

**A Multi-level Analysis of the
Interactions between Vectors, Hosts, and Habitats of EEEV**

by

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Abstract

Eastern equine encephalitis virus (EEEV) is a rare and dangerous mosquito-borne pathogen with an elusive pattern of occurrence across its range in North America. The primary basis of our understanding of its transmission cycle between the vector *Cs. melanura* and avian reservoir hosts stems from studies in the northeastern United States. In this dissertation, I draw upon a range of innovative statistical approaches to study the transmission of EEEV in the southeastern United States in relation to the ecology of EEEV vectors and avian reservoir hosts. I estimate the dispersal distance of *Culex erraticus*, a mosquito species potentially involved in transmission of the virus, using a novel approach rooted in Bayesian statistics and borrowed from the seed dispersal literature. I also consider the distribution of avian reservoir hosts of the virus and their habitat associations, with the goal of estimating rates of utilization of avian host species by EEEV mosquito vectors. Such estimates of host utilization have great potential utility in revealing the relative contribution of various host species to transmission of the virus. Indeed, I provide evidence that the most highly preferred host of *Cs. melanura*, the common yellowthroat (*Geothlypis trichas*), has the strongest support of influencing transmission as a dilution host among species considered. Finally, I develop a model to predict rates of contact of avian hosts with *Cx. erraticus* based on host characteristics using data on host traits available from the ornithological literature. Together these results of my studies provide a strong basis for the future development of predictive models for occurrence of the virus and provide a framework for

future research of the transmission of this virus in the Southeast, and potentially throughout its range in North America.

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I. ESTIMATION OF DISPERSAL DISTANCES OF CULEX ERRATICUS
IN A FOCUS OF EASTERN EQUINE ENCEPHALITIS VIRUS IN THE SOUTHEASTERN
UNITED STATES

Abstract

Patterns of mosquito dispersal are important for predicting the risk of transmission of mosquito-borne pathogens to vertebrate hosts. We studied dispersal behavior of *Culex erraticus* (Dyar & Knab), a potentially significant vector of eastern equine encephalitis virus (EEEV) that is often associated with foci of this pathogen in the southeastern United States. Using data on the relative density of resting adult female *Cx. erraticus* around known emergence sites in Tuskegee National Forest, Alabama, we developed a model for the exponential decay of the relative density of adult mosquitoes with distance from larval habitats through parameterization of dispersal kernels. The mean and 99th percentile of dispersal distance for *Cx. erraticus* estimated from this model were 0.97 km and 3.21 km per gonotrophic cycle, respectively. Parameterized dispersal kernels and estimates of the upper percentiles of dispersal distance of this species can potentially be used to predict EEEV infection risk in areas surrounding the TNF focus in the event of an EEEV outbreak. The model that we develop for estimating the dispersal distance of *Cx. erraticus* from collections of adult mosquitoes could be applicable to other mosquito species that emerge from discrete larval sites.

Introduction

Urbanization and the accompanying modification of natural landscapes are increasing human exposure to mosquito-borne pathogens (Norris 2004, Pimentel et al. 2007, Patz et al.

2008). Predictions of such increases follow directly from Pavlovsky's theory of the natural nidity of transmissible diseases, which states that transmission of a vector-borne pathogen to humans occurs via association with the natural focus of the pathogen, with the focus defined as the specific conditions of habitat and geography that allow for maintenance of the natural transmission cycle of the pathogen (Pavlovsky 1966). Development adjacent to natural habitats such as wetlands increases the frequency of association between humans and isolated pathogen foci, either through the encroachment of human populations on foci or through areal expansion of foci themselves via habitat alteration that creates novel breeding sites for mosquito vectors (Norris 2004). Delineation of the geographic boundaries of mosquito-borne disease foci is thus necessary for accurate quantification of the degree of spatial association between humans and disease-causing pathogens.

Such delineation is particularly relevant in the case of eastern equine encephalitis virus (EEEV), a mosquito-borne pathogen that exhibits relatively stable foci and that is the most severe of the arboviral encephalitides in the United States. The human mortality rate of persons infected with EEEV is 30 - 40% (Whitley and Gnann 2002). Survivors of infection suffer mild to severe neurological damage and commonly require expensive, long-term medical care (Villari et al. 1995). Horses and gamebirds are also commonly infected with the virus and experience mortality rates over 80% (Scott and Weaver 1989).

Although it is an extremely pathogenic disease of humans and horses, EEEV is one of the rarer viral encephalitides causing clinical infections in the United States. The rarity of EEE cases may be due, in part, to the geographic isolation of the virus from areas of human habitation. EEEV is endemic to freshwater swamp habitats where its primary enzootic vector, *Culiseta melanura* (Coquillett), and avian reservoir hosts are sympatric (Scott and Weaver 1989). As

such, delineation of the boundary zones surrounding these foci is critical to identifying high-risk areas as population growth and expansion in the Southeast potentially lead to human encroachment on EEEV foci (Wear and Greis 2001, Alig et al. 2004).

In the southeastern United States, the mosquito species *Culex erraticus* (Dyar & Knab) has recently been identified as a bridge vector that may play a key role in the transmission of EEEV to humans and horses (Cupp et al. 2003, Cupp et al. 2004, Cohen et al. 2009). This mosquito is a moderately competent vector of EEEV and feeds on both birds and mammals (Chamberlain et al. 1954, Hassan et al. 2003). Its typical larval habitat in the southeastern United States is permanent bodies of fresh water overgrown with surface plants (Horsfall 1955). These larval habitats can overlap areas where EEEV is endemic, and in such areas, relative densities of adult *Cx. erraticus* are high compared to other potential bridge vector species of the virus (Cupp et al. 2003, Cupp et al. 2004).

A previous study of the dispersal behavior of *Cx. erraticus* indicated that it is a long-distance flier with a maximum flight range of 1.4 - 2.2 km and a mean dispersal distance of 0.73 (+/-0.61) km (Morris et al. 1991). One approach to delineating the boundaries of areas where humans would be at risk of EEEV infection is to buffer all larval sites in an EEEV focus with a distance equivalent to the upper limit of the maximum flight range of *Cx. erraticus*. Flight distance estimates for *Cx. erraticus* reported by Morris et al. (1991) were based upon a mark-release-recapture approach, but these results must be viewed with caution for several reasons. First, the mosquitoes used in the study were not dispersing from their natural emergence or oviposition site, but from an arbitrary location in the middle of a wastewater treatment facility. Because the flight range of mosquitoes is known to vary with habitat (Silver 2008), mosquitoes released in this environment may display different patterns of dispersal relative to mosquitoes

dispersing from a natural emergence site. Second, mosquitoes were released in the morning in an open, sunny area. *Culex erraticus* seeks resting sites during the morning hours to avoid desiccation (Gray et al. 2010) and would likely undertake an initial movement in search of such a suitable resting site. Finally, marking, trapping, and handling mosquitoes may alter mosquito dispersal behavior (Silver 2008). These potential complications with the previous mark-release-recapture study highlight the need for the development alternative approaches to estimating dispersal distances of *Cx. erraticus*.

In the current study we developed new estimates of the dispersal distance of *Cx. erraticus* that do not rely on mark-release-recapture methods. Because *Cx. erraticus* typically oviposits in rather large, easily located bodies of water, we were able to identify the most likely sites of emergence in a study area in central Alabama. We then parameterized a dispersal kernel for this species using the straight-line distances between the sampling sites where adult females were collected and their putative sources of emergence. Dispersal kernel parameterization is an approach to the study of dispersal in other organisms, most notably angiosperms. We develop a novel application of such “seed dispersal” models to the study of dispersal of *Cx. erraticus*. Our goal was to both estimate the dispersal distances of *Cx. erraticus* and to assess the general utility of “seed dispersal” models for studying mosquito dispersal and predicting relative mosquito densities on a local scale.

Materials and Methods

Field Methods

We estimated the dispersal distances of female *Cx. erraticus* using data on the relative density of adult and larval mosquitoes collected between 2006 and 2009 in Tuskegee National

Forest (TNF) in Macon County, Alabama. TNF has served as the site of an ongoing study of the vector and vertebrate host dynamics of EEEV since 2001 and is described more fully in Cupp et al. (2003). Briefly, the study site encompassed a 28-km² circular area centered on a core wetland complex located approximately 3.0 km from the town square of the city of Tuskegee (32°38'40"N, 85°25'59"W).

In 2006 - 2008, we sampled the adult mosquito population within the study site by aspirating resting mosquitoes from artificial shelters. In 2006, we used a variety of shelter types including fiber pots, resting boxes, and 50-gallon plastic cans, but in 2007 and 2008 we exclusively used 50-gallon black plastic cans, the most attractive type of shelter for resting mosquitoes in TNF (Burkett-Cadena et al. 2008). Because the shelters used in 2006 varied in attractiveness to female mosquitoes, the data we used for the 2006 analysis were derived only from fiber pots and resting boxes, which showed no difference in attractiveness (Burkett-Cadena et al. 2008). Restricting mosquito samples to the same type of collecting container within any year assured that attractiveness of shelters ultimately used in our analyses varied between, but not within, years.

The number and locations of the adult sampling sites also varied between years. In 2006, six sampling sites were spaced approximately 0.43 km along each of five transects radiating out 3.0 km from a point at the center of the study site (Figure 1). In 2007 and 2008, seven sampling sites were located every 0.19 km along six transects radiating 1.5 km (Figure 2). We excluded six sampling sites, which were either moved between 2007 and 2008 or were adjacent to private lands on which we were unable to sample for larval mosquitoes. As such, the data used in our analyses originated from 26 of the 31 sampling sites where fiber pots and resting boxes were used for collection in 2006, and 41 of the 43 sampling sites from 2007 and 2008.

Adult mosquitoes were collected between March and October, with regular sampling occurring between June and September. In 2006, regular sampling consisted of seven sampling intervals, each of which was two weeks in length and during which one mosquito collection was made at all 26 sampling sites. In 2007 and 2008, we collected mosquitoes once at all 41 sampling sites during each of 15 sampling intervals, with each sampling interval lasting one week. Results of any sampling outside of these regular sampling intervals were excluded from our analyses, so that the number of mosquito samples collected is constant across sites sampled in the same year. Following collection in the field, mosquitoes were transported to the laboratory, anesthetized with CO₂, and sorted on a chill table by species and gender.

We also sampled all permanent ponds within the study site for mosquito larvae to identify *Cx. erraticus* larval habitats, and hence the sites of emergence of the population under study. We censused the ponds from mid-July to mid-September in 2009 by repeatedly dip-sampling along the perimeter of each pond. Thirty dips were taken at 100-m intervals along the perimeter of each pond using a larval dipper. All larvae collected from each sampling point on a pond perimeter were transported back to the lab, heat-killed, and sorted by species.

Dispersal Distance Estimation

Our approach to estimating the dispersal distances of *Cx. erraticus* was based on fitting a model of exponential decay with distance to *Cx. erraticus* relative density data from adult sampling sites. The model we used was a special case of the general set of models originally developed to estimate the number of seeds arriving at seed traps from multiple source trees distributed throughout a landscape (Clark 1998, Clark et al. 1998, Clark et al. 1999). Such seed dispersal models specify the number of seeds at trap i as originating from a Poisson distribution

with the mean and variance parameter λ_i equal to the product of trap area A_i and the sum, over all source trees $j=1, \dots, n_i$ that are located within the maximum seed dispersal distance of the trap, of the product of b_{ij} , the estimated fecundity of each source tree j , a parameter γ , and $g(d_{ij})$, a probability density function (pdf) for seed arrival from each source, to an infinitely small area centered on the location of the trap. $g(d_{ij})$ is understood to be a dispersal kernel (Nathan and Muller-Landau 2000), with a functional form that varies with the species for which dispersal is being modeled. Formally,

$$Y_i \sim \text{Pois}(\lambda_i)$$

$$\lambda_i = A_i \sum_{j=1}^{n_i} \gamma b_{ij} g(d_{ij})$$

There is a clear correspondence between the problem of estimating the number of seeds collected at a trap after dispersal from their parent trees and that of estimating the relative density of adult mosquitoes collected at a sampling site after dispersal from their larval habitats. As such, we adapted the above-formulated model to achieve the latter goal. In the model that we developed for *Cx. erraticus* dispersal, Y_i is a random variable of the total number of female *Cx. erraticus* collected over the course of the 2006 sampling season at an adult sampling site. While males were occasionally collected at the adult sampling sites, we restricted our analysis to dispersal of female adults. Ponds throughout the TNF that had at least one larva collected during the first sixty dips of sampling, the number of dip samples taken at the smallest pond, were designated as suitable larval habitats. These ponds were classified as the sources of dispersing female adults, and hereafter they will be referred to as *larval ponds*. We estimated the distance between all adult sampling sites and all larval ponds by delineating the perimeter of each pond using the GPS coordinates of larval sampling points to create a polygon shapefile for each pond

in ArcGIS v.9.2. We then calculated the Euclidean distances between all adult sampling sites and larval pond centroids using UTM coordinates.

A rigorous approach for estimating larval pond “fecundity”, or rate of productivity of adult *Cx. erraticus*, was not available. Therefore, we developed a series of models with productivity parameterized in various manners: assumed constant across ponds (constant), proportional to pond area (area), proportional to pond perimeter (perim), proportional the number of larvae collected in the first sixty dips (larv), proportional to pond area*the number of larvae in the first sixty dips (area*larv), or proportional to pond perimeter*the number of larvae in the first sixty dips (perim*larv).

The functional form of the dispersal kernel we used in the model was that of the exponential described in Clark et al. (1999) :

$$g(d_{ij}) = \frac{1}{2\pi\beta^2} \exp\left(-\left(\frac{d_{ij}}{\beta}\right)\right).$$

This dispersal kernel models the rate of decay of the density with distance from the source as exponential. Parameterization of this part of the model effectively allows for estimation of the mean and percentiles of the dispersal distance of *Cx. erraticus*. Specifically, 2β is an estimate of mean dispersal distance (Clark et al. 1998, Cousens et al. 2008). Percentiles of dispersal distance were estimated by first converting the dispersal kernel $g(d)$ to the distance pdf $f(d)$ (Cousens et al 2008):

$$f(d) = \frac{d}{\beta^2} \exp\left(-\left(\frac{d}{\beta}\right)\right).$$

We then calculated the median, 95th, and 99th percentiles of $f(d)$ through integration using the rectangle method with subintervals that were 1 meter in length. We used an exponential form for

the dispersal kernel because it has received the most support from empirical studies of insect dispersal (Gratton and Vander Zanden 2009).

Finally, counts of *Cx. erraticus* at adult sampling sites were often zero, so that the Negative Binomial was a more appropriate distributional assumption for these data than the Poisson (Chi-Square Test of Goodness-of-Fit – Poisson: $\chi^2 = 304.36$, $df = 12$, $p < 0.001$; Chi-Square Test of Goodness-of-Fit – Negative Binomial: $\chi^2 = 5.90$, $df = 11$, $p = 0.88$). As such, we modeled the number of females collected at each adult sampling site as following a Negative Binomial (p, k) distribution, whereby k is a dispersion parameter and p is a function of the mean. Formally, the models we used were of the form:

$$Y_i \sim \text{NB}(p_i, k)$$

$$p_i = \frac{k}{k + \mu_i}$$

$$\ln(\mu_i) = \ln(A_i) + \ln \sum_{j=1}^m \gamma b_j \frac{1}{2\pi\beta^2} \exp\left(-\left(\frac{d_{ij}}{\beta}\right)\right)$$

$$= \alpha + \ln \sum_{j=1}^{n_j} b_j \exp\left(-\left(\frac{d_{ij}}{\beta}\right)\right)$$

where

$$\alpha = \ln(A) + \ln\left(\gamma \frac{1}{2\pi\beta^2}\right), A_i = A \text{ for all sampling sites } i=1, \dots, n$$

In these models, α and β are the two parameters to be estimated, d_{ij} is the distance in meters between adult sampling site i and larval pond j , b_j is the variable serving as the proxy measure of larval pond productivity, the index i runs over all sampling sites $1, \dots, n = 26$, and the index j runs over all larval ponds $1, \dots, m = 15$.

We conducted modeling in a Bayesian framework, whereby inference about parameters is based upon examination of their posterior distributions. We used Markov Chain Monte Carlo (MCMC) algorithms to sample from the posterior distribution of the unknown parameters, circumventing the need for their explicit derivation (Gilks et al. 1996). We ran these algorithms using R2WinBUGS, the implementation of WinBUGS in R. We set three chains to run for 2000 iterations with a burn-in period of 1000 iterations. The prior distributions were uniform (-1000,1000) for α , uniform(0,5000) for β , and gamma (0.001,0.001) for k . Initial values for the parameters were drawn from Uniform (-10,10) and Uniform (0,500) distributions for α and β , respectively, while the initial value for k was set to 1, 2, or 3. Convergence diagnostics were assessed using the Coda package. We based model selection on minimization of the Deviance Information Criterion (DIC).

After selecting a final model for the 2006 data, we validated the model by applying it to 2007 and 2008 adult sampling sites. We used the 2007 and 2008 adult sampling sites for validation because they differed in location from the ones used in model development. Because the counts of *Cx. erraticus* at the adult sampling sites were summed over a different number of visits to each sampling site in 2007 and 2008 compared to 2006, we used Spearman's Rank Correlation Coefficient to assess model performance (Guisan and Zimmermann 2000).

Additionally, we were interested in the performance of the predicted relative density of mosquitoes from the model compared to another possible correlate that could be used to rank areas in terms of their relative densities. Specifically, we considered the number of overlapping larval buffers at a sampling site, using the mean dispersal distance derived from the model as the buffer radius-length, as this other possible correlate. We estimated the Spearman's Rank correlation coefficient between the relative density of *Cx. erraticus* and this variable, and then

compared both correlation coefficients using the Test of Two Correlated Correlation Coefficients (Meng et al. 1992).

Finally, we were interested in the relative performance of variables representing the total number of overlapping buffers of *any* radius-length in predicting the ranking of areas in terms of relative density of *Cx. erraticus*. As such, we created a set of variables similar to the last one described above, with buffer radius-lengths differing between variables by increments of 100 meters. We then estimated Spearman's Rank Correlation Coefficient between each of these variables and relative density of *Cx. erraticus* in 2007 and 2008.

Results

We collected a total of 2900 resting female *Cx. erraticus* from the adult sampling sites throughout TNF between 2006 and 2008. Seven hundred and seven (24.4%) of these individuals were excluded from our analyses because they were collected during sampling intervals when not all traps were visited, yielding a total of 205 individuals used in dispersal distance models developed using data from 2006 and 1988 individuals used in model validation and correlation analyses using the 2007-2008 data.

In 2006, when the adult sampling sites were distributed throughout an area radiating out 3.0 km from the core site (Figure 1), we collected an average 7.88 (SD=11.73, n=26) females/sampling site over the seven two-week intervals used in the analysis (Figure 3). The distances between adult sampling sites where resting females were collected and the nearest larval pond ranged from 0.107 km to 1.946 km.

In 2007 and 2008, when the adult mosquito collections were focused within the circular area radiating out only 1.5 km from the core site (Figure 2), we collected an average of 48.49

females/sampling site (SD=56.08, n=41) over the 15 weeks used in our analyses (Figure 3). All adult sampling sites in the study area had individuals present during at least one visit, and were distributed at distances ranging from 0.05 to 1.04 km from the nearest larval pond.

In total, 787 *Cx. erraticus* larvae were collected, with an average of 3.47 larvae/30-dip sample (SD=6.64, n=227). Of the 21 ponds we sampled, 15 were found to have *Cx. erraticus* larvae present during the first sixty dipo, and were thus classified as larval ponds (Figure 1).

Chains used in the model development all indicated a satisfactory degree of convergence after 2000 iterations, as the Gelman-Rubin statistics for parameter values in each model were all less than 1.10. Average values of samples from the posterior distributions of α ranged from -8.61 to 2.16, from 483.46 to 1682.38 for β , and from 0.44 to 0.82 for k . Overall, model deviances ranged from 136.65 to 148.59 (Table 1). DIC of the models ranged from 137.33 to 145.03, with the area model, the one that used the area of the larval pond of origin as a measure of productivity, selected as the best-fitting model based on its lower DIC compared to the other five models (Table 1).

The estimate of α based on the mean of samples from its posterior distribution in the area model was -6.37 with a 95% credible interval of (-8.08,-4.80). The dispersal parameter, β , was estimated as 483.46 with a 95% credible interval of (258.53,1058.95). The mean value of the k for the area model was 0.82, with a 95% credible interval of (0.34,1.70) (Table 1, Figure 4). The estimated mean dispersal distance of *Cx. erraticus* (2β) was 0.966 km. The median, 95th, and 99th percentiles for dispersal distance were estimated as 0.811 km, 2.291 km, and 3.206 km, respectively.

