Hosts or habitats: What drives the spatial distribution of mosquitoes?

NATHAN D. BURKETT-CADENA,1,3,† CHRISTOPHER J. W. MCCLURE,2,4 LAURA K. ESTEP,2,5 AND MICKY D. EUBANKS1,6

1Department of Entomology and Plant Pathology, Auburn University, Auburn, Alabama 36849 USA
2Department of Biological Sciences, Auburn University, Auburn, Alabama 36849 USA

Citation: Burkett-Cadena, N. D., C. J. W. McClure, L. K. Estep, and M. D. Eubanks. 2013. Hosts or habitats: What drives the spatial distribution of mosquitoes? Ecosphere 4(2):30. http://dx.doi.org/10.1890/ES13-00009.1

Abstract. Understanding the factors that influence the distribution of vectors is critical to assess the risk of disease transmission across landscapes. For mosquitoes, existing spatial models use only habitat elements (hydrologic and floristic) to predict the distribution of disease vectors within the landscape, ignoring the potential importance of the distribution of hosts. We tested the hypothesis that the distribution of hosts would better explain the spatial distribution of mosquitoes than habitat variables using Information Theory and Poisson Regression. We analyzed the abundance of ectotherm-biting mosquitoes and their hosts from 37 points over a 28-km² area in Tuskegee National Forest, Alabama, USA. We compared models built using information regarding habitat, hosts, or both for their ability to explain the abundance of Culex peccator and Culex territans, two mosquito species that specialize on reptiles and amphibians. Models built using both host and habitat information were the best models for both species, suggesting that information regarding the distribution of mosquito hosts greatly improves models of mosquito distributions. Moreover, when differences in habitat makeup among stations were accounted for, mosquitoes were found to be significantly more abundant at locations with greater host abundance. Since most mosquitoes feed on only a limited range of hosts and successful blood feeding is one of the most crucial bottlenecks in the life cycle of mosquitoes, it follows that mosquitoes would aggregate in areas of greatest host density. Habitat influences the availability of suitable larval development sites, and to some extent, distribution of hosts. Our analysis, however, shows that within the landscape the distribution of host animals is a crucial factor in determining the spatial distribution of vectors. This has clear and important implications for determining which areas are at a greater risk for zoonotic arboviruses and is a potentially transformative paradigm for understanding the factors that influence the spatial distribution of disease vectors.

Key words: Culex; ectotherm; GIS; mosquito-borne disease; spatial epidemiology; vector ecology.

INTRODUCTION

The geographical range of a vector-borne pathogen is confined to the distribution of its vectors (Pavlovsky 1966, Reisen 2010). Vectors, however, are not uniformly distributed within...
their overall geographical range, but are aggregated within the landscape (Smith et al. 2004, Zhou et al. 2004, Ellis 2008). The spatial pattern of disease risk, therefore, is similarly patchy, with greatest risk in areas with more pathogen-infected vectors (Ostfeld et al. 2005, Eisen and Eisen 2008). For some mosquito-borne pathogens, such as eastern equine encephalomyelitis virus (EEEV), the distribution of the pathogen is inextricably linked to the presence of the enzootic vector mosquito (*Culiseta melanura* in the case of EEEV [Morris et al. 1980, Howard et al. 1983]). For this reason, understanding the factors that drive the spatial distribution of vectors is critical to our understanding of the spatial ecology of vector-borne pathogens.

A number of habitat (biotic and/or abiotic) characteristics have been shown to be important in predicting mosquito abundance and/or community composition (Reisen 2010). Land cover/vegetation indices (Moncayo et al. 2000, Diuk-Wasser et al. 2006, Rochlin et al. 2008), canopy cover (Moncayo et al. 2000), soil properties (Fatz 1998), elevation (Reisen et al. 1995, Jacob et al. 2010), and hydrology (Shaman et al. 2002), are just a few of the known environmental variables which have been shown to be associated with the local abundance of a particular mosquito species. Diuk-Wasser et al. (2006) demonstrated the predictive capabilities of GIS for mosquitoes by using remotely sensed landscape elements to model relative abundances of arbovirus vectors, then testing the validity of their models using trapping data from 31 sites, over a three-year period. Their models correctly classified 62.5–81.3% of trapping sites, with respect to low or high mosquito abundance, for the four vector species considered. Since landscape elements are also associated with the occurrence of vertebrate hosts of mosquitoes, such as birds (McClure et al. 2011), amphibians (Bosakowski 1999), and snakes (Steen et al. 2012), it is logical to test whether or not mosquitoes simply occur in preferred habitat, or if they actually seek out areas with higher host densities.

In the current study we investigated the relative importance of habitat and host distribution for determining the distribution of adult female mosquitoes within the landscape. We collected field data on the abundance of amphibian- and reptile-biting mosquitoes and their hosts at 37 sampling stations, and then tested whether data regarding hosts or habitat better explained the abundance of mosquitoes at sampling sites.

**Methods**

**Study system**

We desired a study system composed of (1) a group of hosts which are abundant, easy to census and relatively sedentary; (2) parasites which are mobile and specialize on selected hosts; and (3) a study site in which hosts and parasites are distributed across a habitat mosaic. We chose to work with reptiles and amphibians (ectotherms) and the mosquitoes that feed upon them. Ectotherms are abundant in most ecosystems (Pough 1980, Gibbons et al. 2006), and can be surveyed visually (Manley et al. 2006) as well as auditorily (USGS 2008 North American Amphibian Monitoring Program).Mosquitoes are quite mobile and thus able to move in response to their environment (in contrast to wingless parasites such as ticks which have limited mobility). Two mosquito species that were common at our study site, *Culex territans* and *Culex peccator*, specialize on reptiles and amphibians (Cupp et al. 2004, Burkett-Cadena et al. 2008). Tuskegee National Forest, AL, where the study was conducted, is a patchwork of habitat types, including open marsh, gum swamp, hardwood bottomland, mixed pine and deciduous forest, upland pine forest and open field (Cupp et al. 2003, McClure et al. 2011).

