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Vector-Host Interactions Governing Epidemiology of West Nile Virus in Southern California

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Abstract. Southern California remains an important focus of West Nile virus (WNV) activity, with persistently elevated incidence after invasion by the virus in 2003 and subsequent amplification to epidemic levels in 2004. Eco-epidemiological studies of vectors-hosts-pathogen interactions are of paramount importance for better understanding of the transmission dynamics of WNV and other emerging mosquito-borne arboviruses. We investigated vector-host interactions and host-feeding patterns of 531 blood-engorged mosquitoes in four competent mosquito vectors by using a polymerase chain reaction (PCR) method targeting mitochondrial DNA to identify vertebrate hosts of blood-fed mosquitoes. Diagnostic testing by cell culture, real-time reverse transcriptase-PCR, and immunoassays were used to examine WNV infection in blood-fed mosquitoes, mosquito pools, dead birds, and mammals. Prevalence of WNV antibodies among wild birds was estimated by using a blocking enzyme-linked immunosorbent assay. Analyses of engorged *Culex quinquefasciatus* revealed that this mosquito species acquired 88.4% of the blood meals from avian and 11.6% from mammalian hosts, including humans. Similarly, *Culex tarsalis* fed 82% on birds and 18% on mammals. *Culex erythrothorax* fed on both birds (59%) and mammals (41%). In contrast, *Culex stigmatosoma* acquired all blood meals from avian hosts. House finches and a few other mostly passeriform birds served as the main hosts for the blood-seeking mosquitoes. Evidence of WNV infection was detected in mosquito pools, wild birds, dead birds, and mammals, including human fatalities during the study period. Our results emphasize the important role of house finches and several other passeriform birds in the maintenance and amplification of WNV in southern California, with *Cx. quinquefasciatus* acting as both the principal enzootic and "bridge vector" responsible for the spillover of WNV to humans. Other mosquito species, such as *Cx. tarsalis* and *Cx. stigmatosoma,* are important but less widely distributed, and also contribute to spatial and temporal transmission of WNV in southern California.

INTRODUCTION

Mosquito-borne viruses pose ongoing threats to human and animal health. Most of these viruses are zoonotic, and including West Nile virus (WNV), are maintained in enzootic cycles involving ornithophilic mosquitoes and avian hosts. Disease outbreaks occur when the virus overflows into humans and domesticated animals, usually after intense enzootic amplification during warm weather. Detailed knowledge of the host-feeding patterns of mosquito populations in nature is an essential component for evaluating their vectorial capacity and for assessing the role of various vertebrates to serve as reservoir hosts of vector-borne viruses. Since the introduction of WNV into New York City in 1999, subsequent spread across the continental United States, and to southern California in 2003, studies have focused on the eco-epidemiological factors influencing virus transmission; however, limited studies have been conducted on the vector-host interactions.

The epidemics of WNV in southern California were initiated with a few human cases in 2003, followed by intense amplification and epidemics in 2004,[1] subsidence to maintenance levels, and then recrudescence in 2008.[2–4] West Nile virus initially was isolated from *Culex tarsalis* Coquillett,[1] the primary enzootic and epidemic vector of established arboviruses including Western equine encephalitis and St. Louis encephalitis viruses[5] in the region. However, other competent and efficient mosquito vectors, including *Culex quinquefasciatus* Say, *Culex stigmatosoma* Dyar, and *Culex erythrothorax* Theobald, have also been repeatedly found infected with the virus, and contribute to the transmission of WNV where they are locally abundant.[6,7]

To evaluate the role of various mosquito species in supporting virus transmission among wild birds and for infecting humans, we identified the main hosts of *Culex* mosquito species implicated in the transmission of WNV in southern California. Blood-fed mosquitoes were collected from 2006 to 2008 from Orange, Riverside, and San Bernardino Counties; the vertebrate sources of these blood meals were identified by sequencing polymerase chain reaction (PCR) products of the mitochondrial cytochrome *b* gene. The results of these studies are presented and interpreted in conjunction with concurrent avian, mosquito, and arbovirus surveillance activities in the region.

MATERIALS AND METHODS

Study area. The study area was located in Orange and the western portions of Riverside and San Bernardino Counties (33.38° to 34.17° N, −117.22° to −118.11° W) in southern California (Figure 1). Most of the area's estimated human population of 4.1 million lives in a large metropolitan region consisting of 43 cities and 45 unincorporated communities that stretches from the coastal Santa Ana and Saddleback Valleys in Orange County to the inland San Bernardino and Corona Valleys of western San Bernardino and Riverside Counties. In general, the study area is highly urbanized, with a few remnants of agricultural and undisturbed natural landscapes interspersed within highly fragmented residential and commercial developments. The region experiences a warm

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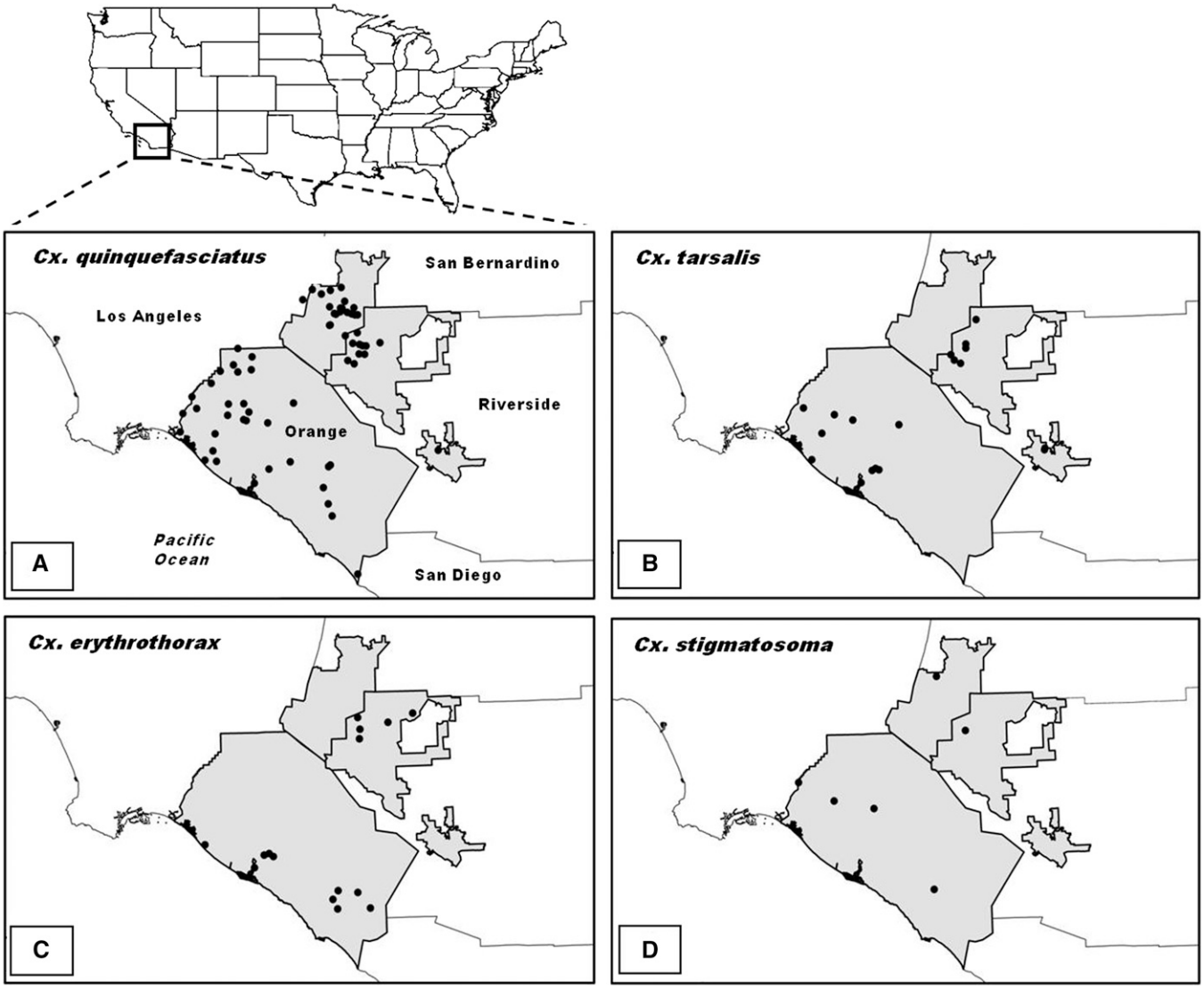

FIGURE 1. Collection sites of blood-fed (**A**) *Culex quinquefasciatus* ($N = 69$ sites), (**B**) *Culex tarsalis* ($N = 18$ sites), (**C**) *Culex erythrothorax* ($N = 15$ sites), and (**D**) *Culex stigmatosoma* ($N = 6$ sites) in Orange, Riverside, and San Bernardino Counties, 2006–2008.

