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Host Selection by *Culex pipiens* Mosquitoes and West Nile Virus Amplification

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Abstract. Recent field studies have suggested that the dynamics of West Nile virus (WNV) transmission are influenced strongly by a few key super spreader bird species that function both as primary blood hosts of the vector mosquitoes (in particular *Culex pipiens*) and as reservoir-competent virus hosts. It has been hypothesized that human cases result from a shift in mosquito feeding from these key bird species to humans after abundance of the key birds species decreases. To test this paradigm, we performed a mosquito blood meal analysis integrating host-feeding patterns of *Cx. pipiens*, the principal vector of WNV in the eastern United States north of the latitude 36°N and other mosquito species with robust measures of host availability, to determine host selection in a WNV-endemic area of suburban Chicago, Illinois, during 2005–2007. Results showed that *Cx. pipiens* fed predominantly (83%) on birds with a high diversity of species used as hosts (25 species). American robins (*Turdus migratorius*) were marginally overused and several species were underused on the basis of relative abundance measures, including the common grackle (*Quiscalus quiscula*), house sparrow (*Passer domesticus*), and European starling (*Sturnus vulgaris*). *Culex pipiens* also fed substantially on mammals (19%; 7 species with humans representing 16%). West Nile virus transmission intensified in July of both years at times when American robins were heavily fed upon, and then decreased when robin abundance decreased, after which other birds species were selected as hosts. There was no shift in feeding from birds to mammals coincident with emergence of human cases. Rather, bird feeding predominated when the onset of the human cases occurred. Measures of host abundance and competence and *Cx. pipiens* feeding preference were combined to estimate the amplification fractions of the different bird species. Predictions were that approximately 66% of WNV-infectious *Cx. pipiens* became infected from feeding on just a few species of birds, including American robins (35%), blue jays (17%, *Cyanocitta cristata*), and house finches (15%, *Carpodacus mexicanus*).

INTRODUCTION

In many parts of North America, mosquitoes from the *Culex pipiens* complex transmit West Nile virus (WNV) among individuals comprising diverse bird communities in a variety of landscapes.[1,2] West Nile virus has had local and regional impacts on bird populations,[3–5] yet just a few bird species, capable of being infected with WNV and then becoming infectious (competent hosts), may be responsible for most WNV maintenance and amplification.[6,7] These so-called super-spreader bird species, such as American robin (*Turdus migratorius*), are typically widespread, but are often not the dominant species in a community. The ornithophilic *Cx. pipiens* mosquito may demonstrate a preference for these super-spreader bird species. When *Culex* spp. feeding patterns are analyzed temporally, several studies have identified a shift in feeding from birds to mammals, which may enhance human epidemics.[8–10]

The contribution of a bird species to West Nile virus transmission depends on its host competence, which is a function of the magnitude and duration of viremia,[1,11,12] host-contact rates,[13,14] and survival rates. Host-contact rates are a function of vector feeding preferences[15] and relative abundance of susceptible hosts. Bird species with high reservoir competence with potential importance for transmission, such as American crow (*Corvus brachyrhynchos*[11]), are now understood to be less important, as shown by the observation that WNV transmission continues even where crow densities have been reduced[4] and because crows do not appear to be major hosts for *Culex* spp. mosquitoes.[16] Extensive serosurveys of avian communities have documented the presence of antibodies to WNV to identify spatial and temporal patterns of transmission.[17–23] However, serologic studies are limited because they quantify exposure rates only within the surviving fraction of the population that can be captured.[24] Such studies offer only limited insight into the actual contribution of different bird species to transmission. Identifying the role of different species in transmission through the integration of reservoir competence and mosquito feeding preferences has only been evaluated in the mid-Atlantic United States[6] and in Memphis, Tennessee.[7]

Mosquito host selection has been measured using forage ratios,[25] human blood index,[26] feeding index,[15] and feeding preference[6] but studies using these indices rarely incorporate fine-scale surveys of host availability. Host availability is a function of ecologic, biologic, and behavioral factors that influence the probability of a host being exposed to a mosquito.[27] Ecologic factors important for host availability include the night-time roost size, location, and height of a bird species. Biologic factors, such as host body mass and anti-mosquito behavior, also affect host selection.[28–31]

In the present study, we tested whether *Cx. pipiens* mosquitoes feed selectively on certain avian hosts and avoid others, and whether these potential variations affected WNV transmission patterns in a known focus of arbovirus transmission.[32–34] By incorporating measures of host selection based upon assessment of host availability, we tested whether American robins are overused relative to other common species. Furthermore, we examined whether temporal patterns reflect a shift in feeding preferences from birds to mammals

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coincident with the onset of human WNV cases. Finally, we modeled the amplification fraction (a measure of the number of infectious *Cx. pipiens* resulting from each bird species) to predict the relative contributions of different bird species to WNV maintenance and amplification.

MATERIALS AND METHODS

Study sites. Sampling sites were in suburban southwest Chicago, Illinois (Cook County; 87°44′W, 41°42′N) and included 11 residential sites and four semi-natural sites (three cemeteries and a wildlife refuge) in 2005 and an additional 10 residential sites and 1 natural site (a forest preserve) in 2006. In 2007, we returned to 10 of the same residential sites and 4 natural sites and added 5 residential sites. Selection criteria for study sites were previously described.[35] Human WNV case data, including date of onset and location, were provided by the Illinois Department of Public Health without personal identifiers. Human cases considered in this report occurred within a 5-km buffer around the 15 field sites in 2005, 26 field sites in 2006, and 19 field sites in 2007. Spatial data were processed using the ArcGIS 9.2 software (Environmental Systems Research Institute, Redland, CA).

Mosquito collections, species identification, and WNV infection rates. Mosquitoes were sampled from each study site once every two weeks from mid-May through mid-October in 2005–2007, using CO_2-baited Centers for Disease Control and Prevention (CDC) (Atlanta, GA) miniature light traps, CDC gravid traps baited with rabbit pellet infusion, and battery-powered backpack aspirators. Mosquitoes were identified to species morphologically[36] and blood-fed individuals were separated from gravid and unfed individuals. Non-bloodfed mosquitoes were pooled and tested for WNV RNA using reverse transcription, quantitative polymerase chain reaction (PCR).[35] For blood-fed mosquitoes, the abdomens were removed (see below), and the carcasses were tested for WNV RNA individually as above. Maximum likelihood estimates for infection rates were calculated using the Pooled Infection Rate version 3.0 add-in[37] in the program Excel (Microsoft, Redmond, WA). Blood-fed *Culex* spp. mosquitoes were identified to species using a PCR-based method.[38]

Blood meal analysis. The relative amount of blood in the abdomens from blood-fed mosquitoes was scored with the Sella scale (1 = unfed; 2–6 = partial to full blood meal; 7 = gravid[39]). Using sterile technique, we removed the abdomen from each specimen, transferred it to a microcentrifuge tube, and DNA was extracted from it (DNeasy Tissue Kits; Qiagen, Valencia, CA). Extracted DNA served as template for a series of PCRs using primer pairs complementary to nucleotide sequences of the vertebrate cytochrome b (*cyt b*) gene as follows. Each sample was tested in two reactions using two separate primer pairs, one termed avian a (5′-GAC TGT GAC AAA ATC CCN TTC CA-3′ and 5′-GGT CTT CAT CTY HGG YTT ACA AGA C-3′;) and the other termed mammal a (5′-CGA AGC TTG ATA TGA AAA ACC ATC GTT G-3′ and 5′-TGT AGT TRT CWG GGT CHC CTA-3′).[40] The Failsafe PCR System (Epicentre Biotechnologies, Madison, WI) was used, and conditions consisted of an initial denaturation for 3.5 minutes at 95°C, followed by 36 cycles consisting of denaturation (30 seconds at 95°C), annealing (50 seconds at 60°C), extension (40 seconds at 72°C), and a final extension for 5 minutes at 72°C. Amplicons were visualized by electrophoresis (E-gel system; Invitrogen, Carlsbad, CA), scored by band intensity (0 = no product; 5 = bold product), and purified (QIAquick PCR Purification Kits; Qiagen).

