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Avian host community structure and prevalence of West Nile virus in Chicago, Illinois

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Abstract Vertebrate host diversity has been postulated to mediate prevalence of zoonotic, vector-borne diseases, such that as diversity increases, transmission dampens. This "dilution effect" is thought to be caused by distribution of infective bites to incompetent reservoir hosts. We quantified avian species richness, avian seroprevalence for antibodies to West Nile virus (WNV), and infection of WNV in *Culex* mosquitoes, in the Chicago metropolitan area, Illinois, USA, a region of historically high WNV activity. Results indicated high overall avian seroprevalence and variation in seroprevalence across host species; however, there was no negative correlation between avian richness and *Culex* infection rate or between richness and infection status in individual birds. Bird species with high seroprevalence, especially northern cardinals and mourning doves, may be important sentinels for WNV in Chicago, since they were common and widespread among all study sites. Overall, our results suggest no net effect of increasing species richness to West Nile virus transmission in Chicago. Other intrinsic and extrinsic factors, such as variation in mosquito host preference, reservoir host competence, temperature, and precipitation, may be more important than host diversity for driving interannual variation in WNV transmission. These results from a fine-scale study call into question the generality of a dilution effect for WNV at coarser spatial scales.

Keywords Dilution effect · Spatial scale · Sentinel species · Reservoir host · Host competence

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Introduction

Since its original introduction to the United States in 1999, West Nile virus (WNV) has become the dominant mosquito-borne viral infection of humans in North America (Lanciotti et al. 1999; McLean 2006). Transmission is maintained through a cycle between bird reservoirs and mosquito vectors, primarily in the genus *Culex*, with humans, horses, and other mammals incidentally infected (Hayes 1989; Komar et al. 2003). Infection with WNV has been responsible for human and equine morbidity and mortality (Bernard et al. 2001; Petersen and Roehrig 2001) and

regional and local declines of bird populations (Naugle et al. 2004; Rocke et al. 2005; LaDeau et al. 2007).

Increased diversity of vertebrate hosts has been hypothesized to decrease disease transmission of zoonotic, vector-borne pathogens through a so-called "dilution effect," where cumulative addition of incompetent reservoir species dampens prevalence of infection by reducing contact rates between vectors and the more competent species of reservoir hosts (Ostfeld and Keesing 2000a). The hypothesis assumes that vectors bite incompetent and competent hosts nonselectively. The dilution effect was originally proposed for Lyme disease (Ostfeld and Keesing 2000b), but dilution is a viable hypothesis for other vector-borne zoonotic disease systems as well (Holt et al. 2003; Peixoto and Abramson 2006), including WNV (Ezenwa et al. 2006; Swaddle and Calos 2008). Indeed, prevalence of WNV infection in mosquito and bird populations may be modulated by heterogeneity in vector or reservoir competence and contact rates between birds and mosquitoes (Woolhouse et al. 1997; Komar et al. 2003; Turrell et al. 2005; Kilpatrick et al. 2006). Recent research has focused on the relationship between host community structure (i.e., species richness and relative abundance of individual species) and prevalence of WNV and other arboviruses (reviewed by Keesing et al. 2006; Kilpatrick et al. 2006,), as well as effects of avian population age structure (Hamer et al. 2008). These relationships remain unclear, however, because under certain circumstances high host diversity apparently provides a suite of competent hosts that allow for persistence and intensification of arbovirus transmission, even given increased frequency of reservoir incompetent hosts (Norman et al. 1999; Gilbert et al. 2001; Keesing et al. 2006).

We investigated the relationship between bird community structure and prevalence of WNV transmission in birds and mosquitoes in Chicago, Illinois, a region of historically high WNV transmission (Ruiz et al. 2004). By the end of 2006, 1,465 human cases of illness from WNV had been reported in Illinois, with the majority of these occurring in the Chicago metropolitan area (Illinois Department of Public Health (2008)). Intensive simultaneous collection of bird and mosquito data across two transmission seasons and across a wide range of urban habitats at a fine scale provides an ideal opportunity for addressing the following research questions: (1) Is avian richness negatively correlated with prevalence of infection in bird and mosquito populations? (2) what bird species display the highest WNV seroprevalence rates in the Chicago area? We hypothesized that increasing species richness of avian hosts decreases overall WNV prevalence. We therefore predicted an inverse relationship between host richness and infection rate in mosquitoes and between richness and infection status in birds.