Application of the model to the validation dataset gave strong support to the model, as *Cx. erraticus* relative density at 2007 and 2008 adult sampling sites was significantly associated

with the total height at the sampling site of the overlapping dispersal kernels centered on each larval pond and weighted by pond area ($r_s=0.689$, 95% CI = (0.477, 0.820), $n=41$, $p < 0.0001$, Figure 5). The rank correlation between *Cx. erraticus* relative density and the number of overlapping larval buffers of radius-length equal to the mean dispersal distance were also significantly associated ($r_s=0.597$, 95% CI= (0.347, 0.761) , $p < 0.0001$, $n =41$) (Figure 6). Overall, there was no evidence to suggest that the strengths of association between these two variables—overlapping dispersal kernel height weighted by pond area and the number of overlapping buffers—and *Cx. erraticus* relative density were significantly different from one another ($Z= 1.04$, $p > 0.10$, $n=41$). Associations between ranks of *Cx. erraticus* relative density and the number of overlapping larval buffers at a site were positive for all other radius-lengths considered other than mean dispersal distance. However, these associations were only significant ($p < 0.05$) for buffers of radius-lengths less than 2500 m. All associations were weaker than that between the rank of *Cx. erraticus* relative density and predicted relative density based on the best-fitting model parameterized by 2006 data (overlapping dispersal kernel height weighted by pond area) (Figure 6).

Discussion

The distance that a mosquito can disperse from its site of emergence is of critical importance in studies of arboviruses. The conventional mark-release-recapture approach to the study of mosquito dispersal has the potential to bias estimates of dispersal distance because animals are often captive reared and/or released in an unfamiliar area that is not necessarily suitable habitat (Silver 2008). In this study we used a modeling approach based on sampling of wild *Cx. erraticus* to estimate the dispersal distances of mosquitoes emerging from natural

wetlands during a gonotrophic cycle. Despite concerns about overestimation of dispersal distances based on mark-release-recapture of lab-bred mosquitoes, our estimate of the average dispersal distance of 0.967 km is close to the mean dispersal distance of 0.73 (+/-0.61) km for *Cx. erraticus* based on mark recapture (Morris et al. 1991). Moreover, our results confirm that *Cx. erraticus* is a strong flier, given that most mosquito species studied typically disperse a no more than few hundred meters during appetential flight (Service 1997).

Knowledge of dispersal patterns can be used to predict the relative abundance of *Cx. erraticus* anywhere in the area of the study, as demonstrated by the strong association between the two correlates that we derived based on modeling results, total height of overlapping dispersal kernels weighted by pond area and number of overlapping buffers with radius length equal to the mean dispersal distance. For vector control, accurate estimates of mosquito abundance have obvious utility. Knowledge of mosquito abundance across a landscape could help formulate a strategy for adulticide application, and it could also be used to predict changes in relative density of *Cx. erraticus* under various scenarios of larvicide applications to larval ponds.

Estimates of dispersal distance resulting from the current study could also potentially be used to delineate boundary zones around the TNF focus outside of which a potentially infected bridge vector—i.e. a female ovipositing in a pond that overlaps an area of enzootic transmission between *Cs. melanura* and avian hosts—has a low probability of dispersing. For example, based on our results of the current study, the probability that a *Cx. erraticus* female will disperse further than 3.21 km from an oviposition site is 0.01. However, exposure to the bite of a bridge vector such as *Cx. erraticus* will be a function of not only of the probability of dispersal to a given distance of a single vector, but also the total number of vectors dispersing such that a proposed

radius ~3 km for the TNF EEEV boundary zones could still involve an unacceptable exposure risk. Risk assessment from a human health perspective is beyond the scope of this paper, but we have provided important information for the development of such assessments.

Whether or not *Cx. erraticus* proves to be an important component in EEEV transmission in the southeastern United States, in this study we have demonstrated that models originally developed to model seed dispersal are adaptable to the problem of estimating mosquito dispersal. Mark-release-recapture studies are subject to biases resulting from the use of captive bred animals, and studies of wild mosquitoes emerging from natural wetlands should be preferable for estimating the movement patterns of mosquitoes. While we applied the seed dispersal modeling approach to a species of mosquito that breeds in relatively discrete areas that are easily identifiable, it could potentially be applied to mosquito species that have a more continuous distribution of breeding habitat, by representing sites of emergence as the centroids of pixels classified as suitable breeding habitats in raster images and using associated attribute data on productivity of the habitats represented by those pixels (Brown et al. 2008, Jacob et al. 2009).

The accuracy of our estimates of the mean and upper percentiles of the dispersal distance of *Cx. erraticus* is contingent upon three assumptions. First, a critical assumption is that all potential larval ponds of *Cx. erraticus* that could contribute adults to the population were identified. While we feel confident in our inventory of source ponds, some of the adults that we captured could have emerged from ponds outside of our sampling area or from small aquatic habitats. Minimally, we identified all of the larval ponds near each sampling site. Thus, we are confident that our model included the primary sources of, and all of the sources that make a significant contribution to, adults for each sampling site.

The second assumption is that the measure of pond productivity used in the final model (larval pond area) is proportional to the number of adults that originate from that pond. Factors such as availability of aquatic vegetation and predator densities could affect productivity. However, as ponds in TNF are all relatively homogenous, being relatively shallow with vegetation scattered across their surface, the use of pond area as a proxy for mosquito production seems reasonable. Without more information on habitat needs of larval *Cx. erraticus* with which to assess pond quality and more data on the characteristics of each pond in the study area, there is no simple means to improve upon the use of pond area as a proxy for mosquito production.

Our final assumption stems from the reduction of the areal extent of each larval pond down to representation by its centroid alone, so the dispersal distance may have been overestimated given dispersal of adults from any point between a pond centroid and its edge. However, the average distance between each larval pond's centroid and larval sampling points along its edge was only 0.063 km, a negligible distance relative to the estimated mean dispersal distance.

In conclusion, use of models originally developed to estimate seed dispersal distances appears to be a powerful approach to characterizing the dispersal of *Cx. erraticus* given knowledge solely of the location of larval habitats and adult sampling sites. Evidence of the utility of this approach is the strong association between overlapping dispersal kernel heights predicted from the model and relative density when the model was applied to new sampling site locations. Our approach could be used in characterizing dispersal for other mosquito vector species, leading to more accurate predictions of their spatial distribution and thus the effective areas where vertebrate hosts are at risk of exposure to the pathogens they transmit.

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Table 1. Mean (95% credible intervals) for parameters and deviance of the models developed for *Cx. erraticus* abundance at 2006 adult sampling sites in Tuskegee National Forest, Alabama, with DIC of the models also presented. Model names correspond to the variable used as a metric for larval pond productivity in each model, as explained in the Methods section.

Model	α	β	k	deviance	DIC
constant	2.16 (0.09,3.66)	600.81 (288.23,2198.90)	0.69 (0.28,1.41)	140.56 (136.70,150.10)	138.34
larv	-1.45 (-3.03,1.09)	1682.38 (323.92,4640.25)	0.44 (0.21,0.81)	148.59 (144.40,155.30)	145.03
area	-6.37 (-8.08,-4.80)	483.46 (258.53,1058.95)	0.82 (0.34,1.70)	136.65 (133.30,144.20)	137.33
perim	-3.89 (-5.81,-2.42)	523.57 (278.45,1478.63)	0.76 (0.31,1.54)	138.94 (135.3,147.80)	138.86
area*larv	-8.61 (-10.83,-6.94)	628.58 (273.42,2678.75)	0.62 (0.26,1.23)	142.05 (138.20,150.20)	138.66
perim*larv	-6.94 (-8.86,-4.82)	1224.30 (302.30,4536.65)	0.48 (0.22,1.02)	147.20 (142.70,154.00)	141.56

Figure 1. Map of the EEEV study area in the TNF study area during 2006. Forest boundaries are shown in green, and the political boundary of the city of Tuskegee is shown in black. Black circles represent the locations of the adult sampling sites in 2006, and the pink circles mark the centroids of all larval ponds of *Cx. erraticus*.

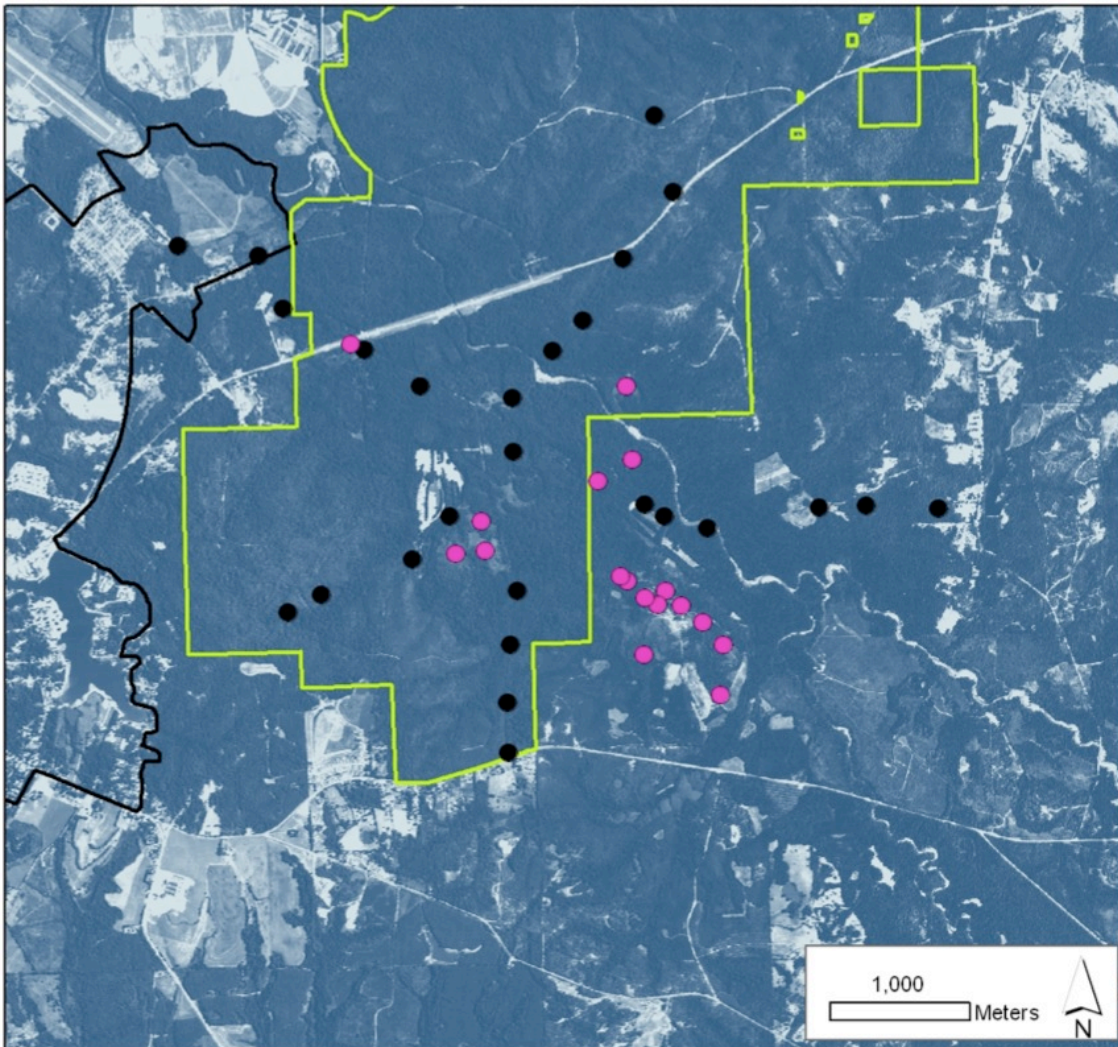


Figure 2. Map of the EEEV study area the TNF study area during 2007 and 2008. The dotted green line delineates the area beyond which a *Cx. erraticus* female that emerges from any of the outer larval sites in the TNF has less than a 0.05 probability of dispersing. Circles represent the locations of adult sampling sites in 2007 and 2008, with sites with a low rate of capture of *Cx. erraticus* coded as light pink, and sites with high rates of capture coded as red. Classification of sampling sites is based on whether the total number of *Cx. erraticus* adult females was below or above the median count among all sampling sites during 2007-2008. The total height of dispersal kernels as parameterized in the *area* model centered on each larval pond, weighted by pond area, and overlapping in each pixel is shown with equal interval symbology ranging from lowest (white) to highest (dark green).

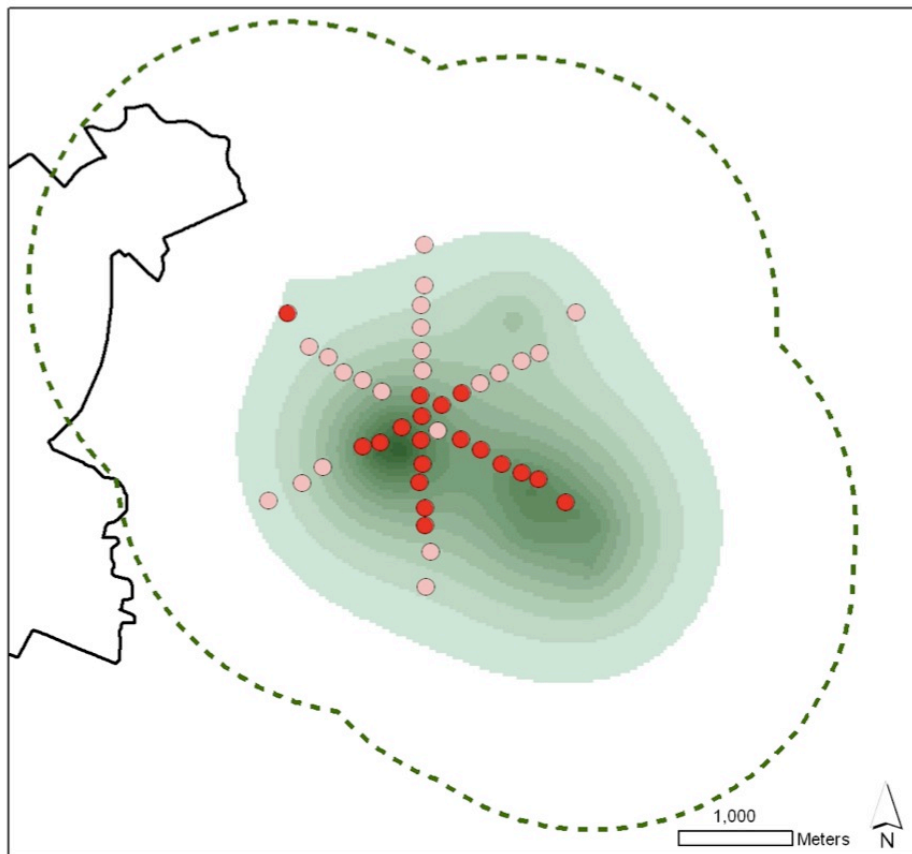


Figure 3. Histograms of the total number of female *Cx. erraticus* collected at adult sampling sites in the TNF study area in either 2006 or 2007 and 2008 cumulatively.

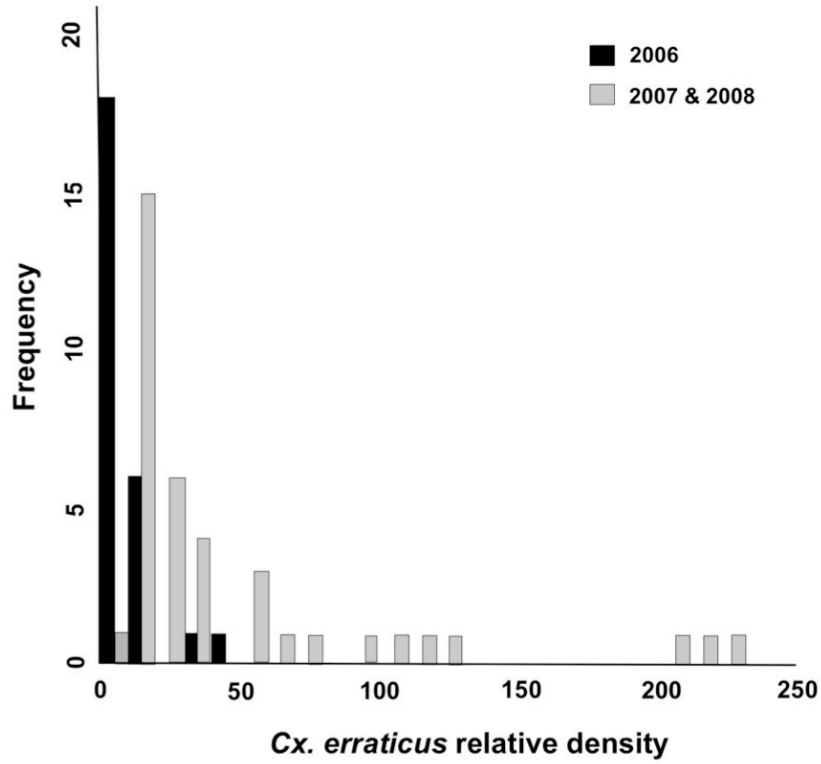


Figure 4. Density and trace plots of MCMC samples from the posterior densities of parameters in the best-fitting model for *Cx. erraticus* abundance at 2006 adult sampling sites, Model area. Three chains were run for 2000 iterations with a burn-in period of 1000. A thinning interval of three was used, so that the number of samples shown is 334.

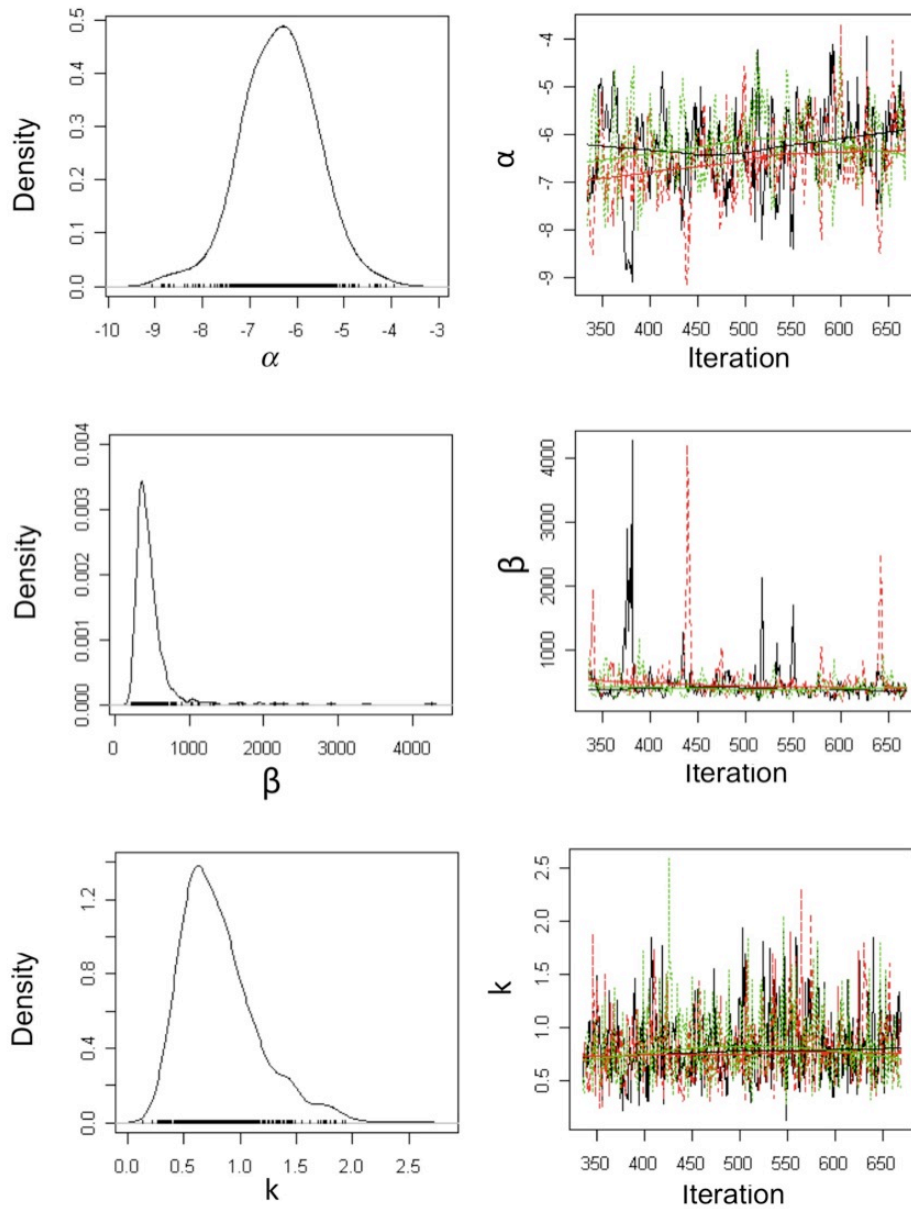


Figure 5. Scatterplot of the total number of *Cx. erraticus* females collected at 41 adult sampling sites between 2007 and 2008 in the TNF study area versus the total height of dispersal kernels as parameterized in the *area* model centered on each larval pond, weighted by pond area, and overlapping at a site.