Nucleotide sequences of amplicons were obtained by direct sequencing (ABI Prism 3700 DNA Analyzer; Applied Biosystems, Foster City, CA). Sequences were subjected to BLAST search in GenBank (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi). Returns to searches were evaluated as follows. Each chromatogram was inspected (Chromas Lite software; Technelysium Pty. Ltd., Tewantin, Queensland, Australia) for sequence quality and presence of double-nucleotide peaks, which may indicate blood from more than one vertebrate species in the blood meal.[41] Samples that produced an amplicon in one or the other reaction and a satisfactory match by BLAST were accepted as the likely host of origin, typically with 99% sequence match. Samples that did not produce an amplicon after the first two reactions, and amplicons that yielded ambiguous sequences (low-quality or double-nucleotide peaks), were subjected to a third PCR using the BM primer pair (5′-CCC CTC AGA ATG ATA TTT GTC CTC A-3′ and 5′-CCA TCC AAC ATC TCA GCA TGA TGA AA-3′) under reaction conditions described above.[40,41] Samples that did not produce an amplicon or yielded ambiguous sequences in the third reaction (BM primer set) were subjected to a final round of PCR using a primer pair designed for reptiles and amphibians (i.e., herp) (5′-GCH GAY ACH WVH HYH GCH TTY TCH TC-3′ and 5′-CCC CTC AGA ATG ATA TTT GTC CTC A-3′).[42] Reaction conditions for the herp primer pair consisted of an initial denaturation for 2 minutes at 95°C, followed by 55 cycles consisting of denaturation (45 seconds at 94°C), annealing (50 seconds at 50°C), extension (1 minute at 72°C), and a final extension for 7 minutes at 72°C. Nucleotide sequences from amplicons of the BM and herp PCRs were similarly obtained and submitted for BLAST, and the likely host was determined by best match to the GenBank database. A blood meal was classified as mixed if two different species were identified in two separate PCRs from the same template and when chromatograms from each PCR demonstrated double-nucleotide peaks.

Sterile technique was used during preparation and handling of abdomens and for DNA extraction. Instruments were autoclaved and subjected to at least one hour of germicidal light prior to use. Negative controls were used during all steps (DNA extraction, PCRs, PCR product clean-up, and sequencing) to monitor for contamination. Positive controls of known-origin blood (16 species of birds, 8 species of mammals, and 2 species of amphibians) were processed and correctly identified with the above procedures. Species selected as controls were known to occur in the study region, and included American robin, American goldfinch (*Carduelis tristis*), brown-headed cowbird (*Molothrus ater*), blue jay (*Cyanocitta cristata*), European starling (*Sturnus vulgaris*), pied-billed grebe (*Podilymbus podiceps*), house sparrow (*Passer domesticus*), red-winged blackbird (*Agelaius phoeniceus*), wood thrush (*Hylocichla mustelina*), northern cardinal (*Cardinalis cardinalis*), song sparrow (*Melospiza melodia*), warbling vireo (*Vireo gilvus*), house finch (*Carpodacus mexicanus*), gray catbird (*Dumetella carolinensis*), orchard oriole (*Icterus spurius*), common grackle (*Quiscalus quiscula*), human (*Homo sapiens*), raccoon (*Procyon lotor*), domestic cat (*Felis catus*), white-footed mouse (*Peromyscus leucopus*), striped skunk (*Mephitis mephitis*), fox squirrel (*Sciurus niger*), eastern cottontail (*Sylvilagus floridanus*), Virginia opossum (*Didelphis*

virginiana), American toad (*Bufo americanus*), and American bullfrog (*Rana catesbeiana*). DNA was extracted from 5 μL of either whole blood or from blood clots to simulate a similar quantity of blood in a mosquito abdomen.

Bird survey. Local bird abundance was quantified at each site twice in 2005 and 2006 using survey point counts as previously described.[43] Briefly, five points were established in each residential site and eight in each natural site. We conducted all surveys between 0.5 hours before sunrise and 4.0 hours after sunrise (5:30 AM–10:00 AM) on days with no precipitation and wind speed less than 24 km/hour. Surveys were conducted between June and mid-July, corresponding with the peak avian breeding season in the region. In 2005, five of 11 residential and all four natural sites were surveyed. In 2006, all 21 residential and five natural sites were surveyed. Five-minute unlimited radius point counts were conducted at each survey point, distance to each observed bird was recorded, and density of each species and total avian density were estimated using Program Distance 5.0.[44]

In 2005, wild birds were captured using 36-mm mesh nylon mist-nets (Avinet, Inc., Dryden, NY) at each site six times at three-week intervals from mid-May to August and at five-week intervals in September and October. In 2006, the same rotation schedule was observed but eight additional residential sites were included. In 2007, 10 residential sites and three natural sites were sampled. Birds were identified to species, weighed, measured, aged, and sexed, and banded with numbered U.S. Fish and Wildlife Service leg bands (U.S. Department of Interior Bird Banding Laboratory, Federal Bird Banding Permit #06507). All fieldwork was carried out under appropriate collecting permits with approvals from the Institutional Animal Care and Use Committee at Michigan State University, Animal Use Form No. 2/03-152-00 and University of Illinois at Urbana-Champaign Animal Use Protocol No. 03034.

Calculation of host preference. Host feeding preferences for birds were calculated using the Manly resource selection design II index,[45] a ratio in which the use of resources is measured for individual mosquitoes and host availability is measured at the population level. Statistics were estimated using the adehabitat package in Program R.[46] The Manly selection ratio uses relative density as the measure of host availability (density-based selection ratio; $\hat{w}_i$) and was calculated for *Cx. pipiens*, *Cx. restuans*, and comparatively for *Cx. pipiens* from residential and natural sites as follows

$$\hat{w}_i = \frac{\text{proportion of utilized bird species } i}{\text{proportion of available bird species } i} = \frac{o_i}{\hat{\pi}_i}$$

A selection ratio of one represents the condition when mosquito feeding on host *i* is in equal proportion to estimated availability. A selection ratio greater than one represents overuse (i.e., more frequent feeding than expected by chance), and a ratio less than one represents underuse (i.e., less frequent feeding than expected by chance). The standard error of $\hat{w}_i$ was estimated as follows

$$\text{SE}\ (\hat{w}_i) = \sqrt{\left(\frac{o_i}{\hat{\pi}_i}\right)^2 * \left[\left(\frac{\text{var}\, o_i}{o_i^2}\right) + \left(\frac{\text{var}\hat{\pi}_i}{\hat{\pi}_i^2}\right)\right]}$$

The available resource units (i.e., birds by species) were estimated and the total number of census points (n = 145) was used to calculate the variance of $\hat{\pi}_i$ for a conservative measure of host availability (var $\hat{\pi}_i = \hat{\pi}_i * (1 - \hat{\pi}_i)$/sum (available hosts = 145)). Overuse or underuse for a host species was considered statistically significant when the 95% confidence interval (CI) did not overlap unity.

