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Culex Flavivirus During West Nile Virus Epidemic and Interepidemic Years in Chicago, United States

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Abstract

Culex flavivirus (CxFV) is an insect-specific flavivirus infecting *Culex* mosquitoes, which are important vectors of West Nile virus (WNV). CxFV and WNV cocirculate in nature and coinfect *Culex* mosquitoes, including in a WNV ''hotspot'' in suburban Chicago. We previously identified a positive association between CxFV and WNV in mosquito pools collected from suburban Chicago in 2006. To further investigate this phenomenon, we compared the spatial and temporal distribution of CxFV during an interepidemic year (2011) and an epidemic year (2012) for WNV. Both viruses were more prevalent in mosquito pools in 2012 compared to 2011. During both years, the CxFV infection status of mosquito pools was associated with environmental factors such as habitat type and precipitation frequency rather than coinfection with WNV. These results support the idea that WNV and CxFV are ecologically associated, perhaps because both viruses respond to similar environmental drivers of mosquito populations.

Keywords: Culex flavivirus, epidemiology, virus ecology, West Nile virus

Introduction

West Nile virus (WNV) is a mosquito-borne flavivirus in the Japanese encephalitis virus serocomplex, which also includes the closely related subtype Kunjin virus, St. Louis encephalitis virus (SLEV), Usutu virus, and others. WNV is maintained in nature in an enzootic transmission cycle between competent mosquito vectors and avian hosts, in particular, passerines (order Passeriformes) (Hayes et al. 2005, Turell et al. 2005, Kilpatrick et al. 2006). Following its emergence in New York State in 1999, WNV spread steadily westward across the continental United States causing epidemic and epizootic disease over the subsequent decade (Hayes et al. 2005, Hayes and Gubler 2006). During 2012, the United States experienced a large WNV outbreak with over 5000 human cases and more than 22,000 positive mosquito pools reported (Beasley et al. 2013, USGS 2015). In contrast, during 2011, there were only ~700 human cases and fewer than 10,000 positive mosquito pools reported (USGS 2015).

Geographic foci of transmission, or ''hotspots,'' have been identified for WNV in the United States. One of these is the city of Chicago and its suburbs in Cook County (Illinois) (Bertolotti et al. 2008, Hamer et al. 2008b, 2009). The southwestern suburbs of Chicago have experienced enzootic WNV transmission in all years since it emerged in the region in 2001, and epizootic and epidemic transmission in many years (Bertolotti et al. 2008). Following the same temporal pattern as rest of the United States, Cook County experienced a large WNV outbreak during 2012, with 174 human cases identified and 2766 positive mosquito pools collected, while only 22 human cases and 852 positive mosquito pools were identified in 2011 (USGS 2015).

The primary vectors of WNV are *Culex* species mosquitoes, including *Culex pipiens*, the northern house mosquito. In urban and suburban areas of the upper Midwest, including Chicago, *C. pipiens* is ornithophilic, preferentially feeding on avian hosts, but has the potential to act as a WNV bridge vector between birds and humans (Kilpatrick et al. 2005,

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Hamer et al. 2008a). *Culex* mosquitoes can also carry Culex flavivirus (CxFV). CxFV is an insect-specific flavivirus (ISFV) along with cell fusing agent virus, and Calbertado virus, which are related to, but phylogenetically distinct from, encephalitic or hemorrhagic arboviruses such as WNV, SLEV, and dengue viruses (DENV1-4) that also belong to the genus *Flavivirus* (Moureau et al. 2015). CxFV was first isolated in *Culex* mosquito populations in Japan in 2007 and has since been identified in mosquitoes globally (Hoshino et al. 2007, Morales-Betoulle et al. 2008, Cook et al. 2009, Kim et al. 2009, Bolling et al. 2011, Newman et al. 2011). Like other ISFVs, CxFV does not infect vertebrate cells and is primarily maintained in mosquito populations through vertical transmission (Hoshino et al. 2007, Bolling et al. 2011, Saiyasombat et al. 2011).

Previously, we identified CxFV in *Culex* mosquitoes from the Chicago area and also observed a positive association between WNV and CxFV in *Culex* mosquito pools collected during 2006; WNV-positive mosquito pools were approximately four times more likely to also be CxFV positive than WNV-negative mosquito pools (Newman et al. 2011). Similarly, we identified WNV and CxFV coinfections in 6 of 15 individual *Culex* mosquitoes collected between 2005 and 2009 (Newman et al. 2011). In this study, we examine the occurrence and co-occurrence of WNV and CxFV in suburban Chicago between 2011 and 2012. Comparing infection patterns during 2 years with very different intensities of WNV transmission offers a unique ''natural experiment'' for investigating the association between these two viruses in nature. We also describe additional individual *Culex* species mosquitoes coinfected with CxFV and WNV, which were collected individually from the study site between 2010 and 2012.