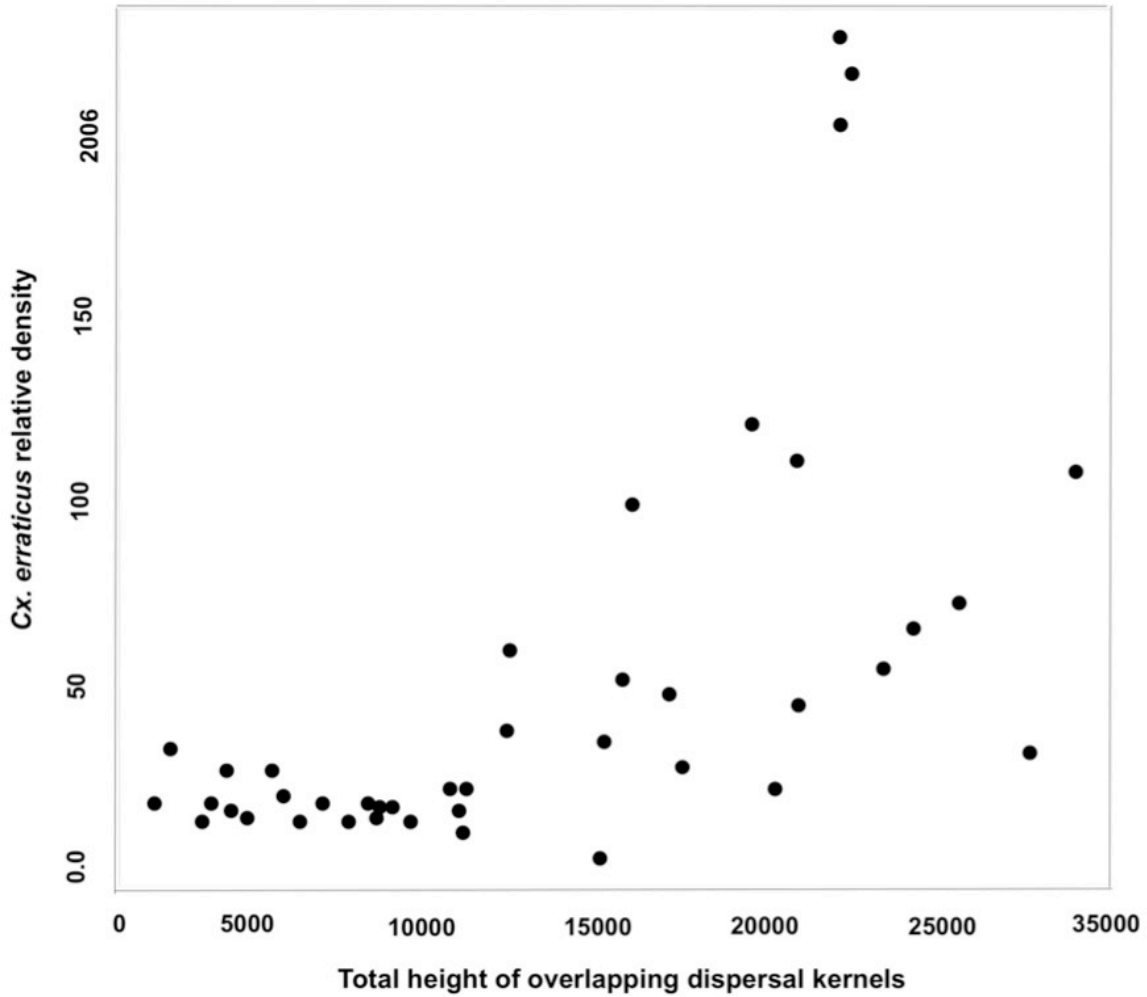
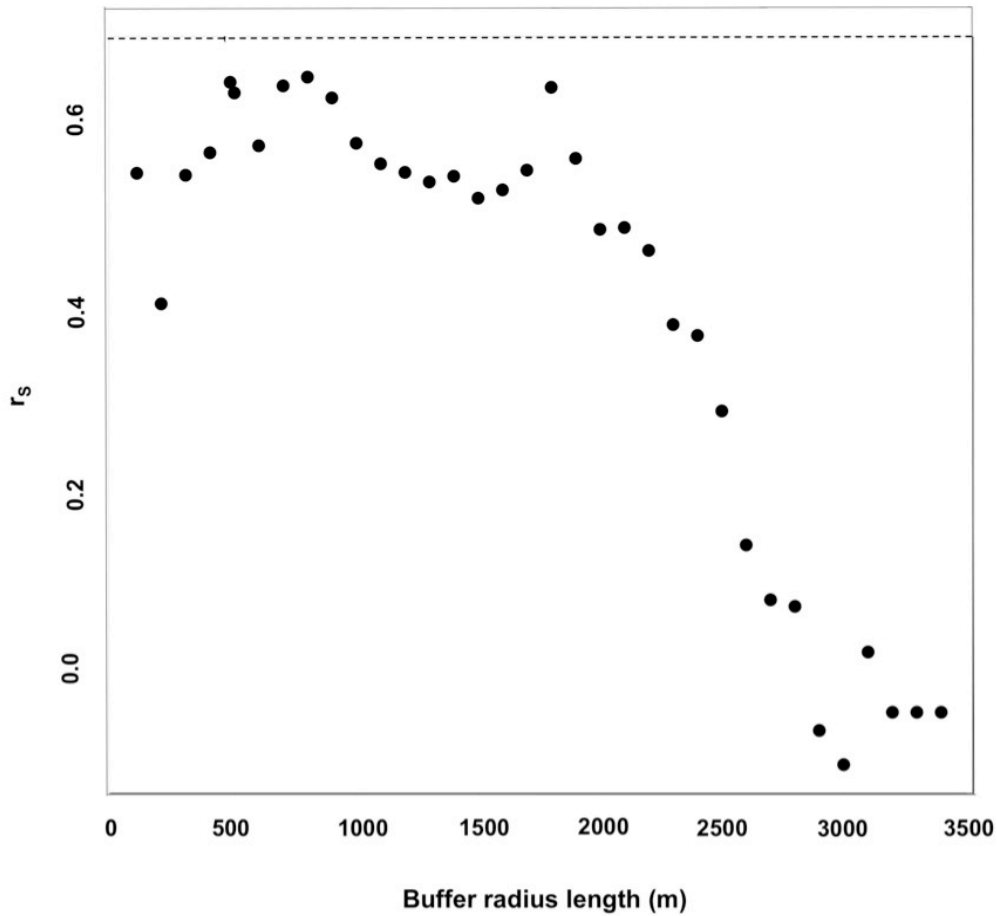


Figure 6. Scatterplot of Spearman's Rank Correlation Coefficient (r_s) for associations between the relative density of *Cx. erraticus* at adult sampling sites in the TNF study area in 2007 and 2008 and the total number of overlapping larval buffers at the site. The dotted line across the top of the figure indicates the r_s (0.69) for the association between the relative density of *Cx. erraticus* at an adult sampling site in 2007 and 2008 and the total height of dispersal kernels as parameterized in the *area* model centered on each larval pond, weighted by pond area, and overlapping at a site.



II. A MUTLI-YEAR STUDY OF MOSQUITO FEEDING PATTERNS ON AVIAN HOSTS IN A SOUTHEASTERN FOCUS OF EASTERN EQUINE ENCEPHALITIS VIRUS

Abstract

Eastern equine encephalitis virus (EEEV) is a mosquito-borne pathogen that cycles in birds, but also causes severe disease in humans and horses. We examined patterns of avian host utilization by vectors of EEEV in Alabama from 2001 to 2009 using blood-meal analysis of field-collected mosquitoes and avian abundance surveys. Northern cardinal (*Cardinalis cardinalis*) was the only preferred host (fed upon significantly more than expected, based on their abundance) of *Culiseta melanura*, the enzootic vector of EEEV. Preferred hosts of *Cx. erraticus*, a putative bridge-vector of EEEV, were American robin (*Turdus migratorius*), Carolina chickadee (*Poecile carolinensis*), barred owl (*Strix varia*), and northern mockingbird (*Mimus polyglottis*). Our results provide insight into the relationships between vectors of EEEV and their avian hosts in the Southeast, and suggest that northern cardinal may be important in the ecology of EEEV in this region.

Introduction

Birds are implicated as the primary reservoir hosts in transmission cycles of many mosquito-borne pathogens.¹ Recent research has emphasized that some bird species are over-utilized relative to their local availability by vector mosquitoes as bloodmeal sources,^{2,3,4,5,6,7} and that

overutilization of certain avian species could influence the transmission of pathogens.⁸ For example, temporal and spatial patterns of American robin (*Turdus migratorius*) abundance have been associated with variability of the rate of human cases of West Nile virus (WNV) and prevalence of WNV in mosquito populations.^{9,6} Such patterns have been attributed to a higher rate of feeding upon American robins by foraging mosquitoes relative to other avian species,¹⁰ which leads to a higher probability of infection of robins, potentially causing robins to function as a superspreader of the virus.³

Studies of avian host utilization by mosquitoes are vital components to elucidating the ecology of arbovirus transmission. To date, however, such studies have been largely restricted to potential vectors of WNV.^{3,4,5,6,7} The extent to which observations from studies of WNV can be applied to other mosquito-borne pathogen systems for which birds serve as reservoir hosts is uncertain, as these pathogens vary in the ecological factors that influence transmission.¹¹

The ecology of the transmission dynamics of eastern equine encephalitis virus (EEEV) is distinct from that of WNV. WNV is a periurban disease for which the primary enzootic vectors in the U.S. are *Culex pipiens* in the Northeast and *Cx. quinquefasciatus* in the Southeast.¹² In contrast, EEEV is endemic to bottomland hardwood swamps, and the primary enzootic vector over most of its range is the ornithophilic mosquito *Culiseta melanura*.¹³ Given the very different habitats in which the two viruses occur, as well as the different vectors that transmit them, it seems likely that other aspects of the ecology of these viruses also differ.

In this study, we assessed patterns of avian host species utilization by mosquitoes by employing data collected over nearly a decade on the East Gulf Coastal Plain in Alabama. We determined the identity of avian blood meals in eight mosquito species: *Culiseta melanura*, *Cx. restuans*, *Aedes vexans*, *Coquillettidia perturbans*, *Cx. erraticus*, *Culex peccator*, *Cx. territans*,

and *Ochlerotatus sticticus*. Five of these species have been implicated in transmission of EEEV, but the avian host preferences of these mosquitoes have not been characterized in detail. *Culiseta melanura* is widely recognized as the primary enzootic vector of EEEV,¹⁴ and *Cx. restuans* has recently been proposed to function as an enzootic vector as well.¹⁵ *Aedes vexans*, *Cq. perturbans*, and *Cx. erraticus* have been proposed to play roles as bridge vectors.^{14,16} *Culex peccator*, *Cx. territans*, and *Oc. sticticus* were also found to have bird blood meals during this study but their roles in transmission of EEEV are uncertain.

In a previous study, we found that, collectively, mosquitoes at a study area in Tuskegee National Forest (TNF) feed on available vertebrate classes, with degree of catholicism varying by mosquito species.¹⁷ In the present study, we focus specifically on characterizing patterns of avian host use by mosquitoes in TNF. A preliminary analysis of the patterns of mosquito feeding on avian species in the same study area was published elsewhere.² That study examined patterns of host use based on bird abundances estimated from a small number of point counts made within a limited portion of the study area. Moreover, the avian abundances used in the earlier analysis were based on estimates that did not account for imperfect detectability of avian species from point count surveys. Here, we analyze multiple years of blood meal data using an improved null model for mosquito utilization of avian hosts relative to availability that accounts for species detectability.¹⁸ Our goal was to produce comprehensive and accurate estimates of forage ratios of avian hosts for mosquito vectors of EEEV.

Materials and Methods

Mosquito Surveys and Blood Meal Source Identification

We studied the blood-feeding patterns of mosquitoes over a nine-year period in a study area in Tuskegee National Forest (TNF) in Macon County, Alabama by regularly collecting

blood-engorged mosquitoes and using DNA analysis to identify the sources of their bloodmeals. This site has been the center of an ongoing study of the ecology of mosquitoes and their interactions with avifauna and herpetofauna in this focus of EEEV since 2001, and it is described more fully elsewhere.^{16,17,19,20} Briefly, the study site is a circular area encompassing 28 km² predominated by a complex of forest, ponds and wetlands. Much of the land is part of TNF, but also extends into adjacent private lands.

Mosquitoes were collected annually between March and October between 2001 and 2009, except in 2005, when no mosquito sampling occurred. Mosquito collection entailed aspiration of individuals from natural resting sites and a variety of container types serving as artificial resting sites immediately surrounding each sampling location, with the container types used varying over the course of the study.^{17,19,20} Following collection, mosquitoes were transported to the laboratory at Auburn University, sorted on a chill table by species, sex, and engorgement status, and stored at -70°C until processing for blood meal identification.

The identity of host blood meals at the study site was determined by specific amplification of a portion of the vertebrate cytochrome B gene, as previously described.^{2,17,21,22} The identity of the amplicons was determined using a combination of heteroduplex analysis and direct DNA sequencing, as previously described.^{2, 5,17}

Avian Surveys and Modeling

We conducted surveys of avian populations at 338 locations spread uniformly throughout the study area from 15 May through June in 2008. We used a systematic sampling design for the surveys, whereby survey points were located 250 m apart on vertices of a grid that covered the study area. Surveys were conducted during two non-overlapping rounds of sampling so that each point was visited twice during the summer. Estimating the probability of detection for each

species requires repeated sampling of the same location, so during each of the two visits to a point count location, three consecutive 4-min counts were conducted. The species identification of all birds seen or heard within 100 m of the point-count location was recorded during each 4-min count period. All sampling sessions occurred between 0500 and 1100 CDT.

Nocturnal bird surveys were also conducted at a subset of 50 of the bird point count locations spaced 500 m apart. Nocturnal surveys were conducted using a combination of silent point counts and audio playback of the target species. Upon arriving at the survey location the observer conducted three consecutive three-minute counts of all individual birds detected within 200 m. The observer then played 20-seconds of chuck-will's-widow (*Caprimulgus carolinensis*) calls followed by a one-minute count period. Next the observer played 20-seconds of whip-poor-will (*Caprimulgus vociferous*) calls followed by a one-minute count period. This procedure was then repeated for eastern screech-owls (*Megascops asio*), barred owls (*Strix varia*), and great horned owls (*Bubo virginianus*) in that order, with each species' call being played followed by a one-minute count period. Care was taken to make sure that the audio recordings were not audible more than 200 m away from the observer. Nocturnal surveys were conducted by a single observer between 15 June and 3 July, 2009 between the hours of 2000 - 2400 and 0400 – 0500 CDT.

We estimated densities of avian hosts at mosquito-sampling sites by applying predictive models of density for each bird species recorded during 2008 and 2009 point counts.¹⁸ We used *N*-mixture models to incorporate heterogeneity in detectability of individual species into models of occupancy and abundance. We modeled mean density of each species as a linear combination of covariates describing the relative abundances of habitat types in 100-m buffers around bird point count locations derived from Alabama Gap Analysis Project (GAP) land cover map²³ and

the National Land Cover Database Tree Canopy Cover Map,²⁴ assuming a Poisson error distribution. While such count data may follow a Negative Binomial distribution, the Poisson distribution has been found to be appropriate for many species detected during our surveys.²⁵ All modeling was carried out using the program PRESENCE²⁶ with further details of model development given elsewhere.²⁷ Predicted densities of nocturnal species were standardized to a 100-m buffer, the area sampled for birds during diurnal avian surveys.

Because of practical considerations, we were forced to exclude a small number of avian species *a priori* from forage ratio calculations. First, house finches (*Carpodacus mexicanus*) were excluded from these analyses because individuals of this species were housed in sentinel cages at the center of the study area from 2002-2004. Avian species from Orders Ciconiiformes (herons) and Pelecaniformes (anhinga) were also excluded because point count methodologies do not provide accurate estimates of their densities²⁸ and we had no means to accurately census for these species. Inadequate numbers of wood ducks (*Aix sponsa*), chickens (*Gallus gallus*), and red-tailed hawks (*Buteo jamaicensis*) were detected to model abundance, and thus these three species were also excluded from forage ratio calculations.

Statistical Analysis

We estimated the rate of use of avian host species identified in blood meals collected between 2001 and 2009 relative to their availability by different mosquito species using the forage-, or selection-ratio approach described elsewhere.^{29,30} With this approach, the ratio of the relative abundance of a host species in the bloodmeal sample to its relative abundance in the avian community is a forage- or selection-ratio. In the current study, the relative abundance of an avian host species in the bloodmeal sample was calculated separately for *Cx. erraticus*, *Cx.*

restuans, and *Cs.melanura*, using bloodmeal abundances summed across all study years. The relative abundance of a host species in the avian community was calculated separately for each of the three focal mosquito species using average estimated densities of avian hosts at all sites where individuals of each mosquito species were collected, respectively (Table 1).

Statistical significance of the forage ratio estimate for an avian species was based on overlap of the 95% confidence intervals of the estimate with the value one.³⁰ An avian species was considered to have been *preferred* if it was overutilized relative to its rate availability to a mosquito species, such that the lower 95% confidence limit for the forage ratio estimate was greater than one. A species was inferred to have been *avoided* if it was underutilized relative to its rate of availability, such that the upper 95% confidence limit for the forage ratio estimate was less than one. An avian species for which the 95% confidence interval for its forage ratio included one was considered to have been *fed upon opportunistically*.³⁰ We additionally estimated forage ratios for each avian host species using blood meals collected strictly between May 1st and August 15th to determine whether or not forage ratio estimates were biased by potentially non-constant relative abundances of avian species between March and October of each year.

The study area in and around TNF represents a rural environment undergoing no wide-scale alteration of habitats and with stable bird populations between years. We thus assumed that the composition of the avian community had been stable over the course of the study period, such that it was reasonable to use point-count surveys in the TNF study area conducted during 2008 and 2009 as representative of the relative abundances of each species in the avian community over the course of the study. To formally test the validity of this assumption, we compared the avian community structure in and around TNF between 2001 and 2009 with data

from Breeding Bird Survey³¹ along the Warrior Stand Route. The Warrior Stand Route runs through the TNF study area and was conducted across the same years as mosquito surveys. This BBS route has been surveyed within five days of the same date under nearly identical weather conditions and by a single observer (GEH) since 1998, so that comparisons of abundances between years are not biased by heterogeneity in detectability of species due to season, weather, time of day, or observer effects.

We created a joint (2001+2009) data set, with record entries indicating the species identification of individual birds observed during the 2001 and 2009 Warrior Stand Breeding Bird Surveys, and the total number of records in the dataset equal to the total number of individuals observed in 2001 and 2009 ($n_{\text{tot}} = 1707$, $n_{2001} = 856$, $n_{2009} = 851$).³² We randomly sampled 856 individuals from this joint data set and assigned them to the first simulated 2001 community, and assigned all remaining 851 individuals in the joint community dataset to the first simulated 2009 community. We then calculated the differences between these two simulated communities of the Shannon Index (H) and the Simpson Index (D), two common diversity indices used to assess community structure.³³ We repeated this randomization and index calculation procedure to yield 10,000 estimates each of the differences in H and D between 2009 and 2001 simulated community-pairs. We then calculated the proportions of the simulated community-pairs for which the absolute value of the differences in D and H were greater than the absolute value of the observed differences in D and H between 2001 and 2009, respectively. We used these proportions as estimates of the p-values for two-tailed tests of the null hypothesis that the avian community structure had not changed along the Warrior Stand Route between 2001 and 2009.³²

Results

A total of 42 avian species were identified as the sources for 528 blood meals from nine species of mosquito in the TNF study area between 2001 and 2009 (Table 2). *Culex restuans* and *Culiseta melanura* fed primarily on perching birds (Order Passeriformes), taking 72.0 and 77.4 % of blood meals from perching birds, respectively and secondarily on herons (Family Ardeidae, Order Ciconiiformes), taking 24.0 and 11.3 % of blood meals from herons, respectively. Other avian hosts of these mosquitoes included yellow-billed cuckoos (Order Cuculiformes), representing 4.0 and 5.66 % of blood meals from *Culex restuans* and *Culiseta melanura*, respectively; and owls (Order Strigiformes), representing 5.66 % of *Culiseta melanura* blood meals. Neither *Culex restuans* nor *Culiseta melanura* were found to feed upon chickens or wild turkeys (Order Galliformes), anhinga (order Pelecaniformes), raptors (Order Falconiformes), ducks (Family Anatidae; order Anseriformes) or hummingbirds (order Apodiformes). *Culex erraticus* fed primarily on herons (64.3%), followed by birds from a wide variety of avian orders, including by perching birds (24.7%), ducks (5.8%), owls (2.4%), gallinaceous birds (1.2%), cuckoos (1.0%), anhinga (0.5%) and hummingbirds (0.2%). The majority of avian bloodmeals were derived from birds that have established breeding populations in central Alabama (Table 2). Those species that do not have breeding populations in central Alabama either over-winter, migrate through (e.g., American bittern *Botaurus lentiginous*), or are domesticated (chicken).

