Host (Avian) Biting Preference of Southern California *Culex* Mosquitoes (Diptera: Culicidae)

Author(s): Taylor Lura, Robert Cummings, Robert Velten, Karin De Collibus, Tim Morgan, Kiet Nguyen, and Alec Gerry

Host (Avian) Biting Preference of Southern California *Culex* Mosquitoes (Diptera: Culicidae)

Taylor Lura,[1] Robert Cummings,[2] Robert Velten,[2] Karin De Collibus,[2] Tim Morgan,[2] Kiet Nguyen,[2] and Alec Gerry[1,3]

ABSTRACT The host preference of a vector mosquito species plays a significant role in determining human and animal risk of infection with mosquito-transmitted pathogens. Host preferences of common southern California *Culex* species for four bird species, American crow (*Corvus brachyrhynchos*), house sparrow (*Passer domesticus*), house finch (*Carpodacus mexicanus*), and mourning dove (*Zenaida macroura*), were examined by determining the proportion of each mosquito species that successfully engorged on each of the four bird species presented equally within a net trap to wild host-seeking mosquitoes. Bloodmeals in engorged mosquitoes captured within the net trap were identified to avian species by using a multiplex polymerase chain reaction assay targeting the *cytochrome b* gene sequence. There were significant differences in host selection by all three *Culex* species captured in numbers sufficient for analysis, with *Culex erythrothorax* Dyar preferentially biting American crows, *Culex tarsalis* Coquillett preferentially biting house sparrows, and *Culex quinquefasciatus* Say preferentially biting house finches. All three *Culex* species demonstrated more frequent engorgement on passerine birds (sparrows, finches, and crows) than the nonpasserine mourning dove. A greater preference for passerine birds might be expected to increase the transmission of pathogens, such as West Nile virus, to which passerine birds are particularly competent hosts.

KEY WORDS *Culex*, host preference, avian, *cytochrome b* gene

Mosquitoes are responsible for the infection of humans and animals by many of the world's most significant pathogens. In the United States, West Nile virus (family *Flaviviridae,* genus *Flavivirus,* WNV), a mosquito-transmitted *Flavivirus,* has significantly affected human and animal health after its introduction in 1999 (Komar 2000) and rapid spread across the continental United States over the next 5 yr (Reisen et al. 2004). WNV is maintained in an enzootic cycle between birds and bird-feeding mosquitoes, with transmission of WNV to humans and horses by mosquitoes that have previously fed on an infected bird (Hayes et al. 2005).

The most competent vectors of WNV are mosquitoes in the genus *Culex* (Turell et al. 2001, 2005; Goddard et al. 2002), most of which are ornithophilic and thus maintain the virus in an avian–mosquito transmission cycle (Burkett-Cadena et al. 2008). Mosquitoes that feed on birds with high WNV viremia are more likely to acquire and transmit virus to a susceptible host during a subsequent feeding event (Goddard et al. 2002, Reisen et al. 2005, van der Meulen et al. 2005). Viremia levels vary by bird species (Komar et al. 2003, Kilpatrick et al. 2007), and mosquito species that feed more often on bird species which develop high WNV viremia may therefore be responsible for greater WNV transmission relative to mosquito species which feed more often on WNV-refractory bird species.

For a mosquito species, the proportion of bloodmeals taken from any one host species relative to other available host species, the "host biting index" (Garrett-Jones 1964, Dye 1986), is typically estimated by the proportion of field-captured engorged mosquitoes containing a bloodmeal identified to the specific host(s) of interest. Under field conditions, the host biting index is a product of the abundance and spatiotemporal distribution of mosquitoes and hosts (i.e., the "host-contact opportunity"), modified by differential mosquito responses to available hosts, resulting in successful engorgement on the host species of interest. In a practical sense, these components of the host biting index are difficult to separate and the relative importance of each component to host-specific engorgement is likely to vary with the composition of host species at times and locations that allow discovery by host-seeking mosquitoes. Among available hosts, mosquito orientation toward and landing on a particular host species, combined with mosquito and host characteristics that determine feeding success, can be considered under the single term "host preference." Host preferences are accepted to determine the range of suitable hosts on which a mosquito

[1] Department of Entomology, University of California, Riverside, CA 92521.
[2] Orange County Mosquito and Vector Control District, Garden Grove, CA 92843.
[3] Corresponding author, e-mail: alec.gerry@ucr.edu.

species will commonly feed (e.g., mammalophilic or ornithophilic feeding preferences), although even at this level there can be considerable plasticity depending upon the availability of hosts (Chaves et al. 2010). However, among suitable and available hosts, the relative influence of host preference to host selection is unclear. Recognition that intrinsic host preferences result in differential host selection among generally suitable hosts may have important implications for understanding pathogen transmission risk and also might provide insight into host-seeking behaviors.

In theory, mosquito host preference can be estimated from host-specific proportional engorgement of field-captured mosquitoes adjusted for local host availability after a census of available hosts to estimate a foraging ratio (Hess et al. 1968). However, host-seeking mosquitoes may not encounter suitable hosts in proportion to human-perceived availability of these hosts in the local habitat. The uneven distribution of both host-seeking mosquitoes and their hosts throughout the available habitat during the time period when mosquitoes are actively host seeking (Bidlingmayer 1971, Edman 1971, Meyer et al. 1991) is likely to result in imprecise estimates of host preference.

Here, we evaluate whether several WNV-competent *Culex* mosquitoes in southern California exhibit differences in their preferences to feed on four common North American bird species when the influences of habitat and host availability were removed. These avian species exhibit high rates of naturally acquired WNV infection, but vary in host competence.