The selection index (w_i) was calculated for *Cx. pipiens* separated by trap type (light, gravid, aspirator), as well as for all individuals combined. Spatial comparison of host selection indices was conducted by calculating the selection index (w_i) for *Cx. pipiens* in residential sites and in natural sites. This analysis separated blood meal results and relative avian densities for residential and natural sites. When calculating feeding preferences, bird species that were not observed as blood meal hosts but were identified in bird surveys were given a blood meal value of one. Bird species observed as blood meal hosts but not identified in bird surveys were given a density equal to the lowest observed bird density, which was 0.0007 birds/hectare.

Amplification fraction. The amplification fraction for each bird species included in the analysis was modeled to integrate host selection ratios and host competence values and to provide a measure of importance for different bird species in the transmission of WNV[6] using a function modified by A. M. Kilpatrick (unpublished data). Competence values were obtained from Kilpatrick and others.[1] The amplification fraction (F_i) represents the estimated proportion of WNV infectious mosquitoes whose infection resulted from feeding on an individual of a certain bird species. It is estimated as the product of the relative avian abundance of host i (a_i), feeding preference of host i (P_i), and competence of host i (C_i), where P_i is a different measure of host selection compared with the Manly selection ratio described above. P_i incorporated the fraction of total avian and mammalian blood meals instead of just avian blood meals.

$$P_i = \frac{\text{fraction of total blood meals from host } i}{(\text{density of species } i \text{ /total avian density}) \ i} = \frac{B_i}{a_i}$$

The probability of each species becoming infected is proportional to the feeding preference, P_i, which changes the amplification fraction to $F_i = a_i \times P_i \times P_i \times C_i$. This expression reduces to $F_i = B_i \times P_i \times C_i$. The amplification fraction was calculated for host availability measures using relative avian densities (F_i). The amplification fraction assumes equal initial seroprevalence, and equal feeding preferences and competence values on adult and juvenile birds. Bird species without a host-competence index were assigned the average competence value for their respective family because more variation occurs between taxonomic families of birds than within them.[6] Because several species did not have a member of its respective family with a known competence value, the average competence for the respective avian order was assigned (Passeriform = 0.773).

RESULTS

Mosquito collections, species identification, and WNV infection rates. A total of 1,483 bloodfed mosquitoes were collected in 2005–2007, representing nine species (Table 1). Identification of *Culex* spp. by PCR resulted in an interpretable result in 91.8% of specimens, where *Cx. pipiens* was the most common *Culex* spp. mosquito (69.2%), *Cx. restuans* next common (22.4%), and the remainder (8.2%) were identified only as *Culex* spp. except

TABLE 1
Number and percentage of blood meals by host class for mosquitoes collected from suburban southwest Chicago, Illinois, 2005–2007

Taxon	Avian (%)	Mammal (%)	Amphibian (%)	Mixed			Total
				Avian–avian (%)	Mammal–mammal (%)	Avian–mammal (%)	
Culex pipiens	488 (80)	98 (16)		6 (1)	4 (1)	15 (2)	611
Cx. restuans	172 (81)	31 (15)	1 (< 1)	3 (1)		6 (3)	213
Cx. salinarius		1 (100)					1
Culex spp.	37 (71)	13 (25)	2 (4)				52
Anopheles quadrimaculatus		2 (100)					2
Culiseta inornata	1 (50)	1 (50)					2
Aedes vexans	15 (11)	111 (80)		1 (1)	9 (6)	3 (2)	139
Coquillettidia perturbans	1 (25)	2 (50)				1 (25)	4
Ochlerotatus triseriatus		5 (100)					5
Oc. trivittatus	1 (7)	13 (93)					14

for two individual *Cx. salinarius*. For all mosquito species of all genera, *Cx. pipiens* predominated in collections (57%), *Cx. restuans* was next in abundance (19%), and *Aedes vexans* (14%) was third in rank abundance. West Nile virus RNA was detected in 14 individual mosquitoes, including 12 *Cx. pipiens* and 2 unidentified *Culex* spp., yielding an infection rate of 18/1,000 in 2005, 7.4/1,000 in 2006, and 8.09/1,000 in 2007.

Blood meal analysis. The hosts of the blood meals of 1,043 (70%) of 1,483 mosquitoes were identified (Table 1). The proportion of reactions yielding amplicons and sequences decreased with increasing Sella score (R^2 = 0.91, degrees of freedom [df] = 4, P = 0.002). Blood meals from *Cx. pipiens* (comprising the bulk of the sample) were identified most commonly as avian (n = 488, 80%), and less commonly but not infrequently as mammalian (n = 98, 16%). A small number were of mixed source (n = 25, 4%, Table 1). Blood meals from *Cx. restuans* were also most commonly (85%) identified to an avian host. Blood meals from *Aedes*, *Anopheles*, and *Ochlerotatus* mosquitoes were primarily identified as mammal hosts (80%, 100%, and 93–100% of blood meals, respectively), but 11% of blood meals from *Ae. vexans* were of avian origin.

Results of BLAST searches of *cyt b* sequences showed that *Cx. pipiens* fed upon 25 avian species with the most common being American robin (48% of avian blood meals), house sparrow (15%), mourning dove (*Zenaida macroura*; 11%), and northern cardinal (8%, Table 2). Results from *Cx. restuans* were similar in the pattern of host feeding, but only 18 bird species were identified. Results showed that among the mammals fed upon by *Cx. pipiens*, the most common were humans (83% of mammalian blood meals), and raccoons (8%, Table 3). Of those blood meals identified as mammalian in *Cx. restuans*, most were from human (84%) but also included raccoon (8%), and eastern cottontail (5%). Mammalian blood meals from *Ae. vexans* were mostly white-tailed deer (*Odocoileus virginianus*; 48%), human (31%), and eastern cottontail (14%). No reptile blood meals were observed and the only amphibian hosts included one *Cx. restuans* and two *Culex* spp. mosquito that were found to have fed upon gray treefrogs (*Hyla versicolor*). Two percent of *Cx. pipiens* with mixed blood meals contained blood from birds and mammals.

Bird abundance. A total of 44 avian species were identified during point count surveys with a total density of 9.66 birds/hectare. House sparrows (4.25 birds/hectare), American robins (2.0 birds/hectare), mourning doves (0.63 birds/hectare), common grackles (0.56 birds/hectare), and European starlings (0.55 birds/hectare) were the most common species. A total of 1,407 birds of 57 species were captured in mist nets in 2005, 1,479 birds of 63 species in 2006, and 1,377 birds of 51 species in 2007. The most commonly captured species were the house sparrow (combined years n = 1,461), American robin (n = 693), American goldfinch (n = 292), gray catbird (n = 277), and northern cardinal (n = 230).

