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WEST NILE VIRUS TRANSMISSION ECOLOGY:
VECTOR-HOST INTERACTIONS

presented by

GABRIEL LEE HAMER

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Edward D. Walker
Major Professor's Signature

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TITLE: WEST NILE VIRUS TRANSMISSION ECOLOGY: VECTOR-HOST INTERACTIONS

By

Gabriel Lee Hamer

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ABSTRACT

WEST NILE VIRUS TRANSMISSION ECOLOGY: VECTOR-HOST INTERACTIONS

By

Gabriel Lee Hamer

Since the introduction of West Nile virus (WNV) to New York in 1999, the virus spread rapidly and became established in much of North America. This arbovirus is maintained in an urban enzootic cycle among *Culex* spp. mosquitoes and birds with occasional spillover to humans. The mechanisms for WNV amplification remain poorly understood and this dissertation investigates several hypotheses associated with transmission among vectors and hosts leading to rapid amplification.

Mosquitoes and birds were collected in southwest suburban Chicago from May to October in 2005-2007. WNV infection in *Culex spp.* mosquitoes, principally *Culex pipiens* and tested using quantitative polymerase chain reaction (PCR), peaked in early August in all three years. The proportion of hatch-year birds (juvenile) captured in mist-nets increased as the season progressed and hatch year bird seroprevalence of WNV antibodies, was 18.5% (100/540) in 2005 and 2.8% (14/493) in 2006. Virus was detected in 11 of 998 bird sera in 2005 and 3 of 1285 in 2006; 11 of the 14 virus positive birds were hatch-year. Significant cross-correlations among these factors indicate a key role for hatch-year birds in the amplification of epizootic transmission of WNV, and in increasing human infection risk by facilitating local viral amplification.

To further explore associations between the vector and host, I conducted a blood meal analysis using PCR and DNA sequencing techniques on bloodfed mosquitoes. Results showed that *Cx. pipiens* fed predominantly (83%) on birds with a high diversity

of species utilized as hosts (25 species). *Cx. pipiens* also fed substantially on mammals (19%; 7 species; humans representing 16%). During a WNV epidemic in 2005, WNV RNA was detected in the head and thorax of a bloodfed *Cx. pipiens* and the blood meal was identified as human. These results fulfill a criterion for incrimination of *Cx. pipiens* as a bridge vector. American robins were marginally overutilized and common grackle (*Quiscalus quiscula*), house sparrow, and European starling (*Sturnus vulgaris*) were underutilized based upon relative abundance measures. West Nile virus transmission intensified in late-July, at times when American robins were heavily fed upon, and then declined when robin abundance declined, 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. Predictions were that—ca. 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*).

Finally, I explored landscape-level patterns of WNV infection in *Culex* spp. mosquitoes for a 3-year database (2004-2006) in the state of Illinois to identify landscape features that predict mosquito infection. I observed variability in the associations among three years but the most parsimonious multivariate model explaining *Culex* spp. mosquito infection rate included elevation and precipitation in 2004, precipitation in 2005, and percent white people and vegetation in 2006. A negative relationship between precipitation and *Culex* infection emerged as the most consistent pattern explaining more variation than any other independent variable. Further multivariate tests reveal a 3-4 week time lag between a lack of rain and an increase in *Culex* infection.

This dissertation is dedicated to my family for their support of all my pursuits.

ACKNOWLEDGEMENTS

I am indebted to my graduate advisor, Dr. Edward Walker, for providing the leadership for my dissertation and for exposing me to a professional model of how to manage a productive academic position, to which I inspire. I also thank other members of my graduate committee: Dr. Jean Tsao for endless conversations about all types of vector-borne diseases; Dr. Dan Hayes for mathematical expertise and the exchange of hunting and fishing stories; and Dr. Uriel Kitron for his 'gut feelings' which I have come to trust.

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I was fortunate to deploy an army of field and lab technicians, including Lisa Abernathy, Giusi Amore, Rachael Atkins, Melanie Bender, Seth Dallmann, Diane Gohde, Mike Goshorn, Jonathon McClain, Mike Neville, Beth Pultorak, Eric Secker, Jennifer Sidge, Timothy Thompson, and Amy Wechsler. I especially thank Tim, Diane, and Jonathon for their exceptional work ethic and independence throughout the duration of this research. I greatly appreciated the guidance and comic relief in the lab from Dr.

Mike Kaufman and Blair Bullard. Dr. Lilian Stark and the Florida Department of Health and Dr. Bill Reisen from the UC Davis Center for Vector-borne Disease Research provided positive control chicken serum, Laura Mosher and the Michigan Department of Community Health provided the positive control NY99 strain of WNV, and the CDC Division of Vector Bourne Infectious Diseases supplied the 4G2 and 6B6C-1 antibodies. I appreciate the assistance from Dr. Goudarz Molaei while establishing the blood meal analysis protocol and from Dr. Marm Kilpatrick for consultation during the host selection analysis. Dr. Linn Haramis and the Illinois Department of Public Health provided access to the Illinois mosquito data. Bill Brown and Jane Messina assisted with gathering and organizing the mosquito infection and weather data. I also thank the numerous municipalities and homeowners that allowed us to conduct our research in suburban Chicago, especially the Village of Oak Lawn for providing field laboratory facilities.

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laboratory diagnostics, and reviewed many first drafts during my program. Sarah also holds our project's record for the number of bloodfed mosquitoes captured in one aspirator cup (n=63!).

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CHAPTER 1

Rapid amplification of West Nile virus: the role of hatch year birds

Abstract

Epizootic transmission of West Nile virus (WNV) often intensifies rapidly leading to increasing risk of human infection, but the processes underlying amplification remain poorly understood. We quantified epizootic WNV transmission in communities of mosquitoes and birds in the Chicago, Illinois (USA) region during 2005 and 2006. Using quantitative PCR methods, we detected West Nile virus in 227 of 1195 mosquito pools (19%) in 2005 and 205 of 1,685 (12%) in 2006; nearly all were *Culex pipiens*. In both years, mosquito infection rates increased rapidly in the second half of July to a peak of 59/1,000 mosquitoes in 2005 and 33/1,000 in 2006, and then declined slowly. Viral RNA was detected in 11 of 998 bird sera (1.1%) in 2005 and 3 of 1,285 bird sera (<1%) in 2006; 11 of the 14 virus positive birds were hatch year birds. Of 540 hatch year birds, 100 (18.5 %) were seropositive in 2005, but only 2.8% (14 of n=493) tested seropositive in 2006 for WNV antibodies using inhibition ELISA. We observed significant time series cross-correlations between mosquito infection rate and: proportion of virus positive birds, proportion of hatch year birds captured in mist-nets (significant in 2006 only), seroprevalence of hatch year birds, and number of human cases in both seasons. These associations, coupled with the predominance of WNV infection and seropositivity in hatch year birds, indicate a key role for hatch year birds in the amplification of epizootic

transmission of WNV, and in increasing human infection risk by facilitating local viral amplification.

Introduction

Since the appearance of West Nile virus (WNV) in New York in 1999 (Lanciotti et al. 1999), the virus has spread rapidly westward across North America, and southward into the Caribbean Basin, Mexico, and Central and South America (Komar and Clark 2006). In the seven years since its establishment, WNV has been responsible for over 20,000 human cases of disease and nearly 1,000 human fatalities in the United States (CDC 2007). Illinois led the nation in human cases (884) and deaths (64) in 2002, was second to California in 2005 (252 human cases and 13 deaths), and ranked sixth in 2006 (211 human cases and 9 deaths). Most human cases in Illinois have been reported from the Chicago region (Gu et al. 2006, IDPH 2007), where infection rates in *Culex* mosquitoes of 60 per 1000 mosquitoes during peak transmission were observed; this rate is much higher than those observed elsewhere (Andreadis et al. 2004, Gu et al. 2004, Ezenwa et al. 2006, Reisen et al. 2006).

The incidence of human cases of West Nile virus infection, when mapped by home address, is highly clustered within urban environments (Ruiz et al. 2004, Watson et al. 2004). In the Chicago metropolitan area, Ruiz et al. (2004, 2007) showed that human WNV incidence was highest in urban areas characterized by medium-density housing, housing constructed in the 1950s, moderate income, and high proportion of white people. Annual variation in incidence has been principally attributed to weather patterns (Andreadis et al. 2004, Shaman et al. 2005); specifically, drought and high temperatures

consistently coincide with years of increased transmission. High temperatures also increase the rate of WNV dissemination in *Culex pipiens*, contributing to amplification (Dohm and Turell 2001). Kilpatrick et al. (2006) suggested that bird community structure, diversity, and presence of particular species such as the American Robin (*Turdus migratorius*) were the main determinants of mosquito infection rate in Maryland and Washington D.C. Thus, presense of avian hosts, competent Culex mosquitoes, and suitable climate provide the general conditions for epidemic transmission of WNV (Day 2001, Andreadis et al. 2004, Gu et al. 2004, Reisen et al. 2004).

The specific associations between hosts and vector that may influence sudden, seasonal WNV amplification remain unknown. Scott and Edman (1991) postulated that avian age structure modulates intensity of arbovirus transmission. Specifically, they speculated that nestling and fledgling birds make especially important contributions to amplification, not just because of the influx of non-immune susceptibles, but also because nestlings and fledging birds are more prone to mosquito bites. We therefore initiated an intensive investigation of WNV epizootic transmission and local viral amplification in a 25 km^2 study area in suburban Chicago, Illinois. This area is known for historic St Louis Encephalitis (SLE) and WNV activity and for a high incidence of human cases of SLE (Zweighaft et al. 1979) and WNV (Ruiz et al. 2004, 2007). We sought to quantify WNV amplification at a local scale, with explicit attention to possible associations among seasonal trends the local abundances of hatch-year birds and timing of infection in birds, mosquitoes, and humans.

Materials and Methods

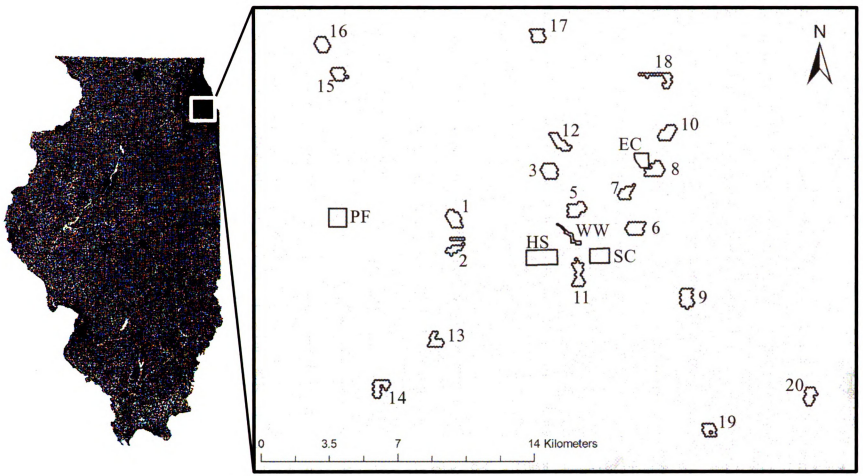
Study Sites

Our study areas were located in southwest suburban Chicago, Illinois (Cook County; 87° 44' W, 41° 42' N; Figure 1.1). Specific residential and natural sites for mosquito and bird sampling were selected by a stratified random design to represent a range of environmental and demographic features. The residential sites were selected to include various levels of income, housing density, and distance to nearest natural area. Using these criteria, in 2005 we established eleven residential sites that encompassed seven municipalities. The four 'green spaces' in the region included three cemeteries and a wildlife refuge. In 2006 we sampled an additional 10 residential sites selected with the same design and one additional green space. Precipitation and temperature conditions from 1873 to 2006 for Chicago were obtained from the National Oceanic and Atmospheric Administration (http://www.crh.noaa.gov/lot/?n=climate) and daily temperature and precipitation were obtained from the National Climatic Data Center (http://cdo.ncdc.noaa.gov/qclcd/QCLCD). Data for human WNV date of onset and spatial occurrence were made available by the Illinois Department of Public Health. Human cases considered in this paper occurred within a 5 km buffer around the 15 field sites in 2005 and 26 field sites in 2006. Spatial data were processed using the ArcGIS 9.0 software (ESRI, Redland, CA).

Field Sampling

Mosquitoes were collected from each of the field sites in 2005 and in 2006 once every two weeks from mid-May through Mid-October. A mosquito trapping session at

Figure 1.1. Map of 26 study sites in southwest suburban Chicago, Illinois. Site labels and letters refer to: (1) Palos Hills – North, (2) Palos Hills – South, (3) Oak Lawn – North, (5) Oak Lawn – Central, (6) Chicago – Mt. Greenwood, (7) Evergreen Park – West, (8) Evergreen Park – North, (9) Blue Island, (10) Chicago – Ashburn East, (11) Alsip, (12) Burbank, (13) Orland Park – North, (14) Orland Park – South, (15) Indian Head Park, (16) Western Springs, (17) Chicago – Midway, (18) Chicago – Marquette Park, (19) Harvey, (20) Dolton, (21) Evanston, (22) Chicago – Rogers Park, (HS) Holy Sepulchre Cemetery, (SC) Saint Casimir's Cemetery, (EC) Evergreen Cemetery, (WW) Wolfe Wildlife Refuge, (PF) Palos Forest Preserve. The two sites north of Chicago (21 and 22) are not visible in the figure.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each site in 2005 consisted of four CO_2 baited CDC miniature light traps (2 elevated into tree canopy; 2 at ground-level), four CDC gravid traps baited with rabbit pellet infusion (Lampman and Novak 1996), and battery-powered backpack aspirators (Meyer et al. 1983). In 2005, elevated light traps captured more *Culex* spp. mosquitoes; therefore, our 2006 sampling consisted of two elevated light traps, 2 gravid traps, and aspirators. Mosquitoes were identified (Andreadis et al. 2005) and pooled into groups of 25 or less, grouped by species, sex, collection site, and date, and placed in 2 mL microcentrifuge tubes. *Culex* individuals that could not be identified to species were grouped as *Culex* complex. The cold chain was maintained while processing and pools were stored at -20 or -80°C prior to testing.

Wild birds were captured with 36 mm mesh nylon mist nets (Avinet, Inc.) from mid-May to mid-October in both years. In 2005, five of the residential sites (Site 1, 5, 7, 10, 11) and all four natural areas were sampled on three-week rotations (slightly longer rotations late in the season) resulting in six visits to each study site. We sampled eight additional residential sites in 2006. Captured birds were identified, weighed, measured, sexed, aged and then released. Age was based on plumage characteristics and yellow gape on base of bill and allowed classification of "hatch year" or "after hatch year" (i.e. adults, see (Pyle 1997). For a few species, some or all individuals were classified as unknown age and/or sex. Birds were marked with numbered USFWS leg-bands (U.S. Department of Interior Bird Banding Laboratory), as authorized by Federal Bird Banding Permit #06507. Blood was sampled by jugular or brachial venipuncture using a 25-gauge tuberculin syringe or a 28-gauge insulin syringe. The volume of blood collected varied by bird size but did not exceed 1% of the bird's body weight or 0.2 mL. Blood was

added to 0.8 ml of BA-1 diluent in a microcentrifuge tube. Blood was stored on ice packs in the field and centrifuged within five hours. Serum and BA-1 was pipetted and placed in a 2.0 ml cryovial; clots and the serum were stored at -20 or -80°C. 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 #12/03-152-00 and UIUC Animal Use Protocol # 03034.

Laboratory Analyses

Mosquitoes were homogenized by adding 1 ml of a 50:50 mixture of phosphate-buffered saline (PBS) and 2X lysis buffer (Applied Biosystems, Foster City, CA) and three # 7 steel shot using a high-speed mechanical homogenizer (Retsch MM 300) for 4 minutes at 20 cycles/second. Each homogenized pool was centrifuged for 2 minutes at 13,000 rpm. RNA was extracted from mosquito pools using an ABI Prism 6100 Nucleic Acid Prep Station following the Tissue RNA Isolation Protocol (Applied Biosystems; P/N 4330252). RNA was eluted in a final volume of 60 uL of elution solution. A region of the WNV RNA envelope gene was detected using real-time, reverse transcription-PCR (RT-PCR) (Lanciotti et al. 2000). The thermocycling was performed on an ABI Prism 9700HT sequence detector at the Research Technology Support Facility at Michigan State University, following the TaqMan One-Step RT-PCR Master Mix Protocol (Applied Biosystems; P/N 04310299).