Materials and Methods

Study Sites. Studies were conducted on 14 nights from 24 April to 29 August 2008 at three wetlands in southern California known by local mosquito control agencies to support high populations of *Culex* mosquitoes. Study sites included two wetlands along the Pacific coast, the 82-ha University of California at Irvine (UCI) freshwater wetlands (33° 39′26.52″ N, 117° 51′19.83″ W) (nine trap nights) and the 121-ha Bolsa Chica (saltwater) Wetlands Reserve (BCWR) (33° 41′5.24″ N, 118° 1′32.15″ W) (three trap nights), and one freshwater wetlands located in the inland southern California desert, the 8,093-ha San Jacinto Wildlife Area (SJWA) (33°52′14.19″ N,117° 7′6.86″W) (two trap nights). Studies also were conducted on 11 nights from 7 October 2008 to 26 June 2009 at an urban site near the Orange County Vector Control District (OCVCD) facility in the city of Garden Grove, CA (33° 46′30.89″ N, 117° 54′15.18″ W), specifically to collect engorged *Culex quinquefasciatus* Say that were captured in very low numbers at the three wetlands locations but are an important vector of WNV in urban southern California (Reisen et al. 2005).

Experimental Design. Blood-fed and unfed mosquitoes were captured within a 3- by 3- by 3-m net trap baited with four species of wild birds. The net trap consisted of a steel frame with a covering of green fabric on top and mosquito netting on all four sides (Fig. 1). The side netting was raised ≈30 cm above

ground level to allow host-seeking mosquitoes to access the interior of the net while restricting their escape after taking a bloodmeal from one of the birds held within the net trap (Roberts 1965, Mitchell et al. 1985).

Birds (adults only) offered to host-seeking mosquitoes were American crow (*Corvus brachyrhynchos*), house sparrow (*Passer domesticus*), house finch (*Carpodacus mexicanus*), and mourning dove (*Zenaida macroura*). All four bird species are commonly found in southern California and vary in average size: 468 g (American crow), 133 g (mourning dove), 28 g (house sparrow), and 21.5 g (house finch) (Cornell Laboratory of Ornithology 2009). House sparrows and house finches were captured using grain-baited, modified Australian crow traps (McClure 1984), and American crows and mourning doves were captured by federal trappers responsible for removing wildlife from the Los Angeles Airport area. House sparrows and house finches were held for only 1 wk, after which they were released at their capture sites, and newly captured birds were used the following week. American crows (*n* = 5) and mourning doves (*n* = 8) were held for the length of the study with individual birds randomly selected for each trap night. To reduce bird stress, individual birds were not used on consecutive trap nights. Between trap nights, crows were maintained in a mosquito exclusion cage (3.0 by 2.5 by 2.5 m) to lower the risk of WNV infection, and mourning doves were kept in individual cages (60 by 60 by 50 cm) to reduce injury to the birds. Food (commercially purchased wild bird seed for the house sparrows, house finches, and mourning doves; cat food, eggs, and earthworms [*Eisenia fetida*] for the crows) and water were provided to the birds ad libitum in the holding and test cages. Collection and handling of birds was conducted in accordance with an approved University of California at Riverside animal use protocol, collection permit 009927 from the State of California Department of Fish and Game, and banding permit 23547 from the federal U.S. Department of the Interior (U.S. Geological Survey).

Trapping Procedure. On each trap night, two birds of each of the four bird species (total of eight birds) were used, with each bird placed into an individual wire mesh cage (50 by 30 by 30 cm with 2.5- by 5-cm mesh size) suspended 1.5 m from the ground on metal rebar support poles (Fig. 1). Bird cages were evenly distributed around the circumference of a 2-m diameter circle centered within the net trap, and individual birds were randomly assigned to a cage and location. Wooden perches and ample food and water were provided in each cage.

Trapping began in late afternoon and the net trap remained in place overnight. Near dawn the next morning, two investigators lowered the sides of the net trap to the ground before entering the net trap to collect mosquitoes captured overnight. Mosquitoes were collected from the interior walls and roof of the net trap by mechanical aspirator and immediately placed on dry ice. Mosquitoes were transported to the laboratory on dry ice to be sorted by sex, bloodmeal

Fig. 1. Net trap with individual birds randomly assigned to wire mesh bird cages spaced evenly around the circumference of a 2-m circle within the net trap. After lowering the net trap sides to contain captured mosquitoes, a collector (T.L.) is collecting mosquitoes from the interior of the net by using a mechanical aspirator. (Online figure in color.)

status (engorged or unfed) and species (Meyer 2003). Only engorged mosquitoes were recorded and retained from collections at the urban OCVCD site. Blood-fed female mosquitoes were placed individually into 1.5-ml microcentrifuge tubes labeled with the collection date, site, and species identification, and then stored at −80°C for later bloodmeal analysis.

Bloodmeal Analysis. The source of the bloodmeal acquired by each engorged *Culex* mosquito was determined by polymerase chain reaction (PCR) to amplify a species specific fragment of the *cytochrome b* (*cyt b*) gene. Because of the capture of very large numbers of *Culex erythrothorax* Dyar on some collection dates, a subsample of this species captured from each location was analyzed.

For each bird species used in this study, PCR primers were developed from a consensus sequence found by aligning all *cyt b* sequences for the species that were available from GenBank (http://www.ncbi.nlm.nih.gov/) in December 2007. A universal reverse primer identical for all four bird species was identified for a conserved region of the *cyt b* gene (5′-AGGGCYAG-KACTCCTCCTAG-3′). Forward primers specific to each bird species were then designed for variable regions of the *cyt b* gene to give amplified fragments that varied in length for each bird species. The forward

primers were as follows: house sparrow (5′-TTCG-TACTAATGCTCTCCCTG-3′), American crow (5′-ATTCCATCCTTACTACTCCATC-3′), house finch (5′-AAACTGGATCAAACAACCCAAC-3′), and mourning dove (5′-ATAATTGCAGGCCTCACCATC-3′), resulting in expected amplified DNA fragments for each bird species of 187, 224, 276, and 319 bp, respectively. Primer specificity was confirmed using whole blood from the wing vein of each bird species as well as field-captured, unengorged *Cx. erythrothorax*, *Anopheles hermsi* Barr & Guptavanji, and *Aedes vexans* Meigen. The best annealing temperature for PCR amplification of DNA fragments for all four bird species was determined to be 56.3°C after amplification over a gradient of temperatures from 55 to 70°C.

