Am. J. Trop. Med. Hyg., 83(3), 2010, pp. 607–613
doi:10.4269/ajtmh.2010.10-0005

Spatial and Temporal Variation in Vector Competence of *Culex pipiens* and *Cx. restuans* Mosquitoes for West Nile Virus

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Abstract. Vector competence, the probability that a vector will transmit a pathogen after feeding on an infected host, is known to vary among vector species, populations, days since feeding, and temperature during the extrinsic incubation period. However, the extent of spatio-temporal variability and consistency in vector competence of populations is not known. We examined vector competence of *Culex pipiens* Linnaeus and *Cx. restuans* Theobald mosquitoes for West Nile virus collected over 3 years from 17 sites to measure spatial and temporal scales of variation in vector competence. We found extreme variation with 0–52% of mosquitoes transmitting West Nile virus at a single site between different sampling periods, and similar variation across populations. However, we also found that within a smaller geographic range, vector competence varied somewhat synchronously, suggesting that environmental and population genetic factors might influence vector competence. These results highlight the spatio-temporal variability in vector competence and the role of local processes.

INTRODUCTION

Vector competence characterizes the likelihood that a vector will transmit a pathogen after feeding on an infected host.[1] It is a critical component influencing pathogen transmission, as can be seen from the fact that it is a linear term in expressions for vectorial capacity and for R_0, the population growth rate of a pathogen.[2–4] Successful infection in mosquitoes must overcome a series of barriers to transmission including the mesenteronal infection barrier,[5] mesenteronal escape barrier,[6] salivary gland infection barrier,[1] and salivary gland escape barrier.[7–9] If pathogens fail to overcome any of these barriers, either because of environmental conditions (e.g., cool temperatures limiting viral replication), or because of interactions between the pathogen and the individual mosquito (including genetic and non-genetic components), the vector will fail to transmit the pathogen.

West Nile virus (WNV) was first detected in the Americas in 1999, and has since spread throughout North and South America where it has infected and been transmitted by a multitude of mosquitoes.[10–12] Several studies have identified key aspects of WNV amplification, including the competence or infectiousness of different avian hosts,[11,13,14] some habitat-specific associations of WNV,[15–18] and the vector competence and feeding patterns of *Culex* mosquitoes, the dominant vectors for WNV between birds, and also to humans in some areas.[19–24] The vector competence of colonized and field populations of mosquitoes for WNV has received substantial attention. It has been found to vary among vector species and genera,[10,25–29] days since feeding,[30–32] strain of WNV,[30–32] and temperature[31,33] during the extrinsic incubation period. Vector competence for WNV also has been shown to vary among mosquito populations of the same species,[25,34–36] with evidence of a genetic basis,[37] and may vary seasonally,[38] but the full extent of the variability and consistency over time of the vector competence of a population is not well characterized.

We therefore sought to determine the extent to which vector competence of free-ranging *Culex pipiens* and *Cx. restuans* mosquitoes varies in space and time. In particular, we mapped variation in vector competence of *Culex* mosquito populations for WNV at several spatial scales and across several seasons and years, and to determine if a genetic basis for vector competence could be identified through the use of microsatellite analysis. To accomplish this effort, *Cx. pipiens* and *Cx. restuans* mosquitoes were collected at geographically and ecologically diverse sites during three transmission seasons, reared under laboratory conditions, and vector competence was measured.

METHODS

Mosquitoes. Field populations of *Cx. pipiens* and *Cx. restuans* were collected as egg rafts from oviposition traps placed overnight in 14 sites from July through September during 2002–2004, in Staten Island, Suffolk County, and Albany County, New York, and in 3 locations in Massachusetts. We aimed to collect egg rafts in July, August, and September at each site in each year, but weather related difficulties (e.g., rainfall during the week of our visit to a site or county) combined with variation in abundance (e.g., *Cx. restuans* abundance decreases in the warmer summer months[29]) prevented us from collecting egg rafts in some months. Sites within a county were 3–5 km apart in Albany County, 8–20 km apart in Suffolk County, 3–4 km apart in Staten Island, and > 10 km apart in Massachusetts.