**Mosquito abundance**

Data on the abundance of female mosquitoes were obtained from weekly collections at 37 sampling stations within the study area (Fig. 1). Sampling stations were spread evenly (~200-m distant) along three 2.4-km transects which intersected at a central station, positioned on the edge of a beaver pond (Fig. 1). Non-attractive methods were used (resting shelters) in order to avoid disrupting the natural distribution by attracting mosquitoes from outside of the immediate vicinities of sampling stations. Mosquitoes were aspirated from resting shelters (trash cans) (Burkett-Cadena et al. 2008a) using a hand-held vacuum between 0700 and 0900 h throughout the season of peak adult activity (May–September 2007). Samples were returned to the laboratory...
for species-level identification using morphological characters of adult females (Darsie and Ward 2005).

We focused our analysis on unfed females because this group (composed mostly of host-seeking females) is the section of the population that is most important from the disease perspective, as potentially infectious vectors. In addition, since unfed females are physiologically geared towards finding a host, it is most plausible that this cohort in particular is influenced by host distribution. Blood-engorged and egg-laden (gravid) females, more concerned with locating sites for resting and/or oviposition than encountering a suitable host, respond differently to environmental cues than do un-fed females (Jones 1981) and were therefore excluded from the analysis.

**Host abundance**

We assessed abundance of potential ectothermic hosts at the same 37 stations where mosquitoes were collected using visual and auditory surveys. Standard herpetological survey methods were used, including visual searches (15-min examination within 30-m radius of each station [Manley et al. 2006]) and auditory surveys (number of vocalizing male frogs within 50-m of each station, estimated when >10 males were vocalizing synchronously) (Weir and Mossman 2005). Each of the 37 stations was surveyed on eight occasions (four daytime and four nighttime visits) from mid-May through early September.

**Habitat assessment**

We calculated the percentage of cover-types within 100-m buffers around each station using the Alabama GAP Land Cover Map (Kleiner et
al. 2007) within ArcGIS (ESRI 2008). Similar land cover classifications were combined to form broader classifications (McClure et al. 2011). Habitat variables used in our analysis were “floodplain forest” (East Gulf Coastal Plain floodplain forest), “pine forest” (evergreen tree plantation, East Gulf Coastal Plain upland longleaf forest loblolly modifier, East Gulf Coastal Plain upland longleaf forest open understory modifier), and “open water”. We also calculated the percent of tree canopy cover within 100 m buffers of each station using the National Land Cover Database Tree Canopy Cover Map (Homer et al. 2004). Buffers of 100 m allowed for sufficient habitat heterogeneity surrounding a point, yet avoided overlap between sampling points. This buffer size is twice that used by Brown et al. (2008), to identify clusters of West Nile Virus vectors in the northeast U.S. and incorporates much of the area within the daily range of flight for Culex mosquitoes (estimated at 80–300 m/day for Culex tarsalis, Reisen and Lothrop 1995).

**Analyses**

We first used an information-theoretic approach to determine support for models describing the distribution of Cx. peccator and Cx. territans (using total mosquito counts as the response variable) using information regarding habitat, hosts, or both. These models used a Poisson error structure in a Generalized Linear Modeling framework using the Laplace approximation with the package nlme (Bates et al. 2011) in R version 2.15.0. We first built models representing all possible combinations of habitat covariates hypothesized a priori to influence mosquito abundance. These covariates included percentage of canopy cover, open water, pine forest, and floodplain forest. Models that had highly correlated covariates (variance inflation factors > 10.0) were not considered. We ranked and compared all models using Akaike’s Information Criterion, corrected for small sample size (AICc) (Hurvich and Tsai 1989, Burnham and Anderson 2002). If the covariates within the model receiving the lowest AICc were a subset of those in a competing model, then that competing model was not considered for inference, as the extra covariates were likely uninformative (Arnold 2010).

We then built models using all possible combinations of host species to fit mosquito abundance, again using total mosquito counts at each station as the response variable. Only those hosts previously reported as fed upon by each mosquito species (Cupp et al. 2004, Burkett-Cadena et al. 2008b) were used as covariates in the analyses. For Cx. territans, covariates (host species) used for model building were the abundance of Bullfrog, Green Treefrog, Bird-voiced Treefrog, Cope’s Gray Treefrog, Spring Peeper, and Green Anole (see Table 1 for scientific names; Cupp et al. 2004, Burkett-Cadena et al. 2008b). We considered the abundance of Southern Leopard Frog, Bullfrog, Green Frog, Copperhead, Cottonmouth, and Plain-bellied Watersnake as covariates for models explaining the abundance of Cx. peccator (Cupp et al. 2004, Burkett-Cadena et al. 2008b). Next we built models for both species of mosquito including information regarding both hosts and habitat representing all combinations of host and/or habitat covariates hypothesized to influence the distribution of each species. We ranked and compared host, and host + habitat models using the same criteria as the habitat models described above. Models with ΔAICc < 2 are considered to have received substantial support (Burnham and Anderson 2002). Because no competitive models in any model set were within 2 AICc units of the best model, we simply compared the best host and habitat models for each mosquito species using AICc. The final step in our information-theoretic approach was to rank the best models for host, habitat and host + habitat by AICc to assess their relative strength. We therefore built models representing all possible combinations of covariates within pools of host only, habitat only, and host and habitat covariates, and then assessed their relative strength using AICc. The AIC-best-model for each analysis will represent the most parsimonious combination of covariates from each pool. Therefore, although the pools of covariates within the host only and habitat only analyses were nested within the pool of covariates available for the host + habitat analysis, the best models from the host only and habitat only analyses may not necessarily be nested within the best host + habitat model.

To avoid double counting of ectotherms, the
maximum count of hosts recorded (Burkett-Cadena et al. 2008b) in a single visit (rather than the total recorded during the study) was used in the analysis, since individuals were not marked or removed. In modeling mosquito distribution, data were aggregated over time, so that the total number of females per station was used in the analysis.