Engorged mosquitoes were homogenized in 180 μl of buffer ATL (DNeasy Blood and Tissue kit, QIAGEN, Valencia, CA) by using an electric motor-driven Kontes pestle (Fisher, Itasca, IL) before DNA extraction following the manufacturer's recommendations and final elution with 200 μl of nuclease-free water. Extracted DNA was then used in a multiplex PCR assay with 1 μl of extracted DNA placed into a 200-μl PCR tube containing PCR Master Mix (Promega, Madison, WI) with 1 μM of the universal reverse and each of the four bird-specific forward prim-

Table 1. Number of blood-fed mosquitoes captured from the interior of a net trap baited with four species of wild birds at three wetlands (UCI, BCWR, and SJWA) and one urban site (OCVCD) in southern California

Mosquito species	Collection site				
	UCI 9 TN[a]	BCWR 3 TN	SJWA 2 TN	OCVCD 11 TN	Total 25 TN
Cx. erythrothorax	712 (325)[b]	47 (25)	54 (54)	0 (0)	813 (404)
Cx. quinquefasciatus	0 (0)	0 (0)	0 (0)	227 (225)	227 (225)
Cx. tarsalis	64 (57)	1 (0)	32 (32)	0 (0)	97 (89)
Total collected	776 (382)	48 (25)	86 (86)	227 (225)	1,137 (718)

[a] TN, number of trap nights at each collection site.
[b] Number blood-fed captured (number tested to determine avian host).

ers in a final reaction volume of 25 μl. The PCR conditions were as follows: initial DNA denaturing at 94°C for 2 min and then 55 cycles of denaturing (94°C for 45 s), annealing (56.3°C for 50 s), and extension (72°C for 1 min), with a final extension of 72°C for 7 min.

Statistical Analysis. Of the mosquito species captured, *Cx. erythrothorax*, *Culex tarsalis* Coquillett, and *Cx. quinquefasciatus* were collected in large enough numbers for statistical analysis. For each *Culex* species captured at the wetlands sites, the relationship between feeding success (proportion engorged of total captured) to overall biting intensity (total number of all *Culex* captured, transformed to $\log_{10} N$) on the same collection night was examined using linear regression, limited to collection periods when ≥10 mosquitoes of the species being analyzed were captured. With a significant relationship between biting intensity and feeding success, biting intensity was subsequently categorized as "high" on nights when ≥50 engorged *Culex* mosquitoes were captured ($n = 3$) and "low" on nights when <50 engorged mosquitoes were captured ($n = 4$) to assess effects of biting intensity on host-specific proportional engorgement. The number of engorged mosquitoes captured during a single collection period with a bloodmeal identified to a specific avian host was determined, converted to a proportion of the total identified bloodmeals for that collection period, and subsequently transformed (arcsine square root $N + 0.05$) for further analysis. For each mosquito species, transformed host-specific proportional engorgement was analyzed using three-way analysis of variance (ANOVA) (PROC ANOVA) for significant variation in the proportion of bloodmeals taken from the available hosts and to determine any significant interactions between host-specific proportional engorgement and collection site or biting intensity. With significant differences in proportional engorgement among hosts, and lacking significant interactions with collection site or biting intensity, the number of bloodmeals identified to a specific avian host was subsequently pooled for each mosquito species across all sites and collection nights and analyzed using a chi-square for differences in proportional engorgement among avian hosts. Differences among mosquito species in host-specific proportional engorgement were examined by Cochran–Mantel–Haenzel relative risk analysis after data transformation ($N + 0.5$) because of a zero count for *Cx. quinquefas-* ciatus fed on mourning doves. All analyses were performed using SAS version 9.2 (SAS Institute, Cary, NC).

Results

In total, 2,298 female mosquitoes (910 engorged) comprising seven species were collected over 14 trap nights in 2008 (mean biting rate, 8.1 mosquitoes per host·night) at the three wetland locations, of which *Cx. erythrothorax* was the most abundant (Table 1). The maximum biting rate for a single night was 47.3 mosquitoes per host at UCI on 12 June 2008. Only 15 mosquitoes of the total collected were species other than *Cx. erythrothorax* or *Cx. tarsalis*, and none of these were engorged. In addition, 227 engorged *Cx. quinquefasciatus* were collected over 11 trap nights at the urban OCVCD location in 2008 and 2009 (mean biting rate, 2.6 mosquitoes per host·night). Blood-fed mosquitoes captured at the OCVCD site were almost exclusively *Cx. quinquefasciatus* (a single engorged *Culex stigmatosoma* Dyar also was captured at this site), and this species achieved a maximum biting rate of 7.9 mosquitoes per host on 12 June 2009. Bloodmeals were identified from 401 of 404 *Cx. erythrothorax*, 87 of 89 *Cx. tarsalis*, and 219 of 225 *Cx. quinquefasciatus*, with blood from two avian hosts identified in one to three individual mosquitoes of each species (Table 2).

The proportion of *Cx. erythrothorax* captured at the wetlands sites that were successful at acquiring a bloodmeal was significantly inversely related to the total number of all *Culex* mosquitoes (fed and unfed) captured during the same collection period ($y = -0.29x + 1.08$; $R^2 = 0.68$; df = 1, 6; $P = 0.02$) (Fig. 2). Although this relationship was similar for *Cx. tarsalis*, it was not significant ($P = 0.19$).