Sites were classified as urban or rural on the basis of Landsat images and/or ground-truthed by field personnel at the beginning of the study.[29] Rural sites were in heavily wooded, lightly used public land with no human dwellings and minimal human disturbance. Urban sites were in residential or heavily built up areas. *Culex pipiens* from a colony in our laboratory established in 2002 from egg rafts collected in Pennsylvania and maintained as described elsewhere[29] were used as an internal control during 2002 and 2003.

For all studies, egg rafts of field and colonized mosquitoes were hatched in Tupperware flats containing tap water and Tetramin slurry, and reared at 26°C with a 16:8 hour light:dark cycle. Experiments on mosquito populations from a given month and site were conducted with mosquitoes from

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multiple (5–20) egg rafts that were combined as larvae after they were identified as *Cx. pipiens* or *Cx. restuans*. Pupating mosquitoes were transferred to an emergence jar. Adults were collected daily, transferred to standard 12″ × 12″ × 12″ mosquito cages, and maintained with water and sugar cubes until six days after the emergence of most of the adults. Mosquitoes were deprived of water and sugar for 24–48 hours prior to testing.

Adult females of colonized and wild-caught mosquitoes were fed on pledgets soaked with 10^{-2} dilution of a WNV-defibrinated blood-sucrose mixture as described,[39] yielding a final blood meal titer of 10^7 plaque-forming units/mL. Engorged mosquitoes were separated and maintained in one-gallon cartons as described above. After 14 days extrinsic incubation at 30°C, capillary transmission assays were conducted as described,[39] and each test group of mosquitoes was frozen for subsequent assay for WNV infection, dissemination, and viral transmission as follows. Mosquitoes were anesthetized with triethylamine, legs were removed, and mouthparts were placed in a capillary tube containing fetal calf serum plus 50% sucrose for *in vitro* transmission assays.[40] After 15 minutes, the contents of the capillary tube were ejected into microfuge tubes containing 0.3 mL bovine albumin in borate-buffered saline. The legs and the body of the mosquito were placed in separate tubes containing 1.0 mL of mosquito diluent (phosphate-buffered saline solution with 20% fetal bovine serum, 100 units/mL penicillin, 100 µg/mL streptomycin, 0.25 µg/mL fungizone, and 10 µg/mL gentamicin). All samples were frozen at –70°C until testing.

Virus. The WNV strain 3356 isolated from an American crow on Staten Island, NY, in 2000 was used in all studies. Presence or absence of infection in mosquitoes was assayed by inoculation of samples onto Vero cell monolayers and monitoring for plaque formation after 96 hr as described.[41] Negative specimens were screened for RNA by using a quantitative real-time reverse transcription–polymerase chain reaction (TaqMan) assay[42,43] to determine whether viral RNA was present in the absence of infectious virus.

Statistical analysis. We evaluated spatio-temporal patterns in vector competence among species, and between populations and temporal samples within populations by using logistic regression (equivalent to a generalized linear model with a binomial distribution and a logit link function) in SPSS version 15.0 (SPSS Inc., Chicago, IL). Each mosquito was treated as a data point, and the species, location (county or site-within-county), urban or rural nature of the site, year, and month that larvae were collected were included as categorical factors in the model. For two sites in Albany county and three sites in Staten Island, we had measurements of vector competence from at least three time points in common. We examined synchrony in temporal variation in the fraction transmitting within a county by using simple Pearson product-moment correlations. In addition, to assess the likelihood of observing the large number of high but mostly non-significant correlations we observed, we performed a randomization test where we generated 10,000 random draws of the same number of estimates of vector competence as observed (3) at two counties (Albany, 2 sites; Staten Island, 3 sites) by using the mean of a binomial variable with the same sample size of mosquitoes as the estimates, and then calculated the probability of observing as high an average correlation as that observed (0.945).