Materials and methods

Study area

We sampled mosquito and bird communities in the Chicago metropolitan area, Cook County, Illinois, from May to October in 2005 and 2006. In 2005, nine study sites were selected on Chicago's south side urban/suburban interface, an area with known clusters of human WNV cases during the 2002 outbreak (Ruiz et al. 2004). Five of these sites were selected based on their residential classification (>35% using land cover mapping, Illinois Department of Agriculture 2008). Residential sites were selected to represent a range of human population densities with varying proximity to large tracts of natural land (US Census Bureau 2000). Additionally, we selected four semi-natural sites that included three cemeteries and a wildlife refuge. In 2006, we used the same selection criteria to select four additional residential sites, which encompassed a larger proportion of the Chicago metropolitan area (Fig. 1).

Estimation of bird species richness and abundance

We established transects of avian point counts at each site. Due to differences in size of sites, transects consisted of five survey points in residential sites and eight survey points in natural sites. Survey points were distributed evenly across

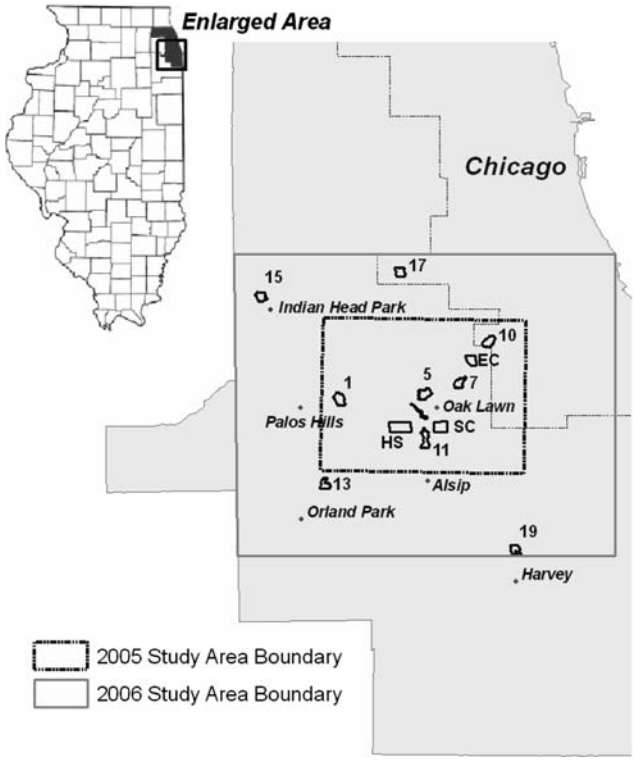

Fig. 1 Map of study area in the Chicago metropolitan region, Cook County, Illinois. Sites 13, 15, 17, and 19 were sampled in 2006, but not 2005; all other sites were sampled in both 2005 and 2006

sites, and points were located at least 0.5 km apart to prevent double counting of birds, in accordance with Breeding Bird Survey protocols (US Geological Survey, North American Breeding Bird Survey). Survey points that were found to be situated in inaccessible, noisy, or high traffic areas were relocated as needed. Each study site was surveyed once in June and once in July, to coincide with the peak avian breeding season in the Chicago area (Kleen et al. 2004). Five-minute unlimited radius point counts were conducted at each survey point (Reynolds et al. 1980), and distance to each observed bird was recorded. We conducted all surveys between 0.5 h before sunrise and 4.0 h after sunrise (0530–1000 a.m.) on days with no precipitation and wind speeds less than 24 km/h.

Collection of bird and mosquito samples

Birds were captured using mist nets (ATX type, 6 or 12-m length, 36-mm mesh, Avinet Inc.). Each site was sampled six times during 2005 and five times during 2006; sites were sampled every 3 weeks between May and August, and every 5 weeks in September and October. All captured birds were identified, aged, sexed, weighed, measured, and marked with US Fish and Wildlife Service bands (US Department of Interior Bird Banding Laboratory), as authorized by Federal Bird Banding Permit no. 06507. We collected blood samples by jugular or brachial venipuncture, using a 25-gauge tuberculin syringe or a 28-gauge insulin syringe. Blood samples did not exceed 0.2 ml or 10% of total bird blood volume. Diluent (BA-1) was added to each blood sample in a 2.0-ml microcentrifuge tube. The amount of diluent added depended upon the volume of the blood sample, such that all samples were later screened for antibodies at a 1:20 dilution (Hamer et al. 2008). Samples were kept cold and then centrifuged within 5 h of collection. Supernatants were transferred to 2.0-ml cryovials; both clots and supernatant were stored at −20 or −80°C.