Culiseta melanura significantly overutilized northern cardinal (*Cardinalis cardinalis*) relative to its rate of availability in the avian community, and thus the northern cardinal was inferred to have been a preferred host of *Cs. melanura*. Ninety-five percent confidence intervals of forage ratios for the 14 other bird species that were identified in *Cs. melanura* blood meals included a value of one, suggesting that these bird species were fed upon opportunistically

(Table 3; Figure 1). Avian species inferred to have been preferred by *Cx. erraticus* included American robin, orchard oriole (*Icterus spurius*), northern mockingbird (*Mimus polyglottis*), wild turkey (*Meleagris gallapavo*), Carolina chickadee (*Poecile carolinensis*), barred owl, and northern cardinal. Carolina wren (*Thryothorus ludovivianus*) and hooded warbler (*Wilsonia citrina*) were both inferred to have been avoided by *Cx. erraticus*. Forage ratios of the remaining 13 species that *Cx. erraticus* fed upon had 95% confidence intervals that included a ratio of one, suggesting that those species were fed upon opportunistically (Table 3; Figure 1). The 95% confidence intervals around the forage ratios of the seven other bird species in blood meal sample from *Cx. restuans* included a ratio of one, indicating that these bird species were also fed upon opportunistically by this mosquito species (Table 3; Figure 1). In general, forage ratio estimates based on expected frequencies of less than five blood meals under the null model of opportunistic feeding should be viewed with caution.³⁰ Avian species with expected frequencies greater than or equal to five in the bloodmeal sample from *Cx. erraticus* were Carolina wren, yellow-billed cuckoo (*Coccyzus americanus*), tufted titmouse (*Baeolophus bicolor*), and northern cardinal, and northern cardinal in the bloodmeal samples from *Cs. melanura*.

Comparisons of the forage ratios estimated from bloodmeal data covering the entire March-to-October mosquito sampling period and bloodmeal data from May 1st and August 15th, when birds are not migrating in east-central Alabama, revealed a high degree of consistency for estimates between the two periods for both *Cs. melanura* and *Cx. erraticus* (Table 4). However, the confidence intervals of orchard oriole, wild turkey, and northern cardinal included one when forage ratios and associated standard errors for these three species were based on the bloodmeals collected strictly between May 1st and August 15th. Comparison between the *Cs. restuans*

samples were not made because only 4 individuals of this species yielding avian-derived bloodmeals were collected between May 1st and August 15th over the eight years of sampling.

The observed difference in the Shannon Index between the 2001 and 2009 data from the BBS Warrior Stand Route was 0.022; the proportion of the community-pairs for which the absolute value of the difference in H exceeded the absolute value of this observed value was 0.624. The observed difference in the Simpson Index between the 2001 and 2009 data from the BBS Warrior Stand Route was 0.003; the proportion of the community-pairs for which the absolute value of the difference in H exceeded the absolute value of this observed value was 0.436. There was thus no evidence to reject the null hypothesis of a stable avian community structure between 2001 and 2009 along the Warrior Stand Route, using either the Shannon or Simpson Index as a measure of avian community structure and an alpha-cutoff of 0.05. Moreover, the rank of species abundances in the 2001 and 2009 samples were positively correlated ($r_{S(49)} = 0.90$, $p < 0.001$). As such, there was strong support for the validity of our assumption that the relative abundances of avian species in the TNF study area estimated from point count surveys during 2008 and 2009 were representative of their relative abundances over the course of the study period.

Discussion

Through comparisons of the sources of mosquito blood meals to the local avian community, we found that putative vectors of EEEV in the Southeast do not feed upon birds opportunistically; rather, these mosquito species use some species of birds more or less than expected based on their relative abundance in the environment. While a number of studies have previously demonstrated similar heterogeneity in mosquito feeding patterns,^{2,3,4,5,6,7,34} our study is the first to demonstrate such heterogeneity at the host-species level for *Cs. melanura*, the

primary enzootic vector of EEEV. Our results provide evidence that the northern cardinal is a preferred host of *Cs. melanura*. As such, the northern cardinal will likely be exposed more frequently to EEEV than other avian species and thus we predict that it plays an important role in ecology of EEEV in the Southeast.

In addition to northern cardinal, ten avian species—common yellowthroat (*Geothlypis trichas*), gray catbird (*Dumetella carolinensis*), eastern towhee (*Pipilo erythrophthalmus*), Louisiana waterthrush (*Parkesia motacilla*), yellow-throated vireo (*Vireo flavifrons*), barred owl (*Strix varia*), hooded warbler (*Wilsonia citrina*), Acadian flycatcher (*Empidonax virescens*), red-eyed vireo (*Vireo olivaceus*), and blue-gray gnatcatcher (*Polioptila caerulea*)—had forage ratio estimates for *Cs. melanura* that were greater than one, suggesting that these species may also be preferred hosts. Three of these species—barred owl, common yellowthroat, gray catbird—were fed upon much more than expected, based on their relative abundances. While the confidence interval for the forage ratios of these ten species included one—making their over-representation in blood meals not statistically significant—standard error calculations were based on sample sizes that were too small to provide reliable estimates of confidence intervals.³⁰ Despite low sample sizes and large standard errors, we suggest that northern cardinal, barred owl, common yellowthroat, and gray catbird have the highest probabilities for playing important roles in EEEV transmission among the avian species for which forage ratios were estimated in the current study.

The established model for EEEV transmission in the northeastern United States, which implicates *Cs. melanura* as the primary enzootic vector of the virus, is commonly extrapolated as an appropriate model for transmission of EEEV throughout North America. Recent studies, however, have suggested that this “northeastern model” may not accurately depict transmission of EEEV in southeastern foci, and that other mosquito species, especially *Cx. erraticus* and *Cx.*

restuans, may be important to enzootic transmission in the southeastern region.^{15,35} If *Cx. erraticus* or *Cx. restuans* play prominent roles in EEEV transmission in the Southeast, inference about avian host preferences of these mosquito species become important. *Culex erraticus* and *Cx. restuans* both had high forage ratios for northern cardinal, and northern cardinal was inferred to be a preferred host species of *Cx. erraticus* when forage ratios were calculated using the entire sample of bloodmeals collected between March and October.

We assumed that the avian community structure is most stable between 1 May and 15 August, the period after spring migration and before late summer dispersal and migration of birds. When forage ratios were based on the restricted samples of bloodmeals collected during this period, neither *Cx. erraticus* nor *Cx. restuans* exhibited significant feeding preferences for northern cardinal. The few bloodmeal samples available during this restricted period for *Cx. restuans* limits our ability to make inferences regarding significant rates of over-and-under utilization of avian hosts by this mosquito species. For *Cx. erraticus*, however, samples sizes of blood meals from between 1 May and 15 August were adequate to make inferences, and we found that the American robin, Carolina chickadee, barred owl, and northern mockingbird are the preferred hosts of *Cx. erraticus*. As such, the northern cardinal may be less important in EEEV transmission compared to these species if *Cx. erraticus* is more important enzootic vector of the virus in the Southeast. These results underscore the need for further research of the relative contribution of different mosquito species to EEEV in this region.

All of our conclusions regarding forage ratios must be considered in light of the fact that we had to exclude some species from our forage ratio analyses because we were unable to accurately census these birds. Notable among these were herons, which comprised a large proportion of the bloodmeals from *Cx. erraticus*. The necessity of excluding herons from our

analysis was unfortunate because herons may also play an important role in the ecology of EEEV in the southeastern United States, and herons comprised a large percentage of avian blood meals in our study. The fraction of total avian blood meals from herons varied by mosquito species, comprising 64.3% percent of the avian bloodmeals of *Cx. erraticus* but just 11.3% percent of *Cs. melanura* blood meals. This difference could reflect contact rates of mosquitoes and herons, given differences in the ecologies of the mosquitoes and birds. *Culex erraticus* breeds in permanent ponds and the densities of *Cx. erraticus* females decline with distance from these breeding sites.²⁰ Herons are waterbirds and thus are also more likely to be found at permanent ponds. *Culiseta melanura*, in contrast, breeds in water pockets associated with buttressed trees and temporary puddles of water created by uprooted trees that occur in swamp habitats,³⁶ but not necessarily near permanent water, and thus may encounter waterbirds less frequently than *Cx. erraticus*.

Given the high proportion of blood meals derived from herons, we would conclude that herons are likely to be frequently exposed to EEEV. Interestingly, in a study of exposure of different avian species to EEEV in Louisiana, 54.8% of heron species tested positive for EEEV neutralizing antibodies, whereas only 26.2% of passerine species were seropositive.³⁷ Notable among the exposure rates of individual species was the high seroprevalence of yellow-crowned night-herons (*Nyctanassa violacea*, 86.1%),³⁷ the second most common avian host of *Cx. erraticus* in the current study. Several studies of defensive behaviors of birds to foraging mosquitoes found that some ciconiiforms, due to their stand-and-wait foraging technique, are highly susceptible to questing mosquitoes.^{38,39,40} Despite the high proportion of blood meals derived from herons, the high seroprevalence of EEEV in wild herons, and evidence that herons are important hosts for many of medically-important mosquitoes, relatively little effort has been

directed towards quantifying the role of ciconiiform birds in the amplification of arboviruses, relative to passerines. Results of the current study underscore the need for further research investigating the role of ciconiiform species in EEEV transmission.

The role that a preferred species play in EEEV transmission follows directly from its forage ratio, i.e. the likelihood that an individual of that species will be fed upon. Because they are more likely to be fed upon in general, highly ranked (preferred) hosts have a higher likelihood of being fed upon by an infected mosquito than lower ranked (less-preferred) hosts. It also follows that such highly ranked hosts, simply because they are a more probable hosts, are more likely to be fed upon by a second uninfected mosquito that subsequently becomes infected. Consequently, assuming uniform reservoir competence (i.e., magnitude and duration of viremia), an individual of a more preferred avian host species should be a more important virus amplifier than an individual from a less-preferred species. All else equal, preferred host species will play a more important role in transmission dynamics than those that are less preferred.

One of the factors that could confound the relationship between vector feeding preference and host importance in virus amplification is reservoir competence. Preferred host species will have a more important role in transmission dynamics only if all avian host species are equivalent in terms of their reservoir competence. Conversely, if a preferred avian species is a poor reservoir host, then that species will act as a dilution host, reducing contact rates between vectors and competent reservoirs.⁴¹ Reservoir competence has been reported for a number of avian species,⁴² but it was not possible for us to infer interspecific differences in reservoir competence of birds based on such estimates. Further research is needed in this area to more accurately assess the influence of variability in reservoir competence of avian hosts on the transmission dynamics of EEEV.

The shift in inference in terms of preferred hosts of *Cx. erraticus* that occurred when forage ratio calculations were based on samples collected either from March through October or between 1 May and 15 August is not surprising given that the month of peak intensity of blood-feeding in this species is August (Fig 2). Individuals of avian host species with a high relative abundance in the *Cx. erraticus* bloodmeal sample collected between March and October may be more preferable hosts compared to individuals of other species, or they may simply have inflated forage ratios caused by changes in the avian community between May and the later weeks of August, when post-breeding dispersal and migration begins. The latter possibility seems highly plausible, because resident birds make up an increasing proportion of the avian community as migratory species leave the study area beginning in August. Thus, northern cardinal and wild turkey, which are not long-distance migrants, are likely to have a greater rate of availability to *Cx. erraticus* from mid August through October than reflected in their relative abundances based on point count surveys conducted during the breeding season. This confounding influence of an underestimated rate of availability of avian hosts was less likely to be present in the calculation of the forage ratio estimates of *Cs. melanura*, as this species has a peak intensity of blood-feeding in TNF in May (Fig 2), when the structure of the avian host community as estimated from point count surveys should be highly stable.

Our goal in conducting this study was to estimate forage ratios for avian species for putative vectors of EEEV. Such forage ratios represent potential proxy measures of the level of exposure to EEEV that individuals of different host species experience. An alternative approach to measuring EEEV exposure is to estimate the seroprevalence or seroconversion rate of EEEV directly in birds or to assay for antibodies of the virus. In studies in Michigan⁴³ and New Jersey⁴⁴, northern cardinals and gray catbirds had high EEEV antibody seroprevalences, when

present in the sample of surveyed birds. Barred owls and common yellowthroats, the species with the highest *Cs. melanura* forage ratios in this study, were not present in samples from either of these studies. Overall, these studies confirm our assertion that northern cardinal, gray catbirds, and potentially barred owls and common yellowthroats, are exposed at a high rate to EEEV, in regions where *Cs. melanura* is the primary enzootic vector of the virus, given evidence from this study of the high *Cs. melanura* forage ratios for these species.

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Table 1. Predicted relative abundances of avian species observed during point-count surveys in TNF and used in forage ratio calculations.

Species	Relative Abundance		
	to <i>Cx. erraticus</i>	to <i>Cx. restuans</i>	to <i>Cs. melanura</i>
Acadian flycatcher <i>Empidonax vireescens</i>	0.025	0.032	0.039
American crow <i>Corvus brachyrhynchos</i>	0.022	0.016	0.015
American robin <i>Turdus migratorius</i>	0.003	0.003	0.003
Barn swallow <i>Hirundo rustica</i>	0.001	0.001	0.001
Barred owl <i>Strix varia</i>	0.006	0.006	0.008
Blue grosbeak <i>Guiraca caerulea</i>	0.004	0.003	0.004
Blue jay <i>Cyanocitta cristata</i>	0.030	0.027	0.027
Blue-gray gnatcatcher <i>Poliopitila caerulea</i>	0.041	0.043	0.042
Broad-winged hawk <i>Buteo platypterus</i>	0.004	0.004	0.004
Brown thrasher <i>Toxostoma rufum</i>	0.017	0.011	0.010
Brown-headed cowbird <i>Molothrus ater</i>	0.009	0.008	0.008
Brown-headed nuthatch <i>Sitta pusilla</i>	0.011	0.008	0.007
Carolina chickadee <i>Poecile carolinensis</i>	0.030	0.030	0.029
Carolina wren <i>Thryothorus ludovicianus</i>	0.059	0.061	0.062
Chipping sparrow <i>Spizella passerina</i>	0.003	0.001	0.001
Chuck-will's-widow <i>Caprimulgus carolinensis</i>	0.001	0.000	0.000
Common grackle <i>Quiscalus quiscula</i>	0.002	0.001	0.001
Common yellowthroat <i>Geothlypis trichas</i>	0.006	0.007	0.008
Downy woodpecker <i>Picoides pubescens</i>	0.022	0.023	0.024
Eastern bluebird <i>Sialia sialis</i>	0.001	0.000	0.000
Eastern kingbird <i>Tyrannus tyrannus</i>	0.001	0.001	0.001
Eastern phoebe <i>Sayornis phoebe</i>	0.001	0.001	0.001
Eastern towhee <i>Pipilo erythrophthalmus</i>	0.011	0.007	0.007
Eastern wood-pewee <i>Contopus virens</i>	0.003	0.003	0.002
Eurasian collared-dove <i>Streptopelia decaocto</i>	0.000	0.000	0.000
Field sparrow <i>Spizella pusilla</i>	0.004	0.004	0.004
Fish crow <i>Corvus ossifragus</i>	0.004	0.004	0.004
Gray Catbird <i>Dumetella carolinensis</i>	0.003	0.003	0.003
Great crested flycatcher <i>Myiarchus crinitus</i>	0.020	0.019	0.019
Hairy woodpecker <i>Picoides villosus</i>	0.004	0.004	0.004
Hooded warbler <i>Wilsonia citrina</i>	0.038	0.042	0.039
Indigo bunting <i>Passerina cyanea</i>	0.016	0.013	0.013
Kentucky warbler <i>Opornis formosus</i>	0.007	0.008	0.008
Louisiana waterthrush <i>Parkeesia motacilla</i>	0.005	0.007	0.008
Mourning dove <i>Zenaidura macroura</i>	0.007	0.006	0.007
Northern cardinal <i>Cardinalis cardinalis</i>	0.111	0.109	0.104
Northern mockingbird <i>Mimus polyglottis</i>	0.003	0.001	0.002
Northern parula <i>Parula americana</i>	0.035	0.045	0.055
Orchard oriole <i>Icterus spurius</i>	0.001	0.000	0.000
Pileated woodpecker <i>Dryocopus pileatus</i>	0.012	0.014	0.015
Pine warbler <i>Dendroica pinus</i>	0.016	0.011	0.009
Prairie warbler <i>Dendroica discolor</i>	0.006	0.004	0.004
Prothonotary warbler <i>Protonotaria citrea</i>	0.005	0.006	0.008
Purple martin <i>Progne subis</i>	0.002	0.002	0.002
Red-bellied woodpecker <i>Melanerpes carolinus</i>	0.079	0.068	0.063
Red-eyed vireo <i>Vireo olivaceus</i>	0.061	0.067	0.062
Red-headed woodpecker <i>Melanerpes erythrocephalus</i>	0.005	0.004	0.004
Red-shouldered hawk <i>Buteo lineatus</i>	0.006	0.007	0.008
Red-winged blackbird <i>Agelaius phoeniceus</i>	0.002	0.001	0.002
Ruby-throated hummingbird <i>Archilochus colubris</i>	0.013	0.013	0.012
Summer tanager <i>Piranga rubra</i>	0.029	0.026	0.024
Swainson's warbler <i>Limnithyris swainsonii</i>	0.004	0.005	0.005
Tufted titmouse <i>Baeolophus bicolor</i>	0.063	0.067	0.065
White-eyed vireo <i>Vireo griseus</i>	0.035	0.036	0.036
Wild turkey <i>Meleagris gallapavo</i>	0.001	0.001	0.001
Wood thrush <i>Hylocichla mustelina</i>	0.012	0.015	0.018
Yellow-billed cuckoo <i>Coccyzus americanus</i>	0.056	0.065	0.069
Yellow-breasted chat <i>Icteria virens</i>	0.007	0.005	0.005
Yellow-shafted flicker <i>Colaptes auratus auratus</i>	0.004	0.005	0.005
Yellow-throated vireo <i>Vireo flavifrons</i>	0.009	0.010	0.010
Yellow-throated warbler <i>Dendroica dominica</i>	0.002	0.002	0.001

Table 2. Total number of blood meals derived from avian species for mosquitoes collected in TNF between March and October from 2001 through 2009.

	<i>Ae. vexans</i>	<i>Cq. perturbans</i>	<i>Cx. erraticus</i>	<i>Cx. peccator</i>	<i>Cx. quinquefasciatus</i>	<i>Cx. restuans</i>	<i>Cx. territans</i>	<i>Cs. melanura</i>	<i>Oe. sticticus</i>
Acadian flycatcher <i>Empidonax virescens</i>			1					2	
American bittern <i>Botaurus lentiginos</i>			23	1		3		1	
American crow <i>Corvus brachyrhychos</i>						2			
American robin <i>Turdus migratorius</i>			14		1				
Anhinga <i>Anhinga anhinga</i>			2						
Barred owl <i>Strix varia</i>			7					3	
Blue grosbeak <i>Guiraca cerulea</i>			2						
Blue jay <i>Cyanocitta cristata</i>			3						
Blue-gray gnatcatcher <i>Polioptila caerulea</i>								2	
Brown-headed cowbird <i>Molothrus ater</i>			2						
Carolina chickadee <i>Poecile carolinensis</i>		1	16						
Carolina wren <i>Thryothorus ludovicianus</i>			1					1	
Chicken <i>Gallus gallus</i>			1						
Common grackle <i>Quiscalus quiscula</i>	2		4						
Common yellowthroat <i>Geothlypis trichas</i>								3	
Eastern screech-owl <i>Otus asio</i>			3						
Eastern towhee <i>Pipilo erythrophthalmus</i>								1	
Great blue heron <i>Ardea herodias</i>	1		141	9		2			
Great egret <i>Ardea alba</i>			1						
Green heron <i>Butorides virescens</i>			25	1		1		2	
Gray Catbird <i>Dumetella carolinensis</i>			3					1	
Hooded warbler <i>Wilsonia citrina</i>			1					2	
House finch <i>Carpodacus mexicanus</i>		1	2		1			1	
Kentucky warbler <i>Opornis formosus</i>			1						
Louisiana waterthrush <i>Parkesia motacilla</i>						2		1	
Northern cardinal <i>Cardinalis cardinalis</i>	3		22		4	6	1	20	1
Northern mockingbird <i>Mimus polyglottis</i>			13		1				
Orchard oriole <i>Icterus spurius</i>			4						
Pied-billed grebe <i>Podilymbus podiceps</i>	1		7						
Pine warbler <i>Dendroica pinus</i>			1						
Red-eyed vireo <i>Vireo olivaceus</i>								3	
Red-tailed hawk <i>Buteo jamaicensis</i>					1				
Ruby-throated hummingbird <i>Archilochus colubris</i>			1						
Summer tanager <i>Piranga rubra</i>						1			
Tufted titmouse <i>Baeolophus bicolor</i>			6			1		2	
White-eyed vireo <i>Vireo griseus</i>	1		5			4		1	
Wild turkey <i>Meleagris gallapavo</i>	2		5						
Wood duck <i>Aix sponsa</i>			17	1					
Wood thrush <i>Hylocichla mustelina</i>			1		1	2			
Yellow-billed cuckoo <i>Coccyzus americanus</i>			4			1		3	
Yellow-crowned night-heron <i>Nyctanassa violacea</i>			75	1				3	
Yellow-throated vireo <i>Vireo flavifrons</i>								1	

Table 3. Forage ratios (95% CI) of the avian species from which blood meals were derived for *Cx. erraticus*, *Cx. restuans*, and *Cs. melanura* between March and October from 2001 through 2009.

