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Fine-scale distribution modeling of avian malaria vectors in north-central Kansas

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ABSTRACT: Infectious diseases increasingly play a role in the decline of wildlife populations. Vector-borne diseases, in particular, have been implicated in mass mortality events and localized population declines are threatening some species with extinction. Transmission patterns for vector-borne diseases are influenced by the spatial distribution of vectors and are therefore not uniform across the landscape. Avian malaria is a globally distributed vector-borne disease that has been shown to affect endemic bird populations of North America. We evaluated shared habitat use between avian malaria vectors, mosquitoes in the genus *Culex* and a native grassland bird, the Greater Prairie-Chicken (*Tympanuchus cupido*), by (1) modeling the distribution of *Culex* spp. occurrence across the Smoky Hills of north-central Kansas using detection data and habitat variables, (2) assessing the occurrence of these vectors at nests of female Greater Prairie-Chickens, and (3) evaluating if shared habitat use between vectors and hosts is correlated with malarial infection status of the Greater Prairie-Chicken. Our results indicate that *Culex* occurrence increased at nest locations compared to other available but unoccupied grassland habitats; however the shared habitat use between vectors and hosts did not result in an increased prevalence of malarial parasites in Greater Prairie-Chickens that occupied habitats with high vector occurrence. We developed a predictive map to illustrate the associations between *Culex* occurrence and infection status with malarial parasites in an obligate grassland bird that may be used to guide management decisions to limit the spread of vector-borne diseases.

Keyword Index: Avian malaria, *Culex*, Greater Prairie-Chicken, hemosporidia, infectious diseases, *Plasmodium*.

INTRODUCTION

Infectious diseases are not uniformly distributed across the landscape. Spatial heterogeneity results from environmental variation that affects the distribution of host species, disease agents, and their potential vectors. Environmental correlates of disease emergence have been broadly applied in the public health sector to predict the distribution of hosts and vectors and infer the potential for transmission and emergence of zoonotic diseases. For example, risk of Lyme disease across North America has been predicted based on the occurrence of ticks as the main vector (Brownstein et al. 2003, Bunnell et al. 2003) and distribution of human cases (Glavanakov et al. 2001, Eisen et al. 2006), both of which are dependent on the underlying biotic and abiotic factors affecting the distributions of vectors and hosts such as landcover, soil type, and temperature.

Spatial patterns of mosquito-borne diseases can be predicted by modeling environmental and climatic factors associated with the vector species (Eisen and Eisen 2011). Predictive maps that explain the potential distribution of these diseases can be used as early warning surveillance systems and as guides for management decisions (Linthicum et al. 2007, Larson et al. 2010). Methods for the development of predictive maps for vector-borne infectious diseases are variable (Kitron 1998, Ostfeld et al. 2005), but generally involve a three-step process which first identifies the occurrence of the vector species (e.g., ticks and mosquitoes), evaluates the potential for interaction with host species (e.g., human, livestock, and wildlife), and finally evaluates the correlation between vector

occurrence and disease in host species. Maps based on vectors to predict the distribution of vector-borne disease have mainly been used at broad spatial scales with relatively coarse resolutions that ignore how fine-scale environmental factors affect transmission dynamics of pathogens across the landscape (Khatchikian et al. 2011). Spatial analysis of vector, host, and pathogen at fine spatial scales may help to elucidate transmission patterns and aspects of disease ecology that would not otherwise be inferred. Historically, the application of predictive maps has been restricted to diseases primarily of human importance (Osnas et al. 2009). Yet, infectious disease is increasingly important as a contributor to population declines and mortality events of wildlife species, and the frequency and geographic distribution of disease outbreaks has increased (Jones et al. 2008). The recognition of the link between wildlife mortality and current changes in land use has led to the increased application of mapping approaches in research on infectious disease of wildlife (Sehgal et al. 2011, Loiseau et al. 2012).

Here, we use a distribution model with high spatial resolution for mosquitoes in the genus *Culex* to investigate the potential transmission of malarial parasites in the genus *Plasmodium* to Greater Prairie-Chickens (*Tympanuchus cupido*; hereafter ‘prairie-chicken’) across a heterogeneous grassland landscape. Changes in rangeland management over the last 30 years are the driver behind the decline of this native grassland bird, however, increased vector abundances associated with the resulting changes in landcover may further exacerbate population decline via further decreases in fitness. Exposure to vector-borne disease agents such as malarial parasites occurs within a narrowly defined window (April-

Study Area

Figure 1. The projected probability of *Culex* spp. occurrence across the grassland habitat of the study area. Probabilities range from low probability (yellow) to high probability (red). Nest locations of *Plasmodium* positive and negative Greater Prairie-Chickens (*Tympanuchus cupido*) are indicated with blue and black circles, respectively. Mosquito sampling locations are indicated with light blue circles.