Mosquito genetic analyses. We examined the population genetics of specimens of *Cx. pipiens* from three WNV challenges in mosquitoes from Staten Island in 2002 and Suffolk in 2002 and 2003. For each of the three challenges, we used all mosquitoes that became infected, and a random subset of mosquitoes that did not become infected from rural sites and urban site 1 from Suffolk, NY and from all four sites in Staten Island, NY (Table 1). We used eight highly polymorphic microsatellite markers as previously described.[44] We used loci CQ11, CQ26, qGT4, pGT9, pGT12, pGT20, pGT46, and pGT53, which have been shown to amplify in populations of the *Cx. pipiens* complex with a low frequency of alleles unique to *Cx. quinquefasciatus*.[45] Analyses of mosquito families have shown that all the microsatellite loci used in this study are inherited in a Mendelian fashion and are not sex-linked.[46,47] Microsatellite loci were amplified and sized as described.[48] We determined whether the allelic frequencies of the population of mosquitoes that after challenge became infected with WNV (susceptible mosquitoes) differed from the population of mosquitoes that did not become infected (resistant) after the 14-day incubation period. We made this determination by calculating F_{ST}, a classical measure of the level of differentiation between the two populations,[49] which examines expected and observed heterozygosity values based on observed allelic frequencies across the full dataset. The calculations were performed independently for 2002 and 2003 comparisons. We also calculated the relative average ancestry in resistant and susceptible *Cx. pipiens* from the two forms of *Cx. pipiens*, form molestus and form pipiens. We used pure populations of the two *Cx. pipiens* forms from prior studies as reference[45] and a Bayesian inference method implemented in the program Structure 2.0.[50]

RESULTS

We examined vector competence in 44 groups of 42.6 ± 13.3 (mean ± SD) *Cx. pipiens* mosquitoes and 24 groups of 23.5 ± 12.1 *Cx. restuans* mosquitoes collected over 3 years at 17 sites across six counties (plus a laboratory colony). The data show that for both species, vector competence is highly variable in space and time with values ranging from 0% to 52% with a coefficient of variation (SD/mean) of 148% for *Cx. pipiens* and ranging from 0% to 24% with a coefficient of variation of 124% for *Cx. restuans* (Tables 1 and 2). For both species, there was no significant difference between sites categorized as urban and rural in the probability of transmitting WNV (urban coefficient = 0.0481 ± 0.2007, z = 0.24, $P = 0.811$, odds ratio [OR] = 1.05, 95% confidence interval [CI] = 0.71–1.55), and this factor was removed from the analysis. In the reduced model, analysis indicated significant differences between years, months, and counties. The site-within-county predictor was marginally non-significant ($\chi^2 = 30.5$, degrees of freedom = 11, $P = 0.073$), and county remained significant ($P = 0.023$), which suggested that variation within county was smaller than between counties, but only marginally. After accounting for differences between locations and time, *Cx. restuans* mosquitoes had a significantly higher (OR = 2.05, 95% CI = 1.1–3.8) probability of transmitting WNV than *Cx. pipiens* (Table 3).

When we examined infection, rather than transmission, *Cx. restuans* were marginally less likely to become infected (OR = 0.76, 95% CI = 0.55–0.105, $P = 0.097$) and marginally more likely to have a disseminated infection (OR = 1.44, 95%

TABLE 1

Fraction of *Culex pipiens* mosquitoes infected and with disseminated infection, transmitting West Nile virus 14 days after feeding on West Nile virus–infected blood with a titer of 10^7 plaque-forming units/mL, and held at 30°C*