Mediterranean climate (Köppen climate classification *Csb* on the coast, *Csa* inland) moderated by easterly winds from the Pacific Ocean, with 30-yr (1971–2000) average annual temperatures and rainfalls ranging from 17.6–19.6°C and 260–375 mm, respectively. The region's weather is typically warm and dry from May to October, with precipitation occurring mainly during November–April. Weather data for this study were obtained from the National Oceanographic and Atmospheric Administration website (http://www.weather.gov/) for Orange County (John Wayne Airport, Santa Ana, CA) and the California Integrated Pest Management website (http://www.ipm.ucdavis.edu/) for Riverside and San Bernardino Counties (cities of Riverside and Ontario, respectively).

Mosquito breeding sites in the urban/suburban habitats consist largely of sources associated with human development, such as water-filled containers, improperly maintained swimming pools and ponds, and slow-flowing water resulting from residential irrigation runoff in street gutters, catch basins, and drainage channels. Riparian corridors and a number of natural and artificially constructed water-treatment wetlands lie next to residential neighborhoods in all three counties, along with dairy wastewater lagoons in some inhabited areas. *Culex quinquefasciatus* is the most abundant and widely distributed mosquito, comprising the majority of adult (> 80%) and larval collections (> 60%) from peridomestic and peripheral habitats in the region.[8–11] *Culex tarsalis* is the second most common vector of arboviruses in the area, has high relative abundance in adult collections from densely vegetated parkland and wetland habitats, and breeds primarily (> 80%) in neglected swimming pools, water conveyance drains, freshly flooded fields, and wetlands.[8–12] *Culex erythrothorax* adults and larvae are focally abundant in the region at freshwater marshes with emergent stands of tule (*Schoenoplectus* spp.) and cattail (*Typha* spp.).[11,13–15] *Culex stigmatosoma* is also found widely, but in low numbers (< 5%) in adult and larval counts.[8–11] Continuous surveillance programs for mosquito-borne diseases conducted by the vector control districts in the study area confirm the findings of earlier investigations on the mosquito fauna and bionomics.[2–4]

Collection of mosquitoes. Mosquitoes were collected weekly from March through November, and variably during the cooler months depending on weather conditions, from 144 permanent locations within the tri-county study area during 2006–2008, as part of the mosquito-borne diseases surveillance programs in the vector control districts (Figure 1). Mosquito collections were made by using modified dry ice-baited, Centers for Disease Control and Prevention (CDC)-style encephalitis vector survey (EVS) traps,[16] operated without lights, gravid traps baited with hay infusion water,[17] and battery-powered, hand-held aspirators from urban/suburban (94), riparian (23), and wetland (27) sites. Collection sites included landscaped vegetation at residential and commercial properties, parks, cemeteries, and wooded riparian and wetland habitats. Mosquito traps were typically set between 10:00 AM and 2:00 PM and picked up the next morning between 8:00 AM and 11:30 AM. Collections of resting blood-fed mosquitoes using aspirators were made from three modified walk-in red boxes ($1 \times 1 \times 2$ m),[18] and porches of several residential properties where mosquito traps were routinely set.

Specimen processing and morphological identification of mosquitoes. Field-collected mosquitoes were transported alive in coolers (4–8°C) with ice packs to the various agency laboratories, knocked-down either with dry ice or triethylamine, identified using taxonomic keys,[19] sorted by species and sex, and enumerated. Specimens with visible blood meals were separated from the collections and transferred to cryotubes labeled with a unique number and held at –70°C in an ultra-low temperature freezer for blood meal identification and detection of WNV.

Non-blooded females were divided into pools of 5 to 50 mosquitoes per species by location, kept at –70°C, and assayed for WNV at the Orange County and West Valley agencies (Orange County and some West Valley specimens, respectively), or shipped overnight on dry ice either to the Center for Vectorborne Diseases (CVEC) Arbovirus laboratory at the University of California, Davis (Northwest and some Orange County and West Valley mosquitoes), or the Marin-Sonoma Mosquito and Vector Control District (Marin-Sonoma) for other West Valley samples.

DNA isolation and blood meal identification from engorged mosquitoes. Mosquito abdomens were removed for blood-meal analysis with the aid of a dissecting microscope under sterile conditions. The DNA was isolated from the abdominal content of engorged mosquitoes individually by using DNA-zol BD (Molecular Research Center, Cincinnati, OH) according to a protocol described elsewhere.[20–22] Mosquito blood meals were identified by PCR-amplification and DNA sequencing of a fragment of mitochondrial cytochrome *b* gene.[20–22] Mixed blood meals of avian and mammalian origin were also identified by using avian- and mammalian-specific primers and direct sequencing of PCR products. Mitochondrial DNA with high copy number and evolutionary rate 5–10 times faster than nuclear genome is a suitable candidate to resolve broader taxonomic groups such as orders and families and differentiate various vertebrate species that serve as the source of blood meals for host-seeking mosquito vectors. The source of the mosquito blood meals was identified by sequence comparison to the GenBank DNA sequence database (NCBI at http://www.ncbi.nlm.nih.gov). Sequences that did not meet the criteria were assumed unknown. These could be caused by the quality of the sequences or the possibility that the blood meals were derived from vertebrates for which cytochrome *b* sequences are not yet available. The performance of the molecular-based assay was validated by isolating DNA from the blood of a number of known vertebrate species, subjecting them to PCR amplification and sequencing.[20]

Detection of WNV in blood-fed mosquitoes. Blood-fed mosquito specimens were tested for the presence of WNV. The head and thorax of individual mosquitoes were screened for viral infection by inoculating mosquito homogenates onto confluent Vero cell cultures as previously described.[23] The RNA was extracted from virus isolates using the viral RNA kit (Qiagen, Valencia, CA) and tested for West Nile virus by real-time reverse transcriptase-PCR (RT-PCR) assays.[24]

Detection of WNV in non-blooded mosquitoes. Pools of non-blooded mosquitoes were assayed for WNV by TaqMan singleplex real-time RT-PCR (ABI 7300 Real-Time RT-PCR System, Applied Biosystems, Foster City, CA) using WNV-specific primers,[24] at the Orange County (2007–2008 mosquitoes) and Marin-Sonoma laboratories (some West Valley specimens), a high-throughput, robotic TaqMan system,[25] at the CVEC facility (some Orange County and West Valley specimens, and all Northwest samples), and a commercial rapid assay—RAMP (Rapid Analyte Measurement Platform, Response Biomedical Corp., Burnaby, British Columbia) for other West Valley samples.

Bird population estimates. Frequency estimates of local avian species (Figure 2) were performed by using the bird population analysis, a project developed by the Cornell Laboratory of Ornithology and the National Audubon Society to track the bird abundance in North America. Frequency estimates were obtained from World Wide Web (http://www.ebird.org), where "Frequency" represents the percentage of checklists reporting the species within a specified date range and region. Observation frequency of avian populations was expressed in decimal format ranging from 0 to 1, respectively, indicating from "absent" to "present" for all observations. The frequency data consist of information obtained on a weekly basis from January 2004 through December 2008 for Orange County only, where most blood-fed mosquitoes (N = 438, 82.7%) were collected. The bird frequency data presents some qualitative information on the occurrence of the individual bird species throughout the region, though it may not provide detailed information on the abundance of the bird species in the study sites.

Surveillance for WNV activity in wild avian populations. West Nile virus activity in the local wild bird population was monitored by antibody serosurveillance in live birds (Orange and Northwest agencies only), and testing dead birds using RT-PCR (all agencies), immunohistochemistry[26] (some Orange County and Northwest samples), or RAMP (some West Valley dead birds) for evidence of WNV infection.

Free-ranging small birds were trapped in grain-baited, modified Australian crow traps[27] and walk-in ground traps on alternate weeks at 10 fixed sites (4 to 5 sites/week) in Orange County and 4 fixed sites (2 sites/week) in northwestern Riverside County; monitored mist nets were used occasionally at selected sites in Orange County to capture American crows and raptors as part of a cooperative study with Bloom Biological, Inc. (http://bloombiological.com/). Birds were identified to species, age, and sex (if possible); newly captured birds were leg-banded with United States Geological Survey (USGS)—issued tags.