	<i>Cx. erraticus</i>	<i>Cx. restuans</i>	<i>Cs. melanura</i>
Acadian flycatcher	0.34 (-0.32 , 1.00)		1.11 (-0.40 , 2.62)
American crow		6.70 (-2.08 , 15.48)	
American robin	44.27 (22.51 , 66.02) **		
Barred owl	10.08 (2.84 , 17.32) *		8.12 (-0.76 , 17.01)
Blue grosbeak	4.55 (-1.70 , 10.79)		
Blue jay	0.86 (-0.10 , 1.83)		
Blue-gray gnatcatcher			1.03 (-0.37 , 2.44)
Brown-headed cowbird	1.84 (-0.69 , 4.37)		
Carolina chickadee	4.60 (2.50 , 6.69) **		
Carolina wren	0.15 (-0.14 , 0.43)		0.35 (-0.33 , 1.04)
Common grackle	19.65 (0.73 , 38.58)		
Common yellowthroat			7.73 (-0.73 , 16.19)
Eastern towhee			3.28 (-3.08 , 9.65)
Gray catbird	8.69 (-1.02 , 18.40)		7.06 (-6.63 , 20.74)
Hooded warbler	0.23 (-0.21 , 0.66)		1.12 (-0.40 , 2.64)
Kentucky warbler	1.15 (-1.10 , 3.40)		
Louisiana waterthrush		15.88 (-4.94 , 36.69)	2.82 (-2.65 , 8.3)
Northern cardinal	1.69 (1.05 , 2.32) *	2.89 (0.98 , 4.81)	4.18 (2.81 , 5.56) **
Northern mockingbird	34.59 (16.86 , 52.32) **		
Orchard oriole	43.63 (1.61 , 85.65) *		
Pine warbler	0.52 (-0.50 , 1.55)		
Red-eyed vireo			1.04 (-0.10 , 2.19)
Ruby-throated hummingbird	0.67 (-0.64 , 1.99)		
Summer tanager		2.00 (-1.81 , 5.81)	
Tufted titmouse	0.81 (0.18 , 1.44)	0.78 (-0.71 , 2.27)	0.67 (-0.24 , 1.58)
White-eyed vireo	1.21 (0.17 , 2.25)	5.86 (0.76 , 10.96)	0.61 (-0.57 , 1.79)
Wild turkey	33.49 (4.77 , 62.22) *		
Wood thrush	0.71 (-0.68 , 2.11)	6.92 (-2.15 , 16.00)	
Yellow-billed cuckoo	0.62 (0.02 , 1.21)	0.81 (-0.74 , 2.36)	0.94 (-0.09 , 1.97)
Yellow-throated vireo			2.24 (-2.10 , 6.58)

* 95% confidence intervals of forage ratios exclude 1.

** 99% confidence intervals of forage ratios exclude 1.

Table 4. Forage ratios of avian species using all bloodmeals collected between March and October, or alternatively, strictly between May and August 15th.

	<u><i>Cx. erraticus</i> Forage Ratio</u>		<u><i>Cs. melanura</i> Forage Ratio</u>	
	<u>Mar - Oct</u>	<u>May - Aug 15th</u>	<u>Mar - Oct</u>	<u>May - Aug 15th</u>
Acadian flycatcher	0.34	0.42	1.11	2.64
American crow				
American robin	44.27 **	46.43 **		
Barred owl	10.08 *	12.24 *	8.12	6.54
Blue grosbeak	4.55	2.79		
Blue jay	0.86	1.05		
Blue-gray gnatcatcher			1.03	0.62
Brown-headed cowbird	1.84			
Carolina chickadee	4.60 **	4.62 **		
Carolina wren	0.15	0.18	0.35	0.43
Common grackle	19.65	24.09		
Common yellowthroat			7.73	6.25
Eastern towhee			3.28	
Gray catbird	8.69	10.51	7.06	
Hooded warbler	0.23	0.28	1.12	1.37
Kentucky warbler	1.15	1.43		
Louisiana waterthrush			2.82	3.43
Northern cardinal	1.69 *	1.61	4.18 **	4.55 **
Northern mockingbird	34.59 **	32.62 **		
Orchard oriole	43.63 *	40.20		
Pine warbler	0.52	0.66		
Red-eyed vireo			1.04	1.27
Ruby-throated hummingbird	0.67	0.84		
Summer tanager				
Tufted titmouse	0.81	0.67	0.67	0.82
White-eyed vireo	1.21	1.49	0.61	0.74
Wild turkey	33.49 *	25.19		
Wood thrush	0.71	0.88		
Yellow-billed cuckoo	0.62	0.57	0.94	0.76
Yellow-throated vireo			2.24	2.73

* 95% confidence intervals of forage ratios exclude 1 (not shown).

** 99% confidence intervals of forage ratios exclude 1 (not shown).

Figure 1. Forage ratios for avian species present in at least two of the total bloodmeal samples collected *Cs. melanura*, *Cx. erraticus*, and *Cx. restuans* in TNF between March and October from 2001 through 2009. Bars show estimated standard errors, and the numbers above bars are sample sizes.

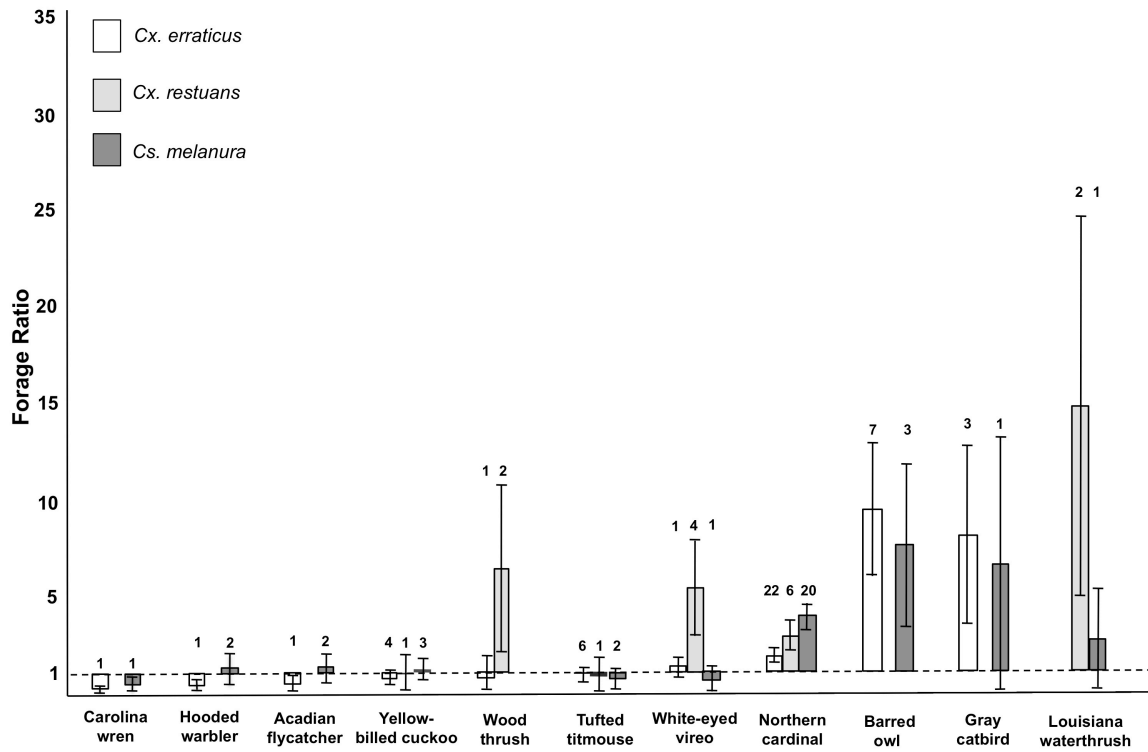
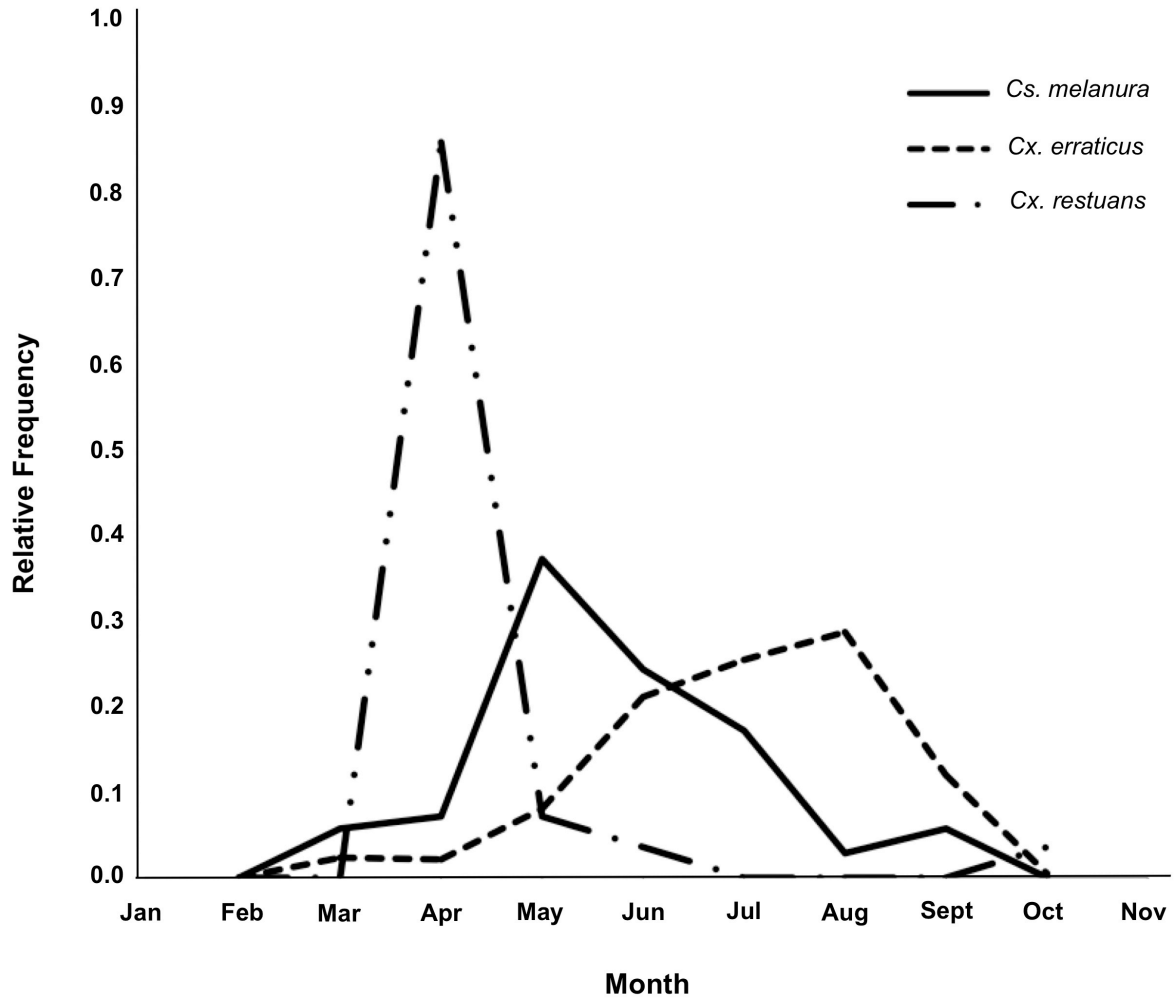


Figure 2. Relative frequencies of blood-engorged *Cs. melanura* (N=70), *Cx. erraticus* (N=1457), and *Cx. restuans* (N=28) collected in TNF each month between 2001 and 2009.



III. DEVELOPING MODELS FOR THE FORAGE RATIOS OF AVIAN HOSTS FOR *CULISETA MELANURA* AND *CULEX ERRATICUS* USING HOST CHARACTERISTICS

Abstract

The relative rates of contact between bird species and mosquito vectors, as measured by forage ratios, suggest that some bird species are used as hosts more than would be expected by chance. While interspecific variation in the rates of utilization of different avian hosts is potentially one of the factors driving spatial and temporal patterns of the occurrence of mosquito-borne pathogens, the ecological factors that might make some birds more or less susceptible to questing mosquitoes have been little studied. We developed linear regression models for two mosquito species, *Culiseta melanura* and *Culex erraticus*, and used multimodel inference to identify avian host characteristics that could be used to predict forage ratios of these two species. We found nesting stratum, body mass, and length of nestling stage of avian host species to be useful for predicting *Cx. erraticus* forage ratios. Nestling stage length received strong support as a predictor in our model of *Cs. melanura* forage ratios. Our results suggest that characteristics of avian hosts may predict relative rates of contact of avian host species with mosquito vectors.

Introduction

Many vector-borne pathogens require both an arthropod host and a vertebrate host to complete their life cycles (Marquardt et al. 2004). For many such pathogens, however, the identity and importance of arthropod vectors is relatively well known, while the suite of vertebrate hosts is

large, variable, and often poorly characterized. Specifically for mosquito-borne pathogens, transmission dynamics may be influenced by heterogeneities amongst vertebrates in their relative rates of contact with mosquito vectors (Kilpatrick et al. 2006). Many mosquito species demonstrate distinct preferences for specific groups of vertebrates, ranging from vertebrate class- to species-level specificity (Boakye et al. 1999; Hassan et al. 2003; Burkett-Cadena et al. 2008). As a consequence of these preferences, groups of vertebrates vary in their individual-specific rates of contact with mosquito vectors.

Variability in individual-specific contact rates operates in conjunction with variability in reservoir competence to produce heterogeneities amongst vertebrate species in their relative contribution to pathogen transmission (Kilpatrick et al. 2006; Kent et al 2009). Heterogeneities amongst avian species in terms of their relative contact rates with mosquitoes may be particularly important to predicting and controlling outbreaks of mosquito-borne disease in the United States, as birds are implicated as reservoir hosts of the majority of mosquito-borne pathogens of public health concern in this country (Gubler et al. 2001).

The rarest, but most severe in terms of morbidity and mortality, of these viruses is eastern equine encephalitis virus (EEEV; Scott and Weaver 1989, Villari et al. 1995). Understanding of the transmission dynamics of EEEV is for the most part restricted to circumstances in which *Cs. melanura* is the primary enzootic vector, as typically occurs in the northeastern US, and where birds are reservoir hosts (Scott and Weaver 1989). In studies of EEEV life cycle, no consideration is generally given to the influences of specific avian host species in transmission; rather, birds are considered as a homogenous class of reservoir hosts. Interspecific variation among avian hosts in contact rates with vector species is ignored despite evidence for heterogeneities among species in contact rate (Hassan et al. 2003; Molaei et al. 2006; Estep et al.

in press) and in reservoir competence (Komar et al. 1999). Thus, studies of EEEV would benefit from a more thorough understanding of contact rates between vectors and passerine species and heterogeneity amongst those species in reservoir competence.

To understand heterogeneity in contact rates between mosquitoes and birds researchers need to know the sources of blood meals from field-collected mosquitoes and the relative abundances of birds in the area. With such data, the researcher can then compare the rate of utilization of avian hosts (relative abundance in the blood meal sample) to their rates of availability (relative abundance in avian community). More formally, a forage ratio (Hess 1968) can be calculated as the ratio of the proportion of blood meals from the hosts to the proportion of the avian community comprised of that species (Manly et al. 2002).

Collection of data necessary to accurately estimate forage ratios for all individual species poses a logistical challenge. Amongst other challenges, the rarer or less-preferred a species is, the fewer will be the blood-engorged mosquitoes that have fed on it, and the lower will be the accuracy of the estimate of its forage ratio. In the extreme case, forage ratios for species that are not present in the study area, but that may be important in transmission in nearby areas, cannot be estimated. Thus, barring intensive sampling over sites spread across a broad geographic area, we currently lack the means to estimate the relative contact rates between vectors of EEEV and all species of avian hosts potentially involved in its transmission.

One possible, but previously unexplored, solution to this problem is the development of a statistical model of forage ratios based on characteristics of the host species. Estimates of characteristics for North American birds, including life-history traits and those relating to habitat utilization are readily available from the ornithological literature. As such, models of forage ratios based on characteristics of avian host species could potentially be applied to almost any

species to estimate its contact rate with vector species without the need for collection and analysis of blood-engorged mosquitoes and avian community surveys.

In the current study, we explored the development of such models. We sought to identify those avian host characteristics for which data are available that are associated with forage ratios of two EEEV vector species that feed regularly on avian hosts in the southeastern United States: *Cs. melanura* and *Cx. erraticus*. *Culiseta melanura* is widely recognized as the primary enzootic vector of EEEV (Scott and Weaver 1989). *Cx. erraticus* has been proposed to play a role as a bridge vector in the southeastern United States (Cupp et al. 2003). As such, insights gained from modeling avian characteristics and feeding preferences of these two mosquito species could potentially be used for predictive model development of EEEV transmission in this region.

Materials and Methods

Inferential Approach and Data Sources

We used a multi-model inferential approach (Burnham and Anderson 2002) to identifying host characteristics associated with forage ratios for each of two mosquito vector species, *Cs. melanura* and *Cx. erraticus*. We first developed a set of candidate general linear models to predict the forage ratios of avian host species using characteristics of those species. To determine whether a host characteristic would be useful for predicting forage ratios, we then used model-averaged estimates of the coefficients of the predictor variables and 85% confidence intervals around those estimates (Arnold 2010). We used unconditional weighted standard errors in calculating confidence intervals, such that predictor variables with confidence intervals that excluded zero were concluded to be useful for prediction (Arnold 2010). The strength of evidence for associations between predictor variables and forage ratios was also considered

through examination of importance weights associated with each predictor variable (Burnham and Anderson 2002).

Forage ratios for each avian species used in model development were estimated from a long-term study of feeding patterns of mosquitoes in Tuskegee National Forest in Alabama (Estep et al. in press). A high proportion of bird species that were recorded during censuses were not detected in any blood meal samples and inclusion of such zero-valued forage ratios would have caused significant violations of linear regression modeling. Thus, we restricted our analysis to those avian species with forage ratios > 0 , and an assumption of this analysis is that the species that we included were representative of the entire avian community. Such species could either have high contact rates with mosquitoes and low relative abundances, or they could have low contact rates with mosquitoes and high relative abundances. Also, we excluded barred owl, a species with a high *Cs. melanura* and *Cx. erraticus* forage ratio estimates, from our analysis. Barred owls are nocturnal sit-and-wait predators, and it seems plausible that they have high forage ratios because they are highly susceptible mosquito hematophagy because of their foraging technique, much like some wading birds (Edman and Kale 1971, Edman et al. 1984), rather than due to the factors considered in our analysis. In total, we used 14 observed forage ratios for development of the *Cs. melanura* model, and 21 observed forage ratios for development of the *Cx. erraticus* model (Estep et al. in press, Appendix I).

We identified seven host characteristics that could influence the contact rate between mosquitoes and avian hosts (Table 1). We based this set of predictor variables partly on their potential association with host attractiveness, defensive behavior, or probability of encounter with mosquitoes, and partly on the availability of information in the ornithological literature. We imputed the number of broods per season for brown-headed cowbird (*Molothrus ater*), a brood

parasite with a wide range of hosts, using the average brood size across all avian species detected during point count surveys in TNF.

The beginning and end of the host-seeking seasons of *Cs. melanura* and *Cx. erraticus* were estimated as the quarter-months during which the 2.5th and 97.5th percentiles of the dates of capture of engorged individuals of each species were captured (Table 1). The beginning and end of the host-seeking season were the first quarter of May and the second quarter of August, respectively, for *Cs. melanura*, and the first quarter of April and the third quarter of September, respectively for *Cx. erraticus*.

Models that included all possible predictor variable combinations comprised the candidate model set for each species. The communal roosting variable was not included in any models in the *Cs. melanura* candidate set because no species for which forage ratio estimates were available roosted communally except in winter. We specified models using the `lm` function in R and used a weighted least squares approach to parameter estimation (R Core Development Team 2008, Kutner et al. 2005). Specifically, we weighted each estimated forage ratio for an avian species by the inverse of the variance of the forage ratio estimate (Estep et al. in press). As such, species with smaller variances about forage ratio estimates received a higher weight of influence in parameter estimation than those with large variances (Kutner et al. 2005). Based on initial model diagnostics, we natural-log transformed the forage ratio variable (response) and the body mass variable (predictor). Variance Inflation Factors were < 10 for predictor variables in all models considered, such that multicollinearity was unlikely to bias parameter estimates (Kutner et al. 2005).