Host preference. The host selection ratio varied among the different avian species found to have been fed upon by *Cx. pipiens* (Table 4). Of the species for which the selection ratio was greater than 1 (indicating overuse relative to availability), the American robin ($\hat{w}_i$ = 2.81) was the only host for which the ratio was statistically significant (95% CI = 1.17–4.46) when calculated for individuals collected with aspirators. American robins were marginally significantly overused when all *Cx. pipiens* were combined (2.26; 95% CI = 0.98–3.54). Of the species for which the selection ratio was less than one (indicating underuse), the statistically significant species were common grackle ($\hat{w}_i$ = 0.06), red-winged blackbird (0.08), American goldfinch (0.09), monk parakeet (*Myiopsitta monachus*; 0.11), house sparrow (0.32), and European starling (0.39). *Culex restuans* feeding preferences displayed similar overall host selection, but no bird species were significantly overused and only three were significantly underused (American goldfinch, 0.22; common grackle, 0.24; and house sparrow, 0.33; Table 5).

Selection ratios for *Cx. pipiens* between residential and natural sites were significantly different (t = 3.67, df = 48, P < 0.001). Overuse was higher for several species in residential sites than natural sites, including mallard (39.9 ± 451, 0.2 ± 0.2; respectively; Table 6) and American robin (2.4 ± 0.4, 1.2 ± 0.2). Underuse was stronger for house sparrow (0.3 ± 0.04, 0.4 ± 0.2) and common grackle (0.1 ± 0.05, 0.3 ± 0.36) in residential sites than in natural sites.

The abundance of American robins captured using mist nets decreased as the summer season progressed, and the abundance of house sparrows in mist nets increased by comparison (Figure 1A). The proportion of *Cx. pipiens* feeding on American robins decreased as the season progressed (Figure 1B), and concomitantly there was an increase in feeding on other avian species, such as house sparrow, mourning dove, and northern cardinal (Figure 1B).

Epidemic curve. A total of 2,753 pools (53,230 individuals) of non-bloodfed *Culex* mosquitoes from 2005–2007 were tested for WNV RNA; 519 (18.9%) of the pools were positive and the peak infection rate (21.9/1,000 individuals) occurred in August. *Culex pipiens* infection with WNV and abundance peaked during the months of August and September, respectively

TABLE 2

Number and percentage of blood meals identified to avian or mixed avian hosts for mosquitoes collected in suburban southwest Chicago, Illinois, 2005–2007*

Host	Fraction of species I in avian community	Mosquito species			
		Culex pipiens (%)†	*Cx. restuans* (%)‡	*Culex* spp. (%)§	*Aedes vexans* (%)¶
American goldfinch	0.0214	1 (< 1)			
American kestrel	0.0001#	3 (1)			
American robin	0.2026	249 (48)	83 (45)	20 (54)	12 (60)
Black-capped chickadee	0.0020	2 (< 1)			
Blue jay	0.0030	14 (3)	2 (1)		
Brown-headed cowbird	0.0028		2 (1)		
Brown thrasher	0.0001#	1 (< 1)			
Cedar waxwing	0.0062	2 (< 1)			
Chicken	0.0001#				1 (5)
Chipping sparrow	0.0080	2 (< 1)			1 (5)
Common canary	0.0001#	1 (< 1)			1 (5)
Common grackle	0.0576	2 (< 1)	3 (2)	2 (5)	
Cooper's hawk	0.0001#	1 (< 1)			
Eastern bluebird	0.0002		1 (1)		
Eastern towhee	0.0002			1 (3)	
European starling	0.0567	12 (2)	11 (6)		
Field sparrow	0.0001#		1 (1)		
Gray catbird	0.0047	2 (< 1)	2 (1)	1 (3)	
House finch	0.0110	34 (7)	8 (4)	1 (3)	1 (5)
House sparrow	0.4400	76 (15)	31 (17)	3 (8)	1 (5)
House wren	0.0030	3 (1)			
Mallard	0.0091		1 (1)		
Mourning dove	0.0650	55 (11)	10 (5)	4 (11)	1 (5)
Northern cardinal	0.0144	43 (8)	19 (10)	4 (11)	
Northern flicker	0.0003	1 (< 1)			
Red-winged blackbird	0.0454	2 (< 1)	4 (2)		
Rock pigeon	0.0095	1 (< 1)			
Scarlet tanager	0.0002	3 (1)	3 (2)		1 (5)
Song sparrow	0.0017	2 (< 1)	1 (1)	1 (3)	
Swainson's thrush	0.0001#	2 (< 1)			
Swamp sparrow	0.0001#		1 (1)		
Turkey	0.0001#				1 (5)
Veery	0.0006	1 (< 1)	1 (1)		
Total avian-derived blood meals		515	184	37	20

* Avian relative abundance provided as the fraction of species i in the avian community (density of species i/total avian density).
† Includes 27 specimens from which double blood meals were identified.
‡ Includes 12 specimens from which double blood meals were identified.
§ *Culex* mosquitos that did not produce a polymerase chain reaction amplicon using the *Culex* spp. primer sets (*Cx. pipiens*, *Cx. restuans*, and *Cx. salinarius*).
¶ Includes 5 specimens from which double blood meals were identified.
# Species was not observed during surveys and was given lowest observed bird density for analysis.

(Figure 2A). Seventy-six human cases of WNV infection were reported within 5 km of the field sites in 2005–2007, and peak date of onset occurred in August (Figure 2B). When human exposure to WNV peaked, there was a high percentage of bird feeding by *Cx. pipiens* and a smaller fraction of feeding on mammals, including humans (Figure 2B).

There was statistically significant temporal variation in the frequency of bird and mammal feeding by *Cx. pipiens* (2×5 contingency table, $\chi^2 = 24.05$, df = 4, $P < 0.0001$) (Figure 2B). Mammal feeding was proportionately higher in June and September, deviating strongly from expectation by chance alone (+24.6% and +51.6% deviation, respectively), and was proportionately lower in July, August, and September, also deviating negatively from chance alone (–16%, –18.1%, and –11.8% deviation, respectively). The variation in bird and human feeding by month was also significant ($\chi^2 = 20.2$, df = 4, $P = 0.0005$) with similar higher feeding on humans in May and September (+37% and +88.5% deviation, respectively).

Amplification fraction. Species-specific amplification fractions were estimated by incorporating the abundance of birds of different species, and their known reservoir competence, into the selection. Results indicate that American robins accounted for 35% of the WNV infections in *Cx. pipiens*, blue jays accounted for 17%, and house finches accounted for 15%, American kestrel (*Falco sparverius*) accounted for 11%, and northern cardinal accounted for 5% (Figure 3). Together,

TABLE 3

Number and percentage of blood meals identified to mammal or mixed mammal hosts for mosquitoes collected in suburban southwest Chicago, Illinois, 2005–2007

Host	Mosquito species			
	Culex pipiens (%)*	*Cx. restuans* (%)†	*Culex* spp. (%)‡	*Aedes vexans* (%)§
Cat	2 (2)			1 (1)
Domestic dog	1 (1)			2 (2)
Human	100 (83)	31 (84)	8 (62)	41 (31)
Opossum	3 (2)			2 (2)
Eastern cottontail		2 (5)		19 (14)
Raccoon	10 (8)	3 (8)	1 (8)	3 (2)
Gray squirrel	3 (2)	1 (3)	1 (8)	
White-tailed deer	2 (2)		3 (23)	64 (48)
Total mammal-derived blood meals	121	37	13	132

* Includes 23 specimens from which double blood meals were identified.
† Includes 6 specimens from which double blood meals were identified.
‡ *Culex* mosquitos that did not produce a polymerase chain reaction amplicon using the *Culex* spp. primer sets (*Cx. pipiens*, *Cx. restuans*, and *Cx. salinarius*).
§ Includes 21 specimens from which double blood meals were identified.