We used blocking enzyme-linked immunosorbent assay (ELISA) for detection of WNV antibodies in bird serum samples (Blitvich et al. 2003). The inner 60 wells of a 96-well EIA/RIA medium binding microtiter plate (Corning Incorporated 3591) were loaded

with a 1:12,000 dilution of 4G2 capturing antibody and coating buffer, and incubated overnight (all incubation at 37°C, humidified with wet paper towel). Plates were washed six times with PBS-Tween 20, pH 7.4, and then wells were blocked with a milk-PBS solution (BIO RAD non-fat dry milk) and incubated for two hours at 37°C. Plates were washed and a 1:50 dilution of WNV antigen and PBS was loaded into wells and incubated for two hours. Plates were washed and 100 µl of field collected serum (1:20 dilution with BA-1) was loaded along with positive and negative controls. The plate was incubated for two hours, washed, and wells were loaded with 1:4,000 dilution of 6B6C-1 monoclonal antibody (MAb) labeled with horseradish peroxidase and milk-PBS. After another two hour incubation and washing, 100 µl of tetramethylbenzidine (Sigma Aldrich, Inc.) were added and then incubated and stopped with 50 µl of sulphuric acid. The reduction in optical density was determined with plate blanks subtracted at a wavelength of 450 nm on an automated plate reader (Molecular Devices). Percent inhibition was calculated as (1 – (TS/CS) x 100), where TS is the optical density of the test serum and CS is the mean optical density of the negative control serum. Two different positive controls and four negative controls were used on each plate. Samples testing positive on the first screen were serially diluted and tested to find the end-point titer.

We tested bird serum samples for the presence of WNV RNA using similar methods described for mosquito pools. We extracted RNA from 100 µl of bird serum in a 1:20 dilution with BA-1 using a protocol developed for the isolation of viral RNA from non-cellular samples on the ABI 6100 nucleic acid prep station (Felton 2003). WNV was detected using RT-PCR as described above.

Data Analysis

Maximum likelihood estimates and 95% confidence intervals (CI) for *Culex* spp. infection rates were calculated by week using the Pooled Infection Rate version 3.0 add-in (Biggerstaff 2006) and Excel (Microsoft 2005). Cross-correlation analyses were used to estimate the correlation between paired time series measured by week. We estimated the correlation of *Culex* spp. mosquito infection with the proportion of virus positive birds, with hatch year and adult bird seropositivity, with the proportion of hatch year birds captured in mist-nets, and with the date of onset of human cases of WNV in the study region. We identified the time lag at which the estimated correlation was maximized. Differences in proportions were compared on frequency data using Pearson's chi-square test. End-point titers for adult and hatch year birds were compared using the Kruskal-Wallis test. All statistical analyses were performed using the R software environment (R Development Core Team, 2004; http://www.R-project.org).

Results

Precipitation and temperature data are shown in Figure 1.2. The mean precipitation in the Chicago region during the months of June, July, and August, 2005 was the third lowest since 1871, and temperatures were the 12th hottest summer on record. In 2006, the mean summer temperature was a little cooler (35th hottest summer on record) and the area received over twice the rain fall during the three month period compared to 2005 (Figure 1.2).

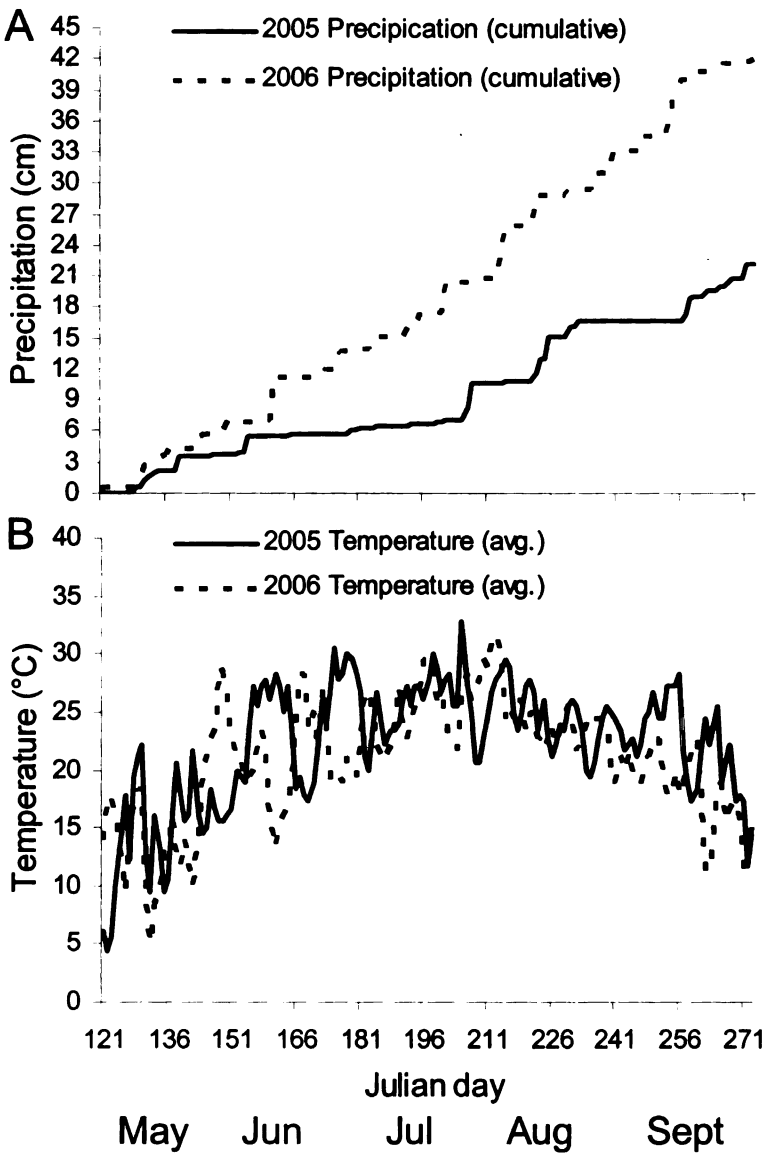

Figure 1.2. Accumulative precipication (cm) and daily average temperature (°C) recorded at Chicago Midway International Airport from May to September in 2005 and 2006.

We collected 21,285 individual mosquitoes which comprised 1,195 pools consisting of 13 mosquito species in 2005, and 24,332 individual mosquitoes which comprised 1,685 and 18 species in 2006. *Culex pipiens* was the dominant mosquito in both years, and the *Culex* spp.. *(Cx. pipiens, Cx. restuans, Cx. tarsalis, Cx. erraticus)* accounted for 79% of the pools in 2005 and for 64% in 2006. *Culex* abundance standardized by mosquitoes per gravid trap increased in mid-July, 2005 and remained steady till late-September (Figure 1.3). In 2006, *Culex* abundance increased earlier in mid-June, and steadily declined till September. We detected West Nile virus in 227 pools (19%) in 2005 and in 205 (12%) in 2006, a significantly smaller proportion ($x^2 = 25$, df = 1, $P < 0.001$). Of the 432 positive pools, all were *Culex* spp. except two in 2005 and five in 2006. The infection rate calculated only for *Culex* spp. mosquitoes reached a peak of 59 (CI_{95} 43.9- 80.2) during week 30 (July 23-29) in 2005 and of 33 (CI_{95} 22.2- 47.7) in week 32 (August 12-18) in 2006 (Figure 1.3). We observed a rapid amplification during weeks 29 and 30 (mid-late July) in 2005 and a lesser amplification during the same time period in 2006.

We captured 1,407 birds of 57 species using mist-nets in 2005 and 1,479 birds of 63 species in 2006. The most commonly captured species were the House Sparrow (*Passer domesticus*; combined years n = 871), the American Robin (*Turdus migratorius*; n = 479), the Gray Catbird (*Dumetella carolinensis*; n = 180), and the Northern Cardinal (*Cardinalis cardinalis*; n = 163). The proportion of hatch year birds in the mist-net samples reached 60% by late-July and remained high (>50%) through mid October in

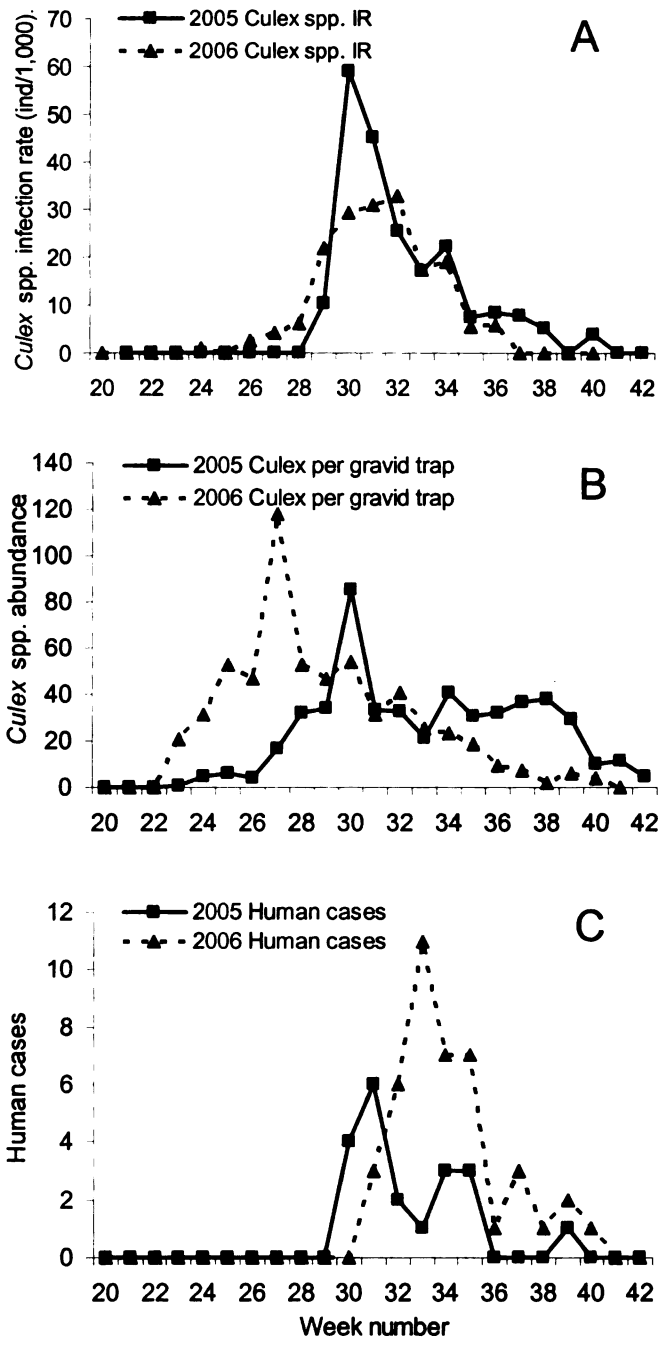

Figure 1.3. Temporal patterns of (A) *Culex* spp. mosquito infection rate, (B) *Culex* spp. mosquito abundance per gravid trap, (C) date of onset of human WNV cases from late-May to mid-October in the southwest suburban Chicago, Illinois region in 2005 and 2006.

both years (Figure 1.4). Proportion of hatch year birds in the mist-nets was significantly cross-correlated with *Culex* infection rate one week later in 2006 ($r = 0.55$, $P < 0.05$).

We collected 1,062 avian blood samples in 2005, of which 225 (21%) tested seropositive for WNV antibodies. In 2005, adult bird seroprevalence was 24.4% (115 of n=471), significantly higher than the hatch year bird seroprevalence of 18.5% (100 of n=540; $x^2 = 4.9$, $df = 1$, $P < 0.05$). In 2006, adult bird seroprevalence of 4.2% (33 of n=792) was not different ($x^2 = 1.17$, $df = 1$, $P > 0.2$) from hatch year bird seroprevalence of 2.8% (14 of n=493). The most abundant seropositive hatch year birds in 2005 were House Sparrow (21% seroprevalence), Northern Cardinal (71%), American Robin (11%), and Gray Catbird (36%). In 2006, the most abundant seropositive hatch year birds were Northern Cardinal (14%) and American Robin (4%), but not House Sparrows (<1%). In both years, seropositive hatch year birds were captured between June 27 and October 16, with a steady increase in the proportion of seropositivity through mid-August. We found a significant cross-correlation between hatch year bird seropositivity and *Culex* infection two weeks later in 2005 and 2006 (Figure 1.5; $r = 0.45$, $P < 0.05$, $r = 0.55$, $P < 0.55$, respectively). One hatch-year song sparrow captured twice at Holy Sepulchre Cemetery tested seronegative for WNV antibodies on July 27, 2005 and then tested seropositive upon recapture on August 17. Hatch year birds averaged significantly higher endpoint titers then adult birds in 2005 (Kruskal-Wallis test, $P < 0.001$) but not in 2006 (Kruskal-Wallis test, $P > 0.5$).

We detected 11 birds (1.1% of 998) in 2005 that were virus positive at the time of capture: 7 House Sparrows, 2 House Finches (*Carpodacus mexicanus*), a Red-winged

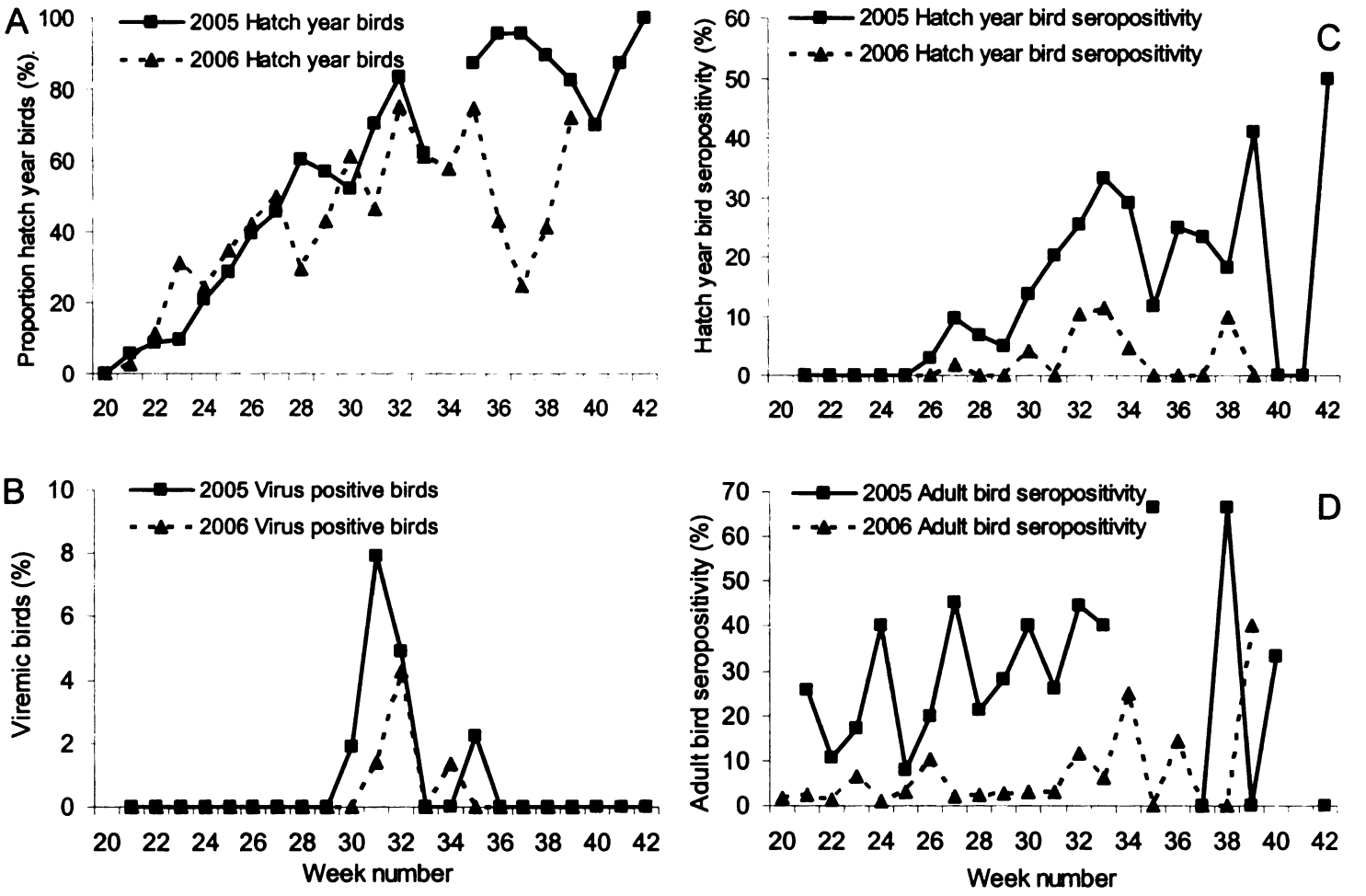

Figure 1.4. Temporal patterns of (A) proportion of hatch year birds in mist-nets samples, (B) virus positive birds, (C) seropositivity of hatch year birds, (D) adult bird seropositivity from late-May to mid-October in the southwest suburban Chicago, Illinois region in 2005 and 2006. Discontinuous data in A and D represent weeks when hatch year or adult birds were not collected.