Materials and Methods

Field and laboratory methods

We collected mosquitoes from southwest suburban Chicago (Cook County), using both CO_2-baited CDC miniature light traps and gravid traps baited with rabbit pellet infusion water. We deployed traps weekly across the study area at 37 fixed locations between 2011 and 2012 (Fig. 1). Traps included 23 CO_2-baited CDC miniature light traps and 14 infusion-baited gravid traps and were grouped for analyses based on habitat type: 15 (5 gravid traps, 10 light traps) in residential neighborhood sites, including yards and commercial properties; and 22 (9 gravid traps, 13 light traps) in urban green space sites, including parks and cemeteries. Mosquitoes were identified and pooled into groups of 50 or fewer by collection date and trap location. *Culex* mosquito pools (combined *C. pipiens* and *Culex restuans*) were collected from the beginning of June through the end of September (MMWR week 23 through MMWR week 39) in 2011 and 2012. Pools were collected from locations in the Village of Oak Lawn, Illinois (41°42′54″N 87°45′12″W), and the Village of Alsip, Illinois (41°40′14″N 87°43′56″W). Mosquito pools were processed for RNA extraction using the MagMAX RNA Isolation kit (Life Technologies, Grand Island, New York) and tested for WNV RNA using real-time RT-PCR as described previously (Hamer et al. 2008b). Extracted RNA was tested for

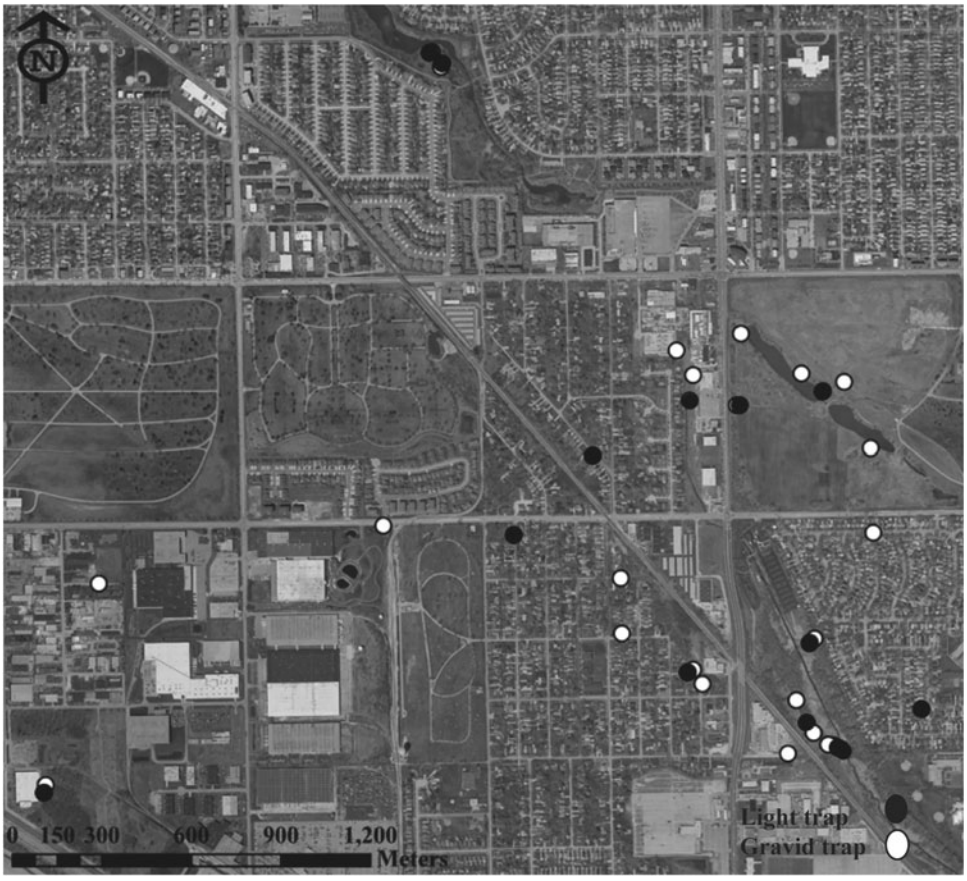

FIG. 1. Map of suburban Chicago study site with 37 locations where traps were deployed in both 2011 and 2012. CO_2-baited CDC light traps are shown as *white circles*, infusion-baited gravid traps are shown as *black circles*.

the presence of CxFV using a previously described CxFV-specific PCR (Newman et al. 2011).