Mosquito and vertebrate host abundances may change throughout the season. We therefore analyzed mosquito and host data to determine whether or not host and mosquito populations are spatially stable throughout the sampling period. For mosquitoes, we first plotted abundance of the two species over the collection season, then compared relative abundances between months with greatest absolute abundance (May and June for *Cx. territans*; July and August, and July and September for *Cx. peccator*) using linear regression (angular transformed proportions). For vertebrate hosts, relative abundances (transformed proportions) among the 37 sampling stations in May were compared to those from July, again using linear regression.

We further examined the effects of host distribution on mosquito abundance by comparing mosquito abundance at stations where hosts were present (n = 20; n = 26) versus those where hosts were absent (n = 17; n = 11) for *Cx. peccator* and *Cx. territans*, respectively. We then used Poisson regression (McElduff et al. 2010) to test whether or not mosquito abundance was significantly greater at stations where hosts were present (alpha = 0.05).

Since presence of absence of hosts at the stations may be affected by habitat, we explored patterns of host and mosquito abundance within each habitat type, for each mosquito species. First, we used K-means clustering to divide the 37 stations into two groups, those with relatively lower amounts of a habitat type within the buffer zone, and those with higher amounts of that same habitat type within the buffer zone. Within each new “minor” or “major” habitat group, we then subdivided the stations into groups of low host abundance (host poor) or high host abundance (host rich), again using K-means clustering. We then developed a Poisson regression model (McElduff et al. 2010) for each of the two habitat groups within each habitat type to test for differences in mosquito counts (O’Hara and Kotze 2010) between host rich and host poor locations (alpha = 0.05).

| Order   | Family         | Latin name         | Common name       | Day | Night | Total |
|---------|----------------|--------------------|-------------------|-----|-------|-------|
| Anura   | Bufonidae      | *Bufo fowleri*     | Fowler’s toad     | 16  | 55    | 71    |
|         | Hylidae        | *Acris (gryllus + crepitans)* | Cricket frogs      | 429 | 797   | 1226  |
|         |                | *Hyla arvalocca*   | Bird-voiced treefrog | 13  | 349   | 362   |
|         |                | *Hyla chrysocelis* | Cope’s gray treefrog | 0   | 36    | 36    |
|         |                | *Hyla cinerea*     | Green treefrog     | 0   | 649   | 649   |
| Microhyliidae | *Pseudacris crucifer* |                     | Spring peeper      | 94  | 5     | 99    |
| Ranidae | *Gastrothylax carolinensis* | Eastern narrowmouth toad | 2   | 5     | 7     |
|         | *Lithobates catesbiana* | Bulfrog            | 2     | 46    | 48    |
|         | *Lithobates clamitans* | Green frog         | 1     | 94    | 95    |
| Caudata | Ambystomatidae | *Ambystoma opacum* | Marbled salamander | 17  | 1     | 18    |
| Squamata| Colubridae     | *Elaphe obsoleta*  | Racer              | 4   | 0     | 4     |
|         |                | *Nerodia erythrogenaster* | Plain-bellied watersnake | 4  | 4     | 8     |
|         | Crotalidae     | *Agkistrodon contortrix* | Copperhead         | 0   | 4     | 4     |
|         |                | *Agkistrodon piscivorus* | Cottonmouth        | 24  | 17    | 41    |
| Phrynosomatidae | *Sceloporus undulatus* | Eastern fence lizard | 8   | 0     | 8     |
| Polychrotidae | *Anolis carolinensis* | Green anole        | 80  | 12    | 92    |
| Scincidae | *Scincella lateralis* | Ground skink       | 24   | 0     | 24    |
| Teiidae  | *Cnemidophorus sexlineatus* | Six-lined race runner | 8   | 0     | 8     |
| Testudines| Emydidae      | *Terrapene carolina* | Eastern box turtle | 28  | 0     | 28    |
|         | Kinosternidae  | *Kinosternon subrubrum* | Eastern mud turtle | 4   | 0     | 4     |

Other species include *Pseudacris nigrita* (4), *Hyla squirella* (1), *Hyla gratiosa* (1), *Eurycea guttolineata* (3), *Eumeces fasciatus* (1), *Cemophora coccinea* (1), *Coluber constrictor* (2), *Diadophis punctatus* (1), *Sterneatherus odoratus* (1), and *Trachemys scripta* (1).
RESULTS

The distribution of habitats, host species, and mosquitoes varied considerably throughout the sampling area. The landscape consists of a mosaic of floodplain and upland habitats dominated by closed canopy forests, and interspersed with wetlands and open fields (Fig. 1). Floodplain and pine forests covered 62.4% and 23.5% of the area within the buffer zones, respectively, but were highly, negatively correlated \((r = -0.87)\). Models containing both of these covariates were not considered as useful for inference. Average canopy cover was high, at 77.3%. Open water contributed relatively little to the area inside buffer zones, at just 6.9% of the total area.

Overall, 2,917 independent observations (visual and auditory) of reptiles and amphibians, belonging to 32 species, were made during the course of the field surveys. The abundances of snakes, lizards, and frogs ranged from low (zero animals detected at 9 stations) to high (29.25 ectotherms at one station) over the course of the study. A diverse assemblage of frogs was detected during surveys, with 12 species belonging to three different families (Table 1). Other amphibians included one toad species and two salamander species (two families). Seven species of snakes (two families), four species of turtles (two families), three lizard species (three families), and three species of skinks (one family) were observed (Table 1).

Abundance of *Culex territans* was greatest in May (Fig. 2A), while abundance of *Culex peccator* peaked in July (Fig. 2A). Despite considerable fluctuation in population size throughout the season, the relative abundance of mosquitoes among the stations showed a strong positive linear relationship. For *Culex territans*, relative abundance among stations in May was significantly associated with that in June (Fig. 2B, \(R^2 = 0.31; P < 0.001\)). For *Culex peccator*, relative abundance among stations in July was significantly associated with that in August (Fig. 2C, \(R^2 = 0.53; P < 0.001\)) and September (Fig. 2C, \(R^2 = 0.53; P < 0.001\)). Host relative abundance at each station was fairly consistent between May and July (Fig. 3, \(R^2 = 0.34; P < 0.001\)), yet varied considerably between stations (Figs. 4 and 5).