TABLE 4
Host-feeding preferences of *Culex pipiens* collected in suburban southwest Chicago, 2005–2007, in total and broken down by trap type

Host	*Cx. pipiens* feeding preference (standard error)			
	Total $\hat{w}_i$	Light trap $\hat{w}_i$	Gravid trap $\hat{w}_i$	Aspirator $\hat{w}_i$
American kestrel*	75.51 (735.08)	161.10 (1,573.70)	75.65 (737.09)	73.62 (719.16)
Swainson's thrush*	50.34 (490.49)	161.10 (1,573.70)	37.83 (369.51)	73.62 (719.16)
Scarlet tanager	34.09 (223.39)	72.72 (480.25)	51.22 (335.70)	33.23 (219.47)
Brown thrasher*	24.17 (245.89)	161.10 (1,573.70)	37.83 (369.51)	73.62 (719.16)
Common canary*	25.17 (245.89)	161.10 (1,573.70)	37.83 (369.51)	73.62 (719.16)
Cooper's hawk*	25.17 (245.89)	161.10 (1,573.70)	37.83 (369.51)	73.62 (719.16)
Ring-necked pheasant†	25.17 (245.89)	161.10 (1,573.70)	37.83 (369.51)	73.62 (719.16)
Hairy woodpecker†	12.96 (91.29)	82.95 (584.27)	19.48 (137.19)	37.91 (267.00)
Eastern towhee†	11.85 (79.86)	75.82 (511.09)	17.80 (120.01)	34.65 (233.56)
Eastern bluebird†	10.75 (69.06)	68.77 (441.99)	16.15 (103.78)	31.43 (201.99)
Blue jay	8.44 (12.88)	3.86 (6.97)	10.88 (16.63)	1.76 (3.18)
Willow flycatcher†	7.17 (37.87)	45.89 (242.37)	10.78 (56.91)	20.97 (110.76)
Common yellowthroat†	6.95 (36.12)	44.45 (231.19)	10.44 (54.29)	20.31 (105.65)
House finch	5.69 (4.58)	5.35 (4.82)	6.03 (4.90)	2.45 (2.21)
Northern cardinal	5.50 (3.87)	3.27 (2.77)	6.72 (4.75)	1.50 (1.27)
Northern flicker†	5.40 (24.87)	34.54 (159.19)	8.11 (37.38)	15.78 (72.75)
Killdeer†	4.68 (20.14)	29.93 (128.90)	7.03 (30.27)	13.68 (58.90)
Eurasian collared-dove†	4.65 (19.95)	29.74 (127.68)	6.98 (29.98)	13.59 (58.35)
Eastern kingbird†	4.04 (16.25)	25.87 (104.01)	6.08 (24.42)	11.82 (47.53)
Great-crested flycatcher†	3.64 (13.91)	23.27 (89.00)	5.46 (20.90)	10.63 (40.67)
Warbling vireo†	3.54 (13.35)	22.63 (85.43)	5.31 (20.06)	10.34 (39.04)
White-breasted nuthatch†	3.29 (12.00)	21.04 (76.78)	4.94 (18.03)	9.61 (35.09)
Veery	3.12 (11.13)	19.98 (71.24)	4.69 (16.73)	9.13 (32.56)
Indigo bunting†	3.09 (10.96)	19.77 (70.15)	4.64 (16.47)	9.04 (32.06)
Eastern wood-pewee†	2.67 (8.84)	17.06 (56.57)	4.01 (13.28)	7.80 (25.85)
Red-eyed vireo†	2.49 (7.99)	15.91 (51.11)	3.74 (12.00)	7.27 (23.36)
Yellow warbler†	2.27 (7.02)	14.55 (44.90)	3.42 (10.54)	6.65 (20.52)
American robin	2.26 (0.39)	0.64 (0.21)	1.80 (0.32)	2.81 (0.50)‡
Song sparrow	2.11 (4.45)	6.75 (15.02)	3.17 (6.69)	3.09 (6.87)
Barn swallow†	1.94 (5.59)	12.44 (35.81)	2.92 (8.41)	5.68 (16.36)
Black-capped chickadee	1.86 (3.71)	5.95 (12.61)	1.49 (2.96)	2.72 (5.76)
House wren	1.82 (2.95)	3.89 (7.04)	0.91 (1.65)	1.78 (3.22)
Blue-gray gnatcher†	1.81 (5.05)	11.58 (32.31)	2.72 (7.59)	5.29 (14.77)
Mourning dove	1.55 (0.53)	1.27 (0.61)	1.74 (0.61)	0.58 (0.28)
Baltimore oriole†	1.12 (2.55)	7.15 (16.29)	1.68 (3.83)	3.27 (7.45)
Gray catbird	0.78 (1.09)	2.49 (3.90)	1.17 (1.63)	1.14 (1.78)
Brown-headed cowbird†	0.65 (1.21)	4.19 (7.77)	0.98 (1.82)	1.91 (3.55)
Cedar waxwing	0.59 (0.75)	1.89 (2.74)	0.89 (1.12)	0.86 (1.25)
American crow†	0.54 (0.93)	3.45 (5.98)	0.81 (1.41)	1.58 (2.73)
Downy woodpecker†	0.53 (0.91)	3.39 (5.85)	0.80 (1.37)	1.55 (2.68)
Chipping sparrow	0.46 (0.54)	1.48 (2.01)	0.69 (0.81)	0.67 (0.92)
European starling	0.39 (0.17)‡	0.21 (0.22)‡	0.39 (0.19)	0.28 (0.19)‡
House sparrow	0.32 (0.05)‡	0.24 (0.08)‡	0.34 (0.05)‡	0.16 (0.05)‡
Mallard†	0.20 (0.27)	1.29 (1.71)	0.30 (0.40)	0.59 (0.78)
Rock pigeon	0.19 (0.25)	1.24 (1.62)	0.29 (0.38)	0.57 (0.74)
Monk parakeet†	0.11 (0.13)‡	0.70 (0.82)	0.16 (0.19)‡	0.32 (0.38)
American goldfinch	0.09 (0.10)‡	0.55 (0.63)	0.13 (0.15)‡	0.25 (0.29)
Red-winged blackbird	0.08 (0.07)‡	0.26 (0.28)	0.12 (0.10)‡	0.12 (0.13)‡
Common grackle	0.06 (0.05)‡	0.20 (0.22)‡	0.10 (0.07)‡	0.09 (0.10)‡

* Species not recorded during avian surveys (given value of the lowest observed bird density).
† Species not observed as a host in the blood meal analysis (given a value of 1).
‡ Statistically significant non-rando m host selection at $P < 0.05$.

these five species accounted for 82% of the WNV-infectious *Cx. pipiens*.

DISCUSSION

The amplification of WNV infection in mosquitoes and bridging of transmission to humans resulting in human infection and disease are intertwined processes whose intensity depends upon the interaction of mosquito vector and vertebrate host populations. On the basis of longitudinal population analyses in three consecutive seasons in the Chicago study region, we have concluded that *Cx. pipiens* functions as both the epizootic and epidemic (i.e., bridge) vector,[47,48] and that the annual flush of nestling and fledgling birds is a causative factor in seasonal amplification.[35] In the present study, two primary questions were considered: first, what birds (or other animals) are serving as the blood hosts of this mosquito vector, and second, does variation in blood host use influence amplification and bridging transmission?