Blackbird (*Agelaius phoeniceus*), and a Northern Flicker (*Colaptes auratus*). Proportion of virus positive hatch year birds (10 of 517) was statistically higher than adult birds (1 of 433; $x^2 = 4.6$, df = 1, $P < 0.05$). In 2006 we detected 3 virus positive birds (0.3% of 1,285): three House Sparrows (two adults and one hatch year). The proportion of virus positive hatch year birds (1 of 495) was not statistically different from adult birds (2 of 791; $x^2 = 0.17$, df = 1, $P > 0.6$). Most (13 of 14) virus positive birds were captured between weeks 30 and 32 (early August) in both years. There was a significant cross-correlation between weekly proportion of virus positive birds and *Culex* infection rate in 2005 (Figure 1.5A; $R = 0.89$, $P < 0.05$) where virus positive birds lagged one week behind mosquito infection. In 2006, this cross-correlation was also significant, but with no time lag (Figure 1.5D; $r = 0.59$, $P < 0.05$).

The Illinois Department of Public Health reported a total of 252 human cases of West Nile virus infection during the 2005 season, and 211 cases in 2006 (IDPH 2007). In 2005, 20 of these cases occurred within a 5 km radius of our study sites between weeks 30 and 39, eight of them occurring during week 31 and 32 (July 30 to Aug 12; Figure 1.2). There was a significant cross-correlation between human case date of onset and *Culex* infection rate with no time lag ($r = 0.85$, $P < 0.05$; Figure 1.5) in 2005. Our larger study region in 2006 contained 42 human cases of WNV, with a peak of 11 occurring in week 33 (Aug 6 to 12). The number of 2006 human cases was significantly correlated with mosquito infection rate and lagged it by 3 weeks ($r = 0.84$, $P < 0.05$).

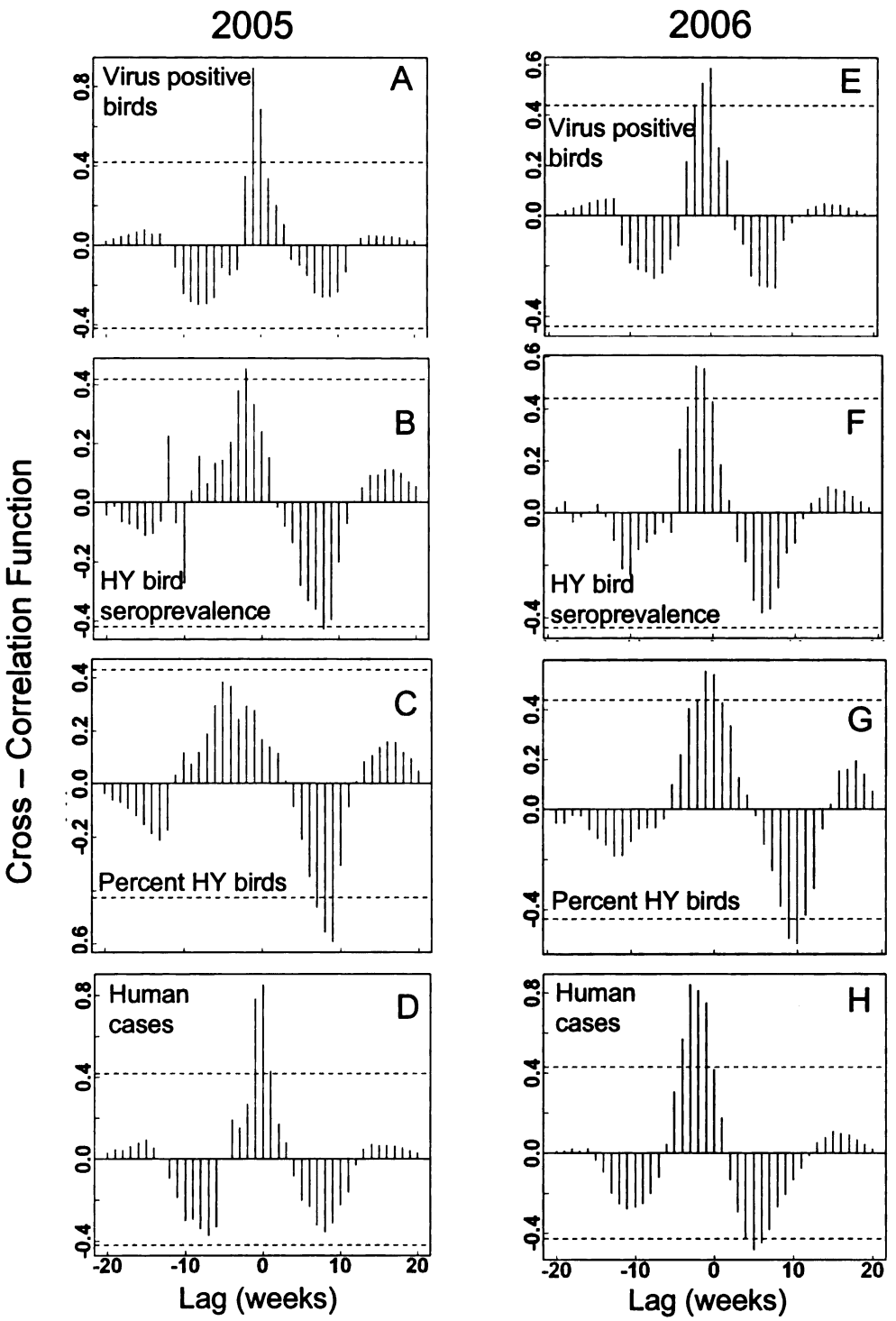

Figure 1.5. Statistically significant correlations between the *Culex* spp. infection rate in 2005 and virus positive birds (A), hatch year bird seropositivity (B), percent hatch year bird captured in mist-nets (C), and date of onset of human cases (D); and between *Culex* infection in 2006 and virus positive birds (E), hatch year bird seropositivity (F), percent hatch year birds captured in mist-nets (G), and date of onset of human cases (H) in southwest suburban Chicago, Illinois.

Discussion

Our results suggest that increases in local abundances of hatch year birds facilitates rapid West Nile virus amplification. Local increases in the relative abundance of hatch-year birds started just before the onset of peak WNV infection in mosquitoes, birds, and humans. We found that the proportion of seropositive hatch-year birds was significantly and positively correlated with *Culex* infection two weeks earlier. Bird and human virus infection were also significantly correlated with *Culex* infection with a lag time of one to three weeks.

Seroprevalence rates observed in samples of hatch year birds in 2005 (18.5%) during an intense epizootic are greater than those reported elsewhere (Nasci et al. 2002 (0-1.2%), Ringia et al. 2004 (4.1%), Beveroth et al. 2006 (5.5%), Gibbs et al. 2006(1.5-3.6%)). These seropositive hatch year birds were exposed to WNV during the 2005 transmission season, as the presence of maternal antibodies is unlikely (Ludwig et al. 1986). Seropositive hatch year birds represent only those that were infected and survived. We did not observe dead birds while in the field. Recovery of dead small bird species is known to be low owing to intensive scavenging on their carcasses and the difficulty of observing them (Wobeser and Wobeser 1992, Ward et al. 2006). Experimental studies show that mortality rates for passerines infected with WNV are high (Komar et al. 2003). This would suggest that many passerine birds, including hatch year birds, are being exposed to WNV, die, and go undetected at our study sites. Seropositive hatch year birds had higher end-point titers than did adults in 2005, probably due to recent exposure of hatch year birds and a waning of neutralizing antibodies (i.e., sero-reversion) in adult birds (Main et al. 1988, Komar 2001). Most of the species of hatch-

year birds captured are known to be local breeders. All but 1 of 77 and all 30 hatch year migrants were seronegative in 2005 and 2006, respectively, indicating that very few of these northern-breeding species are exposed to WNV on their breeding grounds or en route to their stopover in northern Illinois, where they were captured.

Seropositivity rates do not reflect a bird population's force of infection (Komar 2001), but experimental evidence shows that hatch year mourning doves and house finches infected with SLE produce a viremic titer high enough to be infectious (Mahmood et al. 2004). We documented a significantly higher WNV infection in hatch year birds (n=11) compared to adult birds (n=3) with cycle threshold values ranging from 18.9 to 37.3, but our passive capture methods probably under-estimate the proportion of virus positive birds, because many may die. Also, levels of activity and flight habits of infected birds may lower capture probability in mist nets. The importance of young-of-the-year birds in EEE transmission prompted Unnasch et al. (2006) to develop a dynamic transmission model in which vector feeding success and host preference prior to the peak in transmission were shown to be responsible for driving the subsequent peak in EEE viral activity. Our results suggest a similar effect of hatch year birds in WNV amplification. If hatch year birds function as an inflow of new susceptible hosts, the WNV reproduction rate could increase, resulting in an epidemic stage of transmission (Heesterbeek and Roberts 1995, Anderson and May 1991). But as the number of susceptible hosts are exposed and removed, WNV fades out. This speculation could explain our observation that *Culex* abundance continued to be high through September in 2005, but the *Culex* infection rate declined prior to September.

The temporal patterns of mosquito and bird WNV infection and seroprevalence of hatch year birds observed in this study provide insight into the mechanisms of seasonal dynamics of transmission. The peak of virus positive birds followed closely the peak in *Culex* infection in both years. Although the magnitude of these factors changed between the two years, the temporal patterns were remarkably similar as was the timing of events. The cooler and wetter weather in 2006 likely influenced the observed lower mosquito and bird infection rates, lower bird seroprevalence, and fewer statewide human cases. Above average temperatures and below average precipitation have been correlated with increased WNV transmission in North Dakota, Florida, Connecticut, California, and Russia (Andreadis et al. 2004, Reisen et al. 2004, Bell et al. 2005, Shaman et al. 2005). Arbovirus transmission increases with higher temperatures due to the increased dissemination rates in mosquitoes, shorter gonotrophic cycle resulting in female *Culex* refeeding more often, and shorter extrinsic incubation period (Meyer et al. 1990). Drought conditions are favorable for WNV transmission due to increased contact between mosquitoes and amplifying bird hosts at rare water sources (Shaman et al. 2005) or reduced flushing of *Culex* mosquitoes in catchbasins during drought events (Andreadis et al. 2004). The latter is the more likely mechanism operating at our study sites, as fewer mosquito larvae were found in catchbasins following rainfall events (unpublished data).

Intensive simultaneous collection of bird, mosquito, human, and environmental data across an entire transmission season and across several urban site types allowed for unprecedented accuracy in the quantification of longitudinal infection in mosquitoes, birds, and humans. The intensive nature of data collection allowed us to investigate the

hypothesis that increases in hatch-year bird populations are related to seasonal peaks in WNV transmission occurring in a historical foci with above average levels of transmission. Our data suggest that young-of-the-year birds are important amplifying hosts, increasing the susceptible host population. This primed host community triggered by hot and dry conditions leads to rapid amplification resulting epizootics and human epidemics. Continued work in the study area will further clarify the interactions between annual variation in climatic conditions with local transmission dynamics of WNV.

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CHAPTER 2

Hamer, G. L., U. D. Kitron, J. D. Brawn, S. R. Loss, M. O. Ruiz, T. L. Goldberg, and E. D. Walker. 2008. *Culex pipiens* (Diptera: Culicidae): a bridge vector of West Nile virus to humans. Journal of Medical Entomology 45: 125-128.

CHAPTER 2

Culex pipiens (Diptera: Culicidae): a bridge vector of West Nile virus to humans

Abstract

Host-feeding patterns of *Culex pipiens* L. collected in southwest suburban Chicago in 2005 were investigated using PCR and DNA sequencing techniques. *Culex* spp. mosquitoes, most identified to *Cx. pipiens* and the remainder to *Cx. restuans* by PCR, had fed on 18 avian species, most commonly American Robin (*Turdus migratorious*), House Sparrow (*Passer domesticus*) and Mourning Dove (*Zenaida macroura*). Additional bloodmeals were derived from four mammal species, primarily humans and raccoons (*Procyon lotor*). During a West Nile virus (WNV) epidemic in 2005, WNV RNA was detected in heads and thoraces of five *Cx. pipiens* (n = 335, 1.5%) using quantitative PCR. The hosts of these virus-infected, bloodfed mosquitoes included two American Robins, one House Sparrow, and one human. This is the first report of a WNV-infected *Cx. pipiens* mosquito collected during an epidemic of WNV that was found to have bitten a human. These results fulfill a criterion for incrimination of *Cx. pipiens* as bridge vector.

Introduction

West Nile virus (WNV) is now endemic throughout temperate North America, with annual amplification events and regular epizootics and epidemics. While individuals of over 60 mosquito species have tested positive for WNV (CDC, West Nile virus home

page), species in the genus *Culex,* especially *Culex pipiens* L. in the eastern United States north of 36 degrees latitude, have been implicated as the primary enzootic vectors, *i.e.*, those responsible for transmission among bird reservoir hosts (Marra et al. 2004, Turell et al. 2005). Over 23 mosquito species have been implicated as potential bridge vectors, or epidemic vectors, *i.e.*, those responsible for transmission to humans (Marra et al. 2004, Turell et al. 2005). Recent, indirect evidence based on blood meal analysis and theory suggests that *Cx. pipiens* serves as both an enzootic and an epidemic (i.e., "bridge") vector (Apperson et al. 2004, Kilpatrick et al. 2005). The evidence includes data documenting *Cx. pipiens* feeding on both birds and mammals (Apperson et al. 2004), and results of a analytical risk model incorporating data on virus infection and feeding rates on birds, mammals and humans suggesting that *Cx. pipiens* and *Cx. restuans* are responsible for 80% of human WNV infection in the northeastern US (Kilpatrick et al. 2005). However, empirical data demonstrating a virus infected mosquito biting a human being has heretofore been lacking. Our objective was to use blood meal analysis, individual mosquito virus infection detection, and molecular species identification methods to identify enzootic and epidemic mosquito vectors of WNV in an endemic transmission area in suburban Chicago, IL.