Mosquito abundance also varied considerably among stations (Figs. 4 and 5). *Culex peccator* females were collected from 20 of 37 stations (54.0%), with an average of 4.57 females per station (169 total females). *Culex territans* females were more widespread in distribution and more common than *Culex peccator*, being collected from 32 of 37 stations (86.5%), with an average of 8.76 females per station (324 total females).

In our information-theoretic approach, the model including both host and habitat information received all of the model weight for both *Culex territans* and *Culex peccator* (Table 2), indicating substantial support for the hypothesis that mosquitoes choose sites based on hosts in addition to habitat. The best model for *Culex territans* included Bullfrogs, Spring Peepers and Green Anoles as hosts and floodplain forests, and canopy cover as habitat covariates (Table 3). The best model for *Culex peccator* included Bullfrog, Cottonmouth, and Copperhead as hosts and pine forest and open water as habitat covariates (Table 3).

Overall, mosquito abundance was significantly greater in sites with hosts than without hosts (Fig. 6A, B; *Culex peccator*: \(X^2 = 62.89; P < 0.01\); *Culex territans*: \(X^2 = 15.98; P < 0.01\)). This overall pattern of mosquito abundance being greater at sites with greater host richness was also observed within each habitat type (Figs. 7A–D, 8A–D; see Table 4 for values and statistical results). For *Culex peccator*, significantly more females were found at stations with more hosts, regardless of habitat makeup. At stations where floodplain forest was a major component of the habitat (82.81–83.38% of buffer area), for example, females of *Culex peccator* were significantly more abundant (Fig. 7A; \(X^2 = 109.75; P < 0.01\)) at host rich locations (8.92 females/site) than host poor locations (0.3 females/site). *Culex peccator* abundance ranged from 6.15 to 71.8 times more females at host rich vs. host poor sites (Fig. 7A–D, Table 4). For *Culex territans*, the magnitude of the difference in mosquito abundance was not so great, and ranged between 1.34 and 4.14 more females at host-rich than host-poor stations (Fig. 8A–D, Table 4). At stations with dense canopy cover, for example, *Culex territans* abundance was only marginally greater (and not statistically significant) at sites with higher host abundance (1.34 times). At stations with less canopy cover, however, *Culex territans* was 4.14 times more abundant at host-rich than host-poor stations.
When the effect of habitat is ignored, scatter plots of host abundance and mosquito abundance shows that abundance of *Cx. peccator* and *Cx. territans* increases with increasing host richness (Fig. 9A, B).

**Discussion**

Our results indicate that both habitat and hosts are important drivers of the mosquito community, but that within the landscape, host abundance exerts an especially strong effect on mosquito distribution. The magnitude of the effect that host distribution has on mosquito abundance was stronger for *Cx. peccator* than for *Cx. territans*. Nonetheless, abundance of both mosquito species was significantly greater at locations with greater host abundance, regardless of habitat type.

The species of reptiles and amphibians that were the best model predictors of mosquito abundance are also the species most commonly fed upon by those mosquitoes in nature (*Cx. territans*: Bullfrogs and Spring Peepers; *Cx. peccator*: Cottonmouths and Bullfrogs (Table 3; Burkett-Cadena et al. 2008b, Cupp et al. 2004). There are two competing explanations for this result: (1) mosquitoes simply feed upon the most...
Fig. 3. Relative abundances of host animals (reptiles and amphibians) of *Culex territans* and *Culex peccator* between May and July, 2007 at 37 stations (closed circles).

![Graph showing relative abundances of host animals](image)

\[ y = 0.6933x + 0.0082 \]
\[ R^2 = 0.3406 \]
\[ P < 0.001 \]

Fig. 4. Map of spatial distribution of *Cx. peccator* females, hosts and habitat elements at 37 stations in Tuskegee National Forest, Alabama, USA. Host species include Southern Leopard Frog, Bullfrog, Green Frog, Cottonmouth, and Plain-bellied Watersnake (most common hosts for *Cx. peccator* at the site, see Cupp et al. 2004, Burkett-Cadena et al. 2008b).

![Map of spatial distribution](image)
common hosts in their preferred habitat or (2) mosquitoes aggregate around their preferred hosts. Our results support the latter explanation, since areas of each habitat that harbored few or no mosquitoes also had sparse host populations (Figs. 4 and 5). Pine forests, for example, found to be positively associated with *Cx. peccator* abundance (Table 3), were extensive (Fig. 4), but often devoid of *Cx. peccator* females (Fig. 4). This suggests that within an area of suitable habitat,

Table 2. Attributes of the highest-ranking model of mosquito abundance in each of the three model subsets considered: host, habitat, and host-habitat. These models represent the AICc-best combinations of host only, habitat only, and host and habitat covariates. The number of parameters in each model is represented by k and can be calculated by summing the number of covariates in each model and adding one for the intercept. See Methods section for details.

| Model                      | Covariates                                      | k | AICc | ΔAICc | wi |
|----------------------------|-------------------------------------------------|---|------|-------|----|
| *Culex territans* Host and habitat | Bullfrog + Canopy cover + Floodplain            | 4 | 290.54 | 0     | 1  |
| Host only                  | Canopy cover + Floodplain + Water               | 4 | 340.46 | 49.91 | 0  |
| Host only                  | Green anole + Bird-voiced treefrog + Cope’s gray treefrog + Green treefrog + Spring peeper + Bullfrog | 4 | 354.44 | 63.9  | 0  |
| *Culex peccator* Host and habitat | Copperhead + Cottonmouth + Bullfrog + Water      | 5 | 142.71 | 0     | 1  |
| Host only                  | Copperhead + Cottonmouth + Bullfrog             | 4 | 154.9  | 12.2  | 0  |
| Habitat only               | Pine + Water                                    | 3 | 698.08 | 555.38 | 0  |
such as pine forest, mosquitoes congregate in areas with their preferred hosts. Results of within-habitat analysis also support the idea that mosquitoes aggregate in areas of greater host density, because mosquito abundance was significantly greater at stations with greater host richness, regardless whether a habitat type (e.g., floodplain forest) was a relatively minor and major component of the surrounding landscape (Figs. 7 and 8). In only 2 tests (of 13 total) were the differences not statistically significant (Table 4). These results strongly suggest that the distribution of vertebrate hosts is a major driver in the spatial distribution of mosquitoes.