State	Location	Site	Month and year	No.	Fraction infected	Fraction with disseminated infection	Fraction transmitting
MA	Cambridge	Site 1	Aug 2003	36	0.11	0.08	0.03
MA	Cambridge	Site 1	Sep 2003	79	0.10	0.10	0.05
MA	Eastham	Site 1	Aug 2003	16	0.00	0.00	0.00
MA	Eastham	Site 1	Sep 2003	67	0.07	0.03	0.01
MA	Needham	Site 1	Aug 2003	36	0.22	0.11	0.03
MA	Needham	Site 1	Sep 2003	7	0.00	0.00	0.00
NA	Colony	NA	Aug 2002	36	0.00	0.00	0.00
NA	Colony	NA	Sep 2002	50	0.32	0.22	0.06
NA	Colony	NA	Jul 2003	50	0.10	0.08	0.06
NY	Albany	Rural 1	Aug 2003	28	0.18	0.04	0.00
NY	Albany	Rural 2	Sep 2002	32	0.16	0.09	0.09
NY	Albany	Urban 1	Jul 2002	50	0.18	0.08	0.08
NY	Albany	Urban 1	Aug 2002	50	0.00	0.00	0.00
NY	Albany	Urban 1	Sep 2002	50	0.60	0.42	0.28
NY	Albany	Urban 1	Sep 2003	50	0.06	0.02	0.02
NY	Albany	Urban 2	Jul 2002	50	0.08	0.06	0.04
NY	Albany	Urban 2	Aug 2002	50	0.00	0.00	0.00
NY	Albany	Urban 2	Sep 2002	50	0.42	0.16	0.10
NY	Albany	Urban 2	Aug 2003	24	0.00	0.00	0.00
NY	Albany	Urban 2	Sep 2004	35	0.34	0.11	0.06
NY	Albany	Urban 3	Aug 2004	39	0.15	0.08	0.03
NY	Albany	Urban 4	Aug 2004	50	0.12	0.04	0.00
NY	Staten Island	Rural 1	Jul 2002	50	0.38	0.30	0.20
NY	Staten Island	Rural 1	Sep 2002	30	0.20	0.07	0.00
NY	Staten Island	Rural 1	Sep 2003	50	0.18	0.08	0.06
NY	Staten Island	Rural 1	Jul 2004	50	0.20	0.10	0.04
NY	Staten Island	Rural 2	Jul 2002	15	0.33	0.33	0.20
NY	Staten Island	Rural 2	Sep 2002	35	0.40	0.23	0.09
NY	Staten Island	Urban 1	Jul 2002	50	0.46	0.38	0.24
NY	Staten Island	Urban 1	Sep 2002	50	0.18	0.08	0.06
NY	Staten Island	Urban 1	Sep 2003	28	0.21	0.18	0.11
NY	Staten Island	Urban 2	Jul 2002	50	0.80	0.70	0.52
NY	Staten Island	Urban 2	Sep 2002	50	0.30	0.20	0.10
NY	Staten Island	Urban 2	Jul 2003	50	0.22	0.04	0.00
NY	Staten Island	Urban 2	Sep 2003	50	0.04	0.02	0.00
NY	Suffolk County	Rural 1	Jul 2002	45	0.53	0.38	0.22
NY	Suffolk County	Rural 1	Jul 2003	50	0.64	0.22	0.04
NY	Suffolk County	Rural 2	Jul 2002	34	0.00	0.00	0.00
NY	Suffolk County	Rural 2	Aug 2003	32	0.06	0.03	0.03
NY	Suffolk County	Rural 2	Sep 2003	50	0.16	0.06	0.00
NY	Suffolk County	Urban 1	Sep 2003	50	0.00	0.00	0.00
NY	Suffolk County	Urban 1	Aug 2004	50	0.16	0.06	0.02
NY	Suffolk County	Urban 2	Jul 2002	27	0.22	0.15	0.07
NY	Suffolk County	Urban 2	Aug 2003	42	0.10	0.05	0.02

* Mosquitoes were reared from field-collected egg rafts obtained in the month and year indicated. No. = sample size; MA = Massachusetts; NA = not available; NY = New York.

CI = 0.94–2.22, $P = 0.097$) in models with species, year, month, and county, but neither pattern reach statistical significance. However, given infection, *Cx. restuans* was much more likely to have a disseminated infection or transmit (dissemination given infection: OR = 3.33, 95% CI = 1.71–6.49, $P < 0.001$; transmission given infection: OR = 3.01, 95% CI = 1.41–6.45, $P = 0.004$).

