 2003. Host-feeding patterns of potential human disease vectors in the Paraiba Valley region, State of Sao Paulo, Brazil. *J Vector Ecol* 28:74–78.

Hamer GL, Kitron UD, Brawn JD, Loss SR, Ruiz MO, Goldberg TL, Walker ED. 2008. *Culex pipiens* (Diptera: Culicidae): a bridge vector of West Nile virus to humans. *J Med Entomol* 45:125–128.

Hamer GL, Kitron UD, Goldberg TL, Brawn JD, Loss SR, Ruiz MO, Hayes DB, Walker ED. 2009. Host selection by *Culex pipiens* mosquitoes and West Nile virus amplification. *Am J Trop Med Hyg* 80:268–278.

Hanisek G. 2005. Connecticut birds by the season. *The Connecticut Warbler* 25:1–44.

Harbach RE, Harrison BA, Gad AM. 1984. *Culex* (*Culex*) *molestus* Forskal (Diptera: Culicidae): neotype designation, description, variation, and taxonomic status. *Proc Entomol Soc Wash* 86:521–542.

Hayes EB, Komar N, Nasci RS, Montgomery SP, O'Leary DR, Campbell GL. 2005. Epidemiology and transmission dynamics of West Nile virus disease. *Emerg Infect Dis* 11:1167–1173.

Hoff GL, Issel CJ, Trainer DO, Richards SH. 1973. Arbovirus serology in North Dakota mule and white-tailed deer. *J Wildl Dis* 9:291–295.

Huang S, Hamer GL, Molaei G, Walker ED, Goldberg TL, Kitron UD, Andreadis TG. 2009. Genetic variation associated with mammalian feeding in *Culex pipiens* from a West Nile virus epidemic region in Chicago, Illinois. *Vector-Borne Zoonotic Dis* 9:637–642.

Huang S, Molaei G, Andreadis TG. 2008. Genetic insights into the population structure of *Culex pipiens* (Diptera: Culicidae) in the northeastern United States by using microsatellite analysis. *Am J Trop Med Hyg* 79:518–527.

Hutto RL. 1995. The composition of bird communities following stand-replacement fires in northern Rocky Mountain conifer forests. *Conserv Biol* 9:1–19.

Kay BH, Boreham PF, Fanning ID. 1985. Host-feeding patterns of *Culex annulirostris* and other mosquitoes (Diptera: Culicidae) at Charleville, southwestern Queensland, Australia. *J Med Entomol* 22:529–535.

Kay BH, Boreham PFL, William GM. 1979. Host preference and feeding patterns of mosquitoes (Diptera: Culicidae) at Kowanyama, Cape York Peninsula, northern Queensland. *Bull Entomol Res* 69:441–457.

Kent R, Juliusson L, Weissmann M, Evans S, Komar N. 2009. Seasonal blood-feeding behavior of *Culex tarsalis* (Diptera: Culicidae) in Weld County, Colorado, 2007. *J Med Entomol* 46:380–390.

Kilpatrick AM, Kramer LD, Jones MJ, Marra PP, Daszak P. 2006. West Nile virus epidemics in North America are driven by shifts in mosquito feeding behavior. *PLoS Biol* 4:606–610.

Kilpatrick AM, Kramer LD, Jones MJ, Marra PP, Daszak P, Fonseca DM. 2007. Genetic influences on mosquito feeding behavior and the emergence of zoonotic pathogens. *Am J Trop Med Hyg* 77:667–671.

Komar N, Burns J, Dean C, Panella NA, Dusza S, Cherry B. 2001. Serologic evidence for West Nile virus infection in birds in Staten Island, New York, after an outbreak in 2000. *Vector-Borne Zoonotic Dis* 1:191–196.

Komar N, Dohm DJ, Turell MJ, Spielman A. 1999. Eastern equine encephalitis virus in birds: relative competence of European starlings (*Sturnus vulgaris*). *Am J Trop Med Hyg* 60:387–391.

Komar N, Langevin S, Hinten S, Nemeth N, Edwards E, Hettler D, Davis B, Bowen R, Bunning M. 2003. Experimental infection of North American birds with the New York 1999 strain of West Nile virus. *Emerg Infect Dis* 9:311–322.

Main AJ, Anderson KS, Maxfield HK, Rosenau B, Oliver C. 1988. Duration of Alphavirus neutralizing antibody in naturally infected birds. *Am J Trop Med Hyg* 38:208–217.

Martin K. 1973. *Breeding density and reproductive success of robins in relation to habitat structure on logged areas of Vancouver Island, British Columbia* [M.S. thesis]. University of Alberta, Edmonton, Canada. 89 p.

Mitchell CJ, Francy DB, Monath TP. 1980. Arthropod vectors. In: Monath TP, ed. *St. Louis encephalitis*. Washington, DC: American Public Health Association. p 313–379.

Molaei G, Andreadis TG, Armstrong PM, Anderson JF, Vossbrinck CR. 2006. Host feeding patterns on *Culex* mosquitoes and West Nile virus transmission, Northeastern United States. *Emerg Infect Dis* 12:468–474.