Just how ectotherm-biting mosquitoes are able to aggregate in areas with greater host density is not exactly known. A recent study found that seasonal patterns of host use by mosquitoes track the reproductive phenology of the host animals, including frogs (Burkett-Cadena et al. 2011). Aggregation of reproductive phase frogs could theoretically drive aggregation of frog-seeking mosquitoes. Some frog-biting mosquitoes, including *Cx. territans*, are known to be attracted to recordings of frog calls (Borkent and Belton 2006, Bartlett-Healy et al. 2008), providing a potential mechanism for this behavioral pattern.

Our study confirms the findings of others (Roberts et al. 1996, Diuk-Wasser et al. 2006) that habitat is important in determining the suitability of an area for adult mosquitoes, but our study also demonstrates that the aggregation of adult mosquitoes within the landscape is equally driven by the distribution of host animals. When one considers the importance of hosts in the

Table 3. Individual model results (β (SE)) for host and habitat variables used in model selection. Only parameters that were within the best host, habitat, or host + habitat models for each species are included.

| Covariate          | Host and habitat | Habitat only | Host only |
|--------------------|------------------|--------------|-----------|
| *Culex territans*  |                  |              |           |
| Intercept          | -4.36 (0.96)     | -1.78 (0.78) | 1.88 (0.08) |
| Floodplain forest  | 0.31 (0.12)      | 0.58 (0.13)  |            |
| Open water         | 1.79 (0.96)      |              |           |
| Canopy cover       | 5.44 (0.85)      | 3.02 (0.70)  |            |
| Bullfrog           | 0.43 (0.06)      | 1.90 (0.05)  | 0.04 (0.02) |
| Spring peeper      |                  | 0.18 (0.08)  |            |
| Green anole        |                  |              |           |
| *Culex peccator*   |                  |              |           |
| Intercept          | -0.40 (0.22)     | 1.29 (0.13)  | -0.65 (0.22) |
| Pine forest        | 0.70 (0.19)      |              |           |
| Open water         | -6.21 (1.86)     | -5.89 (1.70) |            |
| Bullfrog           | 0.51 (0.08)      |              | 0.47 (0.07) |
| Cottonmouth        | 1.66 (0.08)      |              | 1.74 (0.08) |

Fig. 6. Mosquito abundance (females per site ± SE) as a function of host presence. Females of *Culex peccator* (A) and *Culex territans* (B) were collected weekly from 37 stations, that were also surveyed twice monthly (May–September) for presence of ectotherm hosts, Tuskegee National Forest, Macon County, Alabama, 2007. Significant differences in mosquito abundance, as determined by Poisson regression are denoted by an asterisk. The number of stations within each group is shown over each vertical bar.
mosquito life cycle (most mosquito species cannot reproduce without taking a blood meal), it is less surprising that mosquitoes would congregate in areas with greater host density. Any individuals in a location in which hosts are absent would lose their reproductive potential, a strong selective incentive for aggregating near host populations. Most mosquito species do not feed indiscriminately on the available terrestrial vertebrates in a given area, but instead show “preference” for one group of hosts (often the members of a taxonomic class) (Burkett-Cadena et al. 2008b). That such preference exists is evidence of the importance of particular host species in the life cycle of mosquitoes.

Our results seemingly conflict with those of Lothrop and Reisen (2001), who found that host-seeking females of *Culex tarsalis* (a major vector of arboviruses in the western U.S.) congregated at landscape features and not with large concentrations of potential hosts. Unfortunately, Lothrop and Reisen (2001) used dry ice (carbon dioxide) baited traps to estimate mosquito abundance. The presence of vertebrate hosts has been shown to divert mosquitoes away from dry ice baited traps (Theimann et al. 2011). If mosquitoes were similarly diverted in the study described in Lothrop and Reisen (2001) actual mosquito abundance at host-rich sites could have been underestimated, affecting the validity of

---

**Fig. 7.** Abundance of *Culex peccator* females (mean + SE) as a function of host presence and habitat (two levels) in (A) floodplain forest, (B) open water, (C) pine forest, and (D) canopy cover. Mosquitoes were collected from 37 stations, sampled monthly (May–September) for presence of potential ectotherm hosts, Tuskegee National Forest, Macon County, Alabama, 2007. Habitat and host data are provided in Table 3. Significant differences in mosquito abundance in host-rich and host-poor areas, as determined by Poisson regression, are denoted by an asterisk. The number of stations (n) within each subgroup is shown over each vertical bar of the graph.
their conclusions.

The implications of our study, that mosquitoes aggregate in areas of greater host density, is likely to apply to many, but not all species of mosquitoes, and will need to be examined for other parasitic arthropods, and in different ecosystems. The degree to which host distribution influences parasite distribution is likely to be a function of host breadth (the range of animals fed upon by the parasite), parasite mobility (how far can a particular parasite species travel to encounter a host) and other life history traits of the parasite, particularly breeding site specificity. Mosquito species that show little host preference are less likely to be influenced by the distribution of a particular host group than those with a narrow host range. Ticks (Acari: Ixodidae) require multiple (2–3) blood meals to complete their development, and each blood meal is from a different host animal and usually from quite different host species. Ixodes spp. ticks, vectors of Lyme disease, often parasitize lizards as larvae, small rodents as and nymphs, then parasitize large mammals, including deer, as adults (Nicholson et al. 2009). Ticks cannot fly (wingless), so their ability to encounter a host is limited to how far they can crawl. Given the enormous importance of hosts in their development, it makes sense that ticks would, like Cx. peccator and Cx. territans, aggregate in areas of greater host