Although vector competence was highly variable in space and time (Tables 1–3), there was some marginal evidence that vector competence within a county varied synchronously (Figure 1). We performed correlations between pairs of sites within a county (two in Albany and three in Staten Island) that had at least three time points where both sites were sampled in the same month. Vector competence at the two Albany sites showed a marginally significant correlation (Albany: Figure 1A; r = 0.99, n = 3 samples taken in the same month from both sites; $P = 0.08$; including a fourth point measured in August 2003 at site 2, and September 2003 at site 1; r = 0.99, n = 4, $P = 0.014$). Evidence was slightly weaker at the three Staten Island sites (Figure 1B; rural 1 and urban 1; r = 0.99, n = 3, $P = 0.026$; rural 1 and urban 2: r = 0.89, n = 3, $P = 0.31$, and urban 1 and urban 2: r = 0.91, n = 3, $P = 0.28$). Although these correlations between sites in Staten Island are high, none of these comparisons is significant with a correction for the three comparisons made, most likely because there were only three time points where both sites in each pair were sampled. Nonetheless, the overall probability of observing four correlations (one at Albany with three points, and three at Staten Island for the pairwise comparisons between the three sites) with an average of 0.945 in a randomization test was 0.0033, which suggested that there is evidence of spatial synchrony.

There was some evidence for genetic differentiation between mosquitoes that became infected with WNV after feeding on infected blood (susceptible) and those that did not become infected (resistant) in Suffolk County, NY *Cx. pipiens* mosquitoes (2002: $n_S = 42$, $n_R = 58$, $F_{st} = 0.0149$, $P = 0.047$;

TABLE 2

Fraction of *Culex restuans* mosquitoes infected and with disseminated infection, transmitting West Nile virus 14 days after feeding on West Nile virus–infected blood with a titer of 10^7 plaque-forming units/mL, and held at 30°C*

State	Location	Site	Month and year	No.	Fraction infected	Fraction with disseminated infection	Fraction transmitting
NY	Albany	Rural 1	Sep 2003	12	0.08	0.08	0.08
NY	Albany	Rural 2	Aug 2003	16	0.06	0.06	0.06
NY	Albany	Urban 2	Aug 2003	17	0.06	0.06	0.06
NY	Albany	Urban 2	Sep 2004	12	0.17	0.17	0.08
NY	Albany	Urban 3	Aug 2004	25	0.16	0.04	0.04
NY	Albany	Urban 4	Aug 2004	8	0.00	0.00	0.00
NY	Albany	Urban 5	Aug 2004	38	0.16	0.11	0.03
NY	Albany	Urban 5	Sep 2004	27	0.22	0.11	0.04
NY	Staten Island	Rural 1	Jul 2003	25	0.16	0.12	0.00
NY	Staten Island	Rural 1	Aug 2003	17	0.24	0.24	0.24
NY	Staten Island	Rural 2	Aug 2003	24	0.13	0.13	0.13
NY	Staten Island	Rural 2	Sep 2003	36	0.14	0.11	0.03
NY	Staten Island	Urban 1	Aug 2003	15	0.20	0.07	0.00
NY	Staten Island	Urban 1	Sep 2003	47	0.13	0.04	0.00
NY	Staten Island	Urban 2	Sep 2003	4	0.00	0.00	0.00
NY	Suffolk	Rural 1	Aug 2003	32	0.25	0.19	0.16
NY	Suffolk	Rural 1	Sep 2003	28	0.07	0.04	0.00
NY	Suffolk	Rural 1	Jul 2004	21	0.00	0.00	0.00
NY	Suffolk	Rural 1	Aug 2004	31	0.19	0.10	0.06
NY	Suffolk	Rural 2	Aug 2003	12	0.00	0.00	0.00
NY	Suffolk	Rural 2	Sep 2003	31	0.10	0.06	0.03
NY	Suffolk	Urban 1	Sep 2003	29	0.00	0.00	0.00
NY	Suffolk	Urban 2	Aug 2003	7	0.14	0.14	0.14
NY	Suffolk	Urban 2	Sep 2003	50	0.04	0.04	0.02

* Mosquitoes were reared from field-collected egg rafts obtained in the month and year indicated. No. = sample size; NY = New York.

2003: $n_S = 80, n_R = 64, F_{st} = 0.0203, P = 0.042$) and Staten Island, NY *Cx. pipiens* mosquitoes (only compared in 2002: $n_S = 82$, $n_R = 138$, $F_{st} = 0.0135$, $P = 0.004$). However, there was little evidence of genetic differentiation between mosquitoes that had either disseminated infections or transmitted WNV (F_{st} values for three county-year comparisons = –0.0483 to 0.0092; all P values > 0.21), which was partly caused by the smaller sample size of disseminated and transmitting mosquitoes in these comparisons (Table 1). In addition, genetic differentiation between resistant and susceptible mosquitoes was smaller than differences between Staten Island and Suffolk County populations of mosquitoes ($F_{st} = 0.0327, P = 0.001$).