Infection rates for WNV and CxFV were calculated using the bias-corrected maximum likelihood estimation method to account for variation in pool sizes in the Microsoft Excel add-in (Microsoft, Inc., Redmond, Washington) Pooled Infection Rate version 4.0 (Biggerstaff 2009). Both CxFV and WNV infection rates were compared weekly between years and trap sites using Wilcoxon signed rank tests for nonparametric datasets and Student's *t*-tests for parametric datasets. To evaluate the correlation between WNV infection rate and CxFV infection rate at WNV-positive traps during both years, we calculated Pearson's product-moment correlation coefficient. To account for potential bias in the number of pools tested by trap type, we compared numbers of mosquito pools collected from gravid traps with the number collected from light traps using an unpaired *t*-test. To account for variation in mosquito pool size by trap type, we compared pool sizes collected from gravid traps and pool sizes collected from light traps for both years using unpaired *t*-tests. All statistical analyses were performed in R version 3.0.3 (R Core Team 2014). In all cases, we considered results statistically significant when p values were less than 0.05.

Average seasonal and weekly nighttime temperatures

Nighttime (1700-0800) temperature data were collected from trap locations between 2011 and 2012 using HOBO data loggers (Onset Computer Corporation: Bourne, Massachusetts). Because data loggers were deployed in full sun locations (and were thus susceptible to solar heat effects) we included only nighttime temperatures in our analyses. In addition, nighttime temperatures correspond with increased periods of host and oviposition seeking activity of *Culex* mosquitoes (Reddy et al. 2007). Temperatures were averaged weekly from the beginning of June (MMWR week 23) through the end of September (MMWR week 39). Differences in average nighttime temperatures were compared between 2011 and 2012 using a paired *t*-test.

Average seasonal and weekly precipitation amount and precipitation frequency

Precipitation data were obtained from the nearest National Oceanic and Atmospheric Administration (NOAA) weather station, located at Midway International Airport (KMDW, 41°47′10″N 87°45′09″W) for 2011 and 2012. Total and average weekly precipitation in centimeters were determined for each week in 2011 and 2012 from MMWR week 23 through MMWR week 39. Precipitation frequency was quantified as the number of recorded precipitation events by week from the beginning of June (MMWR week 23) through the end of September (MMWR week 39). Differences in average precipitation were compared between 2011 and 2012 using a Wilcoxon signed-rank test.

Logistic regression

We used logistic regression to evaluate associations between CxFV and WNV status (positive or negative) of mosquito pools and average nighttime temperature and precipitation frequency during the week of collection, habitat type from which a pool was collected, and the trap type. For all models evaluated, we converted CxFV infection status and WNV infection status to dichotomous outcomes (positive = 1, negative = 0). In addition, we included year as a random effect, and we included mosquito pool size as an offset to account for the possible confounding effects of trap type. We based model selection on the minimization of Akaike information criterion (AIC) and the Akaike weight for each model. We evaluated models using the lme4 and AICcmodavg packages (Bates et al. 2015, Mazerolle 2016).

Results

Mosquito collections and virus infection rates

Culex mosquito pool sizes in 2011 and 2012 ranged from 1 to 50 mosquitoes and averaged 8.2 and 9.0 mosquitoes, respectively. There was no significant difference in the number of pools by trap type ($t = 1.62$, df = 35, $p > 0.1$). During 2012, mosquito pool sizes collected from gravid traps (average = 14.3) were larger than those collected from light traps (average = 6.3) ($t = 6.54$, df = 470, $p < 0.001$). However, there was no such difference in the sizes of pools collected from gravid versus light traps during 2011 (average for gravid traps and light traps were 8.98 and 6.29, respectively; $t = 1.09$, df = 544, $p > 0.1$).

Infection rates for both WNV and CxFV at the suburban Chicago study site were higher in 2012 than in 2011 (Table 1). In 2011, the WNV infection rate was 1.1 per 1000 mosquitoes (5 of 546 mosquito pools WNV positive), whereas in 2012, the WNV infection rate was 6.2 per 1000 mosquitoes (25 of 472 mosquito pools WNV positive). CxFV infection rates were approximately two orders of magnitude higher, but followed the same pattern, being 102.1 per 1000 mosquitoes (275 of 546 mosquito pools CxFV positive) in 2011 and 170.2 per 1000 mosquitoes (296 of 472 mosquito pools CxFV positive) in 2012. WNV was identified at 5 of 37 trap locations in 2011 and 16 of 37 trap locations in 2012, while CxFV was identified at all 37 trap locations during both years.