Host-specific proportional engorgement varied significantly among the available hosts for both *Cx. erythrothorax* ($F = 8.25$; df = 3, 20; $P = 0.0009$) and *Cx. tarsalis* ($F = 9.28$; df = 3, 24; $P = 0.0003$), with no significant interactions between host-specific proportional engorgement and biting intensity ($F = 2.38$; df = 4, 20; $P = 0.09$ and $F = 0.93$; df = 4, 24; $P = 0.47$ for *Cx. erythrothorax* and *Cx. tarsalis*, respectively) or collection site ($F = 1.05$; df = 8, 20; $P = 0.44$ and $F = 0.56$; df = 4, 24; $P = 0.70$ for *Cx. erythrothorax* and *Cx. tarsalis*, respectively). *Cx. erythrothorax* fed most often on American crows ($n = 178$, 44.3%, $\chi^2 \geq 35.3$, $P < 0.0001$), whereas *Cx. tarsalis* fed most often on house

Table 2. Identified bloodmeals from engorged mosquitoes captured in a net trap containing eight birds representing four avian species

Avian species	No. of *Cx. erythrothorax* (%)	No. of *Cx. tarsalis* (%)	No. of *Cx. quinquefasciatus* (%)
C. brachyrhynchos	178 (44.3)	19 (21.3)	73 (32.9)
P. domesticus	98 (24.4)	41 (46.1)	53 (23.9)
C. mexicanus	89 (22.1)	24 (27.0)	96 (43.2)
Z. macroura	37 (9.2)	5 (5.6)	0 (0.0)
Total identified bloodmeals	402 (100.0)[a]	89 (100.0)[b]	222 (100.0)[c]
Unidentified bloodmeals	3	2	6
Total mosquitoes tested	404	89	225

[a] Includes one mosquito that fed on both a mourning dove and a house sparrow.

[b] Includes two mosquitoes that contained blood from a house finch and a house sparrow ($n = 1$) or a house finch and an American crow ($n = 1$).

[c] Includes three mosquitoes that contained blood from an American crow and a house sparrow ($n = 2$) or an American crow and a house finch ($n = 1$).

sparrows ($n = 41$, 46.1%, $\chi^2 \geq 7.0$, $P < 0.01$) and *Cx. quinquefasciatus* fed most often on house finches ($n = 96$, 43.2%, $\chi^2 \geq 5.1$, $P < 0.03$) (Fig. 3). All three *Culex* species fed significantly less often on mourning doves relative to all other bird species ($\chi^2 \geq 9.4$, $P < 0.003$). Of particular note, zero of 222 *Cx. quinquefasciatus* bloodmeals were identified to be from a mourning dove.

The probability of feeding on a specific avian species over all others varied significantly among the three *Culex* species (Table 3). For each mosquito species, the probability of feeding on the preferred host (as identified in Fig. 3) was ≈1.5–2 times greater

than the probability that the other *Culex* species would feed on that same host species.

Discussion

Variation in host-specific proportional engorgement by mosquito species in this study was unrelated to habitat or host contact opportunity, because hosts were presented to host-seeking mosquitoes in equal numbers and under identical conditions. Differences in host-specific engorgement as noted are therefore likely to reflect real differences in mosquito orientation toward hosts (Dow et al. 1957, Reisen et al. 1992a, Simpson et al. 2009) and/or host-specific feeding success, perhaps as a result of host defensive behaviors (Edman and Kale 1971, Edman et al. 1974, Nelson et al. 1976, Darbro and Harrington 2007) or other host protective characteristics (e.g., effectiveness of a protective feather "barrier"). Therefore, the term "host preference" in our study combines host orientation behavior and feeding success that together result in successful engorgement on a particular host species relative to other available host species.

What is particularly novel about this study is that mosquitoes were given an unrestricted opportunity to select freely among several avian hosts, including the opportunity to reorient away from a defensive host and toward other nearby hosts, with final host selection measured by engorgement on a particular host species as identified using molecular methods. Previous studies primarily assessed mosquito orientation behavior, with mosquitoes being captured at the first host approached (Dow et al. 1957, Simpson et al. 2009, Suom et al. 2010), often without determining ultimate feeding success on that host. Although there are good reasons to evaluate mosquito orientation toward hosts,
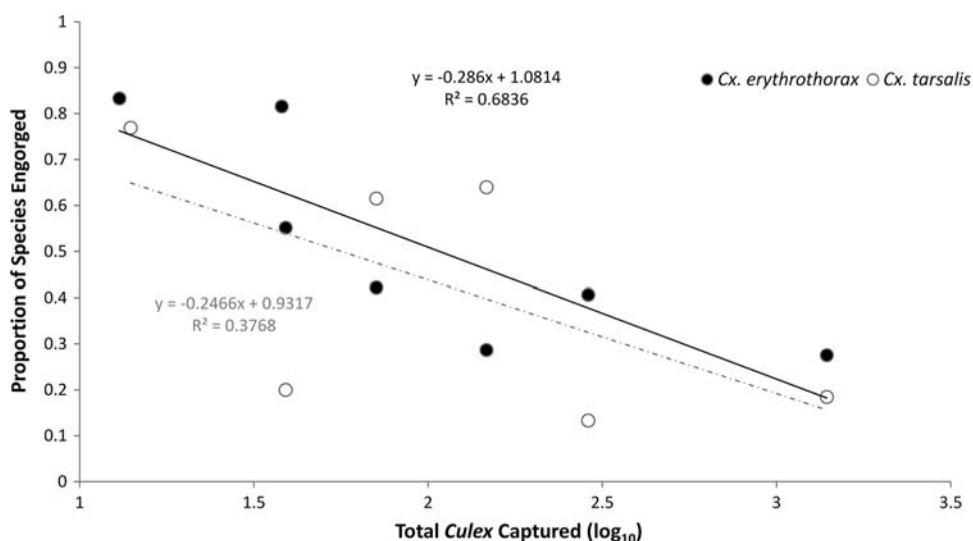