Adult mosquitoes were collected from each of the study sites every 2 weeks from May to October during both field seasons. At each site visit in 2005, adult mosquitoes were collected using four CO_2-baited CDC light traps (two within 2 m of ground level and two in the tree canopy), four CDC gravid traps baited with rabbit pellet infusion (Lampman and Novak 1996), and a battery-powered backpack aspirator (Meyer et al. 1990). The same sampling technique was used in 2006, except ground-level light traps were eliminated, since significantly more *Culex* mosquitoes were captured in elevated traps in 2005. Female mosquitoes were identified to species (Andreadis et al. 2005) and were divided into pools of 25 or fewer individuals. Pools were grouped by date, study site, and species, and were placed in 2.0-ml microcentrifuge tubes in long-term storage at −20 or −80°C until laboratory testing.

Laboratory testing of samples

We estimated avian seroprevalence, since transmission, the passing of disease from one individual to another, is difficult to directly measure in the field. Seroprevalence represents the percentage of a bird population with WNV antibodies at a given time. Not all birds infected with WNV produce an antibody response. Moreover, seroprevalence may depend upon the duration of immunity (Bernasconi et al. 2002; Zinkernagel and Hengartner 2006), persistence or reactivation of infection in the host (Gylfe et al. 2000; Staszewski et al. 2007), and the mortality rate of the affected species. These complications limit interpretation of seroprevalence results; however, it is difficult to gather large samples of birds displaying active WNV infections (Hamer et al. 2008). We used epitope blocking enzyme-linked immunosorbent assay (ELISA) to detect WNV antibodies in bird serum samples (Hamer et al. 2008). Two positive serum controls and four negative serum controls were used as references on each plate. Samples that were positive upon first screening were serially diluted up to 1:640 and retested to determine end point titers.

For mosquito virus testing, 1 ml of a 50:50 mixture of phosphate-buffered saline (PBS) and 2× lysis buffer (Applied Biosystems, Foster City, CA) and three number seven steel shot were added to each tube, and then mosquitoes were homogenized (Retsch MM 300 high-speed mechanical homogenizer, 4 min at 20 cycles/s), followed by centrifugation for 2 min at 13,000 rpm at 4°C. 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 µl of elution solution. These extracts were subjected to real-time, reverse transcription-PCR (RT-PCR) to detect a region of the WNV envelope gene (Lanciotti et al. 2000). The primer-probe set consisted of forward primer 5′-TCAGCGATCTCTCCAC CAAAG-3′, reverse primer 5′-CAGCACGTTTGTCATT G-3′, and probe 6FAM-5′TGCCCGACCATGGG-3′MGB NFQ (Lanciotti et al. 1999). Reactions were carried out using 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). Cycling parameters consisted of 48°C for 30 min for RT, 95°C for 10 min, and 40 cycles of 95°C for 15 s and 60°C for 1 min.

Data analyses

We restricted analyses of avian richness and relative abundance to species that were likely breeding in the region (i.e., migrants or extremely rare species were not considered), nor did we consider waterfowl, gulls, herons, raptors,

and shorebirds, due to their wide-ranging habits and tendency for extensive daily movements in the region. Density estimates for the remaining bird species were derived from detection functions using the program Distance 5.0 (Thomas et al. 2005). Relative abundance of individual species was calculated by dividing species density by total bird density. To meet normality assumptions, all relative abundance values were arcsine-transformed. For species richness, we calculated numbers of species per census point, since the number of points varied among sites. Transformation of richness was unnecessary since the distribution of richness values was approximately normally distributed.

Unequal sampling effort among sites can lead to biased richness estimates. Rarefaction methods address this potential source of bias by using data from a larger sample to estimate richness in sites receiving less sampling effort (Simberloff 1972). Spatial arrangement of survey points in this study resulted in thorough sampling of each study site, and we observed high detectabilities (>80%) during these surveys. At sites with eight survey points, species accumulation saturated within the first five survey points. Differences in survey effort between residential and semi-natural sites were therefore unlikely to bias richness estimates; thus, rarefaction methods were not used for this analysis.