Table 1. Comparison of Maximum Likelihood-Based Estimates of Culex Flavivirus and West Nile Virus Infection Rates (Shown per 1000 Mosquitoes)

Virus	*2011 infection rate (95% CI)*	*2012 infection rate (95% CI)*	*Test statistic (V)*	p
CxFV	102 (93–113)	170 (154–189)	164	<0.001**
WNV	1.1 (0.4–2.5)	6.2 (4.2–9.0)	41	<0.02*

MIL-based infection rates are based on all *Culex* species mosquito pools collected and tested (2011: $N = 546$, 2012: $N = 472$) from June through September, using Wilcoxon signed-rank tests between 2011 WNV interepidemic and 2012 WNV epidemic years in suburban Chicago (95% confidence intervals in parentheses).

* = < 0.05; ** = < 0.01.

CxFV, Culex flavivirus; WNV, West Nile virus.

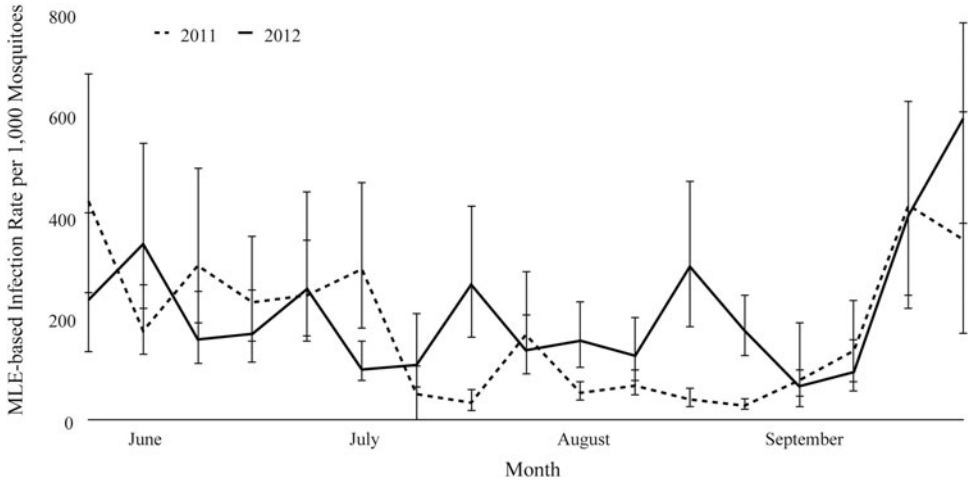

FIG. 2. Maximum likelihood-based estimates of the infection rates of CxFV during each week from June through September in 2011 and 2012. Error bars represent the bias-corrected 95% confidence intervals. CxFV, Culex flavivirus.

The odds of a mosquito pool testing positive for WNV in 2012 was approximately six times higher than in 2011 (OR: 6.1, 95% confidence interval [CI]: 2.3–15.9, $p<0.001$). The CxFV infection rate differed between 2011 and 2012 ($V=164$, $p<0.001$) and varied from week to week during both years (Fig. 2). In addition, the infection rate of CxFV varied significantly among trap locations ($t=2.75$, $df=36$, $p<0.01$). Similarly, WNV infection rate differed between 2011 and 2012 ($V=41$, $p<0.02$) and varied from week to week within and between years (Fig. 3). WNV infection rate also varied significantly between individual traps ($V=41$, $p<0.05$).

During 2012, the trap location with the highest WNV infection rate (28 per 1000 mosquitoes, 95% CI: 6–97) also had the highest CxFV infection rate (799 per 1000 mosquitoes, 95% CI: 429–988). During 2011, the trap location with the highest WNV infection rate (29.8 per 1000 mosquitoes, 95% CI: 2–215) had a CxFV infection rate of 359 per 1000 mosquitoes (95% CI: 147–657), which was intermediate. During 2012, WNV-positive trap locations had higher CxFV infection rates than WNV-negative trap locations ($t=2.47$, $df=21.5$, $p<0.05$). During 2011, the CxFV infection rate at WNV-positive trap locations and WNV-negative trap locations did not differ significantly ($W=65$, $p=0.52$). There was a positive and approximately linear correlation between the WNV infection rate and the CxFV infection rate at WNV-positive traps during 2011 ($t=4.31$, $df=3$, $p=0.023$, $R^2=0.93$), but not during 2012 ($t=1.61$, $df=14$, $p=0.13$, $R^2=0.40$) when the infection rates of both viruses were higher and more variable over time.