We compared the bias-corrected AIC (AICc) between all models in the candidate set for each species and used this criterion in calculating importance weights of models. We performed

model averaging of parameter estimates over all models in the 95% confidence set, i.e. the most highly-ranked models that together comprise 95% of the total AIC weights of all models in the candidates set (Burnham and Anderson 2002). Importance weights for predictor variables in the *Cs. melanura* or *Cx. erraticus* forage ratio models were calculated using all models in the candidate sets for either species *Cs. melanura* or *Cx. erraticus*. As such, all predictor variables had an equal probability of inclusion in models in the set used for calculating importance weights.

Results

Cx. erraticus model

The top-ranked model in the *Cx. erraticus* candidate set included stratum, body mass, nestling, and habitat-edge association as predictor variables and had an importance weight of 0.31 (Table 2). Another model in the *Cx. erraticus* candidate set was competitive with the top-ranked model, having an AICc value that was within two units of the AICc of the top-ranked model and an importance weight of 0.26; this second-ranked model included the four covariates in the top-ranked model, and additionally, availability (Table 2). All models other than the two top-ranked ones had little support as the true model of *Cx. erraticus* forage ratios, given large differences in AICc values from the top-ranked models and low importance weights (Table 2, Burnham and Anderson 2002).

Mid-story and canopy-nesting avian host species had higher *Cx. erraticus* forage ratios than those nesting in lower strata ($\beta_s = 1.95$, LCL = 0.72, UCL = 3.18; Figure 1; Table 3), and stratum had the highest important weight amongst all predictor variable considered for the *Cx. erraticus* models (0.91; Table 3). The direction of association between *Cx. erraticus* forage ratios and body mass was positive ($\beta_m = 0.88$, LCL = 0.18, UCL = 1.01; Figure 2; Table 3), and

the body mass variable had an importance weight of 0.88. The direction of association between *Cx. erraticus* forage ratios and nestling, the variable with the third-highest importance weight of 0.83, was negative ($\beta_n = -0.07$, LCL = -0.12, UCL = -0.01; Figure 3; Table 3). Model-averaged coefficient estimates and associated 85% unconditional confidence intervals of these three top-ranked variables, stratum, body mass, and nestling, suggest that they all could all be used to develop predictive models of the *Cx. erraticus* forage ratios (Table 3). Habitat-edge association and availability, the two variables included in top-ranked models but for which 85% confidence intervals included zero, had coefficient estimates that were positive ($\beta_e = 0.98$, LCL = -0.06, UCL = 2.02, importance weight = 0.75) and negative ($\beta_a = -0.27$, LCL = -0.88, UCL = 0.33, importance weight = 0.38), respectively (Table 3). The two other predictor variables considered in modeling *Cx. erraticus* forage ratios, cavity and roost, had relatively low importance weights (≤ 0.16) (Table 3).

Cs. melanura model

Nestling was the only predictor variable included in the most highly ranked *Cs. melanura* forage ratio model (importance weight = 0.31, Table 4). All other models in the *Cs. melanura* candidate set had AICc values that were either greater than two units of the AICc of the top-ranked model, or within two units of the AICc of the top-ranked models but with little difference in the log-likelihood from the top-ranked model (Table 4). As such, all models other than the top-ranked one had little support as the true model of *Cs. melanura* forage ratios (Burnham and Anderson 2002, Arnold 2010).

The relationship between *Cs. melanura* forage ratios and nestling was negative ($\beta_a = -0.05$, LCL = -0.09, UCL = -0.01), such that species with shorter total periods of availability of nestlings had higher forage ratios (Figure 3). Moreover, the exclusion of zero from its 85%

confidence interval suggests that this variable may be useful for predicting *Cs. melanura* forage ratios. The 85% unconditional confidence intervals for all other variables except the nestling considered in model development included zero (Table 5). The importance weight associated with the nestling variable was 0.83, while the importance weights associated with all other variables were relatively low (≤ 0.30 , Table 5). Directions of associations between forage ratios and host trait variables were consistent with those observed for the *Cx. erraticus* forage ratio models, except in the case of the habitat-edge association and availability variables.

Discussion

Our observations offer the first evidence that the relative rate of contacts between avian hosts and mosquitoes can be predicted from ecological variables. Associations between susceptibility to hematophagy and host characteristics are to be expected because the ecology of both the vector and host will determine whether a mosquito successfully acquires a blood meal from a particular bird species (LoGiudice et al. 2003). The implications of demonstrating specific ecological variables that predict the relative rates of contact between avian hosts and both *Cs. melanura* and *Cx. erraticus*, however, are significant, because forage ratios could be used in elucidating patterns of the occurrence of EEEV in the southeastern United States. Our success at predicting host-vector interactions from environmental variables suggests that pathogen transmission might also be predictable from relatively simple ecological variables.

Mosquitoes often exhibit vertical niche partitioning within habitats (Snow 1955, Swanson et al. 2010), and we expected mosquitoes to encounter bird species that nest within these individual vertical niches more often than species that nest in strata outside of the vector niche. In other words, we expected birds whose vertical niches overlapped those of vector mosquito

species to have inflated forage ratios, such that nesting stratum would be useful in predictive model development. Consistent with this prediction, our results show that nesting stratum can be used to predict *Cx. erraticus* forage ratios of avian hosts. Nesting stratum also received moderate support as an influential variable in the *Cs. melanura* forage ratio models, whereby species nesting in higher strata have higher forage ratios. Although the weight of evidence for an influence of nesting stratum of *Cs. melanura* forage ratios was weak in the current study, the direction of the trend suggests avian hosts species that nest higher up in the forest canopy may be at a greater risk of exposure to both *Cx. erraticus* and *Cs. melanura* hematophagy, and by extension, EEEV. Moreover, our results suggest that the weight of the influence of nesting stratum on forage ratios for avian hosts is different for *Cs. melanura* and *Cx. erraticus*, as would be expected given differences in vertical niche partitioning between these mosquito species.

Variability in vertical distribution of mosquitoes amongst habitats types precluded formation of specific predictions about the direction of association between nesting stratum and forage ratios for avian host species. For example, studies of the vertical distribution of *Cs. melanura* demonstrate a high degree of inter-site variability, with either no association between abundance and trap height detected, or the direction of the detected association dependent upon habitat type (reviewed in Nasci and Edman 1981). In a study in South Carolina with traps suspended at heights of 1.5, 5, and 10 meters proportions of mosquito samples comprised of *Cx. erraticus* increased substantially with lowered trap height (Swanson et al. 2010). However, results of such studies were based on captures of all mosquitoes, not necessarily the subset of females actively seeking hosts. In a study of host-seeking *Cs. melanura*, results were much more clear-cut, wherein traps baited with birds suspended farther from the forest floor attracted more mosquitoes than lower traps, corroborating the direction of the association between stratum

and forage ratio observed in our study (Nasci and Edman 1981). Moreover, a positive association between average hematoparasite load of avian species and nesting stratum was found in surveys of wild-caught birds in Louisiana (Garvin et al. 1997). The explanation for this association was that certain ornithophilic vectors are more common in forest canopies than at lower levels, so species nesting high are exposed to hematophagy thus blood-borne parasites.

The positive association between forage ratio and the body mass of birds in both the *Cx. erraticus* and *Cs. melanura* models was not surprising given that birds with larger body masses have greater rates of output of carbon dioxide (Grubb 1983). Carbon dioxide is one of the primary biochemical attractants for mosquitoes (reviewed in Nicolas and Sillans 1989). Thus, larger bird would be expected to attract questing mosquitoes at a higher rate compared to smaller birds. This basic assumption was supported by a recent study in which the body mass of birds mosquito traps was positively associated with the number of mosquitoes captured at the trap (Suom et al. 2010). However, birds with greater body masses also occupy a greater volume of space, so simply by Brownian motion, mosquitoes are more likely to encounter larger birds. Larger birds may also have higher tolerances to hematophagy (Edman and Scott 1987). Any one of these factors might explain the association between forage ratios and body mass observed in the current study.

The total number of days that nestlings are available, calculated as product of the average number of broods and average nestling stage length, was the variable that received the highest support in the *Cs. melanura* models. Moreover, this variable is likely useful for predicting *Cs. melanura* forage ratios of avian host species. It also received strong support as a variable useful for prediction in the *Cx. erraticus* models. The direction of this association was negative, however— the opposite direction predicted by the hypothesis that nestlings may be particularly

vulnerable to hematophagy by mosquitoes (Blackmore et al. 1958; Kale et al 1952; Griffing et al. 2007; Burkett-Cadena et al. 2010). Reasons for the negative direction of association are unclear. One possibility is that the result is confounded with average nestling length of individuals. Average nestling stage length and the total availability of nestlings were highly correlated (species used for *Cs. melanura* forage ratio models: $r_s(14) = 0.73$, $p < 0.01$; species used for *Cx. erraticus* forage ratio models: $r_s(21) = 0.85$ $p < 0.01$). Average nestling stage length is one of the life-history characteristics associated with average nest predation rate, whereby species at a high risk of nest predation tend to have shorter nestling stage lengths (Martin 1995). As such, a negative association between average nestling and forage ratios would support the inference of predation risk influencing the defensive behavior of avian species, and thus their contact rates with mosquitoes. Alternatively, in species with shorter nestling stages, the risk of hematophagy to recently fledged birds may be particularly high, because such young birds are independent and receive no protection from parents (Burkett-Cadena et al. 2010). If recently fledged birds are preferentially targeted by questing mosquitoes for bloodmeals, as has been suggested previously (Loss et al. 2009), then those species with short nestling stages would be likely to be fed upon by mosquitoes at a high rate.

Habitat is an obvious environmental factor that could link vectors and hosts, so it was not surprising that an association with habitat edge was included as a predictor variable in the top-ranking *Cx. erraticus* forage ratio model. We had predicted that an association with habitat-edge would relate to forage ratio positively in the *Cx. erraticus* model and negatively in the *Cs. melanura* model. The basis for these predictions was a previous study of mosquito microhabitat associations (Bidingmayer 1971), which showed different strengths of habitat-edge association in wooded swamp habitats between *Cs. melanura* and species in the *Culex* (Melaconion)

subgenus, of which *Cx. erraticus* is a member (Darsie and Ward 2005). Specifically, the average number of *Cs. melanura* captured per trap night was higher in traps located in swamps compared to traps located at swamp edges, field edges, or in fields. As such, Bidlingmayer (1971) suggested that *Cs. melanura* is a swamp interior species that avoids swamp edges and the higher levels of illumination associated with them. Conversely, the highest average trap counts of *Culex* (Melanoconion) species were at field edges, followed by traps in fields, at swamp edges, or in swamps (Bidlingmayer 1971). We predicted that if there were similar microhabitat associations of *Cs. melanura* and *Cx. erraticus* in the wetland habitat of TNF, then *Cs. melanura* would have a higher rate of encounter with swamp interior species, resulting in an inflated forage ratio for those species. In contrast, *Cx. erraticus* was expected to encounter swamp- and field-edge avian species at a higher rate than swamp interior species, such that the forage ratio of habitat-edge associated avian species would be inflated. Our results confirmed our predictions, as the direction of influence of habitat-edge association of forage ratios was positive in the *Cx. erraticus* model and negative in the *Cs. melanura* model. Overall, however, the influence of this variable may be relatively small compared to other host trait variables and thus may not be useful for prediction.

Duration of availability of an avian species during the breeding season of *Cs. melanura* or *Cx. erraticus* had relatively low importance weights as a variable useful for predicting forage ratio models for either species. While this variable was included in the second-highest ranking model in the *Cx. erraticus* model set, the 85% confidence intervals included zero for the estimates of the coefficient of this variable, and the direction of association was in the opposite direction as predicted. Moreover, the direction of association of this variable with forage ratios was not consistent between mosquito species models. As such, whether or not an avian host

species was present at the TNF site for the entire mosquito breeding season appears to have had minimal influence on their forage ratio estimates for either mosquito species compared to the influence of other host traits useful for predicting the relative rates of contact between birds and mosquitoes described in this study.

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Table 1. Predictor variables used in for *Cs. melanura* forage ratio and *Cx. erraticus* forage ratio candidate models.

Variable Name	Description	Predicted Direction: <i>Cs. melanura</i> Model	Predicted Direction: <i>Cx. erraticus</i> Model	Data Source
availability	availability status: indicator variable (0 = present for only part of mosquito host-seeking season, 1= present for entire mosquito host-seeking season); assumed present from start of breeding to start of migration (rounded to quarter-month); see text for mosquito host-seeking season dates	pos	pos	Poole et al. 2005 (individual species accounts)
cavity	cavity nesting status: indicator variable (0 = uses nest type other than cavity nest, 1= cavity-nesting)	pos	pos	Erlich et al. 1988
edge	habitat-edge association: average score from 3 sources, where 1 = forest interior species, 2 = generalist species, 3 = edge species	neg	pos	Whitcomb 1981, McIntyre 1995, Bender et al. 1998
mass	body mass (g)	pos	pos	Dunning 2008
nestling	nestling stage length*average number of broods (days)	pos	pos	Erlich et al. 1988, Gough et al. 1998
roost	communal roosting status: indicator variable (0 = does not roost communally on breeding grounds, 1= roosts regularly during breeding season or after breeding season, but before fall migration)		pos	Poole et al. 2005 (individual species accounts)
stratum	nesting stratum: indicator variable (0 = ground-low nesting, 1= mid-story/canopy nesting)	not predicted	not predicted	Gough et al. 1998

Table 2. Bias-corrected AIC (AICc) table for *Cx. erraticus* forage ratio models with moderate or strong support. AICc is the bias-corrected Akaike Information Criterion, K is the number of parameters estimated, Δ_i is the difference in AICc from the model that minimized the AICc, and w_i is the importance weight. All models with $\Delta_i < 7$ are represented.

Model	log(L)	AICc	K	Δ_i	w_i
mass + stratum + edge + nestling	-40.40	98.80	6	0.00	0.31
mass + stratum + edge + nestling + availability	-38.27	99.16	7	0.37	0.26
mass + stratum + nestling	-44.10	102.19	5	3.40	0.06
mass + stratum + edge + cavity + nestling + availability	-37.60	103.21	8	4.41	0.03
mass + stratum + edge + cavity + nestling	-40.37	103.35	7	4.55	0.03
mass + stratum + edge + nestling + roost	-40.40	103.41	7	4.61	0.03
mass + stratum	-46.77	104.04	4	5.24	0.02
stratum + edge + nestling	-45.16	104.31	5	5.52	0.02
mass + stratum + edge + nestling + availability + roost	-38.26	104.52	8	5.73	0.02
stratum	-48.93	105.26	3	6.47	0.01
mass + stratum + cavity + nestling	-43.76	105.51	6	6.71	0.01
mass + stratum + nestling + roost	-43.84	105.69	6	6.89	0.01
stratum + fledgling	-47.62	105.73	4	6.94	0.01

Table 3. Results of model averaging for *Cx. erraticus* forage ratio models and importance weights of predictor variables (weight). MAE is the model-averaged estimate of the variable coefficients and SE is the standard error of that estimate.

<u>Variable</u>	<u>Weight</u>	<u>MAE</u>	<u>SE</u>	<u>85% Confidence Interval</u>	
				<u>Lower</u>	<u>Upper</u>
stratum	0.91	1.95	0.86	0.72	3.18
mass	0.88	0.60	0.29	0.18	1.01
nestling	0.84	-0.07	0.04	-0.12	-0.01
edge	0.75	0.98	0.72	-0.06	2.02
availability	0.38	-0.27	0.42	-0.88	0.33
cavity	0.16	0.08	0.20	-0.20	0.37
roost	0.10	0.04	0.22	-0.28	0.35

Table 4. Bias-corrected AIC (AICc) table for *Cs. melanura* forage ratio models with moderate or strong support. AICc is the bias-corrected Akaike Information Criterion, K is the number of parameters estimated, Δ_i is the difference in AICc from the model that minimized the AICc, and w_i is the importance weight. All models with $\Delta_i < 7$ are represented.

Model	log(L)	AICc	k	Δ_i	w_i
nestling	-17.67	43.73	3	0.00	0.31
stratum + nestling	-16.21	44.86	4	1.13	0.17
cavity + nestling	-17.11	46.66	4	2.93	0.07
(intercept only)	-21.11	47.31	2	3.58	0.05
nestling + availability	-17.48	47.40	4	3.67	0.05
mass + nestling	-17.53	47.50	4	3.77	0.05
edge + nestling	-17.55	47.54	4	3.81	0.05
mass + stratum + nestling	-15.29	48.09	5	4.36	0.03
edge	-19.92	48.24	3	4.51	0.03
stratum + edge + nestling	-15.90	49.30	5	5.57	0.02
stratum + nestling + availability	-15.97	49.44	5	5.71	0.02
availability	-20.54	49.47	3	5.74	0.02
stratum + cavity + nestling	-16.17	49.84	5	6.11	0.01
mass	-20.87	50.15	3	6.41	0.01
stratum	-20.88	50.15	3	6.42	0.01
cavity	-21.06	50.52	3	6.78	0.01

Table 5. Results of model averaging for *Cs. melanura* forage ratio models and importance weights of predictor variables (weight). MAE is the model-averaged estimate of the variable coefficients and SE is the standard error of that estimate.

<u>Variable</u>	<u>Weight</u>	<u>MAE</u>	<u>SE</u>	<u>85% Confidence Interval</u>	
				<u>Lower</u>	<u>Upper</u>
nestling	0.83	-0.05	0.03	-0.09	-0.01
stratum	0.30	0.14	0.24	-0.21	0.49
edge	0.14	-0.03	0.09	-0.15	0.10
availability	0.13	0.02	0.09	-0.10	0.14
mass	0.13	0.02	0.06	-0.06	0.10
cavity	0.13	0.04	0.12	-0.13	0.21

Figure 1. *Cs. melanura*(a) and *Cx. erraticus* (b) forage ratios of avian host species nesting either at ground/low-levels or in the mid-story/canopy. Circle sizes are proportional to observation weights.

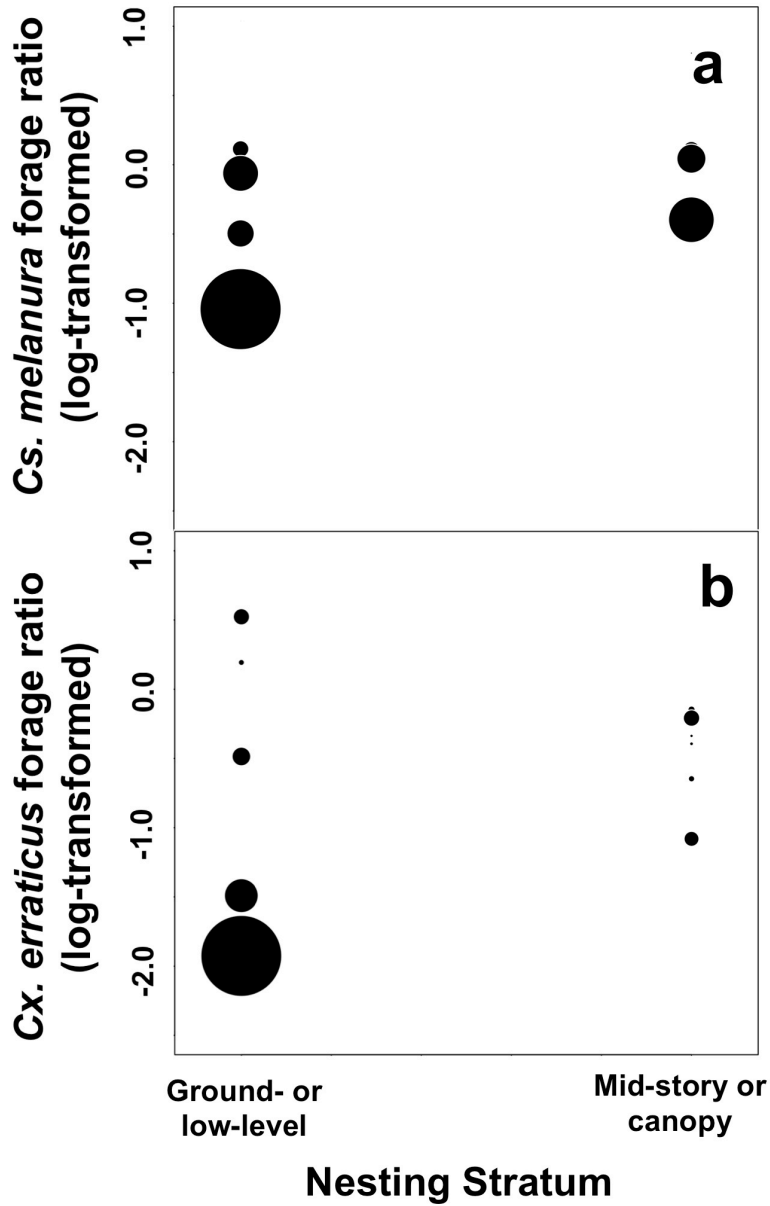


Figure 2. Scatterplot of *Cs. melanura*(a) and *Cx. erraticus* (b) forage ratios for avian host species versus body mass, with best-fit line from simple linear regressions overlain. Circle sizes are proportional to observation weights.