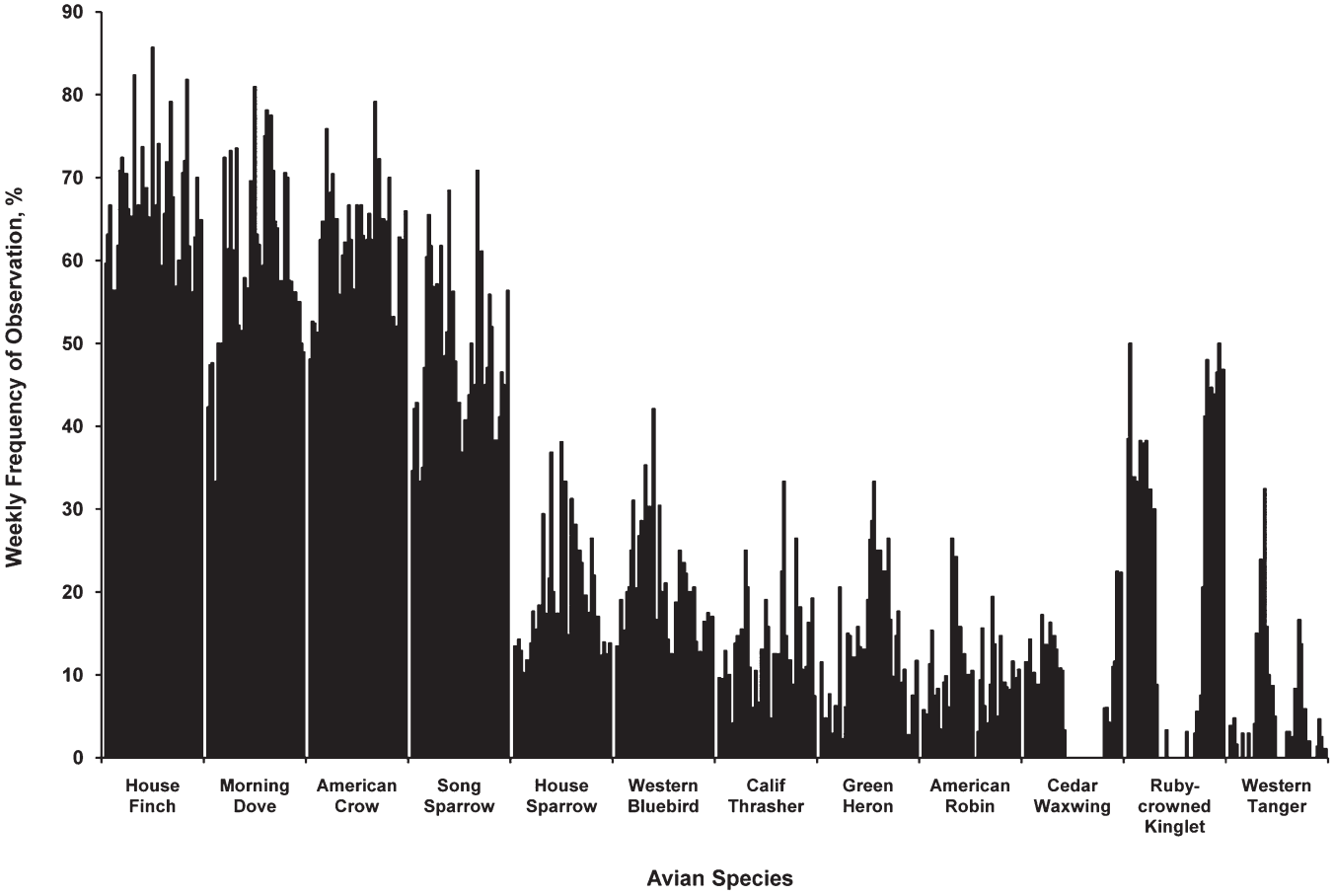

FIGURE 2. Weekly frequency estimates of avian species based on bird population analysis in Orange County, California, 2004–2008.

Birds were quickly bled from the jugular or wing vein with a 1.0-mL syringe and a 28-gauge needle, held briefly to ensure that the puncture wounds were not bleeding, and released at the capture site. Blood for antibody testing (0.2 mL) was dispensed immediately into individually labeled tubes containing 1.8 mL phosphate-buffered saline diluent with 0.75% bovine serum albumin,[28] kept on ice packs, and processed at the Orange County laboratory using a blocking enzyme-linked immunosorbent assay (ELISA) with a baculovirus-Kunjin epitope NS1 recombinant antigen and specific anti-West Nile NS1 monoclonal antibody 3.1112G,[29,30] on heat-inactivated sera. In addition, from August through October 2008, blood (~0.05 mL) from 84 free-ranging birds was added to individually labeled vials containing 1-mL Eagle's Minimum Essential Medium, frozen in the field on dry ice, and evaluated for the presence of active WNV in free-ranging birds by RT-PCR and inoculated Vero and porcine stable equine kidney (PSEK) cell cultures. Confirmation of tissue culture virus isolations was made by *in situ* ELISA using the anti-West Nile NS1 monoclonal antibody 3.1112G.[31,32] Infection data were compared with avian serology results from the same birds as part of the avian surveillance program in Orange County.[33]

Dead birds were collected in response to reports from the public and various animal control agencies by phone calls directly to a vector control district, or to the California Department of Public Health (CDPH) Dead Bird Program Hot Line.[34] Birds collected within 24 hrs of death were processed either locally by each agency or shipped to the California Animal Health and Food Safety (CAHFS) laboratory at the University of California, Davis, for necropsy. Once received, birds were identified to species, oral swabs taken for RAMP assay (West Valley samples), necropsied for kidney or other tissue for WNV testing by either immunohistochemistry (some Orange, Northwest, and CAHFS specimens), or singleplex RT-PCR at Orange County,[33] and CVEC[1] (other specimens from West Valley and Northwest vector control districts), as determined by each agency.

Statistical analysis. Feeding patterns of *Cx. quinquefasciatus*, *Cx. tarsalis*, and *Cx. erythrothorax* on selected host species, and seasonal changes in host-feeding patterns of *Cx. quinquefasciatus* from avian to mammalian species were analyzed by the chi-square (χ^2) test (Abacus Concepts, Inc., 1987; StatView and Graphics; Abacus Concepts, Inc., Berkeley, CA). The WNV infection incidence per 1,000 female mosquitoes was calculated using the maximum likelihood estimation (MLE) method.[35] Annual seasonal (May–October) MLEs were calculated for each of the four *Culex* species tested during 2006–2008.

Ethics statement. Handling of birds was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adhered to principles stated in the Guide for the Care and Use of Laboratory Animals, National Research Council, 1996. The collection, banding, and bleeding

of wild birds was done under State of California Department of Fish and Game Scientific Collecting Permit Nos. 009927 (Orange County), 010415 (Riverside County), and 170628 (Bloom Biological, Inc.); and USGS Master Station Banding, Marking, and Salvage Permit Nos. 23547 (Orange County), 23550 (Riverside County), and 20431 (Bloom Biological, Inc.).

RESULTS

In total, 470,820 female mosquitoes of 14 species were collected from ~144 (94 urban/suburban, 23 riparian, and 27 wetland) locations throughout Orange, northwestern Riverside, and southwestern San Bernardino Counties during 2006–2008. Collections included predominately *Cx. erythrothorax* (N = 253,038, 53.7%), followed by *Cx. quinquefasciatus* (N = 133,450, 28.3%), *Cx. tarsalis* (N = 53,410, 11.3%), and *Cx. stigmatosoma* (N = 8,211, 1.7%). Other mosquito species, including *Culex restuans* Theobald, *Culex thriambus* Dyar, *Culiseta incidens* (Thompson), *Culiseta inornata* (Williston), *Culiseta particeps* (Adams), *Anopheles franciscanus* MacCracken, *Anopheles hermsi* Barr and Guptavanji, *Ochlerotatus washinoi* Lanzaro and Eldridge, *Ochlerotatus squamiger* (Coquillett), and *Ochlerotatus taeniorhynchus* (Wiedemann), were intermittently captured in the traps but accounted for less than 5% (N = 22,711) of the total. The blood-fed mosquitoes examined in this study were from collections made from January 2006 to December 2008, during a period of relatively normal mean annual temperatures (–0.9 to 0.6°C of normal), but below average annual precipitations (–17.3% to –67.9% of normal) for the region.

DNA analysis. Vertebrate hosts were successfully identified from 531 feedings (41.5%) of 1,279 field-collected mosquitoes with visible blood meals representing four *Culex* species (415 of 855 *Cx. quinquefasciatus*, 48.5%; 39 of 77 *Cx. tarsalis*, 50.6%; 65 of 326 *Cx. erythrothorax*, 19.9%; 11 of 20 *Cx. stigmatosoma*, 55.0%; 1 of 1 *Cx. restuans*, 100%). Of the remaining blood-fed mosquitoes, we either did not obtain visible amplification products because of the small size of the blood meal, or the sequencing results were inconclusive to assign a host species.