Average seasonal and weekly nighttime temperatures and precipitation

For each week in 2011 and 2012, we collected between 2000 and 10,500 temperature readings. The average nighttime temperature during 2011 (June to September) was 21.3 (±0.96)°C, while the average nighttime temperature during 2012 was 23.8 (±0.99)°C (Fig. 4), and this difference was statistically significant ($t=4.65$, $df=16$, $p<0.001$). We identified 118 precipitation readings from the beginning of June through the end of September in 2011 and 109 precipitation readings for the same period of time in 2012. The average precipitation for June through September was 0.26 (±0.07) centimeters in 2011 and 0.27 (±0.10) centimeters in 2012 (Fig. 5). Average precipitation did not differ significantly between years ($V=74$, $p>0.1$).

Environmental predictors of CxFV infection status

We evaluated associations between CxFV infection status, WNV infection status, and environmental factors by examining all combinations of five variables (WNV infection status, average nighttime temperature, precipitation frequency, habitat

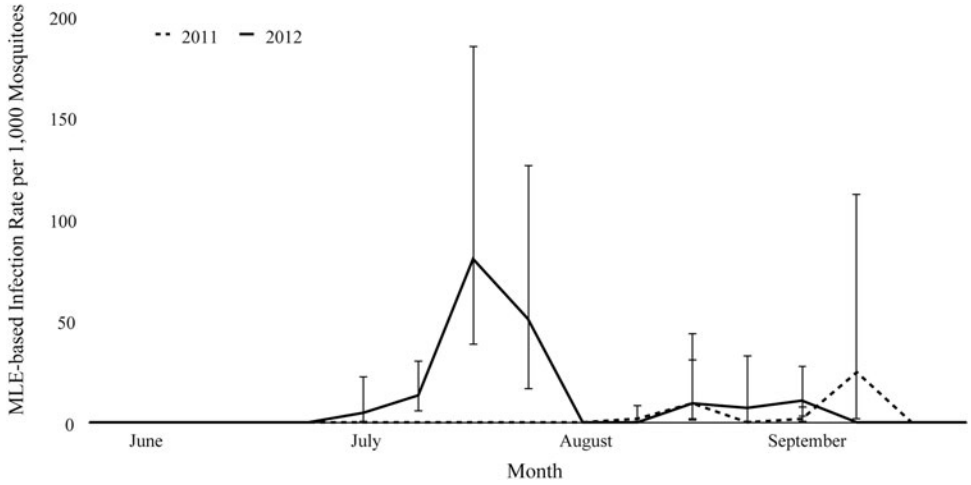

FIG. 3. Maximum likelihood-based estimates of the infection rates of WNV during each week from June through September in 2011 and 2012. Error bars represent the bias-corrected 95% confidence intervals. WNV, West Nile virus.

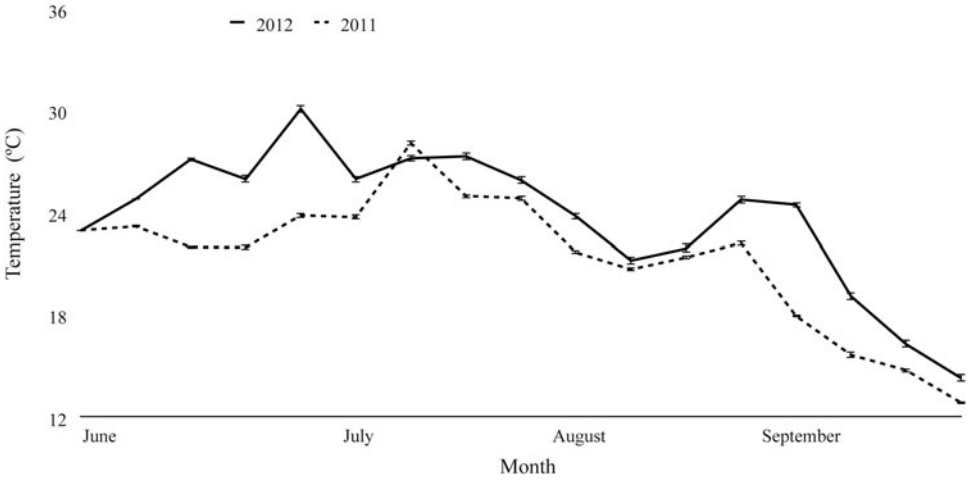

FIG. 4. Average nighttime temperature measured in degrees Celsius (°C) by week (June to September) in 2011 and 2012, collected from 37 fixed mosquito trap locations at the suburban Chicago study site. Error bars represent ±SEM for each week. Average nighttime temperatures were significantly higher in 2012 than in 2011 ($t = 4.65$, df = 16, $p < 0.0001$).

type, and trap type), resulting in 30 competing models (Table 2). All models included year as a random effect and pool size as an offset to account for differences between gravid traps and light traps. The data were best fit (highest Akaike weight) by a model that included nighttime temperature, precipitation frequency, habitat type, and trap type (Table 3). Akaike weights are a conditional probability, representing the relative likelihood of a given model; highest weight corresponds to highest relative likelihood (Wagenmakers and Farrell, 2004). Overall, nighttime temperature had a negative, but not statistically significant, association with the CxFV infection status of a mosquito pool. CxFV infection status was positively associated with precipitation frequency and collection from residential habitats. CxFV infection status was negatively associated with collection from a CDC miniature light trap. Infusion-baited gravid traps are specifically designed to attract *Culex* mosquitoes, which may partially explain this result. WNV infection status was not associated with the CxFV infection status in either 2011 or 2012.