Materials and Methods

We sampled blood-fed mosquitoes in southwest suburban Chicago in 2005 using gravid traps and aspirators at fifteen study sites consisting of eleven residential neighborhoods, three cemeteries, and one wildlife refuge. This region has had a high incidence of human cases of West Nile viral meningoencephalitis since 2002 and of St. Louis encephalitis historically in 1975 (Ruiz et al. 2004). Blood-fed mosquitoes were

processed individually to identify the blood meal source, to identify the mosquito species and to detect WNV. To identify the blood meal source, we first scored the Sella stage of blood meal digestion using an ordinal rating system (Detinova 1962) and digital photography. The abdomen was removed for blood meal analysis and polymerase chain reaction (PCR) identification of the mosquito species, while the thorax and head were retained for RNA extraction and quantitative RT-PCR for virus detection. We amplified the vertebrate mitochondrial cytochrome B gene in the blood meal using four separate PCRs with established primer pairs, purified the amplicon (if present), directly sequenced it, and compared the sequences to those in GenBank (Apperson et al. 2002, Cupp et al. 2004, Molaei et al. 2006). Results of negative controls were acceptable, and all positive controls (blood from 17 species of birds, 9 species of mammals, and 2 species of amphibians) were accurately and consistently identified to species. The same extracted DNA used for blood meal analysis also was used for PCR-based molecular identification of *Culex* species to verify all morphological identifications (Crabtree et al. 1995). A quantitative RT-PCR method was used to detect WNV RNA in the head and thorax using empirically derived crossover thresholds to determine positive samples (Lanciotti et al. 2000, Hamer et al. 2007).

Results

Of 398 blood fed mosquitoes, most (84%) were identified as *Culex* spp. morphologically and as *Cx. pipiens* by PCR. Blood meals of 246 individual *Cx. pipiens* (n = 335, 73.4%) were identified successfully to an avian or mammalian host (Table 2.1). The success of identifying a blood meal was negatively correlated with the Sella score of the abdomen ($r = -0.90$, $df = 4$, $p = < 0.01$). Based on the identified blood meals, *Cx.*

Table 2.1. Blood meal analysis of mosquitoes collected in southwest suburban Chicago in 2005.

	Mosquito Spp.		
Host	*Culex pipiens*	*Culex restuans*	*Culex* spp.[b]
Avian derived bloodmeals (total)	191	27	5
American Robin	69	13	4
Blue Jay	10	2	
House Sparrow	34	5	
Gray Catbird	1	1	
House Finch	14		
Common Grackle	1		
European Starling	2	2	
House Wren	2		
American Kestrel	2		
Northern Cardinal	19	1	
Black-capped Chickadee	2		
Cedar Waxwing	1		
Cooper's Hawk	1		
Mourning Dove	30	2	1
American Goldfinch	1		
Brown Thrasher	1		
Swainson's Thrush	1		
Mallard		1	
Mammal derived bloodmeals (total)	55	8	2
Raccoon	9	2	1
Human	44	6	1
Domestic Dog	1		
Gray Squirrel	1		
Na[a]	89	7	4

[a] No PCR reaction

[b] *Culex* mosquitoes that did not produce a PCR amplicon using the Culex spp. primer sets (*Cx. pipiens*, *Cx. restuans*, *Cx. salinarius*).

pipiens fed primarily on birds (n=191, 57%), with 18 species identified. The most common avian blood sources were American Robin (*Turdus migratorius*; n = 86), House Sparrow (*Passer domesticus*; n = 39), and Mourning Dove (*Zenaida macroura*; n = 33). Mammal feeding accounted for the remaining 28.8% of the *Culex* spp. blood meals, with humans (n = 51) and raccoons (*Procyon lotor*; n = 12) as the most common blood sources. *Cx. restuans* fed on similar avian and mammalian host species as *Cx. pipiens*, but had a higher percentage of avian feeding (n = 27 of 35, 77%).

WNV RNA was detected in five of 335 blood fed *Cx. pipiens* mosquitoes (1.5%) using quantitative RT-PCR (Table 2.2); two additional bloodfed *Culex* spp. mosquitoes tested positive for WNV but did not yield an amplicon after the blood meal analysis PCR. The blood meal host was identified in four of the five WNV-positive *Cx. pipiens*, as follows: American Robin (n = 2), House Sparrow (n = 1) and human (n = 1).

Discussion

Cx. pipiens is commonly considered to be ornithophilic (Marra et al. 2004, Turell et al. 2005), although the percentage of bird feeding varies substantially by region (84-96% feeding on birds in New York [Apperson et al. 2002, 2004); 93% in Connecticut (Molaei et al. 2006); 71% in Tennessee, but only 35% in New Jersey (Apperson et al. 2004); 87% in Maryland and Washington, DC (Kilpatrick et al. 2006); and 57% in this study]. One possible explanation for this variation is substantial substructuring of *Cx. pipiens* populations, with mammal and bird feeding forms and hybrids, identified in Europe and New York (Fonseca et al. 2004, Kent et al. 2007). The structure of the

Table 2.2. Bloodfed mosquitoes collected in suburban Chicago, IL that tested positive for WNV.

Date, 2005	Species	Bloodmeal identification
Jul 19	*Cx. pipiens*	House sparrow
Aug 2	*Culex.* spp.[a]	NA[b]
Aug 18	*Cx. pipiens*	American Robin
Aug 19	*Cx. pipiens*	American Robin
Sep 6	*Cx. pipiens*	Human
Sep 7	*Cx. pipiens*	NA
Sep 13	*Culex* spp.[a]	NA

[a] Mosquito identified morphologically as *Culex* spp. but did not produce a PCR amplicon using *Culex* primer sets (*Cx. pipiens*, *Cx. restuans*, *Cx. salinarius*).

[b] No PCR reaction

population in metropolitan Chicago is not known, but our results show that the mammal feeding rate was comparatively high.

Samples from residential areas such as backyards of homes yielded 75% of the total *Culex* spp. mosquitoes 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, wood lots, and public thoroughfares (Apperson et al. 2002, Apperson et al. 2004, Molaei et al. 2006). Collecting blood-fed mosquitoes in immediate proximity to human habitation could explain our finding of a high frequency of human feeding by *Culex* mosquitoes. Host availability was not quantified in the present study; however, results of blood meal analysis should not be misconstrued as merely reflecting host preferences.

We found that seven of 398 individual bloodfed mosquitoes were infected with WNV for an infection rate of 18 individuals per 1,000 (1.8%). Other research efforts conducted at the same sites in the same year found 227 positive pools out of 1,195 tested, for an infection rate of 11 per 1,000 mosquitoes (1.1%) (Hamer et al. 2007). The Chicago area experienced a WNV epizootic and epidemic in 2005, during a drought, with Illinois ranking second in the US in the total number of human WNV cases (CDC, West Nile virus home page). The high *Culex* spp. infection rate that reached 59 per 1,000 in late July (Hamer et al. 2007), provided an opportunity to identify virus-positive blood-fed mosquitoes and simultaneously to determine the origin of their blood meal. During peak transmission in July 2005, the probability that a WNV-infected *Culex* spp. mosquito fed on a human was 0.01 (i.e., WNV mosquito infection rate of 0.059 x human feeding rate of 0.173).

Incrimination of an epidemic vector of WNV requires demonstration of direct association of an infected vector with humans, a corollary of Koch's postulates. Our study contributes to the substantial evidence that *Cx. pipiens* serves as the enzootic vector of WNV in large parts of North America by showing that virus-infected mosquitoes feed on American Robins and House Sparrows. Moreover, our study implicates *Cx. pipiens* as a bridge or epidemic vector of WNV, because a mosquito with a disseminated virus infection in the head plus thorax was found to have fed on a human, the first recorded observation of such an event. Although the identity, infection, and clinical status of the bitten human are unknown, this finding adds support to the hypothesis that *Cx. pipiens* serves both enzootic and bridge vector. Our experience has been that WNV infection is rather common in *Culex* spp. and *Culex pipiens* in particular, but rare in non-*Culex* mosquitoes. In fact, we have found that non-*Culex* mosquitoes are typically rare during epidemics of WNV at our study site, whether infected or not. Mosquito testing efforts in 2005-2006 in the same region resulted in only 7 positive non-*Culex* pools of 859 tested (an infection rate of 1 per 1,000 mosquitoes), those being one positive pool each of *Aedes vexans* (Meigen), *Anopheles quadrimaculatus* (Say), and *Ochlerotatus triseriatus* (Say), and two positive pools each of *Coquillettidia perturbans* (Walker) and *Ochlerotatus trivittatus* (Coquillett) (Hamer et al. 2007). By comparison, of 2,016 *Culex* spp. pools tested, 425 were positive (estimated infection rate by maximum likelihood method of 12 per 1,000 mosquitoes, a figure comparable to the infection rate of 18 per 1,000 reported here for individually tested mosquitoes). Although we cannot completely exclude the possibility of other mosquito species playing a role as bridge vectors in the Chicago metropolitan area, the finding of a virus-positive *Cx. pipiens* with a human-derived blood

meal combined with the relatively high rate of human feeding by *Cx. pipiens* suggests that control efforts focused on *Cx. pipiens* alone may largely reduce both epizootic amplification and transmission risk to humans.

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CHAPTER 3

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CHAPTER 3

Host selection by *Culex pipiens* mosquitoes and West Nile virus amplification

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 declines. To test this paradigm, we performed a mosquito blood meal analysis integrating host feeding patterns of *Culex pipiens*, the principal vector of WNV in the eastern United States north of the 36 latitude, and other mosquito species with robust measures of host availability, to determine host selection in a WNV-endemic area of suburban Chicago (Illinois), United States during 2005—2007. Results showed that *Cx. pipiens* fed predominantly (83%) on birds with a high diversity of species utilized as hosts (25 species). American robins (*Turdus migratorius*) were marginally overutilized and several species were underutilized based upon relative abundance measures, including common grackle (*Quiscalus quiscula*), house sparrow (*Passer domesticus*), and European starling (*Sturnus vulgaris*). *Culex pipiens* also fed substantially on mammals (19%; 7 species; humans representing 16%). West Nile virus transmission intensified in July of both years, at times when American robins were heavily fed upon, and then declined when robin abundance declined, 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—ca. 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) amongst individuals comprising diverse bird communities in a variety of landscapes[1,2]. WNV 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 the majority of 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 *Culex 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 evidenced 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 WNV antibodies to identify spatial and temporal patterns of transmission[17—23]. However, serological studies are limited, since 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 U.S.[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 ecological, biological, and behavioral factors that influence the probability of a host being exposed to a mosquito[27]. Ecological factors important for host availability include the night-time roost size, location and height of a bird species. Biological factors, such as host body mass and anti-mosquito behavior also impact 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 West Nile virus 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 overutilized relative to other common species. Furthermore, we examined whether temporal patterns reflect a shift in feeding preferences from birds to mammals 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 southwest suburban 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 paper 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 (ESRI, 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 CDC 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, the abdomen was removed from each specimen, transferred to a microcentrifuge tube, and DNA was extracted from it (DNeasy Tissue Kits, Qiagen, USA). 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 of 3.5 min at 95°C,

followed by 36 cycles consisting of denaturation (30 s at 95°C), annealing (50 s at 60°C), extension (40 s at 72°C), and a final extension for 5 min 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,USA).

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, Tewantin, Australia) for sequence quality and presence of double nucleotide peaks, which may indicate the presence of 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 of 2

min at 95°C, followed by 55 cycles consisting of denaturation (45 s at 94°C), annealing (50 s at 50°C), extension (1 min at 72°C), and a final extension for 7 min 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 PCR reactions from the same template and when chromatograms from each PCR demonstrated double nucleotide peaks.

Sterile technique was utilized 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*), 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 hour before sunrise and 4.0 hours after sunrise (0530-1000 A.M.) on days with no precipitation and wind speed less than 24 km/hr. 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, New York) 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. Birds were identified to species, weighed, measured, aged and sexed, and banded with numbered USFWS 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 #12/03-152-00 and University of Illinois at Urbana-Champaign Animal Use Protocol # 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:

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

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 overutilization (i.e. more frequent feeding than expected by chance), and a ratio less than one represents underutilization (i.e. less frequent feeding than expected by chance). The standard error of $\hat{w}_i$ was estimated as follows:

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

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)). Over- or underutilization for a host species was considered statistically significant when the 95% confidence interval did not overlap unity.

The selection index (W_i) was calculated for *Cx. pipiens* seperated 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 ha^{-1}.

Amplification fraction

The amplification fraction for each bird species included in the analysis was modeled in order 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 (pers. comm.). Competence values were obtained from Kilpatrick et al[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 to the Manly selection ratio described above. P_i incorporated the fraction of total avian and mammalian blood meals instead of just avian blood meals.

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

The probability of each species becoming infected is proportional to the feeding preference, P_i, which changes the amplification fraction to $F_i = a_i * P_i * P_i * C_i$. This expression reduces to $F_i = B_i * P_i * C_i$. 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 since more variation occurs between taxonomic families of birds than within them[6]. Several species did not have a member of it's repective family with a known competence value so 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 3.1). Identification of *Culex* sp. 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 for two individual *Cx. salinarius*. For all mosquito species of all genera, *Culex 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 per 1000 in 2005, 7.4 per 1000 in 2006, and 8.09 per 1000 in 2007.

Blood meal analysis

The hosts of the blood meals of 1,043 of 1,483 (70%) mosquitoes were identified (Table 3.1). The proportion of reactions yielding amplicons and sequences declined with increasing Sella score ($R^2 = 0.91$, df = 4, $P = 0.002$). Blood meals from *Cx. pipiens*

Table 3.1. Number and percent of blood meals by host class for mosquitoes collected from suburban southwest Chicago, 2005-2007.

				Mixed			
Taxon	Avian (%)	Mammal (%)	Amphibian (%)	avian-avian (%)	mammal-mammal (%)	avian-mammal (%)	Total
Culex pipiens	488 (80)	98 (16)		6 (1)	4 (1)	15 (2)	611
Culex restuans	172 (81)	31 (15)	1 (<1)	3 (1)		6 (3)	213
Culex 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
Ochlerotatus trivittatus	1 (7)	13 (93)					14

(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 3.1). Blood meals from *Cx. restuans* were also most commonly (81%) 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 *Aedes vexans* were of avian origin.

Results of BLAST searches of *cyt b* sequences revealed 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 3.2). 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 3.3). 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 *Aedes 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.

Table 3.2. Number and percent of blood meals identified to avian or mixed avian hosts for mosquitoes collected in southwest suburban Chicago in 2005-2007. Avian relative abundance provided as the fraction of species i in the avian community (density of species i / total avian density).

Host	Fraction of species i in avian community	Mosquito Spp.			
		Culex pipiens (%)†	*Culex 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		
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

*Species was not observed during surveys and was given lowest observed bird density.
†Includes 27 specimens from which double blood meals were identified.
‡Includes 12 specimens from which double blood meals were identified
§*Culex* mosquitoes that did not produce a PCR amplicon using the *Culex* spp. primer sets (Cx. pipiens, Cx. restuans, Cx. salinarius).
¶Includes 5 specimens from which double blood meals were identified.

Table 3.3. Number and percent of blood meals identified to mammal or mixed mammal hosts for mosquitoes collected in southwest suburban Chicago in 2005-2007.

	Mosquito Spp.			
Host	*Culex pipiens* (%)*	*Culex 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* mosquitoes that did not produce a PCR amplicon using the *Culex* spp. primer sets (*Cx. pipiens*, *Cx. restuans*, *Cx. salinarius*).

§Includes 21 specimens from which double blood meals were identified.

Bird abundance

A total of 44 avian species were identified during point count surveys with a total density of 9.66 birds ha^{-1}. House sparrows (4.25 birds ha^{-1}), American robins (2.0 birds ha^{-1}), mourning doves (0.63 birds ha^{-1}), common grackles (0.56 birds ha^{-1}), and European starlings (0.55 birds ha^{-1}) 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 3.4). Of the species for which the selection ratio was greater than 1 (indicating overutilization 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 overutilized when all *Cx. pipiens* were combined (2.26; CI = 0.98 – 3.54). Of the species for which the selection ratio was less than one (indicating underutilization), 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). *Cx. restuans* feeding preferences displayed similar overall host selection, but no bird species were

Table 3.4. Host feeding preferences of *Cx. pipiens* collected in southwest suburban Chicago in 2005-2007 in total and broken down by trap type. Values in parentheses are standard errors

	Culex pipiens feeding preference			
	Total	Light trap	Gravid trap	Aspirator
	$\hat{w}_i$	$\hat{w}_i$	$\hat{w}_i$	$\hat{w}_i$
American Kestrel†	75.51(735.08)	161.10(1573.70)	75.65(737.09)	73.62(719.16)
Swainson's Thrush†	50.34(490.49)	161.10(1573.70)	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†	25.17(245.89)	161.10(1573.70)	37.83(369.51)	73.62(719.16)
Common Canary†	25.17(245.89)	161.10(1573.70)	37.83(369.51)	73.62(719.16)
Cooper's Hawk†	25.17(245.89)	161.10(1573.70)	37.83(369.51)	73.62(719.16)
Ring-necked Pheasant*	25.17(245.89)	161.10(1573.70)	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)	1.40(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 observed as a host in the blood meal analysis (given a value of 1).
†Species not recorded during avian surveys (given value of the lowest observed bird density).
‡Statistically significant non-random host selection at $P < 0.05$.

significantly overutilized and only three were significantly underutilized (American goldfinsh, 0.22; common grackle, 0.24; and house sparrow, 0.33; Table 3.5).