Culex quinquefasciatus. Analysis of 415 engorged female *Cx. quinquefasciatus* collected with gravid traps (N = 233, 56.2%), EVS light traps (N = 101, 24.3%), and aspirators (N = 81, 19.5%) revealed that 88.2% (N = 366), 9.6% (N = 40), and 2.2% (N = 9) of mosquitoes acquired blood meals from birds, mammals, and from both birds and mammals in mixed blood meals, respectively (Table 1). Overall, 34 avian and mammalian species were represented in 424 feedings by *Cx. quinquefasciatus* from 69 (48 urban/suburban, 12 riparian, and 9 wetland) collection sites. Twenty-five avian species representing 18 families (mostly passeriforms) capable of amplification and supporting WNV transmission were identified as the source of blood. The house finch was the most significant (χ^2 = 4.5–171.6, P < 0.05) source of blood comprising 34.2% (N = 145) and of all vertebrate-derived blood meals, followed by house sparrow, mourning dove, American robin, and American crow. Thrashers (Mimidae), sparrows (Emberizidae), starlings (Sturnidae), thrushes (Turdidae), tanagers (Thraupidae), herons (Ardeidae), and a number of birds from other families, mostly passeriform, infrequently served as hosts. In addition to avian hosts, 9 mammalian species representing 9 families were identified, including 8 human-derived blood meals (1.9% of all vertebrate blood meals).

We identified 9 (2.2%) *Cx. quinquefasciatus* containing mixed blood meals of avian and mammalian origin. Of these, three specimens had acquired blood meals from the house finch mixed with human, desert cottontail rabbit, and domestic cow; three specimens with mixed blood from mourning dove and domestic cat; two specimens with blood from the American robin and domestic cat and dog; and one specimen with blood from cliff swallow and California myotis bat.

In urban/suburban environments, the house finch comprised the largest significant component (N = 101, 34.0%, χ^2 = 5.8–105.8, P < 0.05), whereas in riparian and wetland habitats, house finches (N = 20, 35.7%, and N = 24, 33.8%, respectively) and mourning doves (N = 17, 30.4%, and N = 23, 32.4%, respectively) were fed upon with equal frequency, butsignificantly more than other hosts (χ^2 > 6.62, P < 0.05 and χ^2 > 8.48, P < 0.01, respectively) in these habitats. Throughout the region, there was no difference among urban/suburban, riparian, and wetland habitats in the proportion of blood meals (34.0%, 35.7%, and 33.8%, respectively) acquired by *Cx. quinquefasciatus* from the house finch (Figure 3). *Culex quinquefasciatus* fed upon house sparrows more in urban/suburban (N = 80, 26.9%, χ^2 > 16.9, P < 0.01) than riparian (N = 1, 1.8%) and wetland (N = 1, 1.4%) habitats, whereas mourning doves were fed upon relatively more in riparian (N = 17, 30.4%, χ^2 > 14.3, P < 0.01) and wetland (N = 23, 32.4%, χ^2 > 8.5, P < 0.01) than urban/suburban (N = 40, 13.5%) habitats (Figure 3).

Culex tarsalis. Our analysis of 39 engorged female *Cx. tarsalis* collected by gravid traps (N = 1, 2.6%), EVS light traps (N = 34, 87.2%), and aspirators (N = 4, 10.2%) revealed that 84.6% (N = 33) of specimens had acquired blood meals from birds and 15.4% (N = 6) from mammals (Table 2). No mixed blood meals were identified from this mosquito. Host variability was relatively high, with 16 different avian and mammalian species from 18 (5 urban/suburban, 4 riparian, and 9 wetland) collections sites. Avian hosts were fed upon more often (N = 33, 84.6%, χ^2 = 37.38, P < 0.001) than mammalian sources. Twelve avian species representing 10 families were identified as the source of blood meals for *Cx. tarsalis* (Table 2). Analyses of the species composition of the blood meals revealed that this mosquito species acquired significantly greater numbers of blood meals from mourning doves and house finches (N = 19, 48.7%, χ^2 = 4.04–9.85, P < 0.05) than any other host species (Table 2) in riparian and wetland habitats; however, none of these two bird species were identified as hosts in specimens of *Cx. tarsalis* sampled from urban/suburban habitats. House sparrows, hermit thrushes, and 8 other mostly passeriform birds, were also included as hosts for *Cx. tarsalis*. In addition to the blood meals with avian origin, 4 mammalian-derived meals, including 2 from humans (5.1% of all vertebrate-derived blood meals) collected from urban/suburban sites, were also identified.

Culex erythrothorax. Our analysis of 65 engorged female *Cx. erythrothorax* collected by EVS light traps revealed that 56.9% (N = 37), 40.0% (N = 26), and 3.1% (N = 2) of mosquitoes acquired blood meals from birds, mammals, and from mixed bird blood meals, respectively (Table 3). Host variability was relatively high, with 21 different avian and mammalian species represented in 67 feedings from 15 (2 riparian and 13 wetland) collection sites. Fourteen avian species representing 10 families were identified as the source of blood meals for *Cx. erythrothorax*, including house finches,

TABLE 1

Number and percentage of the avian- and mammalian-derived blood meals identified from *Culex quinquefasciatus* ($N = 415$) in Orange, Riverside, and San Bernardino Counties, 2006–2008

Host	Number/collection method			Species total	% Of total*	SE
	ASP†[1]	CDC‡	GRV§			
Avian						
House finch, *Carpodacus mexicanus*¶	21	32	92	145	34.2[a]	2.3
House sparrow, *Passer domesticus*	55	12	15	82	19.3[b]	1.9
Mourning dove, *Zenaida macroura*¶	1	14	65	80	18.9[b]	1.9
American robin, *Turdus migratorius*¶		9	10	19	4.5[c]	1.0
American crow, *Corvus brachyrhynchos*	2	2	8	12	2.8[c,d]	0.8
California thrasher, *Toxostoma redivivum*	1	1	3	5	1.2[d,e]	0.5
Song sparrow, *Melospiza melodia*		1	4	5	1.2[d,e]	0.5
European starling, *Sturnus vulgaris*		3	1	4	0.9[e]	0.5
Western bluebird, *Sialia mexicana*			3	3	0.7[e]	0.4
Western tanager, *Piranga ludoviciana*			3	3	0.7[e]	0.4
Green heron, *Butorides virescens*		1	1	2	0.5[e]	0.3
White-crowned sparrow, *Zonotrichia leucophrys*			2	2	0.5[e]	0.3
American kestrel, *Falco sparverius*			1	1	0.2[e]	0.2
Blue-throated hummingbird, *Lampornis clemenciae*		1		1	0.2[e]	0.2
Cedar waxwing, *Bombycilla cedrorum*			1	1	0.2[e]	0.2
Cliff swallow, *Petrochelidon pyrrhonota*¶		1		1	0.2[e]	0.2
Common raven, *Corvus corax*			1	1	0.2[e]	0.2
Cooper's hawk, *Accipiter cooperii*			1	1	0.2[e]	0.2
House wren, *Troglodytes aedon*			1	1	0.2[e]	0.2
Lincoln's sparrow, *Melospiza lincolnii*			1	1	0.2[e]	0.2
Rock pigeon, *Columbia livia*		1		1	0.2[e]	0.2
Ruby-crowned kinglet, *Regulus calendula*			1	1	0.2[e]	0.2
Swamp sparrow, *Melospiza georgiana*			1	1	0.2[e]	0.2
Western scrub jay, *Aphelocoma californica*			1	1	0.2[e]	0.2
Wild turkey, *Meleagris gallopavo*			1	1	0.2[e]	0.2
Mammalian						
Domestic dog, *Canis familiaris*¶		4	8	12	2.8[c,d]	0.8
Virginia opossum, *Didelphis virginiana*		6	4	10	2.4[c,d]	0.7
Domestic cat, *Felis catus*¶		5	3	8	1.9[d]	0.7
Human, *Homo sapiens*¶	1	3	4	8	1.9[d]	0.7
Domestic cow, *Bos taurus*¶		7		7	1.7[d]	0.6
California myotis, *Myotis californicus*¶		1		1	0.2[e]	0.2
Desert cottontail, *Sylvilagus audubonii*¶		1		1	0.2[e]	0.2
Mule deer, *Odocoileus hemionus*		1		1	0.2[e]	0.2
Roof rat, *Rattus rattus*		1		1	0.2[e]	0.2
Total 34 species	81	107	236	424		

* Percentages with the same letter (superscript) indicate that the results are not significantly different by chi-square test ($\chi^2 = 4.5–171.6$, $P < 0.05$).
† ASP = aspirator.
‡ CDC = CDC light trap.
§ GRV = gravid trap.
¶ Denotes specimens from which mixed blood meals were identified.

mallards, black-crowned night herons, mourning doves, and a number of other birds. House finches, mallards, black-crowned night herons, dusky-footed woodrats, and roof rats were fed upon with equal frequency, and comprised the majority ($N = 39, 58.2\%, \chi^2 = 5.1–9.2, P < 0.05$) of blood meals in 67 feedings (Table 3). Collectively, *Cx. erythrothorax* fed significantly more on avian ($N = 41, 61.2\%, \chi^2 > 6.72, P < 0.01$) than mammalian sources. Seven species of mammals were among the hosts for *Cx. erythrothorax*. Human-derived blood meal was identified in one mosquito specimen sampled from a wetland habitat comprising 1.5% of all vertebrate-derived blood meals. Two (3.1%) *Cx. erythrothorax* were identified with mixed blood meals of avian origin. Of these, one specimen had acquired blood meals from house finch and mourning dove sources, and one specimen had mixed blood meals of red-winged blackbird and house finch origins. All engorged *Cx. erythrothorax* mosquitoes were collected from riparian and wetland habitats in Orange and Riverside Counties, and no blood-fed specimens were collected from urban/suburban areas.