CxFV and WNV coinfection of Culex *species mosquitoes*

In addition to pooled mosquito samples, we also collected and identified 21 individual *Culex* mosquitoes (species not determined) that were positive for WNV viral RNA (vRNA) between 2010 and 2012, and tested them for coinfection with CxFV. Fourteen of these WNV-positive individual mosquitoes also tested positive for CxFV. Combined with individual *Culex* mosquitoes previously collected from the area between 2005 and 2009 (Newman et al. 2011), 20 out of 36 mosquitoes tested positive for both viruses between 2005 and 2012.

Discussion/Conclusions

At our study site in suburban Chicago, *Culex* mosquito pools were six times more likely to test positive for WNV during an epidemic year (2012) than during an interepidemic year (2011). Similarly, CxFV was 7% more prevalent across the study site in 2012 than 2011. Overall, for both years, the CxFV infection status of a mosquito pool was positively associated with precipitation frequency and collection from residential habitats, and negatively associated with collection from a CDC miniature light trap.

Our results suggest that a correlation between CxFV and WNV at WNV-positive traps during 2011 is not directly causal, but rather reflects common environmental drivers. At traps where WNV-positive pools were collected, CxFV infection rate increased approximately linearly with WNV infection rate. This finding is consistent with our previous

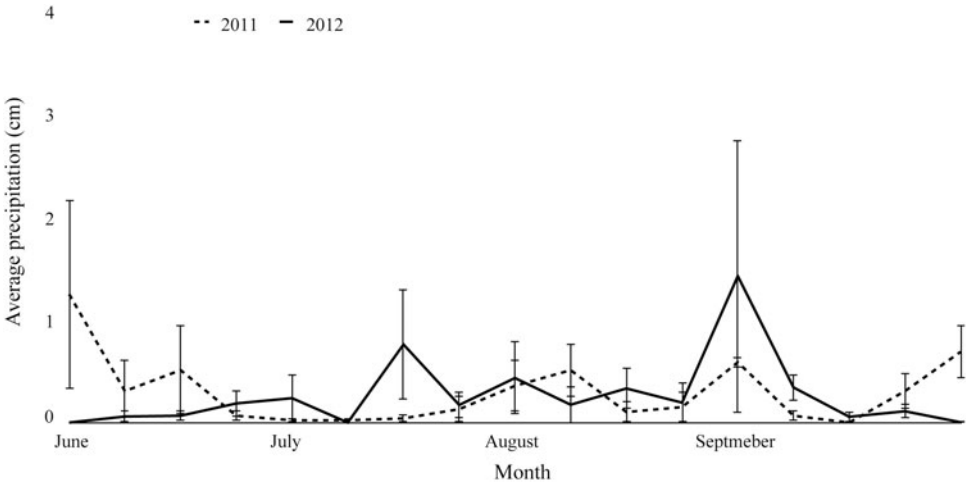

FIG. 5. Average precipitation (cm) by week from June through September in 2011 and 2012 at Midway Airport (KMDW). Error bars represent ±SEM for each week. Overall, there was no difference in average precipitation between years for the time period examined.

Table 2. Candidate Models for Examining the Culex Flavivirus Infection Status of *Culex* Mosquito Pools Collected During the 2011 Interepidemic and 2012 Epidemic Years at the Suburban Chicago Study Site