Our results document extensive feeding of *Cx. pipiens* on humans. This finding is especially striking because this species is thought to rely primarily upon avian hosts for blood. Yet, the results of this study do not support the hypothesis that a shift in *Cx. pipiens* feeding from birds to mammals correlates with elevated human risk of infection, a phenomenon observed elsewhere[7] and attributed to a seasonal decline in

TABLE 5
Host-feeding preferences of *Culex restuans* collected in suburban southwest Chicago, Illinois, 2005–2007

Host	*Cx. restuans* feeding preference $\hat{w}_i$ (standard error)
Scarlet tanager	87.00 (570.02)
Field sparrow*	64.25 (627.45)
Ring-necked pheasant†	64.25 (627.45)
Swamp sparrow*	64.25 (627.45)
Hairy woodpecker†	33.08 (232.96)
Eastern towhee†	30.24 (203.78)
Eastern bluebird	27.43 (176.23)
Willow flycatcher†	18.30 (96.64)
Common yellowthroat†	17.73 (92.18)
Northern flicker†	13.78 (63.47)
Killdeer†	11.94 (51.39)
Eurasian collared-dove†	11.86 (50.91)
Eastern kingbird†	10.32 (41.47)
Great-crested flycatcher†	9.28 (35.49)
Warbling vireo†	9.02 (34.06)
White-breasted nuthatch†	8.39 (30.61)
Veery	7.97 (28.41)
Indigo bunting†	7.89 (27.97)
Eastern wood-pewee†	6.80 (22.55)
Red-eyed vireo†	6.35 (20.38)
Northern cardinal	6.20 (4.48)
Yellow warbler†	5.80 (17.90)
Barn swallow†	4.96 (14.28)
Blue-gray gnatcher†	4.62 (12.88)
House finch	3.42 (2.94)
Brown-headed cowbird	3.34 (5.73)
Blue jay	3.08 (5.11)
Baltimore oriole†	2.85 (6.50)
Song sparrow	2.69 (5.99)
Black-capped chickadee†	2.37 (5.03)
Gray catbird	1.99 (2.78)
American robin	1.92 (0.36)
House wren†	1.55 (2.81)
American crow†	1.37 (2.39)
Downy woodpecker†	1.35 (2.33)
European starling	0.91 (0.41)
Mourning dove	0.80 (0.34)
Cedar waxwing†	0.75 (1.09)
Chipping sparrow†	0.59 (0.80)
Mallard	0.51 (0.68)
Rock pigeon†	0.49 (0.65)
Red-winged blackbird	0.41 (0.26)
House sparrow	0.33 (0.06)‡
Monk parakeet†	0.28 (0.33)
Common grackle	0.24 (0.16)‡
American goldfinch†	0.22 (0.25)‡

* Species not recorded during avian surveys (given value of the lowest observed bird density).
† Species not observed as a host (given a value of 1).
‡ Statistically significant non-random host selection at $P < 0.05$.

TABLE 6
Host-selection ratios for *Culex pipiens* collected in residential and natural study sites in suburban southwest Chicago, Illinois, 2005–2007

Host	*Cx. pipiens* feeding preference (standard error)	
	Residential sites $\hat{w}_i$	Natural sites $\hat{w}_i$
American kestrel*†‡	524.97 (12,372.68)	48.81 (326.50)
Scarlet tanager*†	524.97 (12,372.68)	8.75 (26.01)
Swainson's thrush*†‡	349.98 (8,249.70)	48.81 (326.50)
Brown thrasher†‡	174.99 (4,126.71)	48.81 (326.50)
Cooper's hawk*†‡	174.99 (4,126.71)	48.81 (326.50)
Eastern bluebird*†§	174.99 (4,126.71)	8.27 (24.00)
Eastern towhee*†	174.99 (4,126.71)	9.12 (27.63)
Yellow-shafted flicker*	174.99 (4,126.71)	48.81 (326.50)
Ring-necked pheasant*§	174.99 (4,126.71)	22.50 (103.54)
Swamp sparrow§	174.99 (4,126.71)	48.81 (326.50)
Veery*†§	174.99 (4,126.71)	2.40 (4.27)
Warbling vireo*†§	174.99 (4,126.71)	2.72 (5.04)
Willow flycatcher*†§	174.99 (4,126.71)	5.52 (13.46)
Yellow warbler*§	124.45 (2,475.79)	1.78 (2.87)
Mallard*§	39.92 (450.96)	0.16 (0.17)¶
Barn swallow*§	31.45 (315.66)	1.59 (2.48)
Common yellowthroat*§	27.64 (260.26)	7.06 (19.10)
White-breasted nuthatch*§	26.95 (250.64)	2.87 (5.42)
Hairy woodpecker*§	20.32 (164.36)	25.95 (127.78)
Killdeer*§	20.04 (160.99)	4.65 (10.56)
Eastern kingbird*§	17.15 (127.61)	4.03 (8.65)
Indigo bunting*§	16.96 (125.53)	2.89 (5.46)
Great-crested flycatcher*§	14.47 (99.05)	3.70 (7.67)
Blue jay	10.67 (18.16)	2.75 (3.60)
Baltimore oriole*§	10.11 (58l.15)	0.96 (1.31)
Blue-gray gnatcher*§	7.20 (35.16)	1.84 (2.99)
Northern cardinal	5.19 (3.69)	4.62 (3.45)
House finch	4.95 (3.73)	11.90 (18.13)
Eastern wood-pewee*§	4.87 (19.73)	4.35 (9.63)
Song sparrow*	4.76 (13.48)	1.42 (2.14)
Eurasian collared-dove*†§	4.48 (17.49)	48.81 (326.50)
Black-capped chickadee*	3.96 (10.32)	1.31 (1.93)
Red-eyed vireo*§	3.42 (11.77)	6.41 (16.64)
American robin	2.42 (0.44)	1.20 (0.21)
House wren	1.46 (2.44)	2.40 (4.25)
Mourning dove	1.31 (0.43)	2.57 (1.31)
Gray catbird§	1.04 (2.15)	0.94 (0.89)
Brown-headed cowbird*§	0.98 (1.98)	1.42 (2.14)
Cedar waxwing*	0.88 (1.22)	0.64 (0.80)
Red-winged blackbird*	0.79 (1.04)	0.03 (0.04)
Downy woodpecker*§	0.70 (1.26)	1.50 (2.30)¶
Chipping sparrow*	0.69 (0.86)	0.50 (0.61)
American crow*§	0.55 (0.90)	8.73 (25.92)
European starling	0.38 (0.18)¶	0.29 (0.19)¶
House sparrow	0.30 (0.04)¶	0.44 (0.20)
Rock pigeon*	0.19 (0.24)¶	48.81 (326.50)
American goldfinch§	0.13 (0.15)¶	0.19 (0.20)¶
Monk parakeet*§	0.12 (0.14)¶	0.69 (0.87)
Common grackle*	0.07 (0.05)¶	0.31 (0.36)

* Species not observed as a host in natural sites (given a value of 1).
† Species not recorded during avian surveys (given value of lowest observed bird density).
‡ Species not recorded during avian surveys (given value of lowest observed bird density).
§ Species not observed as a host in residential sites (given a value of 1).
¶ Statistically significant non-random host selection at $P < 0.05$.