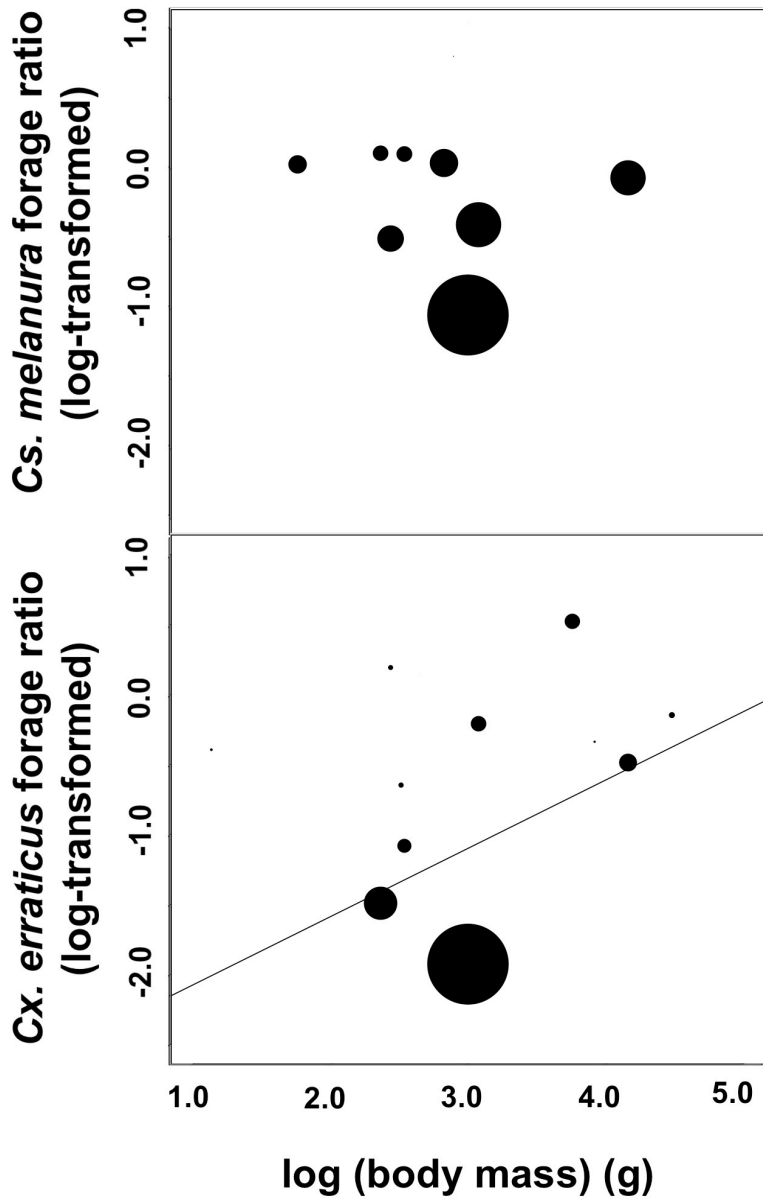
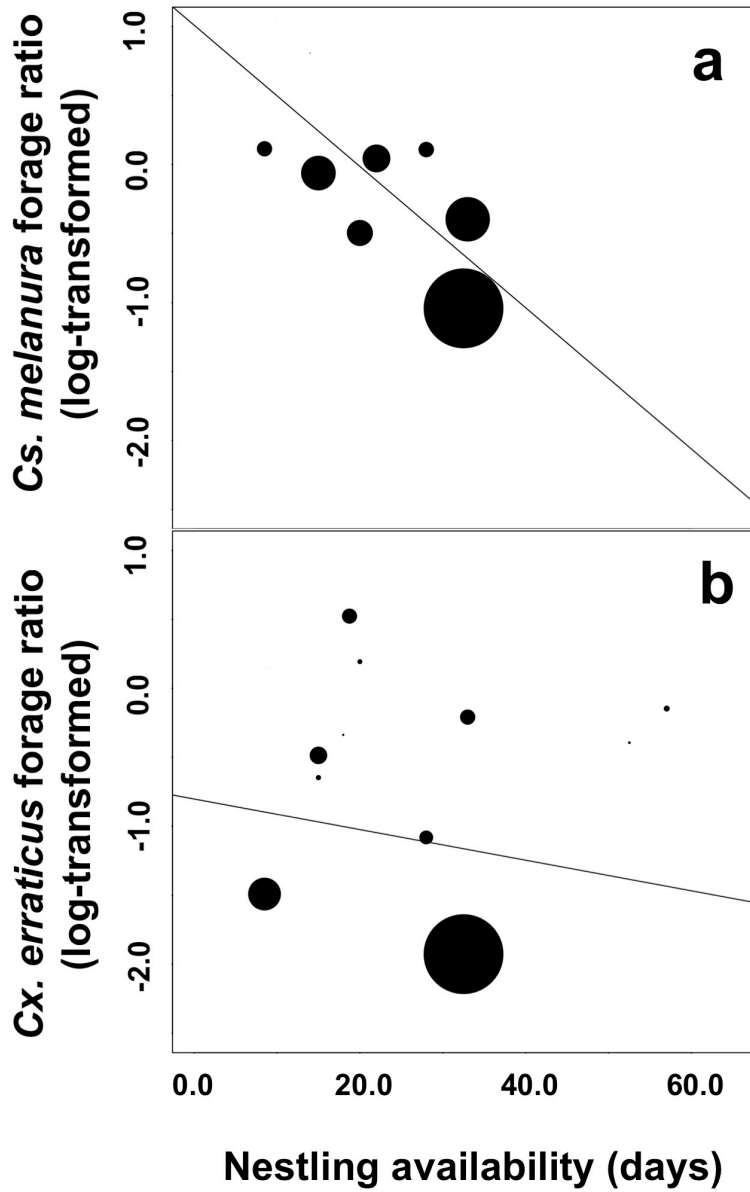


Figure 3. Scatterplot of *Cs. melanura*(a) and *Cx. erraticus* (b) forage ratios for avian host species versus nestling stage length, with best-fit line from simple linear regressions overlain.

Circle sizes are proportional to observation weights.



Appendix I. Forage ratios and host-trait data used in model development.

Species	<i>Cs. melanura</i> forage ratio (standard error)	<i>Cx. erraticus</i> forage ratio (standard error)	<i>Cs. melanura</i> availability	<i>Cx. erraticus</i> availability	Cavity	Edge	Mass	Nestling	Roost	Stratum
Acadian flycatcher <i>Empidonax vireescens</i>	1.11 (0.77)	0.34 (0.34)	0	0	0	1.00	12.6	14.0	0	1
American robin <i>Turdus migratorius</i>		44.27 (11.10)	1	1	0	3.00	78.5	15.0	1	1
Blue grosbeak <i>Guiraca cerulea</i>		4.55 (3.19)	1	0	0	3.00	27.4	9.0	0	1
Blue jay <i>Cyanocitta cristata</i>		0.86 (0.49)	1	1	0	2.00	88.0	19.0	0	1
Blue-gray gnatcatcher <i>Polioptila caerulea</i>	1.03 (0.72)		1	1	0	2.00	5.8	11.0	0	1
Brown-headed cowbird <i>Molothrus ater</i>		1.84 (1.29)	1	1	0	3.00	43.0	10.5	1	0
Carolina chickadee <i>Poecile carolinensis</i>		4.60 (1.07)	1	1	1	1.67	10.0	15.0	0	1
Carolina wren <i>Thryothorus ludovicianus</i>	0.35 (0.35)	0.15 (0.15)	1	1	0	2.50	20.0	13.0	0	0
Common grackle <i>Quiscalus quiscula</i>		19.65 (9.66)	1	1	0	3.00	106.1	18.0	1	1
Common yellowthroat <i>Geothlypis trichas</i>	7.73 (4.32)		1	1	0	2.33	9.5	10.0	0	0
Eastern towhee <i>Pipilo erythrophthalmus</i>	3.28 (3.25)		1	1	0	2.67	40.1	11.0	0	0
Gray Catbird <i>Dumetella carolinensis</i>	7.06 (6.98)	8.69 (4.95)	1	1	0	2.67	35.3	10.5	0	0
Hooded warbler <i>Wilsonia citrina</i>	1.12 (0.77)	0.23 (0.22)	0	0	0	1.00	10.6	8.5	0	0
Kentucky warbler <i>Opornis formosus</i>		1.15 (1.15)	1	0	0	1.00	14.0	9.0	0	0
Louisiana waterthrush <i>Seiurus motacilla</i>	2.82 (2.79)		0	0	0	1.00	19.9	10.0	0	0
Northern cardinal <i>Cardinalis cardinalis</i>	4.18 (0.70)	1.69 (0.32)	1	1	0	2.33	42.7	7.5	0	0
Northern mockingbird <i>Mimus polyglottis</i>		34.59 (9.05)	1	1	0	3.00	48.5	12.0	0	0
Orchard oriole <i>Icterus spurius</i>		43.63 (21.44)	0	0	0	3.00	19.9	12.5	0	1
Pine warbler <i>Dendroica pinus</i>		0.52 (0.52)	1	1	0	1.00	12.3	10.0	0	1
Red-eyed vireo <i>Vireo olivaceus</i>	1.04 (0.58)		1	0	0	1.67	16.8	11.0	0	1
Ruby-throated hummingbird <i>Archilochus colubris</i>		0.67 (0.67)	1	0	0	2.67	3.1	21.0	0	1
Tufted titmouse <i>Baeolophus bicolor</i>	0.67 (0.46)	0.81 (0.32)	1	1	1	1.67	21.6	16.5	0	1
White-eyed vireo <i>Vireo griseus</i>	0.61 (0.60)	1.21 (0.53)	1	0	0	2.33	11.4	10.0	0	0
Wild turkey <i>Meleagris gallapavo</i>		33.49 (14.65)	1	1	0	2.00	6050.0	8.0	1	0
Wood thrush <i>Hylocichla mustelina</i>		0.71 (0.71)	1	0	0	1.33	50.2	12.0	0	1
Yellow-billed cuckoo <i>Coccyzus americanus</i>	0.94 (0.52)	0.62 (0.30)	0	0	0	1.33	64.0	7.5	0	0
Yellow-throated vireo <i>Vireo flavifrons</i>	2.24 (2.22)		0	0	0	2.00	18.0	14.0	0	1

IV. USING ATTRIBUTES OF AVIAN COMMUNITIES TO PREDICT LOCAL ENZOOTIC TRANSMISSION OF EASTERN EQUINE ENCEPHALITIS VIRUS

Abstract

Because eastern equine encephalitis virus (EEEV) is a dangerous pathogen to both humans and horses, there is urgent need to develop models to predict patterns of transmission on a local spatial scale. Here, we develop such a predictive model of EEEV transmission using data on the avian host community. We used data on EEEV seroconversion collected during 2009 from chicken sentinel flocks distributed across Walton County, Florida to develop a logistic regression model of the log-odds of a site having a high versus low rate of EEEV transmission. Covariates considered in model development included linear and quadratic effects for avian community size and the relative abundances of avian host species regarded as preferred hosts of *Culiseta melanura*. The weights of variables associated with relative abundances of focal avian species exhibited a perfect rank-correlation with estimates of how preferred the bird species were as hosts of *Cs. melanura*. While preliminary, these results suggest that presence of hosts that are preferred by *Cs. melanura* and overall reservoir competence for EEEV are factors that figure prominently into local-scale EEEV transmission.

Introduction

Eastern equine encephalitis virus (EEEV) is a highly virulent pathogen with a complex life cycle, involving both a mosquito vector and an avian reservoir host (Morris 1988, Scott and

Weaver 1989). Despite high mortality rates (60-80%), human cases of EEE are quite rare, relative to other arboviruses that occur in the United States (Scott and Weaver, 1989, Villari et al. 1995). An important consequence of such rarity is that human and horse infections are difficult to predict (Letson et al. 1993). Given the severity of cases of EEE in both humans and horses there is an urgent need for the development of models that can predict where and when EEEV will occur.

Despite still incomplete knowledge of the factors that determine occurrence of the virus on a regional scale, progress has been made in the development of predictive models of the temporal occurrence of EEEV at known transmission locales. Specifically, elevations in the weekly minimum infection rates (MIRs) and in the number of infected primary enzootic vectors (*Culiseta melanura*) have been shown to be associated with the number of cases of human eastern equine encephalitis virus in Massachusetts (Hachiya et al. 2007). MIR and detection of positive *Cs. melanura* may be useful for developing an Early Warning System for EEEV, but these variables have not been used to predict where transmission will occur.

While the use of vector MIR's may be useful in forecasting the timing of EEEV transmission, accurate prediction of the spatial distribution will likely depend on attributes not just of vector populations but also of avian communities. Birds are the primary reservoir hosts of EEEV, and thus are important to EEEV transmission (Scott and Weaver 1989). Individuals of different avian host species vary in their rates of contact with mosquitoes, as estimated by forage ratios (Hassan et al. 2003, Estep et al. in press). They also differ in their capacities to replicate the virus following exposure (reservoir competency) and thus in their ability to infect subsequent mosquitoes that feed on them (Komar et al. 1999). Such heterogeneity across hosts with respect to reservoir competences and relative contact rates with vectors can result in variability amongst

sites in rates of EEEV transmission when hosts are have a variable distribution across a landscape.

One means to describe virus transmission is to use a model based on frequency-dependence. Under frequency-dependent transmission, the rate of transmission is directly proportional to I/N , the proportion of the host community comprised of infective individuals, where I = number of infective hosts and N = total number of hosts in the community (Anderson and May 1979). The proportion, or frequency, of infectives in the host population is just an estimate of the probability that a given host that a vector contacts is infective when all hosts have the same probability of contact with a vector. Assuming that it is this probability of a given host that a vector contacts being infective, rather than the proportion of infectives in the host community *per se*, that drives frequency-dependent transmission, the rate of transmission will be most heavily influenced by those species that have high forage rations or extreme reservoir competences (Kilpatrick et al. 2006).

The mode of virus transmission will vary between vector-borne pathogen systems, depending on the behavior of the vector (Antonovics et al. 1995). At one extreme where vectors have short search times for hosts, the rate of transmission will be density-dependent; in other words, it will be proportional to the number of infectives in the host population, rather than the frequency of infectives, as in frequency-dependence. Under density-dependence, the rate of transmission is expected to increase with total size of the host community for a given frequency of infectives (Antonovics et al. 1995). As such, the rate of transmission should increase with total host community size for a given probability of a host that a vector contacts being infective.

At the other extreme of vector behavior where vectors have long search times for hosts, the rate of transmission will be proportional to I/N^2 . In this case, the rate of transmission is

expected to increase initially with increasing host community size. It then plateaus and thereafter exhibits “inverse density dependence”, such that the rate of transmission decreases with increasing host community size for larger communities (Antonovics et al. 1995, Antonovics and Alexander 1992, de Castro and Bolker 2005).

Given that avian species are heterogeneous for the rate of vector contact and reservoir competency, it seems plausible that composition of the local avian community could influence the transmission of EEEV across locations. More specifically, we expect to find support in spatially-explicit models of EEEV transmission for the inclusion of variables representing the relative abundances of species that are highly influential on the probability of a host that a vector contacts being infective, i.e. those species that have high forage ratios and extreme reservoir competences. Additionally, after controlling for the relative abundances of highly influential species, we expect to find support for inclusion of variables of host community size.

To date, no models for predicting local EEEV transmission have incorporated data on avian communities. In this study, we sought to determine whether models based solely on the composition and size of avian communities would be able to predict the likelihood that mosquito-to-bird transmission of EEEV would occur. We made the simplistic assumption that *Cs. melanura* is the primary enzootic vector of the virus and that birds are the only reservoir hosts involved in its transmission. We tested this hypothesis using data derived from seroconversion of sentinel chickens from arbovirus surveillance sites, avian point-counts, and estimates of the relative rates of contact of avian host species with *Cs. melanura* (Estep et al. in press).

Materials and Methods

Data Sources

Data on the enzootic transmission of EEEV used in model development originated from Walton County, FL mosquito control districts. These two districts collectively monitored 24 sentinel chicken flocks in 2009, located throughout the county as part of a long-term arbovirus surveillance program (Moore 1993, Figure 1). Sentinel flocks were comprised of either 2-3 (South Walton district) or 6 (North Walton district) chickens held in outdoor sentinel cages. Mosquito control personnel collected blood samples from all individuals in each sentinel flock weekly and shipped them to the Florida Department of Health for testing for the presence of EEEV neutralizing antibodies via hemagglutinin inhibition and serum neutralization assays (Florida Interagency Arboviral Task Force 2006). Individual chickens with evidence of seroconversion from an EEEV antibody-negative to positive status were sacrificed and replaced with naïve individuals following reporting of test results.

Observational data used as predictor variables in model development originated from point-count surveys of the avian communities around sentinel sites. We assigned four avian survey sites to each sentinel flock, for a total of 96 survey sites (Figure 3). Individual survey sites in the set of four sites surrounding each flock were assigned to points at regular intervals along the perimeter of a buffer with radius-length 250 meters centered on the flock, such that the 2 line segments connecting survey site on opposite sides of the flock intersected and formed right angles at the flock locations. Buffers were rotated by a random angle between 0 and 90 degrees, such that the survey site locations varied amongst flocks but were consistently equidistant from each other within a flock.

Avian survey sites were visited once during June of 2010 by a single observer trained in the vocal and visual identification of avian species that breed in southeastern United States (Bibby et al. 1992). Surveys were conducted between 0500 and 1000 EDT. Each visit was divided into five three-minute sessions during which the identity and location of all birds seen or heard were recorded (Mackenzie and Royle 2005). From these data, the average number of individuals of each species at each avian survey site was calculated. The abundance of each species at each sentinel flock was estimated as the mean of its averaged abundance at each of its four surrounding survey sites.

Analytical Approach

Our analytical approach focused on developing a model of the probability of a site having a high rate EEEV transmission. Sentinel sites where at least one chicken seroconverted in 2009 had higher rates of infection of the susceptible hosts (sentinel chickens) than sites where there were zero seroconversions. By extension, sites where at least one chicken seroconverted had higher rates of EEEV transmission than sites where all chickens remained naïve, as the rate of transmission of a pathogen is directly proportional to the rate at which susceptible hosts becoming infected (Anderson and May 1991). We thus designated sites as either having a low- or high- rate of EEEV transmission based on whether or not at least one chicken seroconverted there.

We used the indicator variable for a site having versus low rate of transmission as the response variable in logistic regression models (1=high, 0=low). We used a multi-model inference approach to our analysis (Anderson et al. 2000, Burnham and Anderson 2002). We specified a set of thirteen candidate models.

The first two models in the candidate set described the log-odds of the sentinel site having a high rate of EEEV transmission as constant (model for the mean; intercept only and no covariates) or dependent on a single covariate, avian community size (total number of birds, averaged across the four point count survey areas surrounding the sentinel site). Each of the next five models in the candidate set were elaborations of the second model that had avian community size as the single predictor variable, whereby the log-odds of the response was modeled as a linear function of avian community size plus the relative abundance of one of five focal avian species. Focal avian species were those that satisfied two requirements (1) avian species inferred to be preferred hosts of *Cs. melanura*, the primary enzootic vector of EEEV, based on forage ratios >1 from published field studies (Estep et al. in press) (2) species that were observed during point count surveys around sentinel flocks in 2010. One species inferred to be a preferred host of *Cs. melanura*, yellow-throated vireo, was not considered in model development because it was present at point count locations surrounding only one sentinel site. As such, models three through seven included the relative abundance of either blue-gray gnatcatcher, common yellowthroat, eastern towhee, northern cardinal, or red-eyed vireo, as these species were present at point count location surrounding at least two sentinel sites and were found to be preferred hosts of *Cs. melanura* (Estep et al. in press)(Table 1).

The eighth model specified the effect of avian community size as quadratic, such that it included the linear and quadratic terms for avian community size to keep the model hierarchically correct. Following the same protocol used for the model that included only a linear effect of avian community size, the last five models were constructed as elaborations as the eighth model. They included the linear and quadratic effects for avian community size and,

individually, the relative abundance of either blue-gray gnatcatcher, common yellowthroat, eastern towhee, northern cardinal, or red-eyed vireo.

Models were specified using the `glm` function in R software using the binomial error distribution (R Development Core Team 2008). The avian community size variable was natural log-transformed, and relative abundance variables were arcsine-square-root-transformed. All variables were centered about their means. Model averaging was performed over the 95% confidence set of models, i.e. those models that had the greatest model weights and that together comprised 95% of the model weight in the final candidate set (Burnham and Anderson 2002). We used 85% confidence intervals about parameter estimate as a basis for inferring whether or not a variable was potentially useful for prediction (Arnold et al. 2010). QAICc values were used in calculating importance weights for variable weights in models in the final candidate set (Burnham and Anderson 2002).