Culex stigmatosoma. Analysis of 11 engorged female *Cx. stigmatosoma* collected by gravid traps ($N = 3$, 27.3%), EVS light traps ($N = 2$, 18.2%), and aspirators ($N = 6$, 54.5%) revealed that all mosquitoes acquired blood meals from avian hosts (Table 4). The avian hosts representing 7 species included house sparrow, house finch, and other bird species (Table 4) from 4 (urban/suburban only) collection sites.

Avian population analysis. Analysis of occurrence and frequencies of wild birds in Orange County is shown in Figure 2. Certain birds, such as the house finch, mourning dove, American crow, song sparrow, and house sparrow were observed throughout the year, with the house finch comprising the highest mean frequency of observation (0.64). The frequency of observance for other species varied. We compared the proportion of *Cx. quinquefasciatus* blood meals on a specific avian species with the frequency of the species in the region. The proportion of mosquitoes from Orange County that had fed on birds such as the house finch, mourning dove, house sparrows, and a few other birds was as expected, based on the occurrence of these birds (Figure 2).

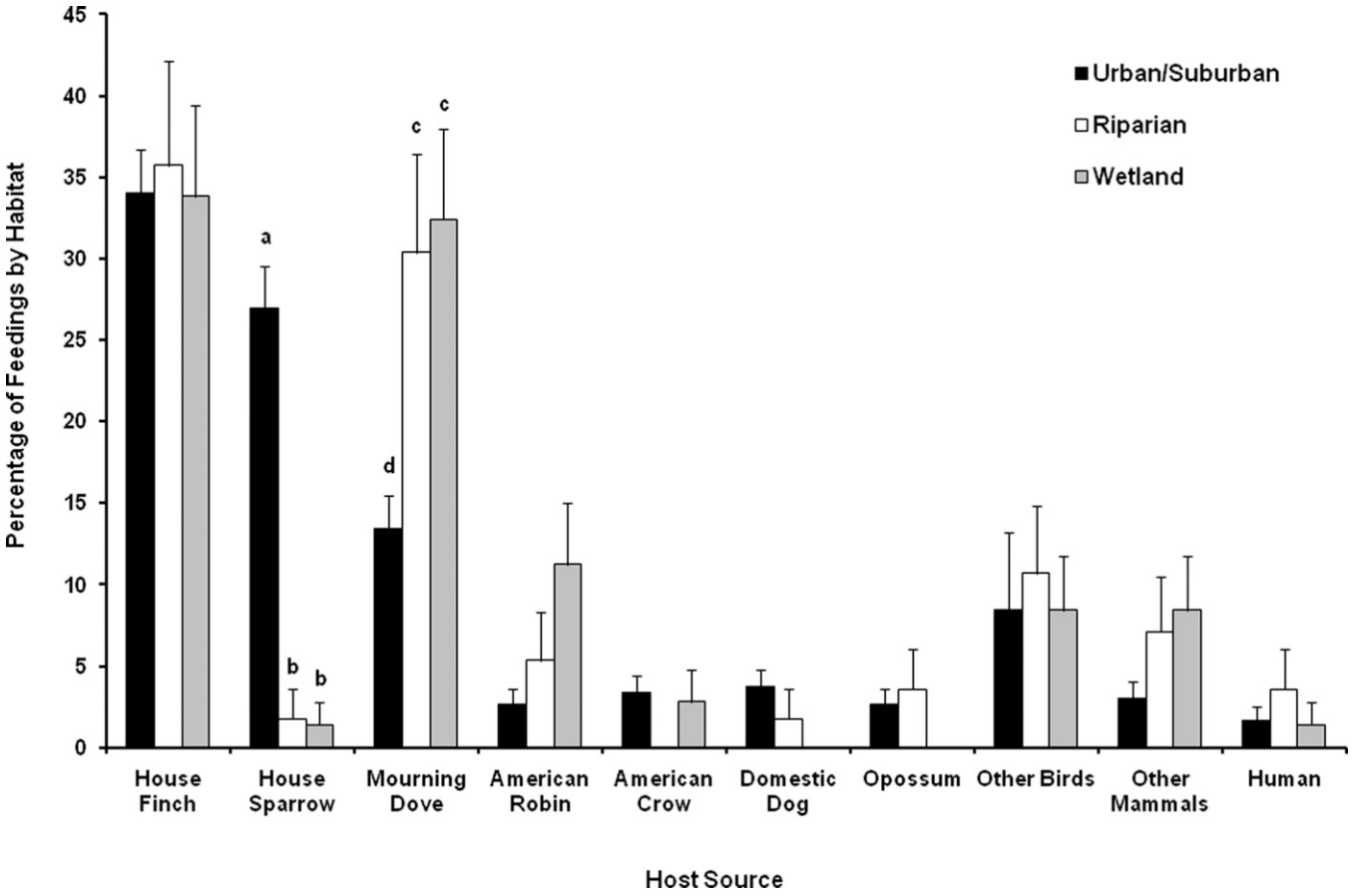

FIGURE 3. Proportions of vertebrate hosts of *Culex quinquefasciatus* in urban/suburban ($N = 297$ feedings), riparian ($N = 56$ feedings), and wetland habitats ($N = 71$ feedings) in Orange, Riverside, and San Bernardino Counties, 2006–2008. Columns not sharing a common letter within each vertebrate species (house sparrow, mourning dove) indicate that the results are significant by habitat (χ^2 test, $P < 0.01$). No significant differences ($P > 0.05$) in relative blood meal compositions by habitat were noted within other species or groups. Vertical bars represent the standard errors.

However, for certain other birds, particularly the American crow, it was substantially lower than expected, based on their estimated frequencies (mean frequency of observation = 0.60). Overall, the American crow was represented in only 14 (2.6%) of 542 mosquito blood feedings from 10 (12.2%) of the 82 collection sites that yielded blood-fed mosquitoes. Although American crows live and roost throughout urbanized areas of California,[36] relatively few mosquitoes fed upon them during this study. In contrast, 49 (59.8%) of the 82 collection sites produced mosquitoes with house finch blood. It is noteworthy that estimated frequencies of various bird species differ temporally and spatially, and may not necessarily represent relative abundance of the various bird species required for the establishment of host-feeding preferences in mosquitoes.

Virus isolations from blooded mosquitoes. Two virus isolates were recovered from individual blood-fed mosquitoes in Vero cell culture, and both were identified as WNV by real-time RT-PCR assays suggesting disseminated infection. The species, date, location, and blood meal source of these WNV-infected mosquitoes are as follows: *Cx. quinquefasciatus* collected on 7/26/06 from Fullerton, containing mourning dove blood and *Cx. quinquefasciatus* collected on 8/5/08 in Anaheim, containing human blood.

Virus recoveries from non-blooded mosquitoes. In total, 222,565 *Culex* mosquitoes in 8,194 pools were assayed for WNV at the Orange County, Marin-Sonoma, West Valley, and CVEC laboratories during 2006–2008. Annual proportions of the *Culex* mosquitoes assayed for WNV were 60,227 in 2,023 pools with 27 positives (1.33%) for 2006; 55,973 in 2,284 pools with 33 positives (1.44%) for 2007; and 106,365 in 3,887 pools with 545 positives (14.0%) for 2008. *Culex quinquefasciatus* comprised 62.8% (N = 139,771) of all *Culex* mosquitoes examined. Average pool size was 27.2 mosquitoes. From this total, 605 WNV-positive pools were obtained, with *Cx. quinquefasciatus* accounting for 480 (79.3%) of all positives. Most (N = 430, 71.1%) of the WNV-positive mosquito pools were collected between July and August. Annual seasonal MLEs for 2006–2008 ranged from 0.59 to 9.52 for *Cx. quinquefasciatus,* 0.30 to 2.75 for *Cx. tarsalis*, 0.08 to 0.31 for *Cx. erythrothorax*, and 0.87 to 22.09 for *Cx. stigmatosoma* per 1,000 mosquitoes tested, respectively, during the study (Table 5).