Fig. 2. Relationship between feeding success of each *Culex* species and the total number of *Culex* captured for each collection period at three wetlands sites. For each *Culex* species, data are limited to collection periods with ≥10 female mosquitoes (fed or unfed) of that species captured in a single collection period.
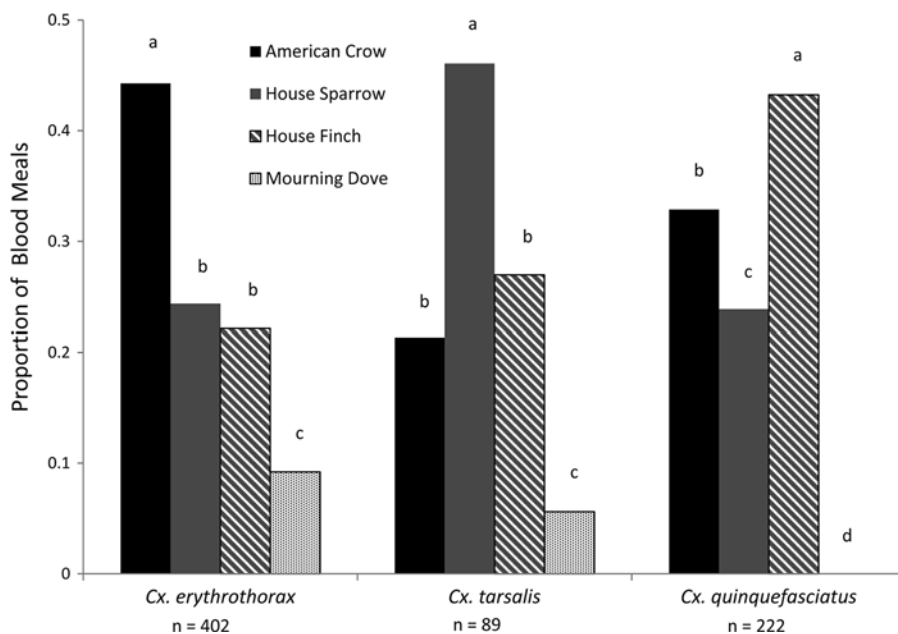
Fig. 3. Frequency of engorgement by *Cx. erythrothorax*, *Cx. tarsalis*, and *Cx. quinquefasciatus* on four bird species offered similarly to wild mosquitoes under field conditions. Within a mosquito species, columns with the same letter are not statistically significant by chi-square analysis ($P > 0.05$).

these orientation behaviors comprise only one component of the host selection process eventually leading to successful engorgement on a particular host. We acknowledge that the limited host species ($N = 4$, birds only) and their spatial arrangement in this study are unlikely to reflect natural conditions and that further studies are needed to assess variation in host preference with other combinations and spatial arrangements of avian and mammalian hosts. Nevertheless, it is clear that *Culex* populations did exhibit a significant preference among the four avian host species offered to them in this study and that this preference was specific to each mosquito species.

All three *Culex* species demonstrated a preference for a different passerine bird species, with a somewhat lower host preference for the remaining passerine species, and the nonpasserine mourning dove being the least preferred host species. For each *Culex* species, the probability of feeding on the preferred host

was considerably greater than the probability that another *Culex* species would feed on the same host species. The differential host preference demonstrated in this study may have important epidemiological implications for pathogens, such as WNV, that persist in an avian–mosquito transmission cycle.

Feeding success was significantly inversely related to increasing biting intensity, perhaps as a result of increasing host defensive behavior (Edman et al. 1974, Nelson et al. 1976). Although host defensive behavior was not evaluated, proportional engorgement on one host species relative to another did not change with increasing biting intensity for either *Cx. tarsalis* or *Cx. erythrothorax*. Biting rate, as measured in this study by the number of engorged mosquitoes captured, was relatively low, averaging 8.1 mosquitoes per host·night at the wetland sites and only 2.6 mosquitoes per host·night at the urban location for all species and nights combined. Considering the range of the host-

Table 3. Relative engorgement probability for mosquito species engorging on each of the four bird species offered similarly to wild host-seeking mosquitoes in southern California

	Engorgement probability ratio (RR)			
	American crow	House finch	House sparrow	Mourning dove
Cx. erythrothorax vs. *Cx. tarsalis*	2.07 (1.37–3.14)	—[a]	0.53 (0.40–0.70)	—
Cx. erythrothorax vs. *Cx. quinquefasciatus*	1.35 (1.09–1.68)	0.51 (0.40–0.65)	—	41.50 (2.56–672.59)
Cx. tarsalis vs. *Cx. quinquefasciatus*	—	0.62 (0.43–0.91)	1.93 (1.39–2.67)	27.26 (1.52–487.82)

Cells indicate the host-specific engorgement probability ratio (95% confidence interval) for the two mosquito species indicated in the row as determined by Cochran–Mantel–Haenzel analysis. The probability ratio (or relative risk [RR]) indicates the increased/decreased likelihood that the first mosquito species, relative to the second mosquito species, indicated in the row heading would feed on the bird species identified in the column rather than the other bird species offered. RR > 1 indicates a greater probability of engorgement, and RR < 1 indicates a reduced probability of engorgement.

[a] —, indicates no significant difference in engorgement probability between the two compared mosquito species.

seeking activity period for these species at the collection sites (Cope et al. 1986, Gerry et al. 2008), it seems unlikely that there would have been many mosquitoes biting the same host at the same time. Biting intensity also seems to have had little effect on successful engorgement once feeding had begun, as nearly all (>99%) of the blood-fed mosquitoes were fully engorged and had taken blood from only one of the available host species. *Culex* with mixed bloodmeals are collected relatively infrequently under field conditions (Tempelis et al. 1967; Molaei et al. 2007, 2010; Thiemann et al. 2011).

The relative abundance of mosquito species captured in this study reflects trapping efforts near wetlands with emergent vegetation that are especially productive for *Cx. erythrothorax* (Cope et al. 1986, Walton and Workman 1998). *Cx. tarsalis* was abundant only near freshwater wetlands, and *Cx. quinquefasciatus* was abundant only at the urban site, as is reported for these species (Bohart and Washino 1978). All three *Culex* species are known to be competent vectors of WNV under laboratory conditions (Goddard et al. 2002, Turell et al. 2005) and have been frequently found infected with the virus in nature (Reisen et al. 2004, Kwan et al. 2010, Molaei et al. 2010).

Under natural conditions, *Cx. tarsalis* feed most frequently on birds (Tempelis and Washino 1967, Tempelis et al. 1967, Reisen et al. 1992b, Wekesa et al. 1997, Kent et al. 2009, Molaei et al. 2010), but will take bloodmeals from both small and large mammals, with increasing mammal feeding associated with greater mosquito density (Nelson et al. 1976, Thiemann et al. 2011). In contrast, *Cx. erythrothorax* is considered to be largely opportunistic and will readily feed on both birds and mammals (Tempelis 1989, Reisen et al. 1992b, Walton et al. 1999, Gerry et al. 2008). *Cx. quinquefasciatus* has been reported to feed predominantly on birds (Hayes et al. 1973, Bohart and Washino 1978), predominantly on mammals (Samuel et al. 2004), or readily on both birds and mammals (Edman 1974; Reisen and Reeves 1990; Reisen et al. 1992b; Niebylski and Meek 1992; Zinser et al. 2004; Molaei et al. 2007, 2010).