We also compared the genetic ancestry for the two forms of *Cx. pipiens* (form pipiens and form molestus) of susceptible and resistant specimens. We found a significant difference in the ancestry of mosquitoes that became infected (average form pipiens ancestry probability 0.76 ± 0.061 [mean ± SE], n = 38) and those that did not become infected (average form pipiens ancestry probability 0.54 ± 0.051, n = 74, by *t*-test on arc-sin square root transformed data, t = 2.51, $P = 0.0014$) in mosquitoes from Staten Island in 2002. However, this difference in ancestry was not apparent in mosquitoes from Suffolk in 2002 (infected, form pipiens ancestry: 0.54 ± 0.077, n = 32; uninfected, form pipiens ancestry: 0.56 ± 0.087, n = 27, t = 0.18, $P = 0.86$).

DISCUSSION

We found substantial spatial and temporal variability in WNV vector competence in *Cx. pipiens* and *Cx. restuans*, in

TABLE 3

Results of binary logistic regression statistical analysis of the probability of a mosquito transmitting West Nile virus*

Predictor	Coefficient	SD	Test statistic (Z or χ^2)	*P*	Odds ratio	95% Confidence interval
Constant	–2.14	0.45	–4.79	< 0.001		
Species (*Culex pipiens*)						
Cx. restuans	0.72	0.32	2.28	0.02	2.05	1.10–3.80
Year (2002)			34.17	< 0.001		
2003	–1.51	0.28	–5.41	< 0.001	0.22	0.13–0.38
2004	–1.48	0.36	–4.13	< 0.001	0.23	0.11–0.46
Month (July)			12.66	0.002		
August	–0.72	0.28	–2.54	0.01	0.48	0.28–0.85
September	–0.68	0.20	–3.34	< 0.001	0.51	0.34–0.76
Location (colony)			15.26	0.018		
Staten Island	0.88	0.45	1.95	0.05	2.40	1.00–5.79
Suffolk	0.22	0.48	0.46	0.65	1.25	0.48–3.21
Albany	0.28	0.46	0.61	0.55	1.32	0.54–3.23
Eastham	–0.07	1.11	–0.06	0.95	0.94	0.11–8.29
Needham	0.63	1.12	0.57	0.57	1.89	0.21–17.00
Cambridge	1.26	0.66	1.91	0.06	3.51	0.97–12.73

* In this analysis, each individual mosquito is a data point. The table shows the relative coefficients for each predictor relative to arbitrary reference levels shown in parentheses. For predictors with more than two groups (year, month, county), the test statistic for the predictor is a Wald's χ^2 with degrees of freedom equal to one less than the number of groups (2, 2, and 6, respectively). Odds ratios give the relative odds of a mosquito transmitting West Nile virus relative to the reference level.