Testing of dead birds. In sum, 1,991 dead birds from Orange, northwestern Riverside, and southwestern San Bernardino counties were processed for WNV (331 in 2006; 339 in 2007; and 1,321 in 2008). Of this number, 973 birds (48.9%) tested WNV-positive by RAMP, immunohistochemistry, or RT-PCR. As observed with the mosquitoes, most (N = 595, 61.1%) of the WNV-infected dead birds were also found between the months of July and August. American crows accounted for 77.2% (N = 751) of the WNV-positive dead birds collected

TABLE 2

Number and percentage of the avian- and mammalian-derived blood meals identified from *Culex tarsalis* ($N = 39$) collected in Orange and Riverside Counties, 2006–2008

Host	Number/collection method			Species total	% Of total*	SE
	ASP†	CDC‡	GRV§			
Avian						
Mourning dove, *Zenaida macroura*¶		11		11	28.2[a]	7.2
House finch, *Carpodacus mexicanus*¶		8		8	20.5[a,b]	6.5
House sparrow, *Passer domesticus*	3	1		4	10.3[b]	4.9
Hermit thrush, *Catharus guttatus*		2		2	5.1[b]	3.5
American crow, *Corvus brachyrhynchos*		1		1	2.6[b]	2.5
American robin, *Turdus migratorius*		1		1	2.6[b]	2.5
Cooper's hawk, *Accipiter cooperii*		1			2.6[b]	2.5
House wren, *Troglodytes aedon*			1	1	2.6[b]	2.5
Mallard, *Anas platyrhynchos*		1		1	2.6[b]	2.5
Rock pigeon, *Columba livia*	1			1	2.6[b]	2.5
Song sparrow, *Melospiza melodia*		1		1	2.6[b]	2.5
Yellow-breasted chat, *Icteria virens*		1		1	2.6[b]	2.5
Mammalian						
Domestic cow, *Bos taurus*¶		2		2	5.1[b]	3.5
Human, *Homo sapiens*		2		2	5.1[b]	3.5
Raccoon, *Procyon lotor*		1		1	2.6[b]	2.5
Roof rat, *Rattus rattus*		1		1	2.6[b]	2.5
Total 16 species	4	34	1	39		

* Percentages with the same letter (superscript) indicate that the results are not significantly different by chi-square test ($\chi^2 = 4.0–9.8$, $P < 0.05$).
† ASP = aspirator.
‡ CDC = CDC light trap.
§ GRV = gravid trap.
¶ Denotes specimens from which mixed blood meals were identified.

through the study years, followed distantly by house finches ($N = 74$, 7.6%), house sparrows ($N = 29$, 3.0%), and mourning doves ($N = 13$, 1.3%). Table 6 shows the WNV-positive rates for the most commonly infected dead bird species.

Prevalence of WNV antibodies in wild birds. In total, 9,889 free-ranging bird samples representing 19 species were collected for antibody titer testing during 2006–2008 by the Orange County and Northwest vector control districts (Table 7). Overall, 414 birds (4.2%) had NS1 antibodies to WNV. West Nile virus antibody rates were highest in captured house finches ($N = 356$, 5.3%), house sparrows ($N = 51$, 2.1%), and rock pigeons ($N = 3$, 1.9%), and significantly higher in after-hatching-year (AHY) ($N = 370$, 5.7%, $\chi^2 = 91.0$, $P < 0.001$) than hatching-year (HY) ($N = 44$, 1.4%) birds. West Nile virus

TABLE 3

Number and percentage of the avian- and mammalian-derived blood meals identified from *Culex erythrothorax* ($N = 65$) collected in Orange and Riverside Counties, 2006–2008

Host	Number/collection method			Species total	% Of total*	SE
	ASP†	CDC‡	GRV§			
Avian						
House finch, *Carpodacus mexicanus*¶		11		11	16.4[a]	4.6
Mallard, *Anas platyrhynchos*		7		7	10.4[a]	3.8
Black-crowned night heron, *Nycticorax nycticorax*		5		5	7.5[a]	3.3
Mourning dove, *Zenaida macroura*¶		3		3	4.5[b]	2.6
Cinnamon teal, *Anas cyanoptera*		2		2	3.0[b]	2.1
Green heron, *Butorides virescens*		2		2	3.0[b]	2.1
Marsh wren, *Cistothorus palustris*		2		2	3.0[b]	2.1
Red-winged blackbird, *Agelaius phoeniceus*¶		2		2	3.0[b]	2.1
Yellow-rumped warbler, *Dendroica coronata*		2		2	3.0[b]	2.1
American robin, *Turdus migratorius*		1		1	1.5[b]	1.5
American wigeon, *Anas americana*		1		1	1.5[b]	1.5
Belding's savannah sparrow, *Passerculus sandwichensis*		1		1	1.5[b]	1.5
Pied-billed grebe, *Podilymbus podiceps*		1		1	1.5[b]	1.5
Wood duck, *Aix sponsa*		1		1	1.5[b]	1.5
Mammalian						
Dusky-footed woodrat, *Neotoma macrotis*		8		8	11.9[a]	4.0
Roof rat, *Rattus rattus*		8		8	11.9[a]	4.0
Desert cottontail, *Sylvilagus audubonii*		4		4	6.0[b]	2.9
Virginia opossum, *Didelphis virginiana*		3		3	4.5[b]	2.6
Coyote, *Canis latrans*		1		1	1.5[b]	1.5
Human, *Homo sapiens*		1		1	1.5[b]	1.5
Mule deer, *Odocoileus hemionus*		1		1	1.5[b]	1.5
Total 21 species		67		67		

* Percentages with the same letter (superscript) indicate that the results are not significantly different by chi-square test ($\chi^2 = 5.1–9.2$, $P < 0.05$).
† ASP = aspirator.
‡ CDC = CDC light trap.
§ GRV = gravid trap.
¶ Denotes specimens from which mixed blood meals were identified.

TABLE 4

Number and percentage of the avian-derived blood meals identified from *Culex stigmatosoma* ($N = 11$) collected in Orange, Riverside, and San Bernardino counties, 2006–2008

Host	Collection method			Species total	% Of total
	ASP†	CDC‡	GRV§		
Avian					
House sparrow, *Passer domesticus*	4			4	36.4
House finch, *Carpodacus mexicanus*	1		1	2	18.2
American crow, *Corvus brachyrhynchos*			1	1	9.1
American robin, *Turdus migratorius*			1	1	9.1
Brown-headed cowbird, *Molothrus ater*		1		1	9.1
Mourning dove, *Zenaida macroura*	1			1	9.1
Western scrub jay, *Aphelocoma californica*		1		1	9.1
Total 7 species	6	2	3	11	

† ASP = aspirator.
‡ CDC = CDC light trap.
§ GRV = gravid trap.

seroprevalence rates in house finches, house sparrows, and rock pigeons paralleled the percentage of dead birds of the same species (7.6%, 3.0%, and 1.0%, respectively) in relative prevalence with WNV.

Of 1,100 dead American crows tested for WNV, 68.3% ($N = 751$) were infected with the virus, but none (0/6) of a small number of captured (living) American crows had WNV antibodies. West Nile virus was recovered in tissue culture from 5 (6.8%) of 74 house finches, indicating ongoing viremia; 2 were found to be WNV-positive for both circulating antibodies and active virus.[34] The WNV RT-PCR TaqMan Ct (critical threshold) values ranged from 16.7 to 36.5 for 8 (10.8%) of the 74 house finch samples (4 of the 8 were also WNV-positive by tissue culture; conversely, one tissue culture WNV-positive specimen tested negative by RT-PCR). No WNV was recovered from any other species (7 house sparrows, one with WNV antibodies; two song sparrows; and one WNV antibody-positive Cooper's hawk), but the numbers were too small and the collection duration too short to infer that other species were not involved in transmission.

DISCUSSION

Our eco-epidemiological study provides insight into the interactions among competent mosquito vectors, avian reservoirs, and incidental hosts, such as humans and/or domestic animals that comprise the WNV infection nidus[37] in southern California. We studied the role of four *Culex* mosquitoes in enzootic and epidemic transmission of WNV and determined their host-feeding patterns from various vertebrate species by taking advantage of sequence homology in mitochondrial genome of vertebrate species. We found that *Culex* mosquitoes feed on a wide range of vertebrate species and display temporal, spatial, and interspecies differences. This information in concert with estimates of local abundance and proportion of infected mosquitoes suggests that a few key species contribute disproportionately to WNV transmission in this region.