Of the bird species presented to wild mosquitoes in this study, American crows are considerably larger than the other bird species and might be expected to produce a higher concentration of host semiochemicals. Dow et al. (1957) demonstrated that larger birds attracted more *Cx. tarsalis* to their immediate vicinity, but this greater attraction did not necessarily result in more successful engorgement. In contrast, Darbro and Harrington (2006) showed no difference in attraction of *Culex pipiens pipiens* L. and *Culex restuans* Theobald to either house sparrows or much larger domestic chickens (*Gallus gallus domesticus*) when these birds were placed in traps suspended within a tree canopy. In our investigation, *Cx. erythrothorax* fed more often on American crows relative to smaller bird species available, but both *Cx. tarsalis* and *Cx. quinquefasciatus* fed significantly more often on the smaller birds (house sparrows and house finches, respectively) compared with the larger American crows and mourn-

ing doves, suggesting factors other than size were responsible for their host preferences. Mosquito orientation toward the individual captive birds separated from successful engorgement was not evaluated in this study.

Interestingly, feeding on mourning doves was very low for all three *Culex* species, even though mourning doves are intermediate in size to other bird species that were fed upon in this study. This finding was surprising given that previous investigations have shown engorgement on mourning doves by *Cx. tarsalis* and *Cx. quinquefasciatus* to be high (Kent et al. 2009; Molaei et al. 2007, 2010), and these birds frequently are infected with WNV in nature (Wheeler et al. 2009, Nguyen et al. 2010). However, host selection by *Culex* mosquitoes as identified in these previous studies may reflect high host-contact opportunity for mourning doves, and perhaps the lack of alternate suitable species nearby, rather than any innate host preference for mourning doves, a sentiment echoed by Kent et al. (2009). Under field conditions, abandonment of a less suitable host species may be limited by low availability of other hosts within the range of detection. The low preference for mourning doves, exhibited by all *Culex* species in the current study, might reflect the proximity of multiple host species allowing host-seeking mosquitoes to readily reorient from doves toward a more attractive or less defensive bird species (Walker and Edman 1985).

Proportional host-specific engorgement by *Cx. erythrothorax* and *Cx. tarsalis* did not vary by collection site, even though sites were separated by ≈50 km and represented three very different wetlands habitats with dissimilar mosquito biting rates. Molaei et al. (2010) reported that the proportion of bloodmeals acquired by *Culex* mosquitoes from selected avian hosts varied with habitat in southern California, but differences in host contact opportunity near mosquito traps are probably responsible for this variation in proportional engorgement (Edman 1971, Patrican et al. 2007, Chaves et al. 2010). All four bird species used in the current study are abundant year-round throughout southern California, so even geographically distant populations of *Culex* species are probably under similar selection pressure to orient toward and feed on these avian hosts.

Host availability at the time and place where mosquitoes are host-seeking is difficult to determine, and estimations of mosquito activity through sampling may provide little direct information on the abundance of mosquitoes within a much broader area where potential hosts may be available. With most animal and mosquito populations nonuniformly distributed throughout the environment because of habitat constraints and social behaviors (Bidlingmayer 1985, Lothrop and Reisen 2001), areawide forage ratios are not likely to provide an accurate measure of host-contact opportunities (Chaves et al. 2010), and they may therefore result in misleading estimates of host preference. Even reasonably comprehensive surveys of the fauna in a local area (as in Kent et al. 2009) could fail to account for variable host and mosquito behav-

iors during the critical host-seeking period, which may alter host contact opportunities in ways that would be difficult to predict. In addition, estimates of proportional host engorgement may be biased by trap locations and trap methods used to capture recently engorged mosquitoes (e.g., Thiemann and Reisen 2012).

There are >300 species of birds in the order Columbiformes (including mourning doves) and >1000 species in the order Passeriformes (including crows, finches, and sparrows) throughout North America, with the four species used in this study being some of the more common and widespread (Cornell Laboratory of Ornithology 2009). Passerine birds typically develop higher WNV viremia of longer duration relative to other bird orders (Komar et al. 2003, Reisen et al. 2005), and the proportion of *Culex* mosquitoes infected with WNV after feeding on an infective bloodmeal is strongly dose dependent (Goddard et al. 2002, Reisen et al. 2005). In the current study, the preference of all *Culex* spp. for the three passerine birds was significantly greater than for the single nonpasserine tested. A greater preference of *Culex* mosquitoes for passerine birds would probably enhance WNV transmission, even when WNV-refractory nonpasserine birds are similarly available in the environment and might otherwise be expected to dilute the pool of competent hosts (e.g., Loss et al. 2009).

Additional studies should be conducted to evaluate whether the reduced preference for mourning doves demonstrated by all three *Culex* species in this study is specific to this avian species or more generally applicable to other nonpasserines. Additional studies to separate the effects of differential orientation behaviors and host-feeding success as components of host preference also are needed, as are further studies to examine host preference across mosquito populations of the same species.

Acknowledgments

References Cited

Bidlingmayer, W. L. 1971. Mosquito flight paths in relation to the environment. I. Illumination levels, orientation and resting areas. Ann. Entomol. Soc. Am. 64: 1121–1131.

Bidlingmayer, W. L. 1985. The measurement of adult mosquito population changes-some considerations. J. Am. Mosq. Control Assoc. 1: 328–348.

Bohart, R. M., and R. K. Washino. 1978. Mosquitoes of California. Division of Agricultural Science, University of California, Berkeley, CA.