| Habitat variable | Relative habitat contribution (% of buffer area) | Relative host abundance | Mosquito abundance | Poisson regression results |
|------------------|-----------------------------------------------|------------------------|-------------------|---------------------------|
|                  | Level  | Mean  | SE    | Level  | Mean  | SE    | Mean  | SE    | Wald X² | P       |
| **Culex peccator** |       |       |       |        |       |       |       |       |         |         |
| Canopy cover     | Minor  | 0     | 0     | Host poor | 0.06 | 0.02 | 0.86 | 0.26 | 21.84   | <0.001 |
|                  | Major  | 4.67  | 0.04 | Host rich | 1.75 | 0.37 | 15.56| 10.9 | 120.82  | <0.001 |
| Floodplain forest| Minor  | 3.72  | 0.04 | Host poor | 0.25 | 0.14 | 0    | 0    |         |         |
|                  | Major  | 83.38 | 0.46 | Host rich | 2.42 | 0.93 | 3.33 | 2.03 |         |         |
| Open water       | Minor  | 64.19 | 0.9  | Host poor | 0.11 | 0.04 | 1.06 | 0.3  | 109.75  | <0.001 |
|                  | Major  | 82.81 | 0.5  | Host rich | 1.99 | 0.37 | 13.55| 8.92 |         |         |
| Pine forest      | Minor  | 7.24  | 0.19 | Host poor | 0.11 | 0.04 | 0.78 | 0.25 | 35.9    | <0.001 |
|                  | Major  | 68.88 | 0.53 | Host rich | 1.82 | 0.36 | 4.8  | 1.99 |         |         |
| **Culex territans** |       |       |       |        |       |       |       |       |         |         |
| Canopy cover     | Minor  | 65.18 | 0.05 | Host poor | 0.13 | 0.06 | 3.2  | 1.37 | 40.28   | <0.001 |
|                  | Major  | 70.24 | 0.06 | Host rich | 3.35 | 0.74 | 13.25| 4.07 | 2.02    | 0.155  |
| Floodplain forest| Minor  | 78.8  | 0.11 | Host poor | 0.07 | 0.02 | 10.1 | 1.69 |         |         |
|                  | Major  | 1.22  | 0.35 | Host rich | 2.09 | 0.8  | 13.5 | 6.52 |         |         |
| Open water       | Minor  | 82.66 | 0.33 | Host poor | 0.12 | 0.03 | 9.17 | 1.57 | 1036.49 | <0.001 |
|                  | Major  | 78.55 | 1.2  | Host rich | 2.93 | 0.58 | 13.33| 3.07 |         |         |
| Pine forest      | Minor  | 4.19  | 0.08 | Host poor | 0.09 | 0.03 | 8.37 | 1.49 | 4.48    | <0.034 |
|                  | Major  | 5.56  | 0.25 | Host rich | 2.93 | 0.58 | 13.33| 3.07 |         |         |
|                  |        |       |       | Host poor | 0.03 | 0.01 | 8.89 | 2.87 |         |         |
|                  |        |       |       | Host rich | .   | .   | .   | .   |         |         |
density, although this hypothesis has yet to be tested. Many other groups of blood-feeding flies, such as sand flies (Diptera: Psychodidae) and black flies (Diptera: Simuliidae), are quite mobile, and species within each group demonstrate varying degrees of host specificity. As in mosquitoes, most other blood-feeding arthropods cannot reproduce without taking a blood meal, suggesting that the patterns found in the current work (that mosquitoes would congregate in areas with greater host density) may apply to many blood-feeding taxa.

Our study is limited by the sensitivity of GIS to detect fine-scale variation in habitat attributes. While we are confident that the habitat covariates investigated here (canopy cover, floodplain forest, pine forest, and open water) are important components of the landscape, there are some habitat attributes that are not well-captured by satellite imagery at the resolution of images from which habitat variables were derived in this study. Of particular note are small wetlands (vernal pools, intermittent streams, floodwater pools) that are not differentiated from other open-water habitats (marshes, ponds). Because each wetland mosquito species breeds in a particular type of wetland, future studies may benefit from assessing habitat at a finer scale. Additionally, our survey methods and data of host and mosquito abundance are biased by

Fig. 8. Abundance of *Culex territans* females (mean ± SE) as a function of host presence and habitat (two levels) in (A) floodplain forest, (B) open water, (C) pine forest, and (D) canopy cover. Mosquitoes were collected from 37 stations, sampled monthly (May–September) for presence of potential ectotherm hosts, Tuskegee National Forest, Alabama, 2007. Habitat and host data are provided in Table 3. Significant differences in mosquito abundance in host-rich and host-poor areas, as determined by Poisson regression, are denoted by an asterisk. The number of stations (n) within each subgroup is shown over each vertical bar of the graph.
differences in detectability of the various species through space and time. Since determining the true absolute abundance of a population is impractical, population size must be inferred from samples from a population in which all individuals are not detectable (due to differences in behavior, physical attributes, or observer bias). Calculating detectability of hosts and mosquitoes may help to ameliorate problems with incomplete enumeration (Williams et al. 2002, Royle and Dorazio 2008) and may be useful in future work further exploring the relationship between distributions of hosts and their parasites.

Our findings, specifically that mosquito abundance is significantly greater in host rich locations than host poor locations, have strong implications for our understanding of the ecology of mosquito-borne diseases. Because spatial distribution of hosts can drive the infection prevalence of zoonotic pathogens (Caraco et al. 2001) as well as the distribution of the vectors (our study), it follows that the spatial distribution of hosts has a profound influence on the nidality of vector-borne pathogens. To our knowledge, this is the first published report to demonstrate a statistically significant relationship between the spatial distributions of mosquitoes and their vertebrate hosts.

ACKNOWLEDGMENTS

Thanks to Katherine Gray and Nathan Click for assistance in the field. Jorge Hersel (TNF Ranger) facilitated permits for working on National Forest lands. Dr. David Bayne permitted access to private lands adjacent to Tuskegee National Forest. Sean Graham contributed helpful suggestions to an early manuscript draft. This work was supported by project # R01AI049724 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

Arnold, T. W. 2010. Uninformative parameters and model selection using Akaike’s Information Criterion. Journal of Wildlife Management 74:1175–1178.