Selection ratios for *Cx. pipiens* between residential and natural sites were significantly different ($t = 3.67$, $df = 48$, $P < 0.001$). Overutilization was higher for several species in residential sites than natural sites, including mallard (39.9 ± 451, 0.2 ± 0.2; respectively; Table 3.6) and American robin (2.4 ± 0.4, 1.2 ± 0.2). Underutilization 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 compared to natural sites.

The abundance of American robins captured using mist-nets declined as the summer season progressed, while the abundance of house sparrows in mist-nets increased by comparison (Figure 3.1A). The proportion of *Cx. pipiens* feeding on American robins declined as the season progressed (Figure 3.1B), while concomitantly there was an increase in feeding on other avian species, such as house sparrow, mourning dove, and northern cardinal (Figure 3.1B).

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 per 1,000 individuals) occurred in August. *Cx. pipiens* infection with WNV and abundance peaked during the months of August and September (respectively; Figure 3.2A). Seventy-six human cases of WNV infection were reported within five kilometers of the field sites in 2005—2007, and peak date of onset occurred in

Table 3.5. Host feeding preferences of *Cx. restuans* collected in southwest suburban Chicago in 2005-07. Values in parentheses are standard errors.

Host	*Culex restuans* feeding preference $\hat{w}_i$
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 observed as a host (given a value of 1).
†Species not recorded during avian surveys (given value of the lowest observed bird density).
‡Statistically significant non-random host selection at $P < 0.05$.

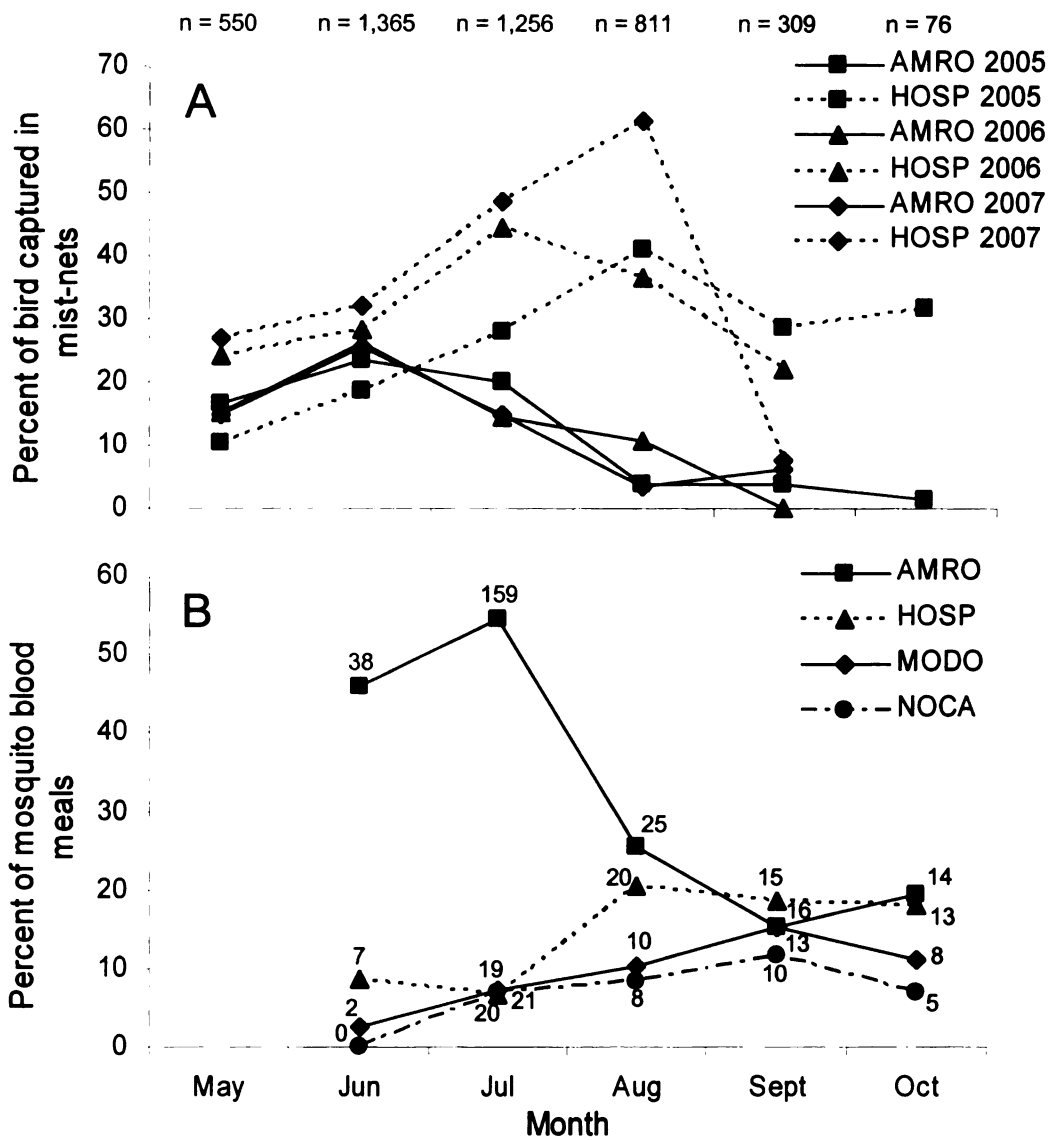

Figure 3.1. Percent of American robin and house sparrow captured in mist-nets in southwest suburban Chicago, IL, 2005—2007 (B). Percent of *Culex pipiens* blood meals derived from American robin, house sparrow, mourning dove, and northern cardinal (B). Total sample size of birds captured in mist-nets for combined year indicated (A) and raw numbers indicated for sample size (B).

Table 3.6. Host selection ratios for *Culex pipiens* collected in residential and natural study sites in southwest suburban Chicago in 2005-07. Blank values indicate zero blood meals were observed from bird species i. Values in parentheses are standard errors

	Culex pipiens feeding preference	
	Residential sites	Natural sites
American Kestrel†‡§	524.97(12372.68)	48.81(326.50)
Scarlet Tanager†‡	524.97(12372.68)	8.75(26.01)
Swainson's Thrush†‡§	349.98(8249.70)	48.81(326.50)
Brown Thrasher‡§	174.99(4126.71)	48.81(326.50)
Cooper's Hawk†‡§	174.99(4126.71)	48.81(326.50)
Eastern Bluebird*†‡	174.99(4126.71)	8.27(24.00)
Eastern Towhee*†‡	174.99(4126.71)	9.12(27.63)
Yellow-shafted Flicker†	174.99(4126.71)	48.81(326.50)
Ring-necked Pheasant*†	174.99(4126.71)	22.50(103.54)
Swamp Sparrow*	174.99(4126.71)	48.81(326.50)
Veery*†‡	174.99(4126.71)	2.40(4.27)
Warbling Vireo*†‡	174.99(4126.71)	2.72(5.04)
Willow Flycatcher*†‡	174.99(4126.71)	5.52(13.46)
Yellow Warbler*†	124.45(2475.79)	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(58.15)	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 residential sites (given a value of 1).
†Species not observed as a host in natural sites (given a value of 1).
‡Species not recorded during avian surveys (given value of the lowest observed bird density).
§Species not recorded during avian surveys (given value of the lowest observed bird density).
¶Statistically significant non-random host selection at $P < 0.05$.

August (Figure 3.2B). 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 3.2B).

There was statistically significant temporal variation in the frequency of bird and mammal feeding by *Cx. pipiens* (2 x 5 contingency table, $X^2 = 24.05$, df = 4, $P < 0.0001$) (Figure 3.2B). 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 ($X^2 = 20.2$, 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 3.3). Together, these five species accounted for 82% of the WNV-infectious *Cx. pipiens*.

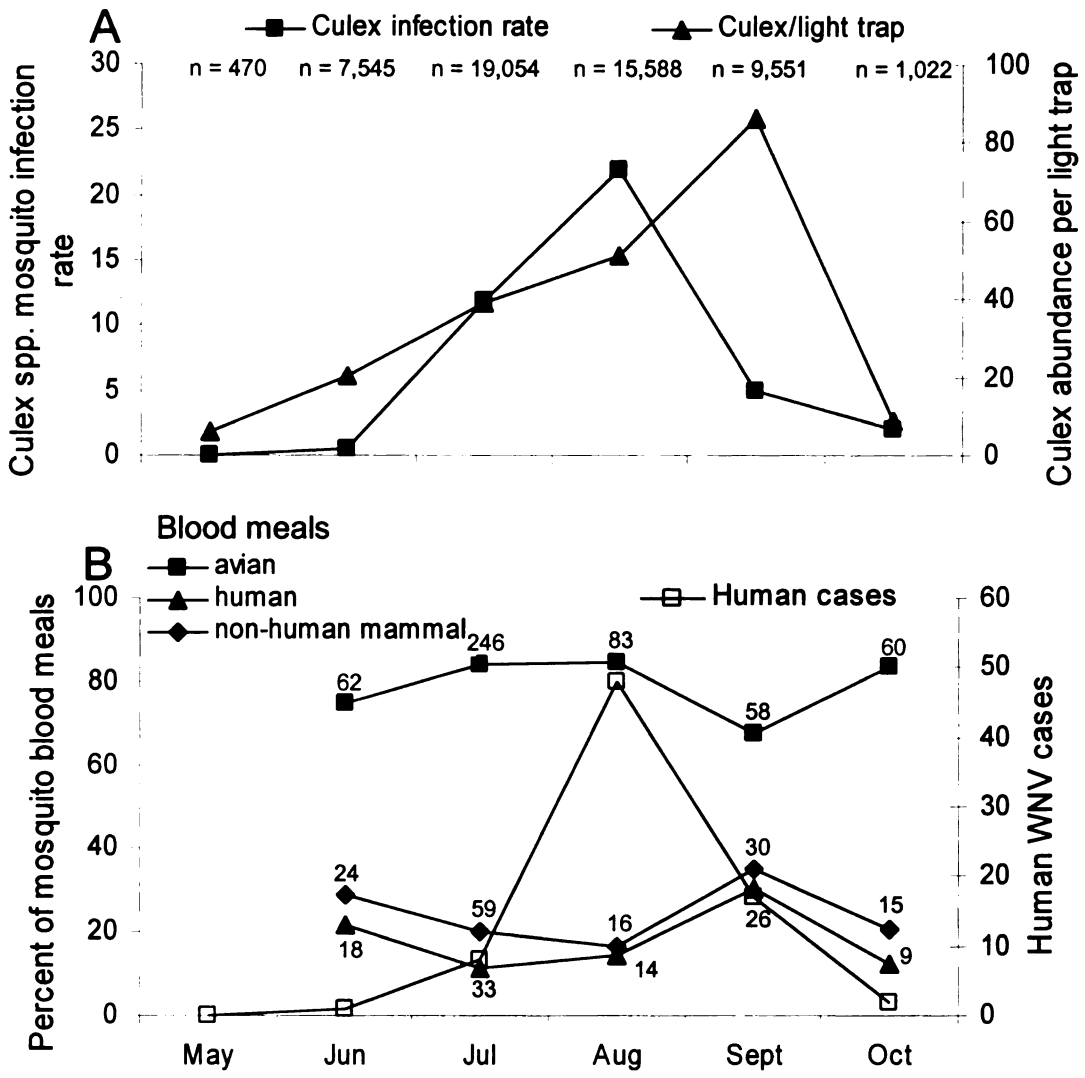

Figure 3.2. Temporal patterns of *Culex* spp. mosquito infection rate and abundance (*Culex* per light trap) in southwest suburban Chicago, IL in 2005—2007 (A). Percent of *Culex pipiens* blood meals derived from birds, humans, and non-human mammals and human West Nile virus case date of onset in the same study sites during the same years (B). 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.

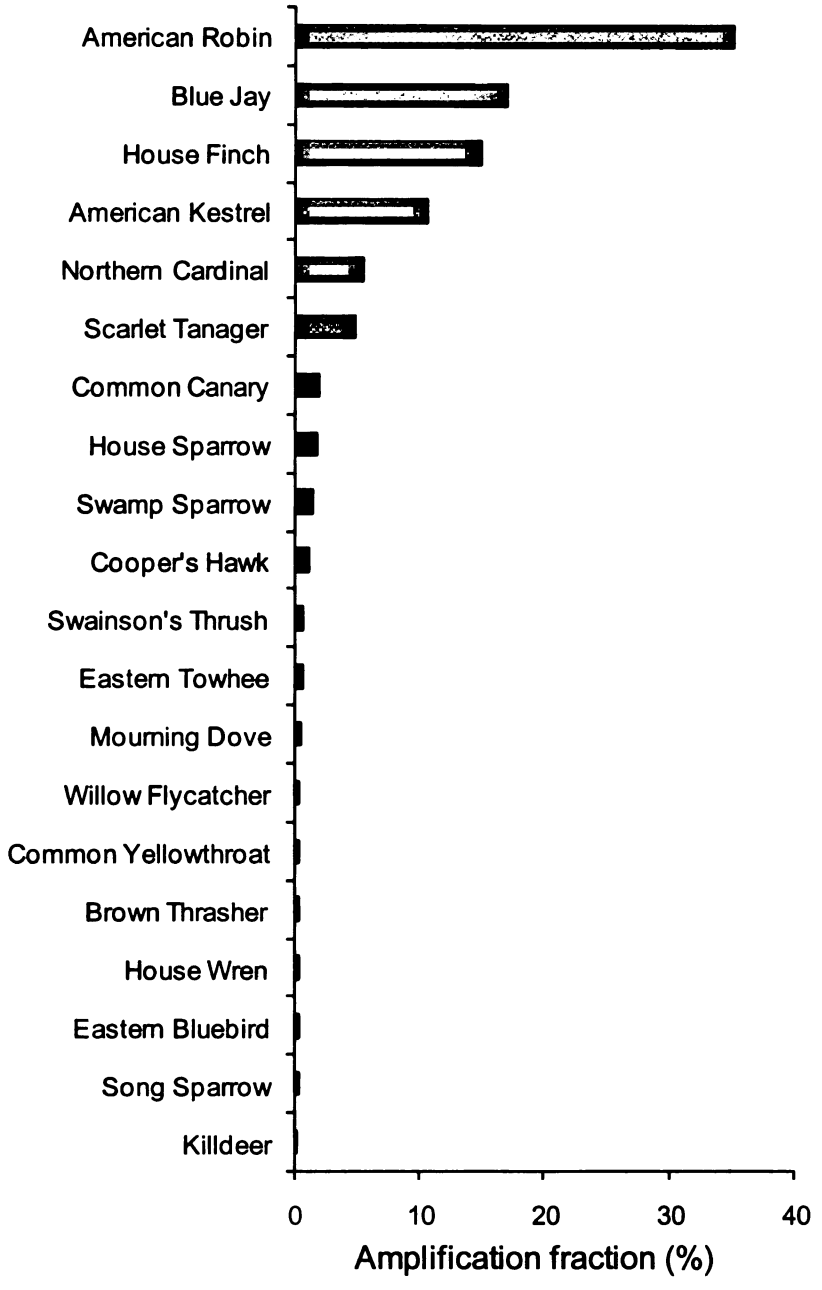

Figure 3.3. Amplification fraction (F_i) represents the fraction of West Nile virus infectious mosquitoes resulting from feeding on that avian host[1,6] (modified by A.M. Kilpatrick, pers. comm.). Species with amplification fractions less than 0.02 are not graphed and include eastern kingbird, black-capped 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.