Our results clearly implicate *Cx. quinquefasciatus* as the primary vector of WNV in this region of southern California. This mosquito was among the most commonly trapped species and the main source of WNV, representing nearly 80% of all WNV-positive mosquito pools. Analysis of blood-fed mosquitoes revealed that *Cx. quinquefasciatus* fed mainly on birds (88%), consistent with earlier studies that have also shown this species feeds predominately on birds in Kern and other areas of southern California.[38,39] This behavioral characteristic would substantially promote enzootic transmission of WNV. In addition, nearly 12% of *Cx. quinquefasciatus* blood meals contained mammalian blood, including humans, highlighting the involvement of this mosquito species as a "bridge vector" in epidemic transmission of the virus as well.

We found opportunistic blood feeding of *Cx. quinquefasciatus* on a diversity of avian and mammalian hosts ($N = 34$), consistent with the results of previous studies.[39–46] Nevertheless, the actual ratio of avian and mammalian feedings varied considerably among various geographic sites. Human-derived blood meals constituted 1.9% of all vertebrate-derived blood meals, indicating that *Cx. quinquefasciatus* readily feeds on humans when accessible. These results are compatible with other studies on the host-feeding patterns of *Cx. quinquefasciatus* from south India,[44] Tucson, Arizona,[45] western Kenya,[47] southwestern Virginia,[48] and Sao Palo, Brazil,[49] but contrast with a recent study in Harris County, Texas, where only 0.4% of engorged specimens contained human-derived blood meals, despite the fact that humans are the most abundant large mammal

TABLE 5

Seasonal (May–Oct) maximum likelihood estimate (MLE) of the West Nile virus (WNV) infection rate per 1,000 with 95% lower and upper confidence intervals (CI) for each *Culex* species from Orange, Riverside, and San Bernardino Counties, 2006–2008

Year	Mosquito species	Mosquitoes tested (May–Oct)	No. of pools	WNV-positive pools	WNV-positive pools/species by month						Seasonal MLE infection rate	95% CI
					May	Jun	Jul	Aug	Sep	Oct		
2006	*Cx. quinquefasciatus*	37,580	1,326	22			3	12	6	1	0.59	(0.38, 0.88)
	Cx. tarsalis	3,377	132	1				1			0.30	(0.02, 1.43)
	Cx. erythrothorax	4,127	104	1				1			0.24	(0.01, 1.18)
	Cx. stigmatosoma	1,145	72	1				1			0.87	(0.50, 4.20)
2007	*Cx. quinquefasciatus*	24,780	1,061	26				5	16	5	1.06	(0.71, 1.54)
	Cx. tarsalis	5,214	221	4			1	1	2		0.77	(0.25, 1.86)
	Cx. erythrothorax	12,112	317	1						1	0.08	(0.01, 0.40)
	Cx. stigmatosoma	804	62	1					1		1.25	(0.07, 6.07)
2008	*Cx. quinquefasciatus*	52,388	2,023	429	3	9	135	173	74	35	9.52	(8.65, 10.46)
	Cx. tarsalis	23,691	741	62	2	2	39	14	4	1	2.75	(2.13, 3.50)
	Cx. erythrothorax	12,806	327	4			2		2		0.31	(0.10, 0.75)
	Cx. stigmatosoma	2,634	156	47		1	21	21	3	1	22.09	(16.58, 29.04)

TABLE 6
WNV infection rates in dead birds from Orange, Riverside, and San Bernardino Counties, 2006–2008

Species	Tested	WNV-positive	% Positive within species (group)	% of all positives
American crow, *Corvus brachyrhynchos*	1,100	751	68.3	77.2
House finch, *Carpodacus mexicanus*	208	74	35.6	7.6
House sparrow, *Passer domesticus*	131	29	22.1	3.0
Mourning dove, *Zenaida macroura*	48	13	27.1	1.3
Rock pigeon, *Columbia livia*	55	10	18.2	1.0
Western bluebird, *Sialia mexicana*	20	9	45.0	0.9
Western scrub jay, *Aphelocoma californica*	14	9	64.3	0.9
Common raven, *Corvus corax*	28	7	25.0	0.7
Sharp-shinned hawk, *Accipiter striatus*	16	4	25.0	0.4
Northern mockingbird, *Mimus polyglottos*	14	4	28.6	0.4
Cooper's hawk, *Accipter cooperi*	28	3	10.7	0.3
Lesser goldfinch, *Carduelis psaltria*	19	3	15.8	0.3
Red-tailed hawk, *Buteo jamaicensis*	12	3	25.0	0.3
Black phoebe, *Sayornis nigricans*	11	3	27.3	0.3
Red-shouldered hawk, *Buteo lineatus*	10	3	30.0	0.3
Nutmeg mannikin, *Lonchura punctulata*	4	3	75.0	0.3
36 species, each with 1–2 WNV-positive samples	162	45	(27.8)	4.6
Others (36 identified species and unidentified birds)	111	0	(0.0)	0.0
Total	1,991	973	48.9	100.0

in the county.[46] The relative paucity of human cases in Harris County, Texas, was interpreted that people in the study area were less exposed to mosquitoes during summer as they reside indoors in air-conditioned facilities during the peak of mosquito activity.[46]

Our analyses of engorged *Cx. tarsalis* revealed that this mosquito species acquired blood meals mainly from birds ($N = 33$, 84.6%) and moderately from mammals ($N = 6$, 15.4%). These results are consistent with a number of studies conducted on blood-engorged *Cx. tarsalis* sampled from Kern County, the Sacramento Valley, and the Los Angeles Basin in California, in addition to analyses in Colorado and Texas.[8,38,50–56] In host preference studies, where a number of vertebrates, including birds, mammals, reptiles, and amphibians, were exposed to host-seeking mosquitoes in bait trap settings, birds were the most attractive hosts for *Cx. tarsalis*[57,58]; a similar host preference investigation found that *Cx. tarsalis* was more likely to feed from house sparrows and house finches than mourning doves (Laura and Cummings, personal communication). Collectively, these findings suggest that *Cx. tarsalis* mosquitoes will acquire blood meals from a variety of available hosts in the environment but may prefer feeding on passerine birds.[59]

Although recognized as a highly competent vector of WNV, *Cx. tarsalis* likely plays a secondary role in transmission of the virus in southern California because of its limited spatial and temporal abundance.[8,9] Seasonal WNV MLEs in *Cx. tarsalis* were comparatively low, ranging annually from 0.3 to 2.75 per 1,000 mosquitoes in the study area; in contrast, seasonal WNV MLEs in *Cx. quinquefasciatus* ranged annually from 0.59 to 9.52 per 1,000 mosquitoes during the study for this widely abundant and more frequently infected mosquito (Table 5).

TABLE 7
West Nile virus (WNV)-seropositive rates in free-ranging birds in Orange and northwestern Riverside Counties, 2006–2008

Species	Number of samples	Number WNV-positive (%)	% Of all positives	Age			
				Number sampled HY*	AHY†	Number WNV-positive HY* (%)	AHY† (%)
House finch, *Carpodacus mexicanus*	6,725	356 (*5.3*)	86.0	2,386	4,339	36 (*1.5*)	320 (*7.4*)
House sparrow, *Passer domesticus*	2,440	51 (*2.1*)	12.3	645	1,795	8 (*1.2*)	43 (*2.4*)
Rock pigeon, *Columbia livia*	178	3 (*1.7*)	0.7	26	152	0	3 (*2.0*)
Brown-headed cowbird, *Molothrus ater*	163	1 (*0.6*)	0.2	10	153	0	1 (*0.7*)
White-crowned sparrow, *Zonotrichia leucophrys*	137	0	0.0	52	85	0	0
Song sparrow, *Melospiza melodia*	113	0	0.0	19	94	0	0
Nutmeg mannikin, *Lonchura punctulata*	46	0	0.0	14	32	0	0
Black-headed grosbeak, *Pheucticus melanocephalus*	37	0	0.0	1	36	0	0
California towhee, *Pipilo crissalis*	22	0	0.0	2	20	0	0
Red-winged blackbird, *Agelaius phoeniceus*	7	0	0.0	4	3	0	0
American crow, *Corvus brachyrhynchos*	6	0	0.0	1	5	0	0
Golden-crowned sparrow, *Zonotrichia atricapilla*	6	0	0.0	4	2	0	0
Rufous-crowned sparrow, *Aimophila ruficeps*	2	1 (*50.0*)	0.2	0	2	–	1 (*50.0*)
Spotted towhee, *Pipilo maculatus*	2	0	0.0	0	2	–	0
Cooper's hawk, *Accipiter cooperii*	1	1 (*100*)	0.2	0	1	–	1 (*100*)
Red-shouldered hawk, *Buteo lineatus*	1	1 (*100*)	0.2	0	1	–	1 (*100*)
Pacific slope flycatcher, *Empidonax difficilis*	1	0	0.0	0	1	–	0
Mourning dove, *Zenaida macroura*	1	0	0.0	0	1	–	0
Sharp-shinned hawk, *Accipiter striatus*	1	0	0.0	0	1	–	0
Total	9,889	414 (*4.2*)	100.0	3,164	6,725	44 (*1.4*)	370 (*5.5*)

*HY = hatching-year.
†AHY = after-hatching-year.