Molaei G, Andreadis TG, Armstrong PM, Bueno R Jr, Dennett JA, Real SV, Sargent C, Bala A, Randle Y, Guzman H, Travassos da Rosa A, Wuithiranyagool T, Tesh RB. 2007. Host feeding pattern of *Culex quinquefasciatus* (Diptera: Culicidae) and its role in transmission of West Nile virus in Harris County, Texas. *Am J Trop Med Hyg* 77:73–81.

Molaei G, Cummings RF, Su T, Armstrong PM, Williams GA, Cheng ML, Webb JP, Andreadis TG. 2010. Vector-host interactions governing epidemiology of West Nile virus in southern California. *Am J Trop Med Hyg* 83:1269–1282.

Montgomery MJ, Thiemann T, Macedo P, Brown DA, Scott TW. 2011. Blood-feeding patterns of the *Culex pipiens* complex in Sacramento and Yolo Counties, California. *J Med Entomol* 48:398–404.

Morris CD, Caines AR, Woodall JP, Bast TF. 1975. Eastern equine encephalomyelitis in upstate New York, 1972–1974. *Am J Trop Med Hyg* 24:986–991.

Nelson RL, Tempelis CH, Reeves WC, Milby MM. 1976. Relation of mosquito density to bird:mammal feeding ratios of *Culex tarsalis* in stable traps. *Am J Trop Med Hyg* 25:644–654.

Niebylski ML, Meek CL. 1992. Blood-feeding of *Culex* mosquitoes in an urban environment. *J Am Mosq Control Assoc* 8:173–177.

Reisen WK, Reeves WC. 1990. Bionomics and ecology of *Culex tarsalis* and other potential mosquito vector species. In: Reeves WC, ed. *Epidemiology and Control of Mosquito-Borne Arboviruses in California, 1943–1987*. Sacramento, CA: California Mosquito and Vector Control Association. p 254–329.

Samuel PP, Arunachalam N, Hiriyan J, Thenmozhi V, Gajanana A, Satyanarayana K. 2004. Host-feeding pattern of *Culex quinquefasciatus* Say and *Mansonia annulifera* (Theobald) (Diptera: Culicidae), the major vectors of filariasis in a rural area of south India. *J Med Entomol* 41:442–446.

Sanogo YO, Kim CH, Lampman R, Halvorsen JG, Gad AM, Novak RJ. 2008. Identification of male specimens of the *Culex pipiens* complex (Diptera:

Culicidae) in the hybrid zone using morphology and molecular techniques. *J Med Entomol* 45:203–209.

Santaella J, McLean R, Hall JS, Gill JS, Bowen RA, Hadow HH, Clark L. 2005. West Nile virus serosurveillance in Iowa white-tailed deer (1999–2003). *Am J Trop Med Hyg* 73:1038–1042.

Savage HM, Aggarwal D, Apperson CS, Katholi CR, Gordon E, Hassan HK, Anderson M, Charnetzky D, McMillen L, Unnasch EA, Unnasch TR. 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. *J Vector-Borne Zoonotic Dis* 7:365–386.

Spielman A. 1964. Studies on autogeny in *Culex pipiens* populations in nature. I. Reproductive isolation between autogenous and anautogenous populations. *Am J Hyg* 80:175–183.

Spielman A. 2001. Structure and seasonality of nearctic *Culex pipiens* populations. *Ann N Y Acad Sci* 951: 220–234.

Tate CM, Howerth EW, Stallknecht DE, Allison AB, Fischer JR, Mead DG. 2005. Eastern equine encephalitis in a free-ranging white-tailed deer (*Odocoileus virginianus*). *J Wildl Dis* 41:241–245.

Tempelis CH, Hayes RO, Hess AD, Reeves WC. 1970. The blood feeding patterns of four species of mosquitoes found in Hawaii. *Am J Trop Med Hyg* 19:335–341.

Tempelis CH, Reeves WC, Bellamy RE, Lofy MF. 1965. A three-year study of the feeding habits of *Culex tarsalis* in Kern County, California. *Am J Trop Med Hyg* 14:170–177.

Thiemann TC, Wheeler SS, Barker CM, Reisen WK. 2011. Mosquito host selection varies seasonally with host availability and mosquito density. *PLoS Negl Trop Dis* 5:e1452.

Trainer DO, Hanson RP. 1969. Serologic evidence of arbovirus infections in wild ruminants. *Am J Epidemiol* 90:354–358.

Whitney E. 1973. Serologic evidence of group A and arthropod-borne virus activity in New York State. *Am J Tropl Mede and Hyg* 12:417–424.

Whitney E, Roz AP, Rayner GA, Deibel R. 1969. Serologic survey for arbovirus activity in deer sera from nine counties in New York State. *J Wildl Dis* 5:392–397.

Zinser M, Ramberg F, Willott E. 2004. *Culex quinquefasciatus* (Diptera: Culicidae) as a potential West Nile virus vector in Tucson, Arizona: blood meal analysis indicates feeding on both humans and birds. *J Insect Sci* 4:20.