Discussion

The amplification of West Nile virus 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 *Culex 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 utilization influence amplification and bridging transmission?

Our results document extensive feeding of *Cx. pipiens* on humans. This finding is especially striking since 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 bird availability (as opposed to some physiological change affecting mosquito feeding patterns)[1]. The initial high rate of feeding on American robin, reported in other studies as well[6,7], was followed by a gradual decline 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* (Table 3.2, 3.3). However, the decline in

feeding on robins was not accompanied by a rise 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 3.1B, 3.2B). Further, 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 WNV infection rate in the *Cx. pipiens* population increases in July and August, some marginal virus transmission to humans occurs owing to the fraction of the *Cx. pipiens* population 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 over- (American robin) or under- (common grackle, starling, house sparrow) utilized 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 ecological 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 overutilization of American

robins, identified as a superspreader species owing to its high reservoir competence, is not the sole determinant of intensification of WNV transmission during amplification. But rather simultaneous underutilization 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 SLEv and WNV[49,50]. Here, there was less feeding on house sparrows than expected based on their abundance, resulting in a lower amplification fraction. By contrast, the less common house finch was predicted to be an important amplifying host (Table 3.5, Figure 3.3). 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]. Further, it would offer an

explanation for why WNV infection in *Culex pipiens* declines 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 overutilization 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 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 employed by this study and others may overlook cryptic blood meals due to 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 overutilization of robins by *Cx. pipiens* collected by aspirators and underutilization 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 is supported by the lower observed sella score, indicating a more complete, less digested blood meal, from aspirators, compared to 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 allowing *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. *Cx. 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 3.1x 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*, revealed more bird-feeding than we typically expect from this mammalophilic mosquito species[16,53,63]. The 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]. Between 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 per 1,000). Given the substantially lower infection rate compared to *Culex* spp. (infection rate of 11.03 per 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 *Aedes vexans* were absent, or present but uninfected, the role of *Aedes vexans* as a primary bridge vector seems unlikely. Indeed, relatively rare virus infection in *Aedes vexans* may reflect occasional feeding on infected robins but not significant vectorial capacity for WNV.

In this paper, we present a modified expression for the amplification fraction (A. M. Kilpatrick, personal communication) a measure of 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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Chapter 4

Landscape determinants of WNV infection in mosquitoes in Illinois, 2004-2006

Abstract

Many studies have investigated the relationship between environmental, demographic, and meteorological features of the landscape with West Nile virus (WNV) transmission. However, most of the studies use response variables such as human case data and vector abundance, which could provide biased measures of WNV transmission on the landscape. This study used Illinois mosquito infection data from 2004 to 2006, to identify landscape features that predict mosquito infection. We observed variability in the associations among three years but the most parsimonious multivariate model explaining *Culex* spp. mosquito infection rate included elevation and precipitation in 2004; precipitation, elevation, impervious surface, percent white, and median human age in 2005; and percent white people and vegetation in 2006. A negative relationship between precipitation and *Culex* infection emerged as the most consistent pattern explaining more variation than any other independent variable. Further multivariate tests reveal a 3-4 week time lag between a lack of rain and an increase in *Culex* infection in 2005. A review of related studies discovers highly variable results for the strength and direction of association between landscape features and WNV transmission. We speculate some of this variation could be explained by the response variable utilized, the geographic scale, and study location.

Introduction

There has been much interest in associating landscape features with arboviral transmission and human risk (Pavlosky 1966). Studies commonly use multiple environmental, demographic, or meteorological features of the landscape to predict thea measure of arbovirus tranmission. The response variable is commonly human disease case data (Brownstein et al. 2003, Ruiz et al. 2004, Ruiz et al. 2007, Brown et al. 2008b, DeGroote et al. 2008), vector abundance data (Diuk-Wasser et al. 2006, DeGroote et al. 2007, Brown et al. 2008a, Reisen et al. 2008, Rochlin et al. 2008), non-human host data (Cooke et al. 2006, Gibbs et al. 2006, Bradley et al. 2008, Pradier et al. 2008), vector infection data (Gu et al. 2006, Ozdenerol et al. 2008), or a combination of vector abundance and human case data (Winters et al. 2008). The goal of these studies to associate landscape features with arbovirus transmission is attractive for several reasons. First, the identification of locations of elevated human risk of exposure or regions of higher arboviral transmission will allow prioritization of vector abatement effort in space and time. Second, once features of the landscape or climatic conditions are identified to be important, human health departments, vector abatement agencies, and landscape architects will be better able to predict, prevent, and control enzootic and epidemic arboviral activity. Finally, assuming the results can be extrapolated to other regions, other public health officials can take appropriate actions to curb human risk of exposure.

To measure arbovirus transmission and make associations with features of the landscape, several problems emerge when using different types of response variables. First, the primary enzootic and epidemic vectors in the eastern U.S., *Culex pipiens*

(Hamer et al. 2008a), will remain within about 0.5 km of the location of production or acquisition of infection (Schreiber et al. 1988, Lapointe 2008). When using human case data, we assume the geocoded address represents the location of exposure, which is not always true (Eisen and Eisen 2007). Just as humans can have a travel history, hosts such as birds have much greater mobility and the sampling location might not represent the location of seroconversion. Also, the probability of human exposure to a pathogen is not uniform across the landscape simply due to the heterogeneous human land use patterns (i.e. residential versus natural areas). Second, human behavior can influence the patterns of exposure (Ostfeld et al. 2005), such as time spent outdoors and use of insect repellent, which could be heterogeneous in the urban environment (Gujral et al. 2007). Third, socioeconomic differences could influence the probability of seeking health care and racial differences (Sierra et al. 2007) might have different natural susceptibility to arboviruses. Even the variable case definition of the clinical diagnosis can result in inconsistency in diagnosis among regions. Finally, when a novel arbovirus arrives, a certain proportion of the human population will be susceptible immunologically (i.e. compromised immune system) and behaviorally (i.e. activities with higher risk of exposure). The exposure of these naive hosts would explain the pattern of high human case rates during the first year of establishment of an arobovirus in different regions of the U.S. Once established, possible herd immunity and behavioral changes could result in the lower observed human case incidence during the years after an arbovirus becomes endemic.

For mosquito-borne arboviruses, not only is the vector abundance important data to collect, but infection data are equally important since the two are not always

proportional in space or time (Andreadis et al. 2004, Hamer et al. unpublished data). Arboviruses such as West Nile virus (WNV) requires both abundant mosquito vectors as well as susceptible birds to amplify and spill over into human hosts (Hamer et al. 2008b). To our knowledge, only two studies have investigated relationships between mosquito infection and landscape features in the WNV system (Gu et al. 2006, Ozdenerol et al. 2008), and only one conducted the analysis at a fine spatial scale. Eisen and Eisen (2007) emphasize the use of crude spatial scales (e.g. county level) obscures fine scale patterns, and utilizing the census tract level is not a biological meaningful unit and introduces the modifiable area unit problem (Kitron 1998), where spatial units of analysis are arbitrary and variable (e.g. census blocks) and many not correspond to biological significance. For these reasons, the fine scale patterns, referring to the a scale relevant to production and dispersal of a mosquito vector, of association between WNV infection in mosquitoes and landscape features are in need of further investigation. We utilized a large 3-year database of mosquito infection with WNV from the state of Illinois to identify relationships with environmental, demographic, and meteorological features of the landscape.

Methods

Mosquito infection

Mosquito trap and WNV testing data for 2004-2006 were gathered from the state-wide database maintained by the Illinois Department of Public Health. Mosquitoes were collected from 1,722 trap locations using light traps, gravid traps, and aspiration (Figure 1). Traps are located primarily in urban areas in residential and semi-natural (e.g.

cemeteries, preserves) locations. Mosquitoes were sorted into pools of variable sizes and tested for WNV using VECTEST, RAMP, and/or qPCR. Most agencies (i.e. mosquito abatement districts) conducted VECTEST or RAMP and then submitted positive samples for qPCR testing. Agencies reported a location and collection date for each pool and trap address information was geocoded to street address. A combination of geocoding methods were used, including ESRI StreetMagUSA, ESRI geocoding (ESRI, Redlands, CA), and manually using Google Earth. Mosquito infection rate was calculated for each trap location for the whole season and for each week (CDC MMWR weeks). Only *Culex* spp. pools (primarily *Cx. pipiens* and *Cx. restuans*) were used in the Minimum Infection Rate (MIR) calculation (Biggerstaff 2006).

Geographic Information System database

We created a GIS database of landscape layers to model mosquito WNV infection rate. Land cover data was obtained from the Illinois Gap Analysis Land Cover Classification (1999-2000) and we re-classified the data to create a binary grid of vegetated (including agricultural areas) and non-vegetated surface. Impervious surface was obtained from the USGS Seamless Data Server which is a measure of any material that prevents the infiltration of water into the soil (Arnold and Gibbons 1996). A digital elevation model was obtained from the USGS Seamless Data Server. The Normalized Difference Vegetation Index (NDVI), a remotely sensed measure of greenness derived from MODIS data, was obtained from the Earth Science Data Interface and the Global Land Cover Facility (University of Maryland) for the time interval of 7/28/05 to 8/12/05. This time-frame was selected since is represents the time during WNV amplification in

mosquitoes in this study. Demographic data was obtained using the United States Census Bureau data at the block group. Temperature and precipitation data was gathered from the United States Geological Survey and the National Oceanic and Atmospheric Administration. To obtain precipitation and temperature data at mosquito trap locations, the stations distributed across Illinois (444 stations in 2004, 451 in 2005, and 456 in 2006) were interpolated using Inverse Distance Weighting in the Geostatistical Analyst (ArcGIS 9.2).

To extract landscape variables for each trap location, we first created a 0.5 km buffer around each trap in order to obtain a mean value around the trap location. The 0.5 km buffer size was chosen because this was the approximate buffer size that Diuk-Wasser et al. (2006) observed the highest relationship between landscape variables and *Cx. pipiens* abundance. We then used Zonal Statistics (Spatial Analyst Tools) to obtain a mean value of vegetation, impervious surface, elevation, and NDVI and then Extract Values to Points to add values to the mosquito trap attribute table (summary statistics in Table 4.1).

Statistical analysis

Spatial autocorrelation and spatial clustering was evaluated for mosquito infection data using Moran's I and Getis-Ord Gi (respectively) in Spatial Statistics Tools (ArcGIS 9.2) at distance thresholds of 0, 2.5, 5, 10, and 20 km.

Associations between *Culex* spp. infection, calculated for the entire season (week 17 to 40), and landscape variables were first explored using univariate linear regressions.

Table 4.1. Summary statistics of dependent and independent variables used in univariate and multivariate models to predict *Culex* spp. mosquito WNV infection in Illinois, 2004-2006. Mean infection rate and meteorologic data were for weeks 17 to 40.

Variable	Mean	St. dev.	Min.	Max.
Dependent				
Mosquito infection rate 2004	3.54	9.66	0.00	100.00
Mosquito infection rate 2005	6.09	12.58	0.00	100.00
Mosquito infection rate 2006	6.24	11.04	0.00	100.00
Independent				
Environmental				
Proportion vegetation (Veg)	0.53	0.31	0.00	1.00
Impervious surface (Imperv)	28.52	19.35	0.00	90.64
Digital elevation model (DEM)	631.50	125.50	314.60	948.00
Normalized Difference Vegetation Index (NDVI)	170.82	31.01	68.86	227.50
Demographic				
Medium age (MedAge)	38.00	6.28	14.20	70.40
Population density (PopDen)	3014.20	4998.90	5.00	46441.00
Proportion white people (PerWhite)	0.84	0.23	0.00	1.00
Meteorologic				
Total precip week 17-40, 2004 (p04ALL)	19.36	4.23	0.00	28.55
Total precip week 17-40, 2005 (p05ALL)	12.87	4.42	0.00	24.96
Total precip week 17-40, 2006 (p06ALL)	21.83	4.31	0.00	35.31
Average temp week 17-40, 2004 (t04ALL)	67.46	2.18	63.78	72.76
Average temp week 17-40, 2005 (t05ALL)	70.32	1.80	72.40	74.81
Average temp week 17-40, 2006 (t06ALL)	68.25	1.89	64.88	73.59

The mosquito MIR dependent variable was log(x+1) transformed to normalize the data. We then used only the variables with significant ($P < 0.05$) positive or negative relationships with infection in stepwise multivariate linear regressions. To minimize collinearity, we included only the variables with a Spearman rank coefficient of less than 0.05 and greater than -0.05. If two variables were significant in the univariate tests but were also collinear, we included the variable with the strongest correlation in the univariate test (largest coefficient). We assessed competing models using Akaike Information Criterion (AIC) for a measure of parsimony (lowest AIC value). Multivariate regressions were also used to assess the time lag between rain and a response in mosquito infection. Statistical analyses were conducted using R (R Development Core Team 2007).

Results

Mosquito infection patterns

A total of 956 mosquito trap locations were sampled in 2004, 892 in 2005, and 893 in 2006 in the state of Illinois (Figure 4.1). *Culex* spp. mosquitoes collected and tested in each year include 13,258 pools (581,651 individuals) in 2004, 13,271 pools (471,031 individuals) in 2005, and 893 pools (15,390 individuals) in 2006. Amplification events occurred in 2005 and 2006 during late-July to a peak weekly infection rate in early August of 10.5 per 1,000 and 16.5 per 1,000 in 2006 (Figure 4.2). The observed

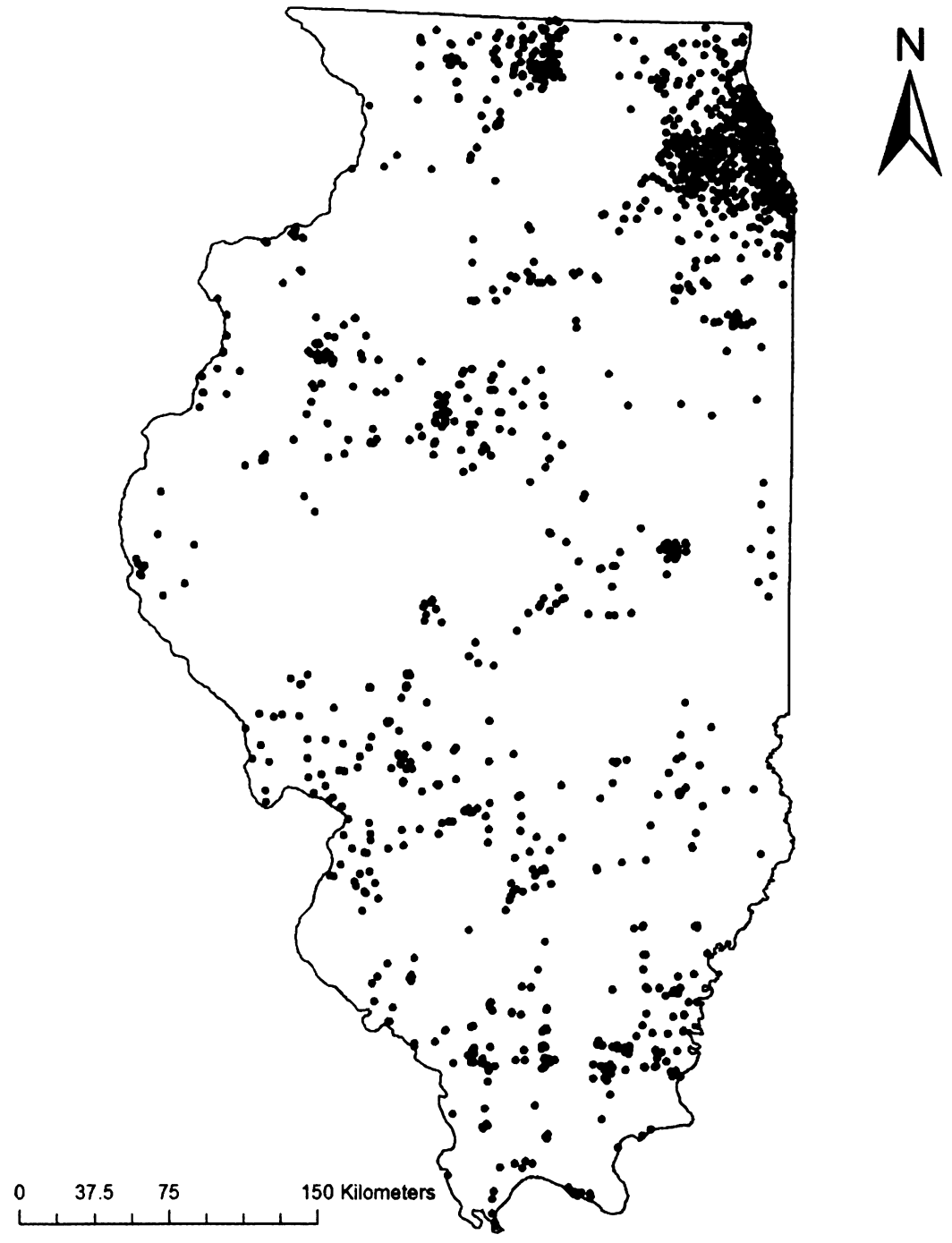

Figure 4.1. Distribution of the 1,722 mosquito trap locations used in the analysis.