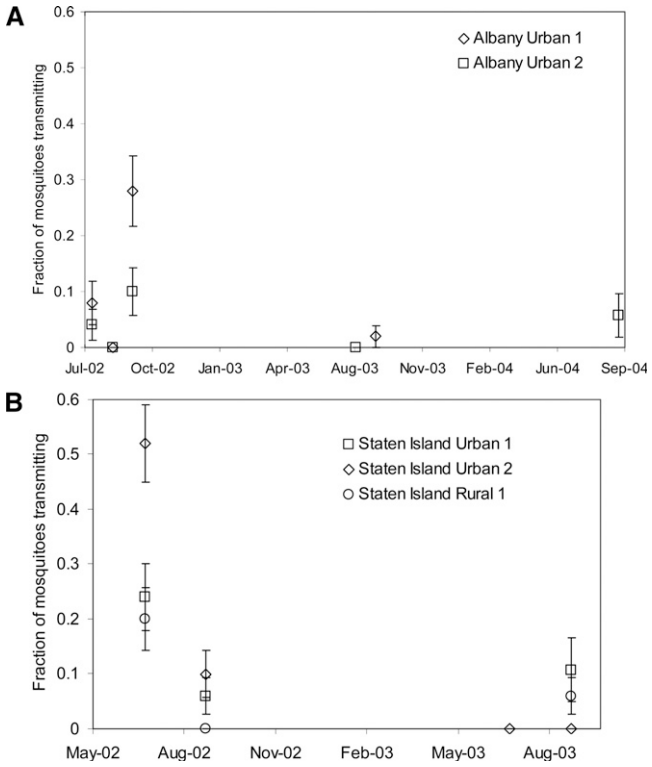

FIGURE 1. Fraction of *Culex pipiens* mosquitoes transmitting West Nile virus (±SE) versus month sampled for two sites in Albany, New York (**A**) and three sites in Staten Island, New York (**B**) where there were at least three estimates of vector competence. Each point represents an average of 45.6 (range = 24–50) mosquitoes. Because only a single site in Suffolk County, New York, was sampled more than twice, data from this county are only shown in Table 1.

agreement with findings of previous studies.[25,34–36] However, in previous studies, few efforts have been made to understand the spatial and temporal scale on which vector competence varied and on the sources of variation. We found that vector competence of *Cx. pipiens* at sites separated by relatively small distances, in this case within a county, varied somewhat synchronously (although sample sizes were small), whereas at larger spatial scales (i.e., between distant counties in New York), variability appeared to be non-synchronous (Figure 1).

The variability in vector competence observed in these experiments over a short time scale (months) and over moderate spatial scales (3–20 km) implies that its contribution to the amplification of WNV is variable, which may make prediction of WNV transmission using surveillance data more challenging. At the same time, the significant difference in vector competence between counties (Table 3) highlights the potential role of vector competence in influencing transmission. Although a previous attempt to find a link between vector competence and WNV transmission intensity in the field was unsuccessful,[34] this might have been because other factors such as mosquito abundance, mosquito feeding patterns, temperature, and acquired immunity were not incorporated into the analysis.

Previously, the most in-depth study of WNV vector competence examined California populations of four species (*Cx. pipiens*, *Cx. tarsalis*, *Cx. quinquefasciatus*, and *Cx. stigmatosoma*) and measured aspects of vector competence over five years.[34] This study demonstrated substantial year-to-year and site-to-site variation in susceptibility to infection but reported less extensive analysis of transmission by these mosquitoes. The investigators found limited evidence of spatial (but no year-to-year) variation in transmission in *Cx. pipiens*, and limited evidence of temporal (but no spatial) variation in *Cx. tarsalis*. They also found no significant evidence that spatiotemporal patterns in susceptibility to infection correlated with intensity of WNV outbreaks in California.

Seasonal variability in vector competence also has been studied in *Cx. tarsalis* for western equine encephalitis virus and St. Louis encephalitis virus.[51,52] These studies showed significant variation in vector competence, with a decrease in susceptibility to infection with increasing temperature in the month of collection for western equine encephalitis virus, and variable transmission rates for both viruses that showed no clear pattern with season. In these studies, and in those just described for WNV, it is unknown on what spatial scale vector competence varied, and the extent to which vector competence has a genetic component.

We identified mosquito genetics as one factor associated with susceptibility to infection, but the pattern was not strong enough to be predictive, and was not apparent when considering transmission. This finding is likely because the markers we used are not physically linked to the locus or loci involved in pathogen susceptibility/resistance. The recent sequencing of the genome of *Cx. quinquefasciatus* (http://www.broadinstitute.org/annotation/genome/culex_pipiens.4/Home.html) may aid in identification of genes that directly influence susceptibility to infection or other aspects of vector competence. Nonetheless, efforts in this direction should also examine gene-by-environment interactions to fully understand variability in vector competence.