Burkett-Cadena, N. D., S. P. Graham, H. K. Hassan, C. Guyer, M. D. Eubanks, C. R. Katholi, and T. R. Unnasch. 2008. Blood feeding patterns of potential arbovirus vectors of the genus *Culex* targeting ectothermic hosts. Am. J. Trop. Med. Hyg. 79: 809–815.

Chaves, L. F., L. C. Harrington, C. L. Keogh, A. M. Nguyen, and U. D. Kitron. 2010. Blood feeding patterns of mosquitoes: random or structured? Front. Zool. 7: 1–11.

Cope, S. E., A. R. Barr, M. J. Bangs, A. C. Morrison, and P. Guptavanij. 1986. Human bait collections of mosquitoes in a southern California fresh water marsh. Proc. Calif. Mosq. Vector Control Assoc. 54: 110–112.

Cornell Laboratory of Ornithology. 2009. Birds of North America. (http://bna.birds.cornell.edu/bna).

Darbro, J. M., and L. C. Harrington. 2006. Bird-baited traps for surveillance of West Nile mosquito vectors: effect of bird species, trap height, and mosquito escape rates. J. Med. Entomol. 43: 83–92.

Darbro, J. M., and L. C. Harrington. 2007. Avian defensive behavior and blood-feeding success of the West Nile vector mosquito, *Culex pipiens*. Behav. Ecol. 18: 750–757.

Dow, R. P., W. C. Reeves, and R. E. Bellamy. 1957. Field tests of avian host preference of *Culex tarsalis* Coq. Am. J. Trop. Med. Hyg. 6: 294–303.

Dye, C. 1986. Vectorial capacity: must we measure all its components? Parasitol. Today 2: 203–209.

Edman, J. D. 1971. Host-feeding patterns of Florida mosquitoes. 1. *Aedes, Anopheles, Coquillettidia, Mansonia and Psorophora.* J. Med. Entomol. 8: 687–695.

Edman, J. D. 1974. Host-feeding patterns of Florida mosquitoes. 3. Culex (*Culex*) and *Culex* (*Neoculex*). J. Med. Entomol. 11: 95–104.

Edman, J. D., and H. W. Kale. 1971. Host behavior: its influence on the feeding success of mosquitoes. Ann. Entomol. Soc. Am. 64: 513–516.

Edman, J. D., L. A. Webber, and A. A. Schmid. 1974. Effect of host defenses on the feeding pattern of *Culex nigripalpus* when offered a choice of blood sources. J. Parsitol. 60: 874–883.

Garrett-Jones, C. 1964. The human blood index of malaria vectors in relation to epidemiological assessment. Bull. W.H.O. 30: 241–261.

Gerry, A. C., T. M. Nawaey, P. B. Sanghrajka, J. Wisniewska, and P. Hullinger. 2008. Comparison of a horse and a carbon dioxide-baited suction trap for collecting hematophagous Diptera in southern California with a discussion of relevance to West Nile virus epidemiology. J. Med. Entomol. 45: 115–124.

Goddard, L. B., A. E. Roth, W. K. Reisen, and T. W. Scott. 2002. Vector competence of California mosquitoes for West Nile virus. Emerg. Infect. Dis. 8: 1385–1391.

Hayes, R. O., C. H. Tempelis, A. D. Hess, and R. C. Reeves. 1973. Mosquito host preference studies in Hale County, Texas. Am. J. Trop. Med. Hyg. 22: 270–277.

Hayes, E. B., N. Komar, R. S. Nasci, S. P. Montgomery, D. R. O'Leary, and G. L. Campbell. 2005. Epidemiology and transmission dynamics of West Nile virus disease. Emerg. Infect. Dis. 11: 1167–1173.

Hess, A. D., R. O. Hayes, and C. H. Tempelis. 1968. The use of the forage ratio technique in mosquito host preference studies. Mosq. News 28: 386–389.

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

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

Komar, N. 2000. West Nile viral encephalitis. Rev. Sci. Tech. Off. Int. Epiz. 19: 166–176.

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

Kwan, J. L., S. Kluh, M. B. Madon, and W. K. Reisen. 2010. West Nile virus emergence and persistence in Los Angeles, California, 2003–3008. Am. J. Trop. Med. Hyg. 83: 400–412.

Loss, S. R., G. L. Hamer, E. D. Walker, M. O. Ruiz, T. L. Goldberg, U. D. Kitron, and J. D. Brawn. 2009. Avian host community structure and prevalence of West Nile virus in Chicago, Illinois. Oecologia 159: 415–424.

Lothrop, H. D., and W. K. Reisen. 2001. Landscape affects the host-seeking patterns of *Culex tarsalis* (Diptera: Culicidae) in the Coachella Valley of California. J. Med. Entomol. 38: 325–332.

McClure, E. 1984. Bird banding, pp. 493–496. Boxwood Press, Pacific Grove, CA.

Meyer, R. P. 2003. Regional guide to the common mosquitoes of California: southern California. Mosquito and Vector Control Association of California, Sacramento, CA.

Meyer, R. P., W. K. Reisen, and M. M. Milby. 1991. Influence of vegetation on carbon dioxide trap effectiveness for sampling mosquitoes in the Sierra Nevada foothills of Kern County, California. J. Am. Mosq. Control Assoc. 7: 471–475.

Mitchell, C. J., R. F. Darsie, T. P. Monath, M. S. Sabattini, and J. Daffner. 1985. The use of an animal-baited net trap for collecting mosquitoes during western equine encephalitis investigations in Argentina. J. Am. Mosq. Control Assoc. 1: 43–47.

Molaei, G., T. G. Andreadis, P. M. Armstrong, R. Bueno, Jr., J. A. Dennett, S. V. Real, C. Sargent, A. I. Bala, Y. Randle, H. Guzman, et al. 2007. Host feeding patterns 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., R. F. Cummings, T. Su, P. M. Armstrong, G. A. Williams, M.-L. Cheng, J. P. Webb, and T. G. Andreadis. 2010. Vector-host interactions governing epidemiology of West Nile virus in southern California. Am. J. Trop. Med. Hyg. 83: 1269–1282.