Culex infection rates (IR) were calculated using maximum likelihood estimation with 95% confidence intervals and the Pooled Infection Rate version 3.0 add-in (Biggerstaff 2006) for Excel (Microsoft 2005). We focused on *Culex* mosquitoes because they have been implicated as important WNV vectors (Turrell et al. 2005), and they comprised >70% of total mosquito captures.

We modeled the association between bird community structure and *Culex* IR at the study site level, using a general linear model with predictor variables, including avian richness, year, seroprevalence (combined for all bird species at the site), and combined relative abundance of bird species with high seroprevalence (i.e., all species with seroprevalence >20% in either 2005 or 2006). This cutoff point corresponded to twice the total avian seroprevalance for the 2 years of study. To identify factors affecting whether individual birds were seropositive, we developed an individual-based logistic regression model with WNV antibody status (1 = seropositive, 0 = seronegative) as the dependent variable. Continuous predictor variables included avian richness and *Culex* IR at the site where the bird was captured. We also coded categorical predictors, species identity and year, as dummy variables to assess whether year of capture or species identity affected the probability of an individual testing seropositive . Finally, we tested for spatial autocorrelation among WNV seroprevalence values using the weighted K-function option in the program point pattern analysis (Chen et al. 2000).

Results

Seroprevalence of bird community and individual species

We collected 2,151 serum samples from a total of 60 species. Due to repeat visits to study sites, 90 of these samples came from recaptured birds. To avoid pseudoreplication, we only considered the first capture event for each bird in the following analysis; thus, we present results based on 2,061 serum samples. Antibodies to WNV were detected in 16 of the 60 species, and seroprevalence for the entire study area and for all age groups and species combined was 20.5% in 2005 and 3.5% in 2006. Seroprevalence of juvenile (hatching year) birds, which reflects new WNV infections during a particular season, decreased from 18.5% in 2005 to 2.4% in 2006. Of 2,030 birds that were aged, seroprevalence was nearly identical for juveniles (11.4%, $n = 948$) and adults (after hatching year; 11.5%, $n = 1,082$).

Seroprevalence varied considerably across species (Table 1). Mourning doves (*Zenaida macroura*), northern cardinals (*Cardinalis cardinalis*), and house finches (*Carpodacus mexicanus*) displayed seroprevalence values greater than 10% in both years. Notably, northern cardinal seroprevalence was 75.8% ($n = 66$) in 2005, decreasing to 20.4% ($n = 49$) in 2006. Though sample sizes were small each year, mourning doves displayed consistently high seroprevalence of 57.1% ($n = 14$) in 2005 and 58.3% ($n = 12$) in 2006. House sparrows (*Passer domesticus*), the most abundant and widespread species in the study area, exhibited a drastic decline in seroprevalence from 23.5% ($n = 302$) in 2005 to 0.0% ($n = 349$) in 2006. Our results indicated a decrease in average numbers of positive mosquito pools and a non-significant decrease of *Culex* IR from 12.32 in 2005 to 9.71 in 2006 ($t = 0.91$, $df = 8$, $P = 0.39$, Table 2). Seroprevalence for a species was not related to the relative abundance of that species (Spearman's rank correlation coefficient, $r = 0.01$, $P = 0.60$).

Predictors of *Culex* infection rate and West Nile virus antibody status in birds

Culex IR was not significantly correlated with year, avian richness, relative abundance of bird species with high seroprevalence, nor to total seroprevalence (Table 3). Likewise, antibody status of individual birds did not depend upon avian richness or species identity (Table 4). Antibody status was primarily a function of year effects, as individual birds were significantly less likely to test seropositive in 2006 than in 2005 (odds ratio = 0.40, $P < 0.01$). Birds from sites with higher *Culex* IR were also more likely to test antibody positive (odds ratio = 582.16, $P < 0.01$, Table 4).