Bartlett-Healy, K., W. Crans, and R. Gaugler. 2008. Phonotaxis to amphibian vocalizations in Culex territans (Diptera: Culicidae). Annals of the Entomological Society of America 101:95–103.

Bates, D., M. Maechler, and B. Bolker. 2011. lme4: Linear mixed-effects models using S4 classes. R package version 0.999375-42. http://CRAN.R-project.org/package=lme4

Borkent, A., and P. Belton. 2006. Attraction of female Uranotaenia lowii (Diptera: Culicidae) to frog calls in Costa Rica. Canadian Entomologist 138:91–94.

Bosakowski, T. 1999. Amphibian macrohabitat associations on a private industrial forest in western Washington. Northwestern Naturalist 80:61–69.

Brown, H., M. Duik-Wasser, T. Andreadis, and D. Fish. 2008. Remotely-sensed vegetation indices identify mosquito clusters of West Nile virus vectors in an urban landscape in the northeastern United States. Vector-Borne and Zoonotic Diseases 8:1.

Burkett-Cadena, N. D., M. D. Eubanks, and T. R. Unnasch. 2008a. Preference of female mosquitoes for natural and artificial resting sites. Journal of the American Mosquito Control Association 24:228–235.

Burkett-Cadena, N. D., S. P. Graham, H. K. Hassan, C. Guyer, M. D. Eubanks, C. R. Katholi, and T. R.
Unnasch. 2008b. Blood feeding patterns of potential arbovirus vectors of the genus Culex targeting ectothermic hosts. American Journal of Tropical Medicine and Hygiene 79:809–815.

Burkett-Cadena, N. D., C. J. W. McClure, R. A. Ligon, S. P. Graham, C. Guyer, G. E. Hill, S. S. Ditchkoff, M. D. Eubanks, H. K. Hassan, and T. R. Unnasch. 2011. Host reproductive phenology drives seasonal patterns of host use in mosquitoes. PLoS ONE 6:e17681.

Burnham, K. P., and D. R. Anderson. 2002. Model selection and multimodel inference: a practical information-theoretic approach. Springer-Verlag, New York, New York, USA.

Caraco, T., M. C. Duryea, S. Glavanakov, W. Maniatty, and B. K. Szymanski. 2001. Host spatial heterogeneity and the spread of vector-borne infection. Theoretical Population Biology 59:185–206.

Cupp, E. W., K. Klingler, H. K. Hassan, L. M. Viguers, and T. R. Unnasch. 2003. Transmission of eastern equine encephalomyelitis virus in central Alabama. American Journal of Tropical Medicine and Hygiene 68:495–500.

Cupp, E. W., D. Zhang, X. Yue, M. S. Cupp, C. Guyer, T. Korves, and T. R. Unnasch. 2004. Identification of reptilian and amphibian bloodmeals from mosquitoes in an eastern equine encephalomyelitis virus focus in central Alabama. American Journal of Tropical Medicine and Hygiene 71:272–276.

Darsie, R. F. Jr., and R. A. Ward. 2005. Identification and geographical distribution of the mosquitoes of North America, North of Mexico. University Press of Florida, Gainesville, Florida, USA.

Diuk-Wasser, M. A., H. E. Brown, T. G. Andreadis, and D. Fish. 2006. Modeling the spatial distribution of mosquito vectors for West Nile virus in Connecticut, USA. Vector-borne and Zoonotic Diseases 6:283–295.

Eisen, R. J., and L. Eisen. 2008. Spatial modeling of human risk of exposure to vector-borne pathogens based on epidemiological versus arthropod vector data. Journal of Medical Entomology 45:181–192.

Ellis, A. M. 2008. Linking movement and oviposition behaviour to spatial population distribution in the tree hole mosquito Ochlerotatus triseriatus. Journal of Animal Ecology 77:156–166.

ESRI. 2008. Arc GIS Version 9.x. Environmental Systems Research Institute, Redlands, California, USA.

Gibbons, J. W., C. T. Winne, D. E. Scott, J. D. Willson, X. Glaudas, K. M. Andrews, B. D. Todd, L. A. Fedewa, L. Wilkinson, R. N. Tsaliagos, et al. 2006. Remarkable amphibian biomass and abundance in an isolated wetland: implications for wetland conservation. Conservation Biology 20:1457–1465.

Homer, C., C. Huang, L. Yang, B. Wylie, and M. Coan. 2004. Development of a 2001 national landcover database for the United States. Photogrammetric Engineering and Remote Sensing 70:829–840.

Howard, J. J., D. E. Emord, and C. D. Morris. 1983. Epizootiology of eastern equine encephalomyelitis virus in upstate New York, USA: V. Habitat preference of host-seeking mosquitoes (Diptera: Culicidae). Journal of Medical Entomology 20:62–69.

Hurvich, C. M., and C. L. Tsai. 1989. Regression and time-series model selection in small sample sizes. Biometrika 76:297–307.

Jacob, B., N. D. Burkett-Cadena, J. C. Luvall, S. H. Parcak, C. J. W. McClure, L. K. Estep, G. E. Hill, E. W. Cupp, R. J. Novak, and T. R. Unnasch. 2010. Developing GIS-based eastern equine encephalitis vector-host models in Tuskegee, Alabama. International Journal of Health Geographics 9:12.

Jones, M. D. R. 1981. The programming of circadian flight-activity in relation to mating and the gonotrophic cycle in the mosquito, Aedes aegypti. Physiological Entomology 6:307–313.

Kleiner, K. J., M. D. Mackenzie, A. L. Silvano, J. A. Grand, J. B. Grand, J. Hogland, E. R. Irwin, M. S. Mitchell, B. D. Taylor, T. Earnhardt, E. Kramer, J. Lee, A. J. McKerrow, M. J. Rubinio, K. Samples, A. Terando, and S. G. Williams. 2007. GAP land cover map of ecological systems for the state of Alabama (Provisional). http://www.auburn.edu/academic/forestry_wildlife/alabama_gap_analysis_project/AL-GAP_land_cover_map_metadata.htm.