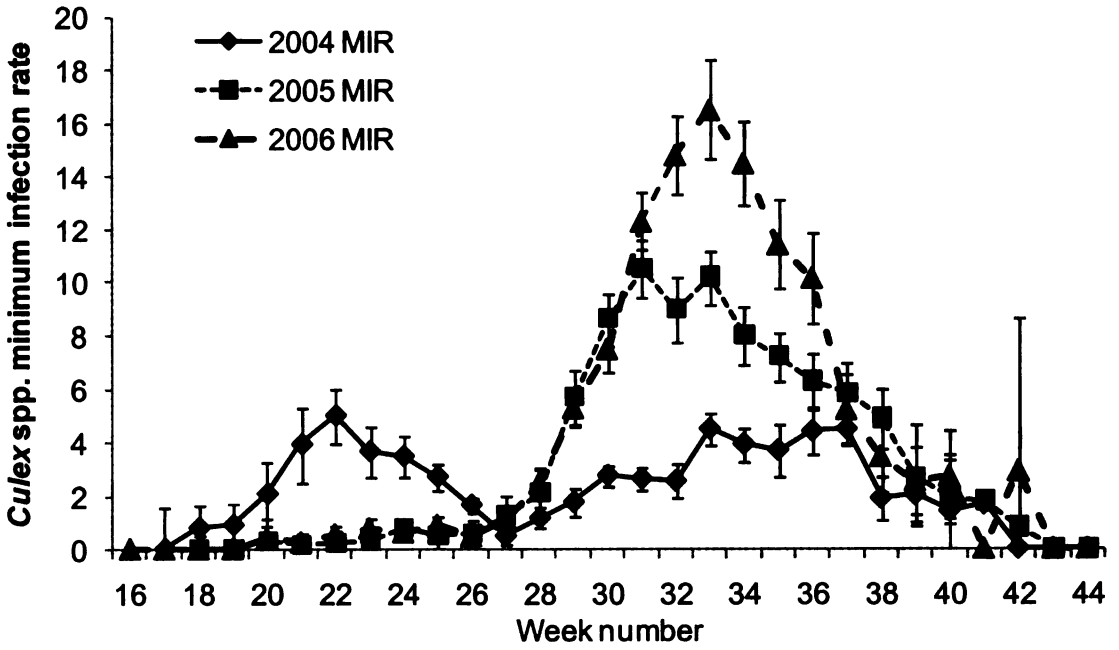

Figure 4.2. *Culex* spp. mosquito infection rate in Illinois from 2005 to 2006.

Table 4.2. Univariate regression coefficients on the associations between *Culex* spp. WNV infection and landscape variables in Illinois, 2004-2006.

Variable	2004	2005	2006
Environmental			
Proportion vegetation (Veg)	-0.270*	-0.977***	-1.723***
Impervious surface (Imperv)	0.004*	0.018***	0.017***
Digital elevation model (DEM)	0.002***	0.003***	0.001^{NS}
Normalized Difference Vegetation Index (NDVI)	-0.001^{NS}	-0.010***	-0.011***
Demographic			
Medium age (MedAge)	-0.006^{NS}	-0.022**	-0.022**
Population density (PopDen)	$< -0.001^{NS}$	< 0.001***	0.001***
Proportion white people (PerWhite)	-0.063^{NS}	-1.77***	-1.556***
Meteorologic			
Total precip week 17-40	-0.048***	-0.135***	0.023*
Average temp week 17-40	-0.180***	-0.309***	-0.123***

Significance: NS, no significance (P > 0.05); *P < 0.05; **P < 0.01; ***P < 0.0001

amplification season in 2004 was an atypical early and late moderate peak in early June and late August.

Tests show that there was significant spatial autocorrelation of *Culex* infection rate in 2005 at all distance thresholds tested, and the highest degree occurred at the 2500m (Moran's Index = 1.59, Z-score = 4.91). There was no spatial autocorrelation for the 2004 and 2006 infection rate data at any distance threshold tested (2500m distance threshold; I = 0.018, Z = 0.81; I = 0.135, Z = 0.44, respectively). Results of the hotspot analysis (Getis-Ord Gi*) showed significant clustering of *Culex* infection rate in 2005 but not 2004 and 2006 (2005 data at 2500m distance threshold; observed G = 0.000072, expected G = 0.000006, Z-score = 6.31).

Landscape determinants of mosquito infection

The results of univariate tests of associations between *Culex* infection rate and environmental, demographic, and metrological variables are summarized in Table 4.2. The proportion of vegetation, NDVI, proportion white people, precipitation, and temperature was negatively related to *Culex* infection with significance for at least two of three years. Factors positively related to *Culex* infection for at least two years include impervious surface, elevation, and population density.

Multivariate linear regression models using factors significant in the univariate tests but not collinear allowed evaluation of several competing models for 2004-2006 *Culex* infection rates (Table 4.3). The most parsimonious model for explaining *Culex* infection for each year was selected using the lowest AIC values. In 2004, the model

Table 4.3. Selected and competing linear regression models for *Culex* spp. mosquito infection rate in Illinois, 2004-2006.

Year	Model	df	r^2	P-value	AIC	Independent variables
2004						
	MIR04ALL1	3, 924	0.120	<0.0001	2633.71	DEM + p04ALL + Veg
	MIR04ALL2*	2, 925	0.121	<0.0001	2631.71	DEM + p04ALL
	MIR04ALL3	1, 926	0.079	<0.0001	2673.51	DEM
2005						
	MIR05ALL1*	5, 870	0.252	<0.0001	2653.14	p05ALL + DEM + Imperv + PerWhite + MedAge
	MIR05ALL2	4, 871	0.251	<0.0001	2653.53	p05ALL + DEM + Imperv + PerWhite
	MIR05ALL3	3, 873	0.251	<0.0001	2655.91	p05ALL + DEM + Imperv
	MIR05ALL4	2, 874	0.249	<0.0001	2656.17	p05ALL + DEM
	MIR05ALL5	1, 890	0.221	<0.0001	2734.17	p05ALL
2006						
	MIR06ALL1	4, 875	0.113	<0.0001	2799.47	PerWhite+Veg+MedAge+p06ALL
	MIR06ALL2	3, 876	0.108	<0.0001	2803.31	PerWhite + Veg + MedAge
	MIR06ALL3*	2, 877	0.109	<0.0001	2801.33	PerWhite + Veg
	MIR06ALL4	1, 878	0.077	<0.0001	2831.54	PerWhite

*Selected models

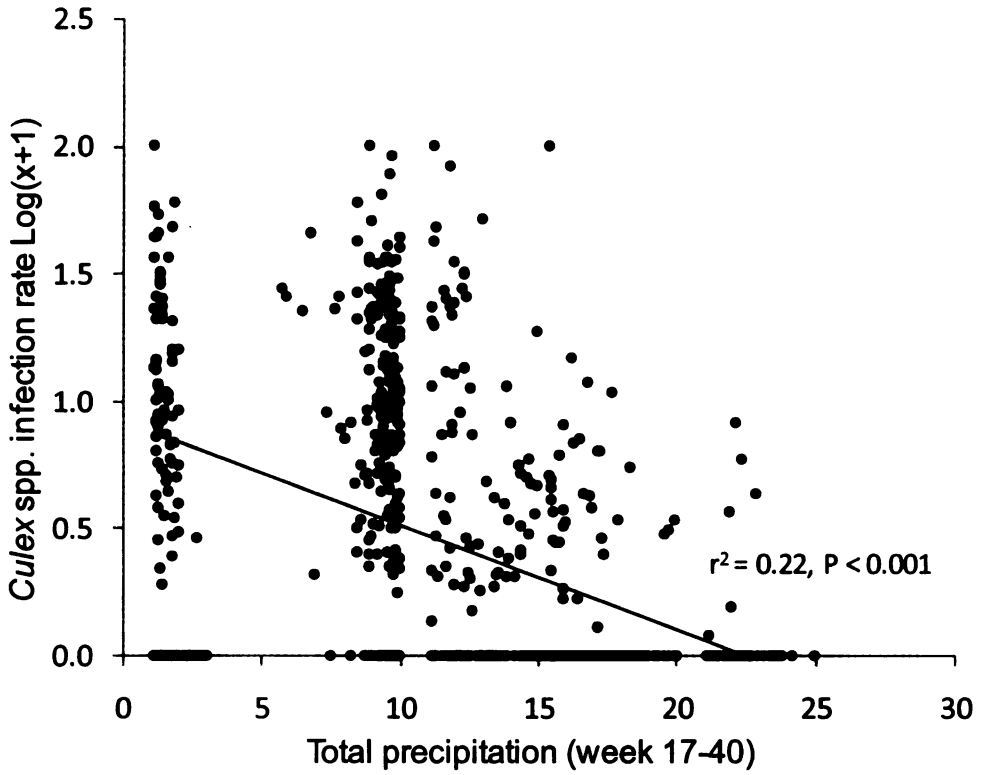

Figure 4.3. Relationship between 2005 *Culex* spp. infection rate and total precipitation (week 17-40) in Illinois.

Table 4.4. Multivariate regression coefficiencts on the relationship between *Culex* spp. infection rate (dependent) and weekly precipitation (independent) in Illinois, 2005.

	2005 weekly *Culex* spp. MIR					
Weekly precip.	29	30	31	32	34	34
17	-0.553	-0.972	-1.366*	-1.029	-0.502	-0.185
18	0.764	1.141	2.254	3.004	-0.104	-1.441
19	-0.261	-0.434	-0.652*	-0.204	-1.010**	-0.275
20	-0.034	0.491	0.502*	0.316	0.784**	0.374
21	1.094	2.875	1.034	-5.559	-1.078	0.949
22	-0.849	0.239	0.974	0.017	0.073	-0.683
23	-0.201	-0.126	-0.224	-0.346	-0.561	-0.361
24	-0.326	-0.298	-0.362	-0.533	-0.281	-0.218
25	-6.247*	-6.267*	-6.954*	-3.290	-4.126	1.449
26	-0.990**	-1.097**	-0.661	-0.371	-0.045	0.134
27	0.260	0.064	0.468	0.837*	0.256	0.716
28	-0.227	-0.521	-0.855**	-0.873***	-0.452	-0.311
29	0.023	-0.246	-0.321	-0.326	-0.483*	-0.049
30		-0.137	-0.267	-0.395**	-0.522***	-0.503**
31			-0.267	-1.327	0.113	0.259
32				-0.254	0.529	0.076
33					-0.447	-0.366
34						0.118

Significance: NS, no significance (P > 0.05); *P < 0.05; **P < 0.01; ***P < 0.0001

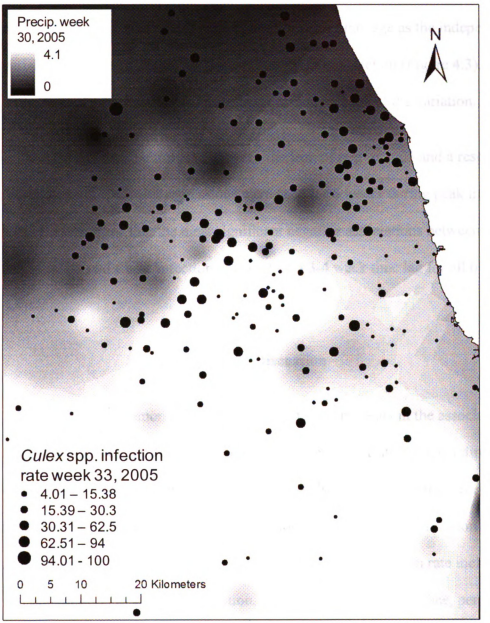

Figure 4.4. Relationship between *Culex* spp. infection rate during week 33, 2005 and total precipitation during week 30, 2005 in the Chicago, Illinois region.

explaining 12% of the variation in *Culex* infection included elevation and precipitation as independent variables. In 2005, the most parsimonius model included precipitation, elevation, impervious surface, percent white, and median age as the independent variables explaining 25.1% of the variation in *Culex* infection (Figure 4.3). In 2006, the proportion of white people and vegetation explained 11% of the variation.

To estimate the time lag between the lack of rain in 2005 and a response in *Culex* infection, multiple linear regressions were used for 6 weeks during peak infection (Table 4.4). Results show that the most significant negative associations between weekly total precipitation and *Culex* infection occurred on a 3-4 week time lag for all 6 weeks (Figure 4.4).

Discussion

This study demonstrates high variability among years in the association of landscape features and *Culex* infection rate of WNV. The strength and direction of the coefficients in the univariate regressions were only consistent for the three years of study for two of nine predictor variables (proportion of vegetation and elevation). Also, the most parsimonious multivariate model explaining *Culex* infection rate included elevation and precipitation in 2004, precipitation, elevation, impervious surface, percent white, and median age in 2005, and percent white people and vegetation in 2006.

The negative association between precipitation and *Culex* abundance and infection supports the results from previous studies observing patterns in the field

(Andreadis et al. 2004, Shaman et al. 2005). The hypothesis explaining this pattern with the leading support is that heavy rain events flush *Culex* spp. mosquito larvae out of catchbasins (Koenraadt and Harrington 2008). The 3-4 week time lag between the lack of rain and an increase in *Culex* infection observed in this study is first time this has been reported to my knowledge. In a much different WNV system vectored by *Culex nigripalpus* in southern Florida, Shaman et al. (2005) identified a 2-6 month time lag between drought and an increase in seroconversion of sentinel chickens and human case date of onset. In our study region, we have observed a 1-3 week time lag between *Culex* infection and bird and human infection (Hamer et al. 2008b). This means that we expect to have a 4-7 week time lag between a dry period and human risk of exposure. According to this result, rain events (or the lack of) during weeks 26-30 (the month of July) are critical to the potential for amplification. This pattern should be further tested to see if this pattern holds true for other years and in other regions.

Other aspects of the results are not expected, such as the negative association of temperature and positive association of elevation with *Culex* infection. These associations imply less *Culex* infection with higher temperatures and lower elevation. Most field and laboratory studies find increased temperatures associated with more infection due to the decreased extrinsic incubation period (Meyer et al. 1990, Dohm et al. 2002, Reisen et al. 2006). Most studies have found a negative association between elevation and infection (DeGroote et al. 2008, Ozdenerol et al. 2008; Table 4.5), which is explained as low, poorly drained areas being favorable to container breeding mosquitoes. However, the differences in these observations might be due to the different geographic extent of scale. In Illinois, the majority of the infection occurs in the northern region

around the city of Chicago, which also has a lower average summer temperature and higher elevation than the rest of the state. Kitron (1998, 2000) acknowledged the issue of geographic scale since relationships are subject to relativity. This issue of geographic scale could partly explain the variable results observed in a summary of the relationships between landscape features and WNV transmission (Table 4.5). Other factors contributing to the variable strength and directions could be temporal scale, response variable, geographic region, and primary enzootic and epidemic vector in study region. For these reasons, caution should be taken when interpreting relationships under different geographic scales and extrapolating predictive maps based on one geographic location to other regions.

Table 4.5. Selective review of relationships between landscape features and West Nile virus (WNV) transmission.