Results

A total of 67 chickens distributed over 15 of the 24 sites monitored seroconverted from a status of naive to a status of positive for EEEV antibodies in 2009, such that 15 sites were designated as having a high rate of EEEV transmission in 2009 and 9 were classified as having a low rate. The average number of chickens that seroconverted over the entire season, calculated strictly over the 15 sites with high rates of transmission, was 4.47 (SD = 4.54 , min = 1, max = 17). High transmission-rate sites occurred throughout Walton County, with 6 of 8 sites classified as high-rate sites in the North Walton County district (north of the Choctawhatchee Bay), and 9 of 16 sites classified as high-rate sites in the South Walton County district (south of the Choctawhatchee Bay; Fig 1).

The average size of the 24 avian communities across sentinel sites based on these average species abundance estimates was 6.02 individuals (SD = 2.48 , min = 3.25, max = 12.40).

Individuals from sixty different avian species were detected during point-count surveys.

Two of the thirteen models in the model set had strong support. The model that included linear and quadratic effects for avian community size was the top-ranking model. The model that included linear and quadratic effects for avian community size and the relative abundance of common yellowthroat ranked second with a ΔQAICc of 1.26. The weights associated with these two top-ranking models were 0.36, 0.19, respectively (Table 2). Overall, the six models that included the quadratic effect of avian community size had higher weights than the models that included only a linear effect for avian community size when weights were calculated over the set of models that included all models except for the model that was found to have a poor fit to the data (le Cessie and van Houwelingen, 1991, Table 2).

Eighty-five percent confidence intervals of the model-averaged parameter estimate over models in the ninety-five percent confidence set for all covariates considered in model development included zero, except for the quadratic effect of avian population size. (Table 3; Figure 3). While the eighty-five percent confidence interval for the effect of the relative abundance of common yellowthroat on the log-odds of the response included zero, its parameter estimate exhibited a strong negative skew (Table 3; Figure 4).

Ranks of importance weights associated with the variables representing the relative abundances of preferred avian host species of *Cs. melanura* were perfectly correlated with the ranks of the forage ratios of those species, such that the null hypothesis of zero correlation between ranks was rejected ($r_s=1.00$, $n=5$, $p=0.01677$; Figure 5).

Discussion

Understanding the transmission dynamics of EEEV and developing a model to predict its occurrence on a local scale is challenging given the potential involvement of multiple mosquito species and a suite of avian hosts that vary in capacity to serve as a reservoir hosts. Our results reflect the complexity of this vector/host/viral system. The relative abundance of no single avian species was found to be useful for prediction of EEEV transmission, but our models suggest that heterogeneities amongst avian species do indeed impact the transmission dynamics of EEEV. This insight provides an important step forward in our understanding of the contribution of the avian community to EEEV transmission and provides a foundation for future modeling efforts.

The top-ranked model in our candidate model set included linear and quadratic terms for avian community size. However, the model that included linear and quadratic effects of avian community size, as well as the relative abundance of common yellowthroat, had a QAICc value within 2 units of the top-ranked model, suggestive of strong support for this model. Models that are competitive with the top-ranked model based on Δ QAICc values, but that are simply elaborations of the top-ranked model with one variable added, require that the log-likelihood values of either model be additionally examined to determine if the more elaborate model has essentially the same log-likelihood as the top-ranked model. In this case of equivalent log-likelihoods between models, the more elaborate model is typically considered to be less competitive (Burnham and Anderson 2002, Arnold 2010). In our analysis, however, the second-ranked model with the common yellowthroat variable has a log-likelihood clearly different from the top-ranked model such that we infer presence of common yellowthroat has a biological influence on EEEV transmission. As such, the model that included linear and quadratic effects for avian community size, as well as the relative abundance of common yellowthroats, is

competitive with the top-ranked model for being the one amongst all models we considered that most closely approximates the true model of EEEV transmission.

It is interesting and probably not coincidental that the common yellowthroat was the species of bird with the highest forage ratio estimate for *Cs. melanura* among all avian species in a study conducted 200 km north in Macon County, Alabama, that were detected in Walton County surveys (Estep et al. in press), as well as the bird species whose relative abundance was inferred to influence EEEV transmission based on our results. The higher the forage ratio estimate of a species, the greater the proportion of *Cs. melanura* bites we would expect to be directed towards that host and away from other hosts. A species with higher forager ratio estimates should have the greatest influence on the probability that vectors are infective and thus on virus transmission. This influence results simply because individuals of species with high feeding index values come into contact more often with *Cs. melanura* than less-preferred hosts.

The common yellowthroat has the highest forage ratio among birds in this region (Estep et al. in press) and is inferred from our results to have a biological influence on transmission. Perhaps just as importantly, however, the ranks of the importance weights for the relative abundances of the four other species considered in our models correlated perfectly with the ranks of their forage ratios. The importance weight of a variable is the probability that the variable would be in the top-ranking model if the same set of models were run on a different dataset (Burnham and Anderson 2002). Overall, the relationship between importance in predicting viral transmission and preference as a host for *Cs. melanura* is strong evidence that heterogeneity amongst avian species in terms of their relative rates of contact has a strong influence on transmission.

Our model also suggests that differences there are differences among avian species in their competence as virus amplifiers. The direction of association between the relative abundance of common yellowthroat and transmission was negative, such that there appears to be a lower rate of transmission at sites where common yellowthroats have a greater relative abundance. This pattern of negative association was also evident for eastern towhees. Such a negative association suggests that common yellowthroats and eastern towhees act as dilution hosts (LoGiudice et al. 2003), diverting bites away from more competent avian species and effectively lowering the probability that a vector feeds upon an infected bird.

In contrast, our model suggests that northern cardinal, red-eyed vireo, and blue-gray gnatcatcher may act to increase the rate of transmission, possibly because they have an above-average reservoir competence. Overall, these results suggest that variation among avian hosts in terms of their relative rates of contact with *Cs. melanura* in combination with their reservoir competence influence EEEV transmission and spatial patterns of variability in rates of transmission. However, it appears that it is the variability amongst avian host species in relative rates of contact with *Cs. melanura* that is more important in determining the magnitude of their influence on transmission than variability in their reservoir competences.

While preliminary, our results provide the strongest evidence to date that heterogeneity amongst avian host species in terms of their rates of contact with *Cs. melanura* and reservoir competence for EEEV influences transmission of the virus. Therefore, any model considers differential contributions of individual host species to transmission and variability in those contributions across geographic locations due to differences in avian community composition has the potential to elucidate patterns and to predict of local-scale variation in EEEV transmission.

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Table 1. Descriptive statistics of the relative abundances of avian species at sentinel sites in Walton County, Florida in 2009. Species represented are those that were inferred to be preferred hosts of *Cs. melanura* in Tuskegee National Forest based on forage ratio estimates > 1 (Estep et al. in press). Alpha codes follow guidelines of the American Ornithological Union's Checklist of North American Birds.

Species	Alpha Code	Forage Ratio	Relative Abundance	Present (# Sites)
Barred owl <i>Strix varia</i>	BDOW	8.12	-	0
Common yellowthroat <i>Geothlypis trichas</i>	COYE	7.73	0.006 (0.010)	8
Gray Catbird <i>Dumetella carolinensis</i>	GRCA	7.06	-	0
Northern cardinal <i>Cardinalis cardinalis</i>	NOCA	4.18	0.142 (0.047)	24
Eastern towhee <i>Pipilo erythrophthalmus</i>	EATO	3.28	0.046 (0.051)	17
Louisiana waterthrush <i>Seiurus motacilla</i>	LOWA	2.82	-	0
Yellow-throated vireo <i>Vireo flavifrons</i>	YTVI	2.24	0.000 (0.002)	1
Hooded warbler <i>Wilsonia citrina</i>	HOWA	1.12	-	0
Acadian flycatcher <i>Empidonax vireescens</i>	ACFL	1.11	-	0
Red-eyed vireo <i>Vireo olivaceus</i>	REVI	1.04	0.006 (0.017)	6
Blue-gray gnatcatcher <i>Polioptila caerulea</i>	BGGN	1.03	0.004 (0.009)	5

Table 2. Summary of attributes of candidate models considered in developing a logistic regression model of the log-odds of a sentinel site having a high- versus low-level of virus activity (>1 chicken seroconverted to EEEV antibodies) in Walton County in 2009. QAICc is the bias-corrected quasi-Akaike Information Criterion, K is the number of parameters estimated, Δ_i is the difference in QAICc from the model that minimized the QAICc, and w_i is the QAICc weight. Goodness-of-fit (GOF) of each models to the data was assessed with the le Cessie and Houwelingen test (le Cessie and Houwelingen 1991).

Model	p-value (GOF Test)	log(L)	QAICc	K	Δ_i	w_i
N + N ²	0.565	-10.152	29.503	3	0.000	0.363
N + N ² + COYE	0.440	-9.330	30.765	4	1.261	0.193
N + N ² + NOCA	0.630	-9.748	31.601	4	2.097	0.127
N + N ² + EATO	0.424	-9.940	31.986	4	2.482	0.105
N + N ² + REVI	0.598	-10.059	32.223	4	2.720	0.093
N + N ² + BGGN	0.564	-10.152	32.409	4	2.905	0.085
N + COYE	0.927	-13.263	35.726	3	6.223	0.016
(intercept only)	NA	-15.878	35.937	1	6.433	0.015
N	0.006	-15.877	38.326	2	8.823	-
N + REVI	0.188	-15.718	40.636	3	11.133	0.001
N + NOCA	0.313	-15.807	40.813	3	11.310	0.001
N + BGGN	0.155	-15.808	40.816	3	11.313	0.001
N + EATO	0.137	-15.854	40.909	3	11.406	0.001

Table 3. Variable weights and model-averaged estimates of intercept and variable coefficients for EEEV models in Walton County, Florida.

Variable	Model-averaged Estimate	Weighted Unconditional SE	85% Confidence Interval		Importance Weight
			Lower	Upper	
Intercept	-1.132	0.775	-2.247	-0.018	1.000
N	-2.194	2.697	-6.077	1.688	0.985
N ²	18.857	9.298	5.472	32.242	0.966
COYE	-2.145	4.180	-8.162	3.873	0.209
NOCA	1.090	2.520	-2.539	4.718	0.128
EATO	-0.307	0.824	-1.493	0.879	0.106
REVI	0.292	0.984	-1.124	1.708	0.094
BGN	0.004	0.911	-1.307	1.315	0.086

Figure 1. Sentinel site locations in Walton County, Florida used in model development. EEEV-positive sites are those where at least one chicken seroconverted to EEEV antibodies in 2009.

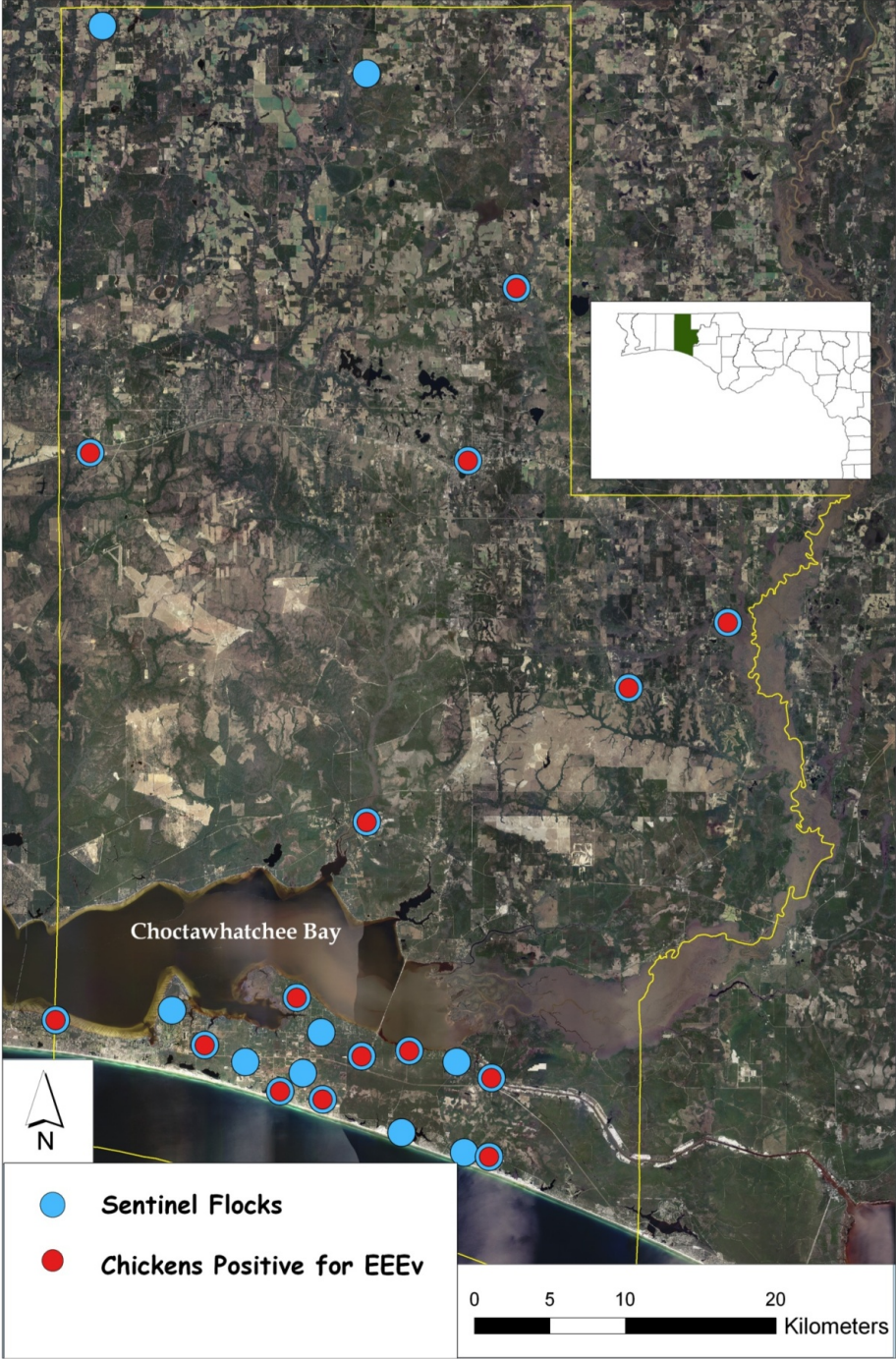


Figure 2. Image of 2009 avian point-count-locations centered on individual sentinel sites in Walton County, Florida.



Figure 3. Inferred rates of EEEV transmission among sentinel sites of variable avian community sizes in Walton County, Florida, with estimated function of the probability of a site having a high level of virus activity overlain. EEEV-negative sites were those where at least chicken seroconverted to EEEV antibodies in 2009, whereas sites where no chickens seroconverted were designated as EEEV-negative sites. The model-averaged estimate and associated 85% weighted unconditional confidence interval was -2.194 (-6.077, 1.688) for the linear effect of avian community size (N; ln-transformed, centered about mean) and 18.857 (5.472, 32.242) for the quadratic effect of avian community size (N²; ln-transformed, centered about mean).

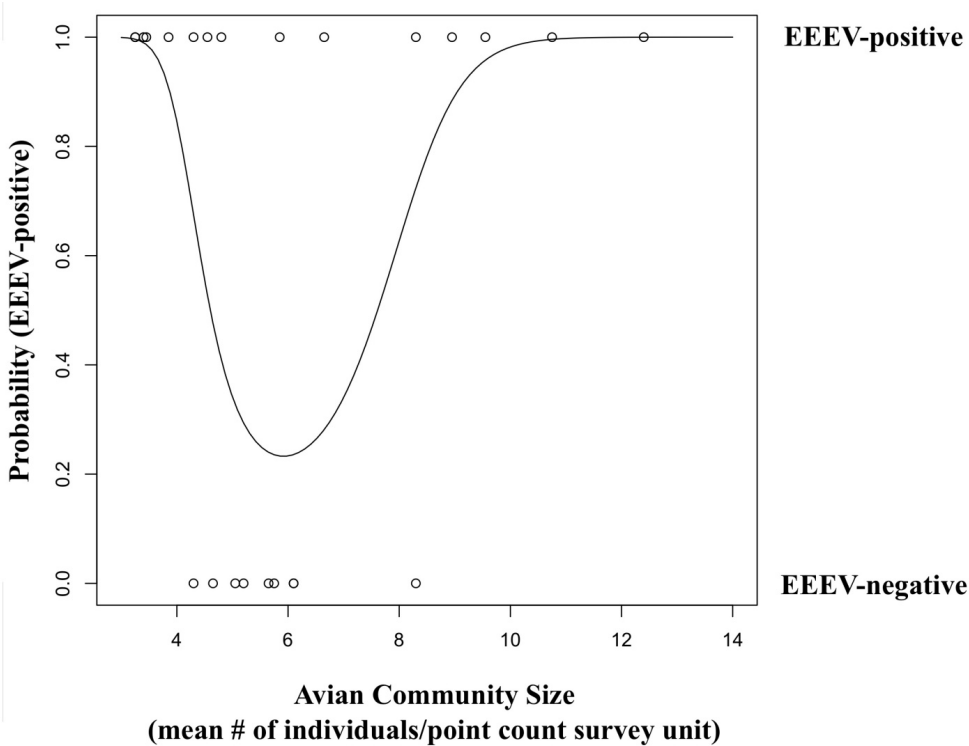


Figure 4. Relative abundances of common yellowthroat at EEEV-positive and EEEV-negative sentinel sites in 2009 in Walton County, Florida. Plots show 1.5*inter-quartile range (whiskers), the interquartile range (box edges), and the median (horizontal line). EEEV-negative sites were those where at least chicken seroconverted to EEEV antibodies in 2009, whereas sites where no chickens seroconverted were designated as EEEV-negative sites. Model-averaged estimates of coefficients for relative abundances are shown in plots, with 85% weighted unconditional confidence intervals.

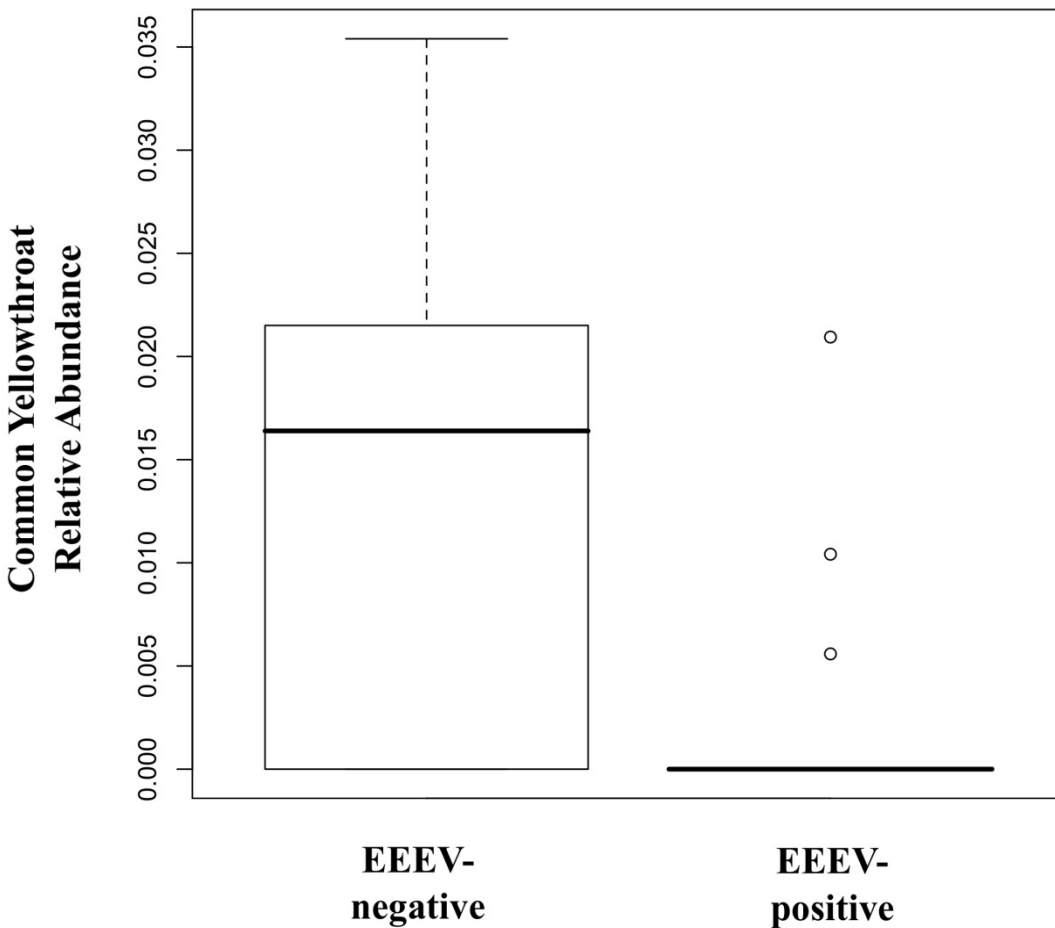


Figure 5. Association between weights associated with variables of relative abundances of avian species vs. estimated forage ratios of those species ($r_S(5) = 1.000$, $p = 0.0167$). Species shown are those inferred to be preferred hosts of *Cs. melanura* based on forage ratio estimates (Estep et al. in press).