We compared the avian community between 2005 and 2006 to determine whether significant changes in richness

Table 1 West Nile Virus seroprevalence for wild bird species testing seropositive by enzyme-linked immunosorbent assay (ELISA) during field sampling in the Chicago, IL, metropolitan area, 2005–2006

Species	2005			2006			Total		
	N	No. pos	Percentage pos	*N*	No. pos	Percentage pos	*N*	No. pos	Percentage pos
Mourning Dove (*Zenaida macroura*)	14	8	57.14	12	7	58.33	26	15	57.69
Northern Cardinal (*Cardinalis cardinalis*)	66	50	75.76	49	10	20.41	115	60	52.17
Common Yellowthroat (*Geothlypis trichas*)	1	0	0.00	3	1	33.33	4	1	25.00
Blue Jay (*Cyanocitta cristata*)	3	0	0.00	2	1	50.00	5	1	20.00
House Finch (*Carpodacus mexicanus*)	36	5	13.89	22	4	18.18	58	9	15.52
Gray Catbird (*Dumetella carolinensis*)	71	16	22.54	60	0	0.00	131	16	12.21
Brown-headed Cowbird (*Molothrus ater*)	12	3	25.00	12	0	0.00	24	3	12.50
European Starling (*Sturnus vulgarus*)	21	4	19.05	33	2	6.06	54	6	11.11
House Sparrow (*Passer domesticus*)	302	71	23.51	349	0	0.00	651	71	10.91
Red-winged Blackbird (*Agelaius phoeneceus*)	36	6	16.67	31	1	3.23	67	7	10.45
American Robin (*Turdus migratorius*)	160	28	17.50	206	9	4.37	366	37	10.11
Baltimore Oriole (*Ictera galbula*)	4	1	25.00	8	0	0.00	12	1	8.33
Song Sparrow (*Melospiza melodia*)	45	3	6.67	43	0	0.00	88	3	3.41
American Goldfinch (*Carduelis tristis*)	41	1	2.44	87	3	3.45	128	4	3.13
Common Grackle (*Quiscalus quiscula*)	12	1	8.33	33	0	0.00	45	1	2.22
Swainson's Thrush (*Catharus ustulatus*)	33	1	3.03	14	0	0.00	47	1	2.13
All species tested	965	198	20.52	1096	38	3.47	2061	236	11.45

Boldface indicates species included in high seroprevalence (>20%) calculation used for general linear model analysis of *Culex* infection rate

Table 2 Summary of mosquito collection and estimates of *Culex* spp. infection rates (using maximum likelihood estimation) for study sites during field sampling in the Chicago, IL, metropolitan area, 2005–2006

Site	2005				2006			
	No. pools	No. pos pools	Infection rate	95% CI	No. pools	No. pos pools	Infection rate	95% CI
1	68	13	9.68	5.42–16.18	69	8	6.47	3.04–12.30
5	58	13	12.54	7.05–20.93	64	14	15.22	8.73–25.06
7	64	13	11.34	6.37–18.94	67	11	9.35	4.96–16.30
10	26	4	9.74	3.20–23.65	20	1	5.84	0.33–29.63
11	68	13	9.99	5.62–16.66	52	10	11.61	6.00–20.68
13					71	8	6.23	2.92–11.85
15					39	8	11.66	5.49–22.25
17					30	2	4.75	0.85–15.75
19					57	11	10.96	5.80–19.15
EC	36	6	9.44	3.91–19.65	100	21	10.64	6.80–16.00
HS	115	31	13.67	9.49–19.19	67	16	15.02	8.96–23.97
SC	68	13	9.71	5.43–16.23	48	11	12.28	6.51–21.47
WW	101	44	24.80	18.29–33.15	49	6	6.18	2.55–12.85
Total study area	604	150	12.32		733	127	9.71	

or community composition contributed to the strong year effects noted in the individual-based model. Though average bird species richness decreased from 21 species (range = 10–32, SD = 8.01) in 2005 to 18 species (range = 9–31, SD = 7.32) in 2006, the change was not statistically significant (paired *t*-test, $t = 2.08$, $df = 8$, $P = 0.07$). Relative abundance of the high seroprevalence species group was also similar between years ($t = 0.98$, $df = 8$, $P = 0.36$), and there were no major changes in species abundance rank. There was no evidence of spatial autocorrelation for

Table 3 Results of general linear model for *Culex* infection rate (IR) as a function of year, avian richness, seroprevalence, and combined relative abundance of highly infected species (i.e., all species with seroprevalence >20% in either 2005 or 2006)