Model	*AICc*	*ΔAIC*	*Akaike weight*
temp[b] + precip[c] + habitat[d] + trap_type[e]	1193.39	0	0.38
WNV[a] + precip + habitat + trap_type	1194.29	0.9	0.24
WNV + temp + precip + habitat + trap_type	1195.42	2.03	0.14
habitat + trap_type	1196.26	2.87	0.09
temp + habitat + trap_type	1196.4	3.02	0.08
WNV + temp + habitat + trap_type	1198.41	5.03	0.03
WNV + habitat + trap_type	1198.27	4.88	0.03
WNV + temp + precip + trap_type	1233.1	39.71	0
WNV + temp + precip + habitat	1276.53	83.14	0
WNV + temp + precip	1308.91	115.52	0
WNV + temp + habitat	1277.97	84.59	0
WNV + precip + habitat	1275.18	81.79	0
temp + precip + habitat	1274.88	81.49	0
WNV + temp + trap_type	1234.46	41.08	0
WNV + precip + trap_type	1232.09	38.7	0
temp + precip + trap_type	1231.09	37.7	0
WNV + temp	1309.13	115.75	0
WNV + precip	1307.69	114.31	0
WNV + trap_type	1234.31	40.92	0
WNV + habitat	1277.34	83.95	0
temp + precip	1307.49	114.1	0
temp + trap_type	1232.49	39.1	0
temp + habitat	1276.46	83.08	0
precip + trap_type	1230.07	36.68	0
precip + habitat	1273.48	80.09	0
WNV	1308.57	115.18	0
Temp	1307.88	114.49	0
Precip	1306.21	112.82	0
trap_type	1232.33	38.94	0
Habitat	1275.75	82.36	0

Year was included as a random effect and pool size was included as an offset in all models to account for differences in average pool sizes between light and gravid traps. Models are arranged by Akaike weights with the model that has the highest weight listed first.
[a]WNV infection status (positive = 1, negative = 0).
[b]Nighttime temperature.
[c]Precipitation frequency.
[d]Habitat (residential or urban green space).
[e]Gravid or light trap.

identification of a positive ecological association between CxFV and WNV in mosquito pools collected in 2006 (Newman et al. 2011). However, our overall finding that CxFV infection status is primarily associated with environmental factors is more consistent with that reported for *Culex quinquefasciatus* from the Southeastern United States (Kent Crockett et al. 2012). Kent Crockett et al. (2012) found no evidence of an association between CxFV and WNV in *C. quinquefasciatus* populations from sites in Georgia, Louisiana, and Mississippi during 2009.

Table 3. Model Parameters for the Top Ranked Models Predicting the Culex Flavivirus Infection Status of Mosquito Pools Collected in 2012 and 2011

Variable	*Estimate*	*SE*	*OR*	*95% CI*	p
Temp	–0.01	0.01	0.99	0.96–1.01	0.34
Precip	0.14	0.06	1.15	1.02–1.30	0.03*
habitat (residential)	1.01	0.16	2.73	1.99–3.75	<0.0001***
trap_type (light)	–1.51	0.17	0.22	0.16–0.31	<0.0001***

* = < 0.05; *** = < 0.001.

We detected CxFV in *Culex* mosquito pools during all weeks from May through October in 2011 and 2012, and although infection rates varied from week to week across the seasons, differences within a given year were not statistically significant (Fig. 2). In a study of CxFV infection in *C. pipiens* and *Culex tarsalis* mosquitoes from Colorado, as well as in another study of CxFV infection in *C. quinquefasciatus* and *C. restuans* mosquitoes in East Texas, infection rates appeared to be seasonal (Kim et al. 2009, Bolling et al. 2011). In Colorado, the CxFV infection rates of *C. pipiens* increased gradually from June to September in 2006, while in 2007, the infection rate was highest in June and decreased gradually in September (Bolling et al. 2011). In Texas, CxFV was detected only during February and March; continued surveillance during warmer periods (April to August) resulted in no detection of CxFV-positive mosquito pools (Kim et al. 2009).

Overall, 2012 was significantly warmer than 2011 (Fig. 4). Temperature is an important factor influencing WNV

transmission and is known to affect the extrinsic incubation period (EIP) of WNV in mosquitoes (Dohm et al. 2002a, Reisen et al. 2006, Kilpatrick et al. 2008). For example, warmer temperatures have been associated with a shorter EIP for the WN02 genotype of WNV in *Culex* species mosquitoes in the United States (Kilpatrick et al. 2008). The warmer weather observed at our study site during 2012 (Fig. 4) may partially explain the increased prevalence of WNV when compared with 2011; however, we did not directly examine the influence of temperature on WNV prevalence in this study. Temperature is also an important factor influencing mosquito abundance (Hayes et al. 2005, Reisen et al. 2010, Chaves et al. 2011). For example, at warmer environmental temperatures, mosquito development may be accelerated and influence temporal abundance (Ewing et al. 2016). However, increasing temperature is also associated with a decreased lifespan in adult *Culex* mosquitoes (Loetti et al. 2011). The effects of temperature on the maintenance of CxFV in natural populations are not known. However, the apparent decrease in CxFV prevalence in some regions during the summer months suggests that temperature may be influencing CxFV prevalence indirectly through mosquito abundance (Kim et al. 2009). Increases or decreases in mosquito abundance on a fine scale could influence the prevalence of a vertically transmitted virus such as CxFV. *Culex* abundance may also be influenced by intermittent larval control and application of adulticides (Village of Alsip 2015), which we did not measure.