We also found some evidence that the genetic ancestry of *Cx. pipiens* mosquitoes (forms pipiens and molestus) differed between mosquitoes that became infected and those that did not become infected. In one of the two comparisons made (Staten Island specimens collected in 2002) susceptible mosquitoes had a much higher probability of form pipiens ancestry. We previously showed that mosquitoes with higher probability of ancestry from form molestus were more likely to feed on mammals. This finding has been recently replicated in the midwestern United States.[53] One possible, but entirely speculative, mechanism that could explain this pattern is that with stronger selective pressure on the virus to replicate more efficiently in vectors, which it encounters more frequently because birds are more competent than mammals,[11] WNV would be more likely to encounter form pipiens mosquitoes. However, mosquitoes are also under selection to avoid infection and the damage to mosquitoes during replication that reduce fitness.[54] Finally, because we only observed a difference in genetic ancestry of susceptible and infected mosquitoes in one of two populations where we examined them, our findings need replication and corroboration and would be strongest if they were made between pure populations of the two *Cx. pipiens* forms.

Across all of our sampling, we found that *Cx. restuans* were more likely to transmit WNV than *Cx. pipiens*, but were marginally less likely to become infected. A previous study in our laboratory found no significant difference in the fraction of all *Cx. pipiens* and *Cx. restuans* mosquitoes transmitting, but also found that of mosquitoes that became infected, *Cx. restuans* were much more likely to have a disseminated infection and to transmit.[29] This finding suggests that there may be more

barriers to dissemination and infection of the salivary glands in *Cx. pipiens* than in *Cx. restuans*. A similar comparison exists between *Culex tarsalis* and *Cx. pipiens*, with the former being more efficient at dissemination and transmission of WNV.[30,32]

One other study examined vector competence in *Cx. restuans* from a single population in Maryland (but did not study *Cx. pipiens*) and found that 100% of mosquitoes became infected and 55% transmitted, but the sample size was too small (11 mosquitoes) to make strong comparisons with our data.[28] Two other studies measured vector competence of field-collected *Cx. pipiens* mosquitoes (from New York) and found 14% and 20% of mosquitoes transmitted WNV, which is well within the range we observed.[26,27]

In our study, different temperatures and other environmental factors at the different sites may have affected the parental generation that laid the egg rafts collected for our experimental vector competence assays physiologically and by altering the genetics and phenotypes of mosquito populations.[55] This possibility was supported by the significant genetic differentiation between counties, and these differences in turn might have influenced vector competence despite our rearing all larvae and maintaining all adults at one temperature (30°C). Although our collections of mosquito populations were not conducted frequently enough to enable determination of influences of temperature or other environmental influences on vector competence, suggestive evidence has been observed in previous studies with seasonal patterns of susceptibility to infection.[51,52]

More broadly, our results suggest that vector competence is not a static intrinsic trait of a particular mosquito population, and spatial variation within a species can be larger than between species (Tables 1 and 2). Instead, our study suggests that vector competence of a mosquito population can vary over time and appears to be dependent on intrinsic and extrinsic influences, such as environmental and genetic factors, and possibly their interaction. This finding is interesting because temperature has received substantial attention as a determinant of the geographic distribution and transmission intensity of particular vector-borne diseases,[56] and vector competence has been suggested as one possible contributing factor.[57–59] It remains to be determined whether temporal variability in vector competence and not just susceptibility to infection can be consistently linked to environmental factors in a predictive manner. Temperature is already known to have an impact on survivorship, feeding frequency, immature developmental rates, and vector competence directly,[31,60,61] all of which affect vectorial capacity. Disentangling the factors that determine the wide variation in vector competence we observed is an important challenge for future research.

Received January 3, 2010. Accepted for publication May 15, 2010.

Acknowledgments: We thank Pam Chin for help rearing mosquitoes; the entire Arbovirus Laboratory insectary staff for help with experimental procedures; H. Brightman for assistance with data management; Timothy J. Lepore Jr. and Dr. Anthony Kiszewski for field collection efforts; Kenli Okada for painstakingly comparing and repeating microsatellite analyses to maintain coherence over the years; and the University of Pennsylvania Sequencing Facility for working with us to optimize microsatellite analysis of the *Cx. pipiens* complex.

Financial support: This study was supported by National Institute of Allergy and Infectious Diseases contract #NO1-AI-25490, Centers for Disease Control and Prevention grant 1RO1AI069217-01, and National Science Foundation grant EF-0914866 as part of the joint National Science Foundation–National Institutes of Health Ecology of Infectious Disease program.

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