Predictor variable	Coefficient	95% confidence interval	*t*	*P*-value
Constant	12.76	−3.54–29.06	1.65	0.12
Year	0.95	−5.41–7.3	0.31	0.76
Avian richness	−1.67	−8.21–4.87	−0.54	0.60
Seroprevalence	20.02	−9.42–49.46	1.44	0.17
Highly infected	−6.69	−15.47–2.1	−1.61	0.13

Table 4 Results of logistic regression model relating avian richness, year, *Culex* infection rate (IR), and bird species identity to antibody status (1 = seropositive, 0 = seronegative) of individual birds captured in the Chicago metropolitan area, 2005–2006

Predictor Variable	Coefficient Estimate	Odds ratio (95% CI)	*P*-value
Constant	−16.7	–	0.99
Year	−0.91	0.40 (0.22–0.73)	<0.01
Richness	0.22	1.24 (0.74–2.09)	0.41
Culex IR	6.37	582.16 (64.06–5290.12)	<0.01
Species[a]	–	–	>0.90

[a] Coding of dummy variables for species identity resulted in a predictor variable for each species tested (not shown in table). None of the species variables were significant predictors of individual antibody status (*P*-values all 0.90 or greater)

seroprevalence in 2005 or 2006 (Fig. 2), which suggests that modeling results were not biased by spatial non-independence.

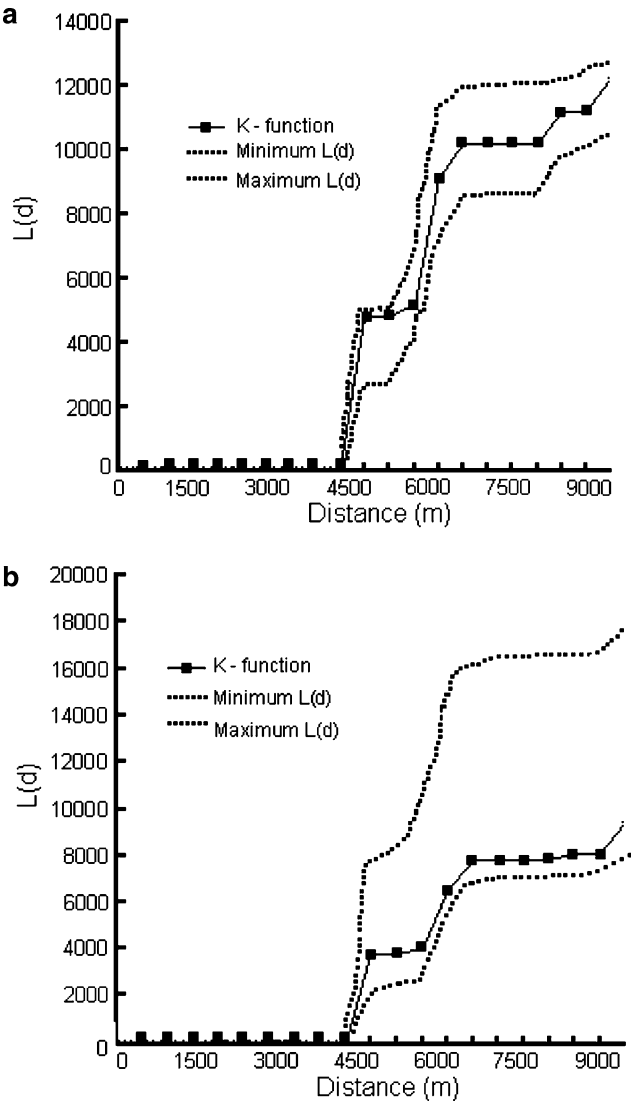

Fig. 2 Weighted K-function for seroprevalence values in 2005 (**a**) and 2006 (**b**). *Dotted lines* indicate the range of *L*(*d*) values generated from Monte Carlo simulations, *solid line* indicates the *K*-function calculated from observed data, and the *x*-axis indicates the distance at which presence of clustering is tested. Since the *K*-function lies completely within the simulation range in both 2005 and 2006, there is no evidence for spatial autocorrelation

Discussion

We found no evidence to support the hypothesis that avian richness is negatively correlated to prevalence of WNV in the Chicago metropolitan area. Relative abundance of individual species with high seroprevalence was also unrelated to mosquito infection rates. Our results indicate high overall avian seroprevalence and variation in seroprevalence across host species. Northern cardinals and mourning doves displayed the greatest seroprevalence values; since they were also widespread and common, these species may be important sentinels for WNV in the Chicago area.