A seasonal shift in the host-feeding pattern of *Cx. quinquefasciatus* and *Cx. tarsalis* has been reported, where during mid- to late-summer, a greater proportion of mosquitoes acquired blood meals from mammals in comparison with birds,[52] which was interpreted as reflective of a decline in bird abundance. This behavioral feature may potentially enhance the role of these species in WNV transmission to incidental hosts, such as humans, horses, and perhaps other mammals. Analyses of the seasonal proportions of blood meals acquired by *Cx. quinquefasciatus* from various vertebrate hosts in this study showed no significant temporal changes in the host-feeding pattern of this mosquito species, and the relatively low sample size of engorged *Cx. tarsalis* precluded a comprehensive assessment in this regard. Care should be exercised in interpreting host-feeding patterns of the mosquito species in relation to bird abundance. The bird frequency data obtained from the online source provide some information on the relative occurrence of various bird species in the region; however, it may not be sufficient for thorough analyses of host-feeding preference.

Our analyses of blood-engorged *Cx. erythrothorax* showed an opportunistic feeding, where almost 57% had obtained blood meals from birds, 40% from mammals, and 3% had mixed blood of two bird species. Earlier studies have shown that *Cx. erythrothorax* is focally abundant in wetland/riparian habitats with dense emergent vegetation,[13,60] where it feeds readily on both birds and mammals.[52,61] Therefore, this species could serve as a potential "bridge vector" of WNV from viremic birds to humans in proximity to wetland habitats. *Culex erythrothorax* is a competent vector for WNV,[6] and the virus has been occasionally isolated from field-collected adults and larvae.[62] Nevertheless, *Cx. erythrothorax* is considered to be a more important nuisance mosquito for humans,[63] and domestic animals,[59] than a major vector of WNV. This mosquito species feeds relatively infrequently on competent avian hosts, making the probability of virus uptake and transmission low. Seasonal MLEs for *Cx. erythrothorax* were the lowest among all *Culex* tested, ranging annually from 0.08 to 0.31 per 1,000 mosquitoes during the study (Table 5).

Our analyses of a relatively small sample size of engorged *Cx. stigmatosoma* revealed that all had acquired blood meals from avian hosts. This is consistent with findings of other studies reporting that this species feeds almost exclusively on birds.[8,14] Seasonal WNV MLEs for *Cx. stigmatosoma* were the highest among the four *Culex* species examined during this study, ranging annually from 0.87 to 22.09 per 1,000 mosquitoes (Table 5), confirming laboratory studies indicating that *Cx. stigmatosoma* is the most competent vector for WNV,[6] of species tested in California. Earlier researchers postulated that because of its early spring abundance, frequent feeding on passeriform birds, and a high level of vector competence, *Cx. stigmatosoma* could play a primary role in early season amplification of arboviruses in southern California.[59] However, our study showed that this species was not an early season indicator of WNV activity, and often lagged virus-positive *Cx. quinquefasciatus* and *Cx. tarsalis* by at least 1 month. Additionally, unlike *Cx. quinquefasciatus*, *Cx. stigmatosoma* was not found to be consistently infected throughout the season (Table 5). Similar results were obtained in a recent study in neighboring Los Angeles County.[4] Although breeding habitats for *Cx. stigmatosoma* are found in residential areas, its low abundance in larval and adult collections, and the lack of feeding on mammalian hosts, particularly humans, probably limits its involvement in epidemic transmission of WNV.

Our analysis revealed that two passeriform birds, house finches and house sparrows, frequently served as important sources of blood meals for all four mosquito species (especially *Cx. quinquefasciatus*), where between 16.9% and 34.2% of all vertebrate-derived blood meals were from the house finch and between 10.3% and 36.4% were from house sparrows (excluding *Cx. erythrothorax*). A recent study on the blood-feeding habit of *Cx. quinquefasciatus* populations collected from Harris County, Texas, showed a relatively high prevalence of feeding on these same avian species, where 3.0% and 3.2% of vertebrate-derived blood meals were from house finches and house sparrows, respectively, though in the latter study, mourning doves were the most frequent hosts, constituting 18.3% of the total blood meals.[46] House finches and house sparrows are abundant and widely distributed in peridomestic habitats in southern California and together, they represented 53.5% of the blood meals acquired by *Cx. quinquefasciatus*, the most frequently infected WNV vector in the region. These birds also comprised nearly 98% of all WNV-seropositive birds captured in this study (Table 7), reconfirming prior studies in southern California.[4,64] The relatively high WNV-seroprevalence rate in AHY house finches (7.4%) compared with HY birds (1.5%) (Table 7) probably reflects a shift toward greater immunity in older, surviving avian hosts after several years of exposure to WNV in California.[65] As competent amplifying hosts, house finches and house sparrows likely play a highly significant role in supporting WNV transmission in this region.[4,7,66]

An earlier study has suggested that intense WNV infections in American crows and other corvids as amplifying hosts are important for infecting *Cx. quinquefasciatus* to facilitate virus transmission to humans in periurban habitats.[67] Corvid birds with higher viremias have been considered important in introducing and enhancing WNV activity to *Culex* mosquitoes, and to avian populations with moderate susceptibility to infection.[7] Elevated infections of American crow populations have been reported from southern California,[67] and western scrub jays in Kern County have been shown to have elevated seroprevalence rates by the end of summer, even though most infected individuals do not survive after experimental infection.[7] Our blood meal analysis revealed that *Cx. quinquefasciatus* feeds rarely on members of the Corvidae family, despite frequent detection of WNV infection in dead crows during this study (Table 6). Other studies have shown that American crows can acquire infection through various means other than mosquito bites, such as oral ingestion of the virus in aqueous solution, consumption of infected bird carcasses,[20,68] and physical contacts with infected feces and bird lice.[69] American crows have been shown to be highly susceptible to WNV infection under laboratory conditions with high mortality rates,[68,70,71] and may serve as important sentinel hosts for WNV activity because of their large size and association with human residential areas.[72,73]

An enhanced interaction in host utilization between *Cx. tarsalis* and the mourning dove was noticed in this study, where 28.2% of all vertebrate-derived blood meals were from this species as the most common source of blood. Similarly, other mosquito species, *Cx. quinquefasciatus*, *Cx. stigmatosoma*, and *Cx. erythrothorax*, displayed frequent feedings on mourning doves (18.9%, 9.1%, and 4.6%, respectively). Our findings are consistent with the result of a recent study on the host-feeding

pattern of *Cx. tarsalis,* where between 36% and 53% of blood meals during the months of June and July were from two dove species, the mourning dove and the Eurasian collared dove in Colorado.[56] Similar findings have been reported on the preference of *Cx. tarsalis* for doves as the source of blood meals from Colorado.[74,75] The potential role that doves play in enzootic cycling of WNV is unclear. The reservoir competence value, expressed as the duration and magnitude of infectious-level viremias, has been reported to be relatively low for the mourning dove[7,68,76]; however, this species has been shown to be frequently infected with the virus in nature and could be zooprophylactic by diverting host-seeking mosquitoes from competent host species.[77] Furthermore, intense feeding pressure on doves may compensate for the relatively poor competence of adult doves,[56,68,78] as determined in laboratory-controlled infection experiments.

CONCLUSION

Our results show that WNV is maintained and amplified in a transmission cycle involving predominantly *Cx. quinquefasciatus*, and passeriform birds, primarily house finches and house sparrows in southern California. In addition to passeriform birds, a pronounced interaction between *Culex* mosquitoes, particularly *Cx. tarsalis* and columbiform (doves) birds, was also noted. The preponderance of WNV-infected *Cx. quinquefasciatus* identified in conjunction with vector competence, opportunistic host-feeding, and abundance, particularly in residential habitats in southern California,[8,9,39,79] incriminates this mosquito species as the principal mosquito vector of the virus in southern California,[4] with other *Culex* mosquitoes also contributing to temporal and spatial transmission of WNV in the region. Because of their association with human hosts for blood feeding, both *Cx. quinquefasciatus* and *Cx. tarsalis* are involved in transmission of WNV to humans, in addition to their role in enzootic transmission of the virus among birds. Infected birds and mosquitoes can further disseminate the virus within residential habitats, where a secondary cycle involving *Cx. stigmatosoma* and peridomestic passeriform birds may establish and advance amplification of the virus.[64] Several anthropogenic factors, such as additional mosquito production from abandoned swimming pools in economically distressed neighborhoods[80] and limited diversity in avian populations,[81] may potentially increase vector-host interactions in southern California, and offset dampening of WNV transmission that might be caused by increased avian diversity, an eco-epidemiological factor defined as the "dilution effect."[82,83]

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