Predictor variable	Response variable	Geographic unit of analysis	Geographic extent	Strength and direction	Reference
Environmental					
Proportion vegetation	*Culex spp.* infection	Buffer around trap	State	neg***	This study
	Human cases	Census tract	County (2)	pos**	Ruiz et al. 2002
Percent forest	Human cases	County	State (8)	neg**	Brown et al. 2008
	Human cases	Census block	State	neg**	DeGroote et al. 2008
	Culex pipiens abundance	Buffer around trap	State	neg^{NA}	Diuk-Wasser et al. 2006
Impervious surface	*Culex* spp. infection	Buffer around trap	State	pos***	This study
Distance to riparian corridor	*Culex* infection	Trap	District (3)	neg**	Gu et al. 2006
NDVI	Infected mosquitoes	30m^2 grid	County	pos^{NA}	Ozdenerol et al. 2008
	Culex spp. infection	Buffer around trap	State	neg***	This study
	Human cases	Census tract	City	pos*	Brownstein et al. 2002
Slope	Human and bird cases	Zip code	State	neg*	Cooke et al. 2006
	Infected mosquitoes	Grid (30m^2)	County	neg^{NA}	Ozdenerol et al. 2008
Elevation	Infected mosquitoes	Grid (30m^2)	County	neg^{NA}	Ozdenerol et al. 2008
	Culex spp. infection	Buffer around trap	State	pos***	This study
	Human cases	Census block	State	neg**	DeGroote et al. 2008
Stream density	Human cases	Census block	State	pos**	DeGroote et al. 2008
	Human and bird cases	Zip code	State	neg*	Cooke et al. 2006
Heterogenious ag. areas	Equine serology	Grid (5km^2)	Regional park	pos*	Pradier et al. 2008
Road density	Human and bird cases	Zip code	State	pos**	Cooke et al. 2006
	Human cases	Census block	State	neg**	DeGroote et al. 2008
	Human cases	Census block	State	neg**	DeGroote et al. 2008
Percent urban land use	Human cases	County	State (8)	pos**	Brown et al. 2008
	Avian serology	Capture location	County (4)	pos*	Bradley et al. 2008
	Bird serology	Buffer around sample	State	pos*	Gibbs et al. 2006
	Human cases	Census block	State	neg**	DeGroote et al. 2008
Percent agriculture	Human cases	Census block	State	pos**	DeGroote et al. 2008

Significance: NA, not available; *P < 0.05; **P < 0.01; *** P < 0.0001

Table 4.5. Continued.

Predictor variable	Response variable	Geographic unit of analysis	Geographic extent	Strength and direction	Reference
Demographic					
Medium age	*Culex* spp. infection	Buffer around trap	State	neg**	This study
	Human cases	Census tract	County (2)	pos**	Ruiz et al. 2002
Housing density	Human cases	Census tract	County (2)	neg**	Ruiz et al. 2002
	Bird serology	Buffer around sample	State	neg**	Gibbs et al. 2006
Population density	Human cases	Census block	State	neg**	DeGroote et al. 2008
	Culex spp. infection	Buffer around trap	State	pos***	This study
Proportion white people	Human cases	Census tracts	County (2)	pos**	Ruiz et al. 2002
	Culex spp. infection	Buffer around trap	State	neg***	This study
Percent black population	Infected mosquitoes	Grid ($30m^2$)	County	pos^{NA}	Ozdenerol et al. 2008
Income	Infected mosquitoes	Grid ($30m^2$)	County	neg^{NA}	Ozdenerol et al. 2008
	Human cases	Census tract	County (2)	pos**	Ruiz et al. 2002
1950-1959 era housing	Human cases	Census tract	County (2)	pos**	Ruiz et al. 2002
1940-1960 era housing	Human cases	Census tract	County (mult.)	Pos^{NA}	Ruiz et al. 2007
Meteorologic					
Precipitation	*Culex tarsalis* abundance	Buffer around trap	County	pos**	Winters et al. 2008
	Culex spp. infection	Buffer around trap	State	neg***	This study
Precipitation - evaporation	Human and bird cases	Zip code	State	pos**	Cooke et al. 2006
Temperature	*Culex tarsalis* abundance	Buffer around trap	County	pos**	Winters et al. 2008
	Culex spp. infection	Buffer around trap	State	neg***	This study

Significance: NA, not available; *P < 0.05; **P < 0.01; ***P < 0.0001

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Chapter 5

Synthesis of project results and future research directions

Introduction

The introduction of West Nile virus (WNV) into North America in 1999 generated renewed public and scientific interest in arboviruses. Scientists who once studied Saint Louis Encephalitis and Eastern Equine Encephalitis shifted gears to study the rapidly emerging flavivirus novel to North American vectors and hosts. These scientists were well equipped to understand the basic principles of arbovirus transmission that guided quick and efficient studies to explore this new system and address basic research questions. A search for the term "West Nile virus" in Web of Science generated 3,664 records from 1943 to 2008, and 3,302 (90%) of those records were published from 1999 to 2008.

The renewed funding opportunities for studying WNV generated a cohort of students beginning their professional careers studying various aspects of this arbovirus. This system provides a model for studying disease pathology, epidemiological modeling, viral genetics, and transmission ecology. Although each arbovirus differs in various aspects, exposure to the fundamentals of disease ecology will provide new professionals the ecological, molecular, and analytical tools to continue to study the now-established WNV as well as future arboviruses and vector-borne pathogens.

This dissertation project was embedded within a collaborative effort studying the eco-epidemiology of West Nile virus emergence in urban areas. The goal of this greater project, funded by the National Science Foundation and National Institute of Health dual program in the Ecology of Infectious Diseases, was to study the process of invasion, establishment, and cycling of WNV in urban environments. We aimed to quantify this process using descriptive and predictive models, and to use a multidisciplinary, spatially realistic, comparative approach to associate disease patterns with risk factors and the biological process underlying these patterns. My dissertation research complemented the greater project by investigating host and vector interactions that facilitate WNV amplification.

Summary of results

Amplification

Collectively, this dissertation discovers a number of mechanisms necessary for the amplification of WNV in our study region. The climatic factors associated with the intensity of WNV transmission that we observed are consistent with other studies (Andreadis et al. 2004, Shaman et al. 2005), including less precipitation and higher temperatures in years of increased transmission. The only exception to this was my finding of higher temperature associated with increased transmission at the state-level, which is interpreted with caution due to the scale of the analysis (Chapter 4). The pattern of increased transmission with less rain, which is often contrary to popular belief, is due to the effect of rain on the vectors of WNV. Our own unpublished data as well as others (Koenraadt and Harrington 2008) demonstrates that *Culex* spp. mosquitoes are flushed

out of catch basins from heavy rain events. Reduced productivity of the primary vector reduces the amplification potential. The observation of increased disease transmission during increased temperatures is hypothesized to be related to shorter vector breeding cycles and a decreased extrinsic incubation period (EIP) in mosquitoes with higher temperatures (Reisen et al. 2006). The EIP, defined as the measure of time from ingestion of an infectious blood meal to the time that the mosquito is capable of delivering an infectious bite, decreases due to an increased dissemination rate as the virus passes through the mid-gut and becomes a systemic infection in the mosquito (Dohm et al. 2002).

Although much of the inter-annual variation in amplification intensity is due to climatic factors, this study also identified a number of biological and ecological factors necessary for amplification. A longitudinal study (Chapter 1) identified seasonal patterns of viral activity in mosquitoes, birds, and humans. This study demonstrated that the flush of hatch year birds during the breeding season was coincident with the amplification event. Additionally, viral activity peaked in birds about one week behind the peak in mosquito infection, and 79% of the individual birds testing virus positive were hatch year birds. The peak in human exposure of WNV occurred three weeks following the peak in mosquito infection, demonstrating the amplification process resulting in spill-over into humans. The emergence of these hatch year birds, which are immunologically susceptible hosts, provides fuel for the amplification process resulting in human risk of exposure.

We further explored how young birds contribute to transmission and amplification in a parallel study. We collected blood samples from nestling birds to identify if the interaction between nestling birds and mosquitoes facilitates amplification (Loss et al. in press). Although nestlings have been important amplification hosts in other arbovirus systems (Scott et al. 1990, Unnasch et al. 2006), this study found that nestling birds are not a focus of amplification events. Of the 194 nestling blood samples, only one tested virus positive and one tested seropositive. Also, a mosquito trapping experiment did not detect significantly more *Culex* spp. mosquitoes in traps mounted to active nest boxes than in control traps. Collectively, we identified the increase in hatch year birds in June-July is important for providing susceptible hosts, yet nestling birds are not largely responsible for amplification. We therefore speculate that fledgling birds, having left the nest and up to a few weeks old, are the individuals hosts largely responsible for amplification.

Vector and host incrimination

To further our understanding of the amplification process, we implemented a mosquito blood meal analysis. Identifying the vertebrate blood meal host from mosquitoes in our study region offers a number of opportunities, and this became a large portion of my dissertation research. The blood feeding patterns alone are not that informative, since host availability will dictate feeding opportunities. Instead, we quantified avian host availability using bird point count surveys which allowed us to calculate the *Culex pipiens* feeding preference. We identified the American robin as the only species significantly over-utilized, while several species were under-utilized,

including common grackle, house sparrow, and European starling. Using reservoir competence values derived in other studies, we were able to determine the amplification fraction for each bird species, which is defined as the number of infectious mosquitoes resulting from feeding on a given species. This allowed us to rank the bird species in order of relative importance to amplification, and found American robin (35%), blue jay (17%), and house finch (15%) as the top three species.

Additionally, the blood meal analysis allowed investigation of the various mosquito species and their role in the transmission cycle. We found that 80% of *Culex pipiens* and 81% of *Culex restuans* had avian-derived blood meals. This finding confirms previous reports that these two species are ornithophilic, and the primary species responsible for enzootic transmission and amplification. We are also able to incriminate the mosquito species responsible for transmitting WNV from birds to mammals (i.e. bridge vectors). Previous studies incriminate *Aedes vexans* as a bridge vector, due to their competence as a WNV vector, abundant populations, and tendency to feed on mammals, including humans (Turell et al. 2005, Molaei and Andreadis 2006). We identified 11% of *Aedes vexans* with avian-derived blood meals, and the remaining 89% with mammal-derived blood meals, which demonstrates the potential for this species to obtain WNV from an infectious bird and then feed on humans. However, using the three years of mosquito trapping data, we found very low infection rates for *Aedes vexans* (0.34 per 1,000) compared to *Culex* spp. mosquitoes (11.3 per 1,000). Moreover, we identified substantial human feeding by *Culex pipiens*, including a WNV positive individual containing a human-derived blood meal (Chapter 2). This provides direct evidence that *Culex pipiens* is capable of serving as a bridge vector.

An extension to the blood meal analysis study was performed in collaboration with Ted Andreadis at the Connecticut Agricultural Experiment Station. In our Chicago study region, we observed higher rates of mammal feeding by *Culex pipiens* than expected, and we hypothesized that genetic substructuring accounted for the difference in avian and mammal feeding. The individual *Culex pipiens* collected in 2005-2006 with identified hosts (n = 346) in this study were investigated using a microsatellite analysis of 10 polymorphic markers (Huang et al. in press). The individual *Cx. pipiens* with mammal-derived blood meals had significantly higher ancestry and proportion of hybrids with the *Cx. pipiens molestus* form. *Cx. pipiens molestus* has been identified in London, New York, and Washington DC, and is characterized as having higher inclination for mammal feeding and breeding and living in underground urban structures (Harbach et al. 1984, Byrne and Nichols 1999, Fonseca et al. 2004, Kilpatrick et al. 2007). This finding further suggests that feeding preferences have a genetic basis and may explain the relatively high rates of human WNV incidence in Chicago, Illinois relative to other mid-western and eastern cities where *Cx. pipiens* is the dominant vector.

Future research directions

Fortunately, the collaborative project, initially funded for three years, was renewed for another five years which will allow continued investigation of arbovirus transmission, amplification, and evolution using the WNV system in the urban environment. In 2008, we conducted a field season with reduced effort which will eventually provide eight consecutive seasons of empirical data collected at a fine spatial scale in an urban focus of transmission. Our initial phase of investigation in our study

region allowed us to uncover a number of patterns and mechanisms of amplification using mostly pooled data among our study sites. However, based on patterns of mosquito infection and abundance and avian infection, seroprevalence, and abundance, we see high variation among our studies sites. These patterns lead us to hypothesize that amplification events occur at very small spatial scales where mosquitoes and birds come into contact and are not static in space or time. This shifting mosaic of hot spots for WNV transmission is likely related to bird and mosquito movement and distribution patterns, and our initial study was not designed to address this.

The next phase of research will study bird movement and roosting behavior using radio telemetry, especially for fledgling American robins, which we have identified as the most important amplification host in our study region. Additionally, it will be equally important to study *Culex* mosquito distribution and movement. *Culex* relative densities will be assessed in a randomized trapping strategy and movement, dispersal, and source of productivity will be assessed using a mark and re-capture design. Instead of using the traditional fluorescent dust to mark individual adult mosquitoes, we plan to use novel stable isotope techniques. We plan to treat catchbasins producing *Culex* mosquitoes with food labeled with unique stable isotopes. Then the labeled adults would be captured in a trap array surrounding the study site to detect direction and distance of movement. With detailed knowledge of bird and mosquito movement and distribution, we will be able to test our hypothesis that amplification events occur at very small spatial scales. This research has the potential for novel insights into the WNV system that could allow management intervention to break these local amplification events that spill over into regional patterns of elevated transmission resulting in human exposure.

West Nile virus as a model arbovirus

Part of the motivation for the continued funding of this project is not only to learn more about WNV, but to also learn more about this system as a model to be able to predict and react to the emergence of a future arbovirus novel to North America. We are in a unique situation to have learned a great deal about local WNV transmission in our study region during the first phase of the project, and expect to gain additional and potentially novel findings in the next phase.

The key elements of WNV transmission, amplification, and evolution that we have gained and continue to pursue are fundamental to this disease system. Eventually, our empirical data will be utilized in an epidemiological model to describe how the virus circulates among mosquito vectors and avian hosts with spill-over into humans. These individual-based compartmental models, describing avian hosts as being susceptible, infected, and removed (SIR), have the ability to simulate transmission under different conditions to observe the results (Wonham et al. 2004, Shaman 2007). This quantitative tool will give us a valuable perspective on the WNV system. Not only will parameters we derive in the field and gather from literature be utilized to test hypotheses, but unknown parameters can be estimated by fitting the model to empirical data. Repeated simulations of this model under multiple conditions and parameters will allow examination of the distribution of outcomes, such as extinction, maintenance, or amplification of WNV. These simulation models will offer the opportunity to identify the most effective forms of management intervention, reducing amplification and spill-over into humans. Ultimately, a simulation model that could be implemented in real time

in June and July could allow parameter setting based on gathered information (temperature, precipitation, *Culex* abundance and infection, etc.) to predict where, when, and to what degree WNV amplification might occur (Theophilides et al. 2003).

The value of developing a model to describe West Nile virus transmission is the ability for the model to be generalized and adapted to other disease systems. If a new pathogen emerges in the United States, we will have the ability to quickly adapt a model with new compartments and parameters for a different disease system. For example, an emerging arbovirus resulting in epidemics in Europe and South Asia is chikungunya (Powers and Logue 2007). Chikungunya is an alphavirus, related to Eastern Equine Encephalitis, transmitted among a mosquitoes and primates. The principal vectors are *Aedes albopictus* and *Aedes aegypti*, both of which are established in the southeastern U.S. In sylvatic transmission cycles in Africa, the virus is transmitted among mosquitoes and non-human primates, but urban cycles involve humans as the main reservoir host. Unlike WNV, humans are capable of developing an infectious titer of chikungunya, able to re-infect a mosquito. The U.S. appears to be a receptive environment making the establishment of an urban cycle plausible, especially in the south-eastern U.S.

Although chikungunya has a number of differences with WNV, we would quickly be able to adapt a simulation model for the disease system, which would allow insights into management strategies to minimize or eradicate the agent. Our understandings of WNV transmission, amplification, and evolution and the diverse tools we use to study this system will be valuable lessons and skills to apply to future vector-borne diseases.

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