The importance of scale and other ecological factors affecting WNV transmission

As evidenced by viral sequence data, WNV transmission and evolutionary dynamics operate in response to fine-scale environmental and anthropogenic features of the urban landscape (Bertolotti et al. 2008). This study was conducted at a finer spatial scale than the majority of previous studies, which typically address the dilution effect at county, state, and regional scales (reviewed by Keesing et al. 2006; Swaddle and Calos 2008). Dilution effects were modeled at fine spatial scales in New York (Schmidt and Ostfeld 2000), and Ezenwa et al. (2006) also focused on the scale of local study sites in Louisiana. Our fine-scale results from the Chicago metropolitan area cast doubt on whether increased host biodiversity has a net effect on WNV prevalence. Without corroborating evidence of similar phenomena

operating at this mechanistic scale, coarse scale diversity-prevalence relationships may be merely correlational, not causative.

Ezenwa et al. (2006) indicate that high diversity of non-passerine species (i.e., raptors, waterfowl, etc.) may be responsible for diluting WNV transmission in Louisiana; however, the authors also failed to detect a relationship between WNV prevalence and passerine diversity. Generalizations stating that non-passerine species are important for diluting WNV transmission fail to consider the relative paucity of data for competence of this species group. Experimental infection studies suggest that passerines (i.e., songbirds) are generally more competent than non-passerines (Komar et al. 2003; Kilpatrick et al. 2007); however, further research would be necessary to fully document the competence of many non-passerine species.

Exclusion of non-passerine species groups from this study limits the scope of our inference; however, we provide two lines of evidence suggesting that non-passerines are not central to WNV transmission in our study area. First, gulls, waterfowl, and raptors were usually observed flying high overhead, but they were rarely noted near the ground. These transitory species are therefore unlikely to interact with local WNV vectors. Second, during a simultaneous study to assess mosquito feeding preferences in the same study sites, passerines comprised 85% of avian blood meals from *Culex pipiens* mosquitoes (Hamer et al. 2009, in press). If non-passerines were key WNV hosts, we would expect to find evidence that mosquitoes fed upon them often. Thus, our primary focus on passerine species is warranted, since we found no evidence suggesting that non-passerine species are important to WNV transmission in our study area.

The dilution hypothesis assumes frequency-dependent transmission (i.e., the biting rate remains at a constant frequency regardless of host density); mosquito-borne disease transmission is also typically assumed to be frequency-dependent. In density-dependent systems (i.e., where biting rate varies with host density; Dobson 2004), high host diversity may have no effect or increase transmission, since increases in host diversity lead to more contacts between infected vectors and susceptible hosts. Though we found no apparent association between species richness and prevalence in this study, our results do not necessarily imply that WNV transmission in the Chicago area is density dependent. These findings highlight the possibility that extrinsic factors and variation in vector and host competence may be more important than avian community structure for determining variation in WNV prevalence. WNV transmission is also likely dependent upon the vector-to-host density ratio, with lower ratios corresponding to lower WNV prevalence. Research to address these factors will further clarify fine-scale dynamics of WNV transmission.

Four conditions have been established as necessary for the dilution effect in vector-borne diseases (Ostfeld and Keesing 2000a). Discrepancies from these conditions may partially explain why we found no evidence for a relationship between richness and WNV prevalence. In accord with the first condition, primary WNV vectors display generalist host preferences, feeding on multiple host species (Tempelis 1975; Molaei et al. 2006); however, since *Culex* mosquitoes appear to prefer to feed on some bird species while avoiding others (Kilpatrick et al. 2006; Hamer et al. 2009, in press), increased host diversity may not divert mosquito bites away from optimal WNV hosts. The second condition requires infection occurring primarily via vector-borne transmission. While there is some evidence for non-vector-borne modes of transmission [i.e., from parent to offspring in mosquitoes (Komar 2001) and from bird to bird (McLean et al. 2001)], the principal route of WNV transmission is between birds and mosquitoes. The third condition, that host competence varies among species, is well accepted and documented in laboratory studies (Komar et al. 2003), but it is unclear how competence varies intraspecifically.