bird availability (as opposed to some physiologic change affecting mosquito feeding patterns).[1] The initial high rate of feeding on American robin, also reported in other studies,[6,7] was followed by a gradual decrease in feeding on American robin (also reported in other studies[7,40]) supporting an interpretation of a broadly opportunistic strategy of *Cx. pipiens* where host availability of preferred hosts dictates the apparent feeding patterns reflected by blood meal analysis. This interpretation is supported by the similarity in feeding patterns exhibited by *Cx. restuans* (Tables 2 and 3). However, the decrease in feeding on robins was not accompanied by an increase in feeding on humans and other mammals, but rather by an increase in feeding on other bird species, in particular house sparrows, mourning dove, and northern cardinal (Figure 1B and 2B). Furthermore, the trend at the beginning and near the end of the season (June and September) was for a relatively higher frequency of feeding on mammals, but during the amplification events and dates of onset of human cases, frequency of feeding on mammals was actually significantly lower than the full season average and birds were the more frequent hosts. From these patterns, we conclude that the risk of human infection (i.e., bridging transmission) relates not to a shift in the bird: mammal ratio of feeding frequency, but rather to the amplification process itself. As the WNV infection rate in the *Cx. pipiens* population increases in July and August, some marginal virus transmission to humans occurs because of the fraction of the *Cx. pipiens* population

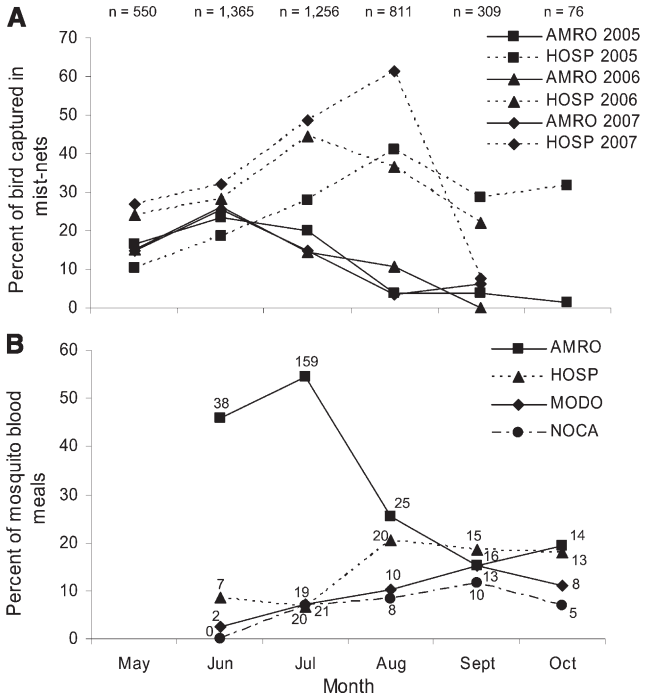

FIGURE 1. **A**, Percent of American robin and house sparrow captured in mist-nets in southwest suburban Chicago, Illinois, 2005–2007. **B**, Percent of *Culex pipiens* blood meals derived from American robin, house sparrow, mourning dove, and northern cardinal. Total sample size of birds captured in mist-nets for combined year are indicated in **A** and raw numbers are indicated for sample size in **B**.

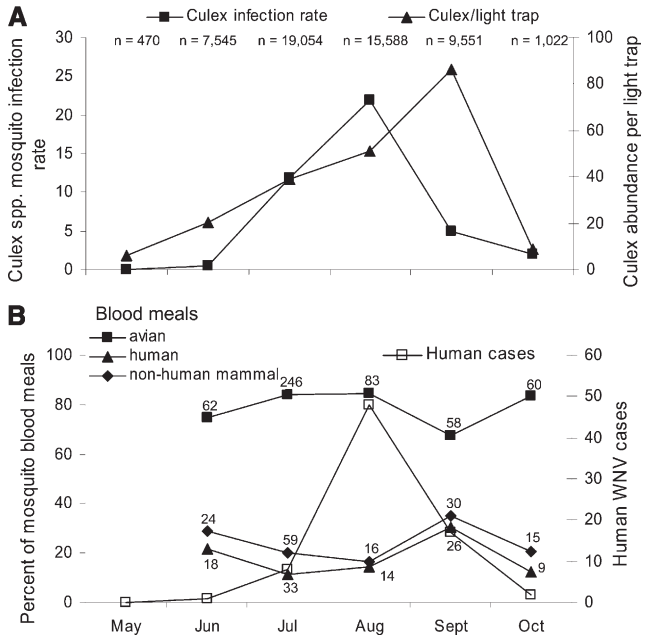

FIGURE 2. **A**, Temporal patterns of *Culex* spp. mosquito infection rate and abundance (*Culex* spp. per light trap) in southwest suburban Chicago, Illinois, 2005–2007. **B**, Percent of *Cx. pipiens* blood meals derived from birds, humans, and non-human mammals and human West Nile virus case date of onset in the same study sites during the same years. Raw numbers in **A** indicate total numbers of *Culex* spp. mosquitoes captured and tested. Mosquitoes captured in light traps are a subset of the total. Raw numbers in **B** indicate raw number of blood meals.

that during that time period bites humans. Given the sharp coincidence of amplification and dates of onset of human infection, interventions directed at processes promoting amplification seem paramount, especially those initiated immediately prior to and during generation of the epizootic curve.

Although host selection by *Cx. pipiens* and other *Culex* spp. was influenced by host availability, our analyses indicated that certain common species of birds were overused (American robin) or underused (common grackle, starling, house sparrow) relative to their abundance. The null hypothesis that *Cx. pipiens* selects avian blood hosts on the sole basis of relative availability was rejected. The behavioral and ecologic explanations for these patterns are unknown, but could relate to relative tendency of birds to aggregate into roosts, the position and structure of nests, the host-defensive behavior of nestlings and fledglings, and olfaction cues. Our results indicate that overuse of American robins, identified as a superspreader species because of its high reservoir competence, is not the sole determinant of intensification of WNV transmission during amplification. Simultaneous underuse of certain common species that have rather poor predicted reservoir competences (starlings and red-winged blackbirds in particular) similarly contributes to WNV amplification. This study indicates the house sparrow plays a minor role in amplification events although other studies have indicted this species as an important host for both St. Louis encephalitis virus[49] and WNV virus.[50] Here, there was less feeding on house sparrows than expected on the basis of their abundance, resulting in a lower amplification fraction. In contrast, the less common house finch was predicted to be an important amplifying host (Table 5 and Figure 3). It is also important to note that competence values used to calculate the amplification fraction are an aggregate of 11 primary research papers in which birds were experimentally infected.[1] Many avian species have yet to be the subject of such experimental studies, and many published competence values are based on small samples sizes of infected birds (e.g., American robin, n = 2). This limitation emphasizes the need for more experimental studies to complement field studies.

The presence of alternate avian hosts, after feeding on robins wanes, suggests that those birds might actually serve a zooprophylaxis function, as has been suggested for non-human mammal hosts (dogs, horses, and deer) in diverting infectious mosquitoes away from humans.[40,51] The same could be true for abundant avian hosts, especially ones with poor reservoir competence, which would serve to dampen transmission. This observation has important implications in the measure of host community competence and in understanding the so-called dilution effect.[43,52] Furthermore, it would offer an explanation for why WNV infection in *Cx. pipiens* decreases in August when temperatures are still supportive of transmission and birds remain generally available.