Higher frequency of precipitation events was positively associated with CxFV infection status (Table 3). Increased precipitation can influence mosquito abundance (Koenraadt and Harrington 2008, Gardner et al. 2012). Low precipitation and high mean daily temperatures were associated with high *Culex* larval abundance in catch basins at our site during 2010 (Gardner et al. 2012). However, whether this is also observed during a particularly hot year like 2012 is not known (NOAA 2012, 2015). Our finding that CxFV infection status was positively associated with precipitation frequency in 2011 and 2012 may be related to the potential for increased availability of oviposition habitat following rainfall events during hot and dry periods, although we did not measure mosquito productivity in catch basins in this study.

Mosquito pools collected from residential habitats (properties of individual homeowners and businesses) were more likely to be CxFV positive than pools collected from urban green spaces (parks and cemeteries). This finding is consistent with our previous findings (Newman et al. 2011). In this study, pools collected from residential habitats were almost thrice more likely to be infected with CxFV compared to pools collected from urban green spaces in both 2011 and 2012. Warmer and drier conditions, such as those that occurred overall during 2012 (NOAA 2012, 2015), might reduce potential oviposition habitat in urban green spaces, whereas in residential areas, human water usage (swimming pools, planters, and bird baths) might maintain a more consistent breeding habitat. In particular, storm water catch basins are abundant in the residential neighborhoods of the suburban Chicago landscape and are important *Culex* oviposition sites (Gerry and Holub 1989, Gardner et al. 2012).

Overall, our results suggest that WNV and CxFV respond to similar ecological drivers, such as precipitation frequency and habitat type. Weather patterns and climate variability are known to influence mosquito abundance, competence, and arbovirus infection rates (Reisen et al. 2006, 2010, Vaidyanathan and Scott 2007, Chaves et al. 2011, Wang et al. 2011). These factors have historically been important for explaining differences in mosquito infection rates with WNV between years (Ruiz et al. 2010). Our results suggest that the same may be true for CxFV and our previous finding of an association between CxFV and WNV may be environmentally mediated.

To date, relatively few studies have examined CxFV and WNV coinfection in *Culex* species mosquitoes or mosquito cell culture, and results have been inconsistent. Kent et al. (2010), found no difference in WNV replication kinetics in *Aedes albopictus* C6/36 cells or in *C. quinquefasciatus* sequentially infected with a strain of CxFV, but did identify an increase in WNV transmission in a strain of *C. quinquefasciatus* when WNV was coinoculated with CxFV. However, CxFV is vertically transmitted and likely precedes WNV infection (Saiyasombat et al. 2011). Conversely, in a study of WNV transmission in a laboratory population of *C. pipiens* naturally infected with CxFV, WNV dissemination was delayed in coinfected individuals, but no significant difference in transmission was observed (Bolling et al. 2012). In that study, however, the mosquito colonies were from different geographic regions and may have differed in vector competence (Bennett et al. 2002, Vaidyanathan and Scott 2007). In this study, we report a total of 20 CxFV and WNV coinfected *Culex* species mosquitoes collected from 2005 to 2012 from our field site. These results show that WNV/CxFV coinfection is common in nature, although they do not indicate whether the two viruses infect the same cells or modify infectivity in the mosquito.

Viruses may affect mosquitoes in ways that influence transmission. For example, CxFV is efficiently transovarially transmitted in *C. pipiens*, whereas WNV is not (Baqar et al. 1993, Dohm et al. 2002b, Goddard et al. 2003, Saiyasombat et al. 2011). The effects of primary CxFV infection on the transovarial transmission of WNV are currently unknown. Similarly, no studies to our knowledge have investigated the potential influence of CxFV infection on mosquito feeding behavior. Infection of *Ae. aegypti* with another flavivirus, dengue virus, has been shown to influence feeding behavior, potentially reflecting infection of the mosquito nervous system (Platt et al. 1997). We recently found that CxFV infection alters the flight activity of *C. pipiens* collected from our study site in suburban Chicago by reducing the overall activity (Newman et al. 2016). How this reduction in flight activity influences coinfections with WNV and the results we report in this study is unknown. The potential behavioral effects of CxFV on *Culex* mosquitoes and the indirect effects on WNV transmission have yet to be examined. Our results suggest that CxFV and WNV respond to similar environmental drivers in nature, but they do not preclude the possibility of virus–virus interaction within mosquitoes.

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Author Disclosure Statement

No competing financial interests exist.

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