The fourth condition of the dilution hypothesis states that optimum hosts are common and widespread. To test this criterion, we calculated a Pearson correlation between host competence and relative abundance of each species. We used host competence values from studies of experimental infection (Komar et al. 2003; Komar et al. 2005), where competence was defined as the product of susceptibility, infectiousness to vectors, and duration of infection. The relationship between host competence and relative abundance was weak ($r = 0.11$), suggesting that optimal hosts are not necessarily common in the Chicago study area. If optimal hosts are uncommon, they are more likely to be present in species-rich communities than species-poor communities (Davies et al. 2000); therefore, greater diversity would enhance rather than dilute transmission.

Annual weather fluctuations appear to be more important than bird community structure for driving variation in WNV transmission in the Chicago area and may partially explain the strong year effects noted in these analyses (see also Epstein and Defillipo 2001; Platonov et al. 2001; Bell et al. 2005; Shaman et al. 2005). Above average heat and drought characterized the Chicago summer of 2005, but rainfall was much greater in 2006. The 2005 season was favorable for creating *Culex* breeding sites and increasing productivity, since hot and dry conditions prevent flushing of *Culex* larvae from storm water catch basins. Intense heat also shortens breeding cycles and decreases extrinsic incubation periods in mosquitoes (Reisen et al. 2006). Further study of temperature and precipitation will help clarify the relationship between climate and WNV transmission.

Overall seroprevalence and importance of individual host species

Combined results for all bird species indicate that the bird community is still displaying WNV seroprevalence at a high rate, 5 years after the initial emergence of WNV in the Chicago area. Despite a significant decline in overall seroprevalence in 2006, total average seroprevalence for the study was a relatively high 11.5%. Studies in Florida in 2000 (Godsey et al. 2005), Georgia in 2000–2004 (Gibbs et al. 2006), and statewide surveys of Illinois in 2002 (Ringia et al. 2004) and 2001–2004 (Beveroth et al. 2006), indicate seroprevalence values considerably lower than those reported here. Studies reporting greater seroprevalence than those in this study were generally conducted in areas of recent WNV emergence (e.g., New York in 2000 and 2001, Komar et al. 2001a, b). Amplification and persistence of WNV transmission is a function of multiple interactive factors. Causes for high-level persistence of WNV in the Chicago area may include temperature and moisture regimes that are favorable for amplification, water drainage systems that support mosquito overwintering and breeding, spatial and temporal patterns of *Culex* mosquito abundance, or landscape features, such as extensive green space, that allow high contact rates between competent vectors and hosts (Ruiz et al. 2004).

Our results indicate that northern cardinals and mourning doves experienced unusually high seroprevalence levels. Cardinals have displayed high WNV seroprevalence in other regions (Komar et al. 2001b; Gibbs et al. 2006); however, the 76% seroprevalence from 2005 in our study is the highest documented for this species. Mourning dove seroprevalence was also much lower in other studies (Ringia et al. 2004; Gibbs et al. 2006). Though our results indicate that high seroprevalence in mourning doves and cardinals is unrelated to their abundance, these species meet the criteria of Gibbs et al. (2006) as optimal WNV sentinels; both species are widespread, easily captured, closely associated with humans, and exhibit an antibody response and low mortality rate after WNV infection. House sparrows, the most abundant and widespread species, appear to be poor sentinels for WNV, since all house sparrows tested in 2006 ($n = 349$) were seronegative. Moreover, estimates of host selection in the study area suggest that *Culex* mosquitoes avoid feeding on house sparrows (Hamer et al. 2009, in press).

Conclusion

Findings from this study strongly suggest that avian community structure is unrelated to prevalence of WNV in the Chicago metropolitan area. These results from a fine-scale study call into question whether increased host diversity has a net effect on WNV prevalence at coarse spatial scales. Understanding factors related to the dynamics of WNV transmission, such as variation in vector and host competence and mosquito feeding preference, will clarify causes for variation of WNV transmission. Dynamic models that incorporate these ecological details, while simultaneously considering climatic features, such as temperature and precipitation, will improve upon models that consider only a single aspect of the transmission system.

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