Lotro, H. D., and W. K. Reisen. 2001. Landscape affects the host-seeking patterns of Culex tarsalis (Diptera: Culicidae) in the Coachella Valley of California. Journal of Medical Entomology 38:325–32.

Manley, P. N., B. Van Horne, J. K. Roth, W. J. Zielinski, M. M. McKenzie, T. J. Weller, F. W. Weckerly, and C. Vojta. 2006. Multiple species inventory and monitoring technical guide. General Technical Report WO-73. U.S. Department of Agriculture, Forest Service, Washington, D.C., USA.

McClure, C. J. W., L. K. Estep, and G. E. Hill. 2011. Using public land cover data to determine habitat associations of breeding birds in Tuskegee National Forest, Alabama. Southern Journal of Applied Forestry 4:199–209.

McElduff, F., M. Cortina-Borja, S. Chan, and A. Wade. 2010. When t-tests or Wilcoxon-Mann-Whitney tests won’t do. Advances in Physiology Education 34(3):128–133.

Moncayo, A. C., J. D. Edman, and J. T. Finn. 2000. Application of geographic information technology in determining risk of eastern equine encephalomyelitis virus transmission. Journal of the American Mosquito Control Association 16:28–35.

Morris, C. D., R. H. Zimmerman, and J. D. Edman. 1980. Epizootiology of eastern equine encephalo-
myelitis virus in upstate New York, USA: II. Population dynamics and vector potential of adult Culiseta melanura (Diptera: Culicidae) in relation to distance from breeding site. Journal of Medical Entomology 17:453–465.

Nicholson, W. L., D. E. Sonenshine, R. S. Lane, and G. Uilenberg. 2009.Ticks (Ixodida). Pages 483–532 in G. R. Mullen and L. A. Durden, editors. Medical and veterinary entomology. Academic Press, Burlington, Massachusetts, USA.

O’Hara, R. B. and D. J. Kotze. 2010. Do not log-transform count data. Methods in Ecology and Evolution 1:118–122.

Ostfeld, R. S., G. E. Glass, and F. Keesing. 2005. Spatial epidemiology: an emerging (or re-emerging) discipline. Trends in Ecology and Evolution 20:328–336.

Patz, J. A. 1998. Predicting key malaria transmission factors, biting and entomological inoculation rates, using modelled soil moisture in Kenya. Tropical Medicine and International Health 3:818–827.

Pavlovsky, E. N. 1966. Natural nidality of transmissible diseases, with special reference to the landscape epidemiology of zooanthroposes. [Translated from Russian.] University of Illinois Press, Urbana, Illinois, USA.

Pough, F. H. 1980. Advantages of ectothermy for tetrapods. American Naturalist 115:92–112.

Reisen, W. K., H. D. Lothrop, S. B. Presser, M. M. Milby, J. L. Hardy, M. J. Wargo, and R. W. Emmons. 1995. Landscape ecology of arboviruses in southern California: Temporal and spatial patterns of vector and virus activity in Coachella Valley, 1990-1992. Journal of Medical Entomology 32:255–266.

Reisen, W. K., and H. D. Lothrop. 1995. Population ecology and dispersal of Culex tarsalis (Diptera: Culicidae) in the Coachella Valley of California. Journal of Medical Entomology 32:490–502.

Reisen, W. K. 2010. Landscape epidemiology of vectorborne diseases. Annual Review of Entomology 55:461–483.

Roberts, D. R., J. F. Paris, S. Manguin, R. E. Harbach, R. Woodruff, E. Rejmankova, J. Polanco, B. Wulschleger, and L. J. Legters. 1996. Predictions of malaria vector distribution in Belize based on multispectral satellite data. American Journal of Tropical Medicine and Hygiene 54:304–308.

Rochlin, I., K. Harding, H. S. Ginsberg, and S. R. Campbell. 2008. Comparative analysis of distribution and abundance of West Nile and eastern equine encephalomyelitis virus vectors in Suffolk County, New York, using human population density and land use/cover data. Journal of Medical Entomology 45:563–571.

Royle, J. A. and R. M. Dorazio. 2008. Hierarchical modeling and inference in ecology. Academic Press, San Diego, California, USA.

Shaman, J., M. Stieglitz, C. Stark, S. Le Blancq, and M. Cane. 2002. Using a dynamic hydrology model to predict mosquito abundances in flood and swamp water. Emerging Infectious Diseases 8:8–13.

Smith, D. L., J. Dushoff, and F. E. McKenzie. 2004. The risk of a mosquito-borne infection in a heterogeneous environment. PLoS Biology 2(11):e368.

Steen, D. A., C. J. W. McClure, J. C. Brock, D. C. Rudolph, J. B. Pierce, J. R. Lee, W. J. Humphries, B. B. Gregory, W. B. Sutton, L. L. Smith, D. L. Baxley, D. J. Stevenson, and C. Guyer. 2012. Landscape-level influences of terrestrial snake occupancy within the southeastern United States. Ecological Applications 22:1084–1097.

Theimann, T., B. Nelms, and W. K. Reisen. 2011. Bloodmeal host congregation and landscape structure impact the estimation of female mosquito (Diptera: Culicidae) abundance using dry ice-baited traps. Journal of Medical Entomology 48:513–517.

Weir, L. A., and M. J. Mossman. 2005. North American Amphibian Monitoring Program (NAAMP). Pages 307–313 in M. J. Lannoo, editor. Amphibian declines: conservation status of United States species. University of California Press, Berkeley, California, USA.

Williams, B. K., J. D. Nichols, and M. J. Conroy. 2002. Analysis and management of animal populations. Academic Press, San Diego, California, USA.

Zhou, G., N. Minakawa, A. Githeko, and G. Yan. 2004. Spatial distribution patterns of malaria vectors and sample size determination in spatially heterogeneous environments: a case study in the west Kenyan highland. Journal of Medical Entomology 41:1001–1009.