The differences in host selection in natural and residential sites within our relatively small study region demonstrate the importance of fine-scale variation in host availability. Stronger overuse for mallards and robins in residential sites than in natural sites indicates that *Cx. pipiens* host preference is context specific. The differences in these selection ratios are predicted to have dramatic effects on interpreting the contribution of birds to WNV transmission, and this finding might also provide a mechanism for high rates of transmission in suburban

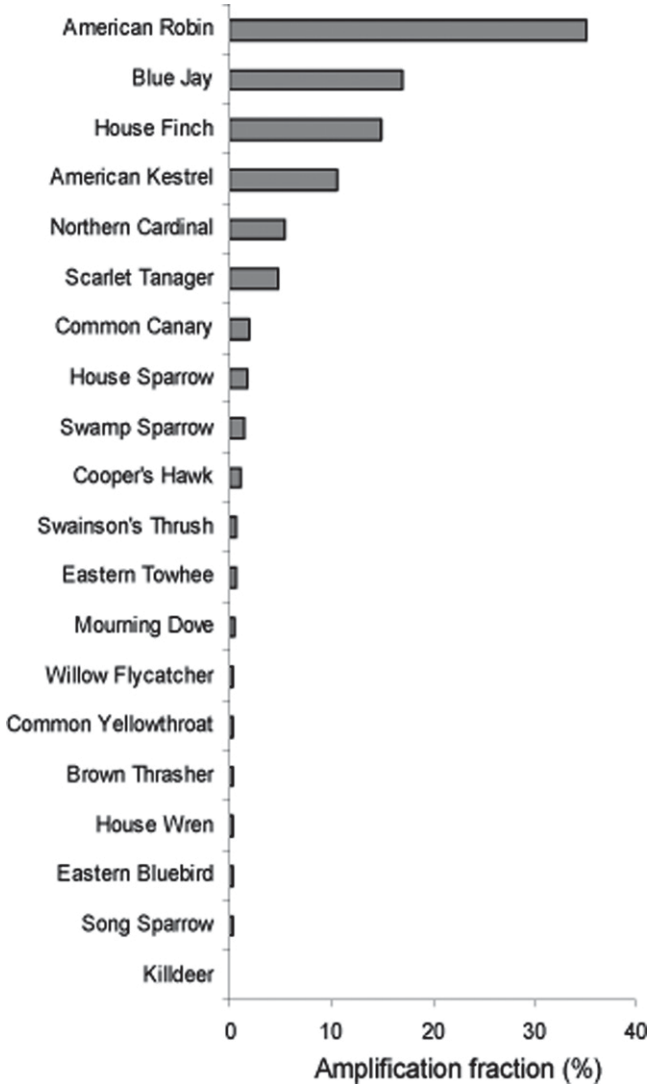

FIGURE 3. Amplification fraction (F_i) representing the fraction of West Nile virus infectious mosquitoes resulting from feeding on that avian host[1,6] (Kilpatrick AM, unpublished data). Species with amplification fractions < 0.02 are not graphed and include eastern kingbird, black-capped chickadee, great-creasted flycatcher, warbling vireo, white-breasted nuthatch, eastern wood-pewee, red-eyed vireo, hairy woodpecker, yellow warbler, barn swallow, blue-gray gnatcher, veery, indigo bunting, American crow, cedar waxwing, chipping sparrow, European starling, northern flicker, baltimore oriole, Eurasian collard-dove, common grackle, gray catbird, American goldfinch, mallard, downy woodpecker, red-winged blackbird, rock pigeon, monk parakeet, ring-necked pheasant, and brown-headed cowbird.

environments, where residential and natural areas are in close proximity.

The percent of avian feeding by *Cx. pipiens* varies considerably by region (35–96%).[7,16,40,53,54] We documented an unusually high rate of human feeding by *Cx. pipiens* (16% of total blood meals). Recent evidence confirms that a portion of this rate variation is genetically based. Specifically, population substructuring appears to exist in the *Cx. pipiens* complex, with an increased affinity for human hosts hypothesized for the *Cx. pipiens molestus* form.[55–58] A second hypothesis for variation in human feeding is host availability. Samples from residential areas such as alleys and residential backyards yielded 79% of the bloodfed *Cx. pipiens* in our study. Other recent blood meal analysis studies with *Cx. pipiens* were done within urban areas, but actual sample sites were parks, uninhabited military forts, sewage treatment plants, golf courses, cemeteries, woodlots, and public thoroughfares.[16,40,53,54] Collecting bloodfed mosquitoes in immediate proximity to human habitation could explain our finding of a high frequency of human feeding by *Culex* mosquitoes, a phenomenon supported by previous studies.[54,59,60]

We found that 4% of *Cx. pipiens* blood meals contained mixed sequences (more than one host species), which concords with a range of 3–8% reported in previous studies.[7,16,59,61] The direct sequencing method used in this study and others may overlook cryptic blood meals because of the amplification of the predominant blood meal, especially for species such as starlings with high anti-mosquito behavior,[62] which would be negatively biased. The overuse of robins by *Cx. pipiens* collected by aspirators and underuse of robins by *Cx. pipiens* collected in light traps suggests that host-seeking individuals with partial blood meals collected by light traps were less likely to contain robin blood than were those with a complete blood meal collected by aspirators. This finding is supported by the lower observed sella score, indicating a more complete, less digested blood meal, from aspirators, compared with those collected in light and gravid traps (3.2, 3.6, 4.1, respectively). Collectively, this supports the hypothesis that robins have relatively low anti-mosquito behavior, which enables *Cx. pipiens* to complete a blood meal.

Concurrent host-feeding and virus detection data for *Cx. pipiens* previously published[47] and the magnitude of bird feeding reinforces the role of *Cx. pipiens* as the primary enzootic vector in the study region. *Culex restuans* could also contribute to early-season enzootic transmission, but based on this sampling effort and molecular species identification, this species appears less important (*Cx. pipiens* are 3.1 times more abundant). The presence of a virus-positive *Cx. pipiens* with a human-derived blood meal demonstrates that this species is capable of being a bridge vector for epizootic transmission.[47] Host-feeding results for *Ae. vexans* showed more bird-feeding than we typically expect from this mammalophilic mosquito species.[16,53,63] Identification of 14% of *Ae. vexans* feeding on birds supports a recent study suggesting the potential role of this mosquito as a bridge vector.[48,64] During 2005–2007, this study collected 784 pools (11,701 individuals) of *Aedes vexans* but only 4 pools were positive for WNV RNA (infection rate of 0.34/1,000). Given the substantially lower infection rate compared with *Culex* spp. (infection rate of 11.03/1,000; 519 positive pools of 2,753), and the occurrence of a not insubstantial number of human cases at times and in places when *Ae. vexans* were absent, or present but uninfected, the role of *Ae. vexans* as a primary bridge vector seems unlikely. Indeed, relatively rare virus infection in *Ae. vexans* may reflect occasional feeding on infected robins but not significant vectorial capacity for WNV.

In this report, we present a modified expression for the amplification fraction (A. M. Kilpatrick, unpublished data), a measure of the avian species-specific contribution to WNV transmission. The finding that 66% (F_i) of WNV infectious *Cx. pipiens* became infected from feeding on viremic American robins (35%), blue jays (17%), and house finches (15%) combined implicates these common urban birds as the major contributors to epizootic transmission of WNV, in particular the force of infection.[65] The finding that these common urban birds may be responsible for WNV amplification provides a mechanism for this *Culex* spp. mosquito-driven disease system

to rapidly adapt to diverse bird communities during invasion and establishment across North America.

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