Nelson, R. L., C. H. Tempelis, W. C. Reeves, and M. M. Milby. 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, M. L., and C. L. Meek. 1992. Blood-feeding of *Culex* mosquitoes in an urban environment. J. Am. Mosq. Control Assoc. 8: 173–177.

Nguyen, K., S. Bennett, R. Cummings, K. De Collibus, C. Fogarty, J. Francisco, C. Herrera, T. Morgan, M. Jozan, A. Tilzer, et al. 2010. Evaluation of West Nile virus activity in Orange County, California, during 2009. Proc. Calif. Mosq. Vector Control Assoc. 78: 64–68.

Patrican, L. A., L. E. Hackett, J. E. Briggs, J. W. McGowan, T. R. Unnasch, and J. H. Lee. 2007. Host-feeding patterns of *Culex* mosquitoes in relation to trap habitat. Emerg. Infect. Dis. 13: 1921–1923.

Reisen, W. K., and W. C. Reeves. 1990. Bionomics and ecology of *Culex tarsalis* and other potential mosquito vector species, pp. 254–329. *In* W. C. Reeves (ed.), Epidemiology and control of mosquitoborne arboviruses in California, 1943–1987. California Mosquito and Vector Control Association, Sacramento, CA.

Reisen, W. K., J. L. Hardy, and S. B. Presser. 1992a. Evaluation of domestic pigeons as sentinels for detecting ar-

bovirus activity in southern California. Am. J. Trop. Med. Hyg. 46: 69–79.

Reisen, W. K., M. M. Milby, S. B. Presser, and J. L. Hardy. 1992b. Ecology of mosquitoes and St. Louis encephalitis virus in the Los Angeles basin of California, 1987–1990. J. Med. Entomol. 29: 582–598.

Reisen, W. K., H. D. Lothrop, R. Chiles, M. M. Madon, C. Cossen, L. Woods, S. Husted, V. Kramer, and J. Edman. 2004. West Nile virus in California. Emerg. Infect. Dis. 10: 1369–1378.

Reisen, W. K., Y. Fang, and V. M. Martinez. 2005. Avian host and mosquito (Diptera: Culicidae) vector competence determine the efficiency of West Nile and St. Louis encephalitis virus transmission. J. Med. Entomol. 42: 367–375.

Roberts, R. H. 1965. A steer-baited trap for sampling insects affecting cattle. Mosq. News 25: 281–285.

Samuel, P. P., N. Arunachalam, J. Hiriyan, V. Thenmozhi, A. Gajanana, and K. Satyanarayana. 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.

Simpson, J. E., C. M. Folson-O'Keefe, J. E. Childs, L. E. Simons, T. G. Andreadis, and M. A. Diuk-Wasser. 2009. Avian host-selection by *Culex pipiens* in experimental trials. PLoS ONE 4: e7861.

Suom, C., H. S. Ginsberg, A. Bernick, C. Klein, P. A. Buckley, C. Salvatore, and R. A. LeBrun. 2010. Host-seeking activity and avian host preferences of mosquitoes associated with West Nile virus transmission in the northeastern U.S.A. J. Vector Ecol. 35: 69–74.

Tempelis, C. H. 1989. Estimation of vectorial capacity: mosquito host selection. Bull. Soc. Vector Ecol. 14: 55–59.

Tempelis, C. H., and R. K. Washino. 1967. Host-feeding patterns of *Culex tarsalis* in the Sacramento Valley, California, with notes on other species. J. Med. Entomol. 4: 315–318.

Tempelis, C. H., D. B. Francy, R. O. Hayes, and M. F. Lofy. 1967. Variations in feeding patterns of seven culicine mosquitoes on vertebrate hosts in Weld and Larimer counties, Colorado. Am. J. Trop. Med. Hyg. 16: 111–119.

Thiemann, T. C., S. S. Wheeler, C. M. Barker, and W. K. Reisen. 2011. Mosquito host selection varies seasonally with host availability and mosquito density. PLoS Negl. Trop. Dis. 5: e1452.

Thiemann, T. C., and W. K. Reisen. 2012. Evaluating sampling method bias in *Culex tarsalis* and *Culex quinquefasciatus* (Diptera: Culicidae) bloodmeal identification studies. J. Med. Entomol. 49: 143–149.

Turell, M. J., M. O'Guinn, and D. J. Dohm. 2001. Vector competence of North American mosquitoes (Diptera: Culicidae) for West Nile virus. J. Med. Entomol. 38: 130–134.

Turell, M. J., D. J. Dohm, M. R. Sardelis, M. L. O'Guinn, T. G. Andreadis, and J. A. Blow. 2005. An update on the potential of North American mosquitoes (Diptera: Culicidae) to transmit West Nile virus. J. Med. Entomol. 42: 57–62.

van der Meulen, K. M., M. B. Pensaert, and H. J. Nauwynck. 2005. West Nile virus in the vertebrate world. Arch. Virol. 150: 637–657.

Walker, E. D., and J. D. Edman. 1985. The influence of host defensive behavior in mosquito (Diptera: Culicidae) biting persistence. J. Med. Entomol. 22: 370–372.

Walton, W. E., and P. D. Workman. 1998. Effect of marsh design on the abundance of mosquitoes in experimental

constructed wetlands in southern California. J. Am. Mosq. Control Assoc. 14: 95–107.

Walton, W. E., P. D. Workman, and C. H. Tempelis. 1999. Dispersal, survivorship, and host selection of *Culex erythrothorax* (Diptera: Culicidae) associated with a constructed wetland in southern California. J. Med. Entomol. 36: 30–40.

Wekesa, J. W., B. Yuval, R. K. Washino, and A. M. deVasquez. 1997. Blood feeding patterns of *Anopheles freeborni* and *Culex tarsalis* (Diptera: Culicidae): effects of habitat and host abundance. Bull. Entomol. Res. 87: 633–641.

Wheeler, S. S., C. M. Barker, Y. Fang, M. V. Armijos, B. D. Carroll, S. Husted, W. O. Johnson, and W. K. Reisen. 2009. Differential impact of West Nile virus on California birds. Condor 111: 1–20.

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