August), during which vector species (*Culex* spp.) are abundant in the environment and female prairie-chickens are nesting (Ganser and Wisely 2013). Female prairie-chickens have limited mobility during incubation and are philopatric to nesting areas among years (McNew et al. 2011). Nesting females are at an elevated risk of being infected with mosquito-borne parasites and pathogens due to the extended periods of time spent incubating both during the day and at night (Winder et al. 2014), which reduces behavioral defense against host-seeking mosquitoes (Valkiunas 2005, Burkett-Cadena et al. 2011).

We evaluated the probability of *Culex* occurrence in the grasslands of the Smoky Hills of Kansas to determine if the potential for exposure to vector-borne parasites at nest locations was greater or less than in other available but unoccupied locations. A low probability of *Culex* occurrence at nest locations would be expected if female prairie-chickens avoided nesting in areas occupied by these vector species. Alternatively, a higher probability of *Culex* occurrence at nests vs other available habitats could be a result of host-seeking behavior (Egizi et al. 2014) and/or shared habitat affiliations between vectors and hosts, facilitating the transmission of malarial parasites. To test if shared habitat affiliations between vectors and hosts were a potential mechanism facilitating transmission of *Plasmodium* parasites, we assessed the association between mosquito and prairie-chicken nesting habitats and compared parasite infection status in females nesting in areas of high or low suitability for *Culex* spp.

MATERIALS AND METHODS

Study area

Our study area encompassed 283 km² and was located in the Smoky Hills of north-central Kansas (39.421944 N, 97.588611 W; Figure 1). The mixed-grass prairie landscape is a sparsely populated rural region with multiple landcover types including row crop agriculture, grasslands, woodlands, water, and roads. The climate is temperate, with moderate rainfall and an annual precipitation of 883 mm, of which nearly half can be attributed to precipitation from May-August.

Data collection

We captured female prairie-chickens with walk-in traps and drop nets at breeding grounds from 2007-2011. We trapped a total of 110 individual female prairie-chickens during a 5-year period (2007: n = 9, 2008: n = 18, 2009: n = 27, 2010: n = 29, 2011: n = 27). At first capture, we fitted each female with a collar-style VHF radio transmitter (Model RI-2B, Holohil Systems Ltd, Ontario, Canada) and collected ~40 µl of blood via toenail clipping. We collected each blood sample in 1 ml of Queen's lysis buffer (Seutin et al. 1991) or Longmire's solution (Longmire et al. 1997), and stored samples at -20° C to preserve DNA for genetic analysis. We monitored radio-collared females via telemetry repeatedly throughout the nesting season to determine the locations of nests.

During the 2011 prairie-chicken nesting season, we collected mosquitoes at 20 randomly selected sampling locations in

Table 1. Environmental variables incorporated in the construction of the probability map of avian malarial vectors.

Environmental variable	Description	Variable ranges within the study area	Contribution to probability map (%)	Permutation importance (%)	References
Distance to water	Distance to the closest water source, calculated using Euclidean distance in meters ^a	0.0-2173.5 m	37.0	32.0	Brown et al. 2008
Elevation	Elevation above sea level in meters ^b	422.7-518.3 m	14.7	11.4	Schurich et al. 2014
Topographic Wetness Index (TWI)	Predicted soil moisture pattern, calculated as the natural logarithm of the ratio between local upslope contributing are and slope (Pathak 2010) ^c	2.0-21.5 units (ranging from low moisture to high moisture)	13.2	13.1	Cohen et al. 2008, Clennon et al. 2010
Distance to woodland	Distance to closest woodland, calculated using Euclidean distance in meters ^a	0.0-2002.8 m	11.8	19.1	Gleiser et al. 2002
Curvature	Indicator for aquatic habitat stability, calculated as the rate-change of slope per unit distance ^c	-1.3-2.0 units (negative values are upwardly convex, positive values are upwardly concave)	7.5	3.0	Mushinzimana et al. 2006
Aspect	Slope direction, measured in degrees ^c	0-360°	6.6	5.3	Schurich et al. 2014
Distance to road	Distance to closest road, calculated using Euclidean distance in meters ^a	0-1590 m	3.8	6.1	Khatchikan et al. 2011
Drainage class	Frequency and duration of wet period, classified on a scale ranging from poorly to excessively drained ^d	1-4 (ranging from poorly to excessively drained)	3.0	6.3	Larson et al. 2010
Slope	Steepness of terrain, measured in degrees ^c	0-14.4°	1.8	3.8	Larson et al. 2010

^aDerived from 2005 Landcover Map of Kansas, Kansas Applied Remote Sensing (KARS; <http://kars.ku.edu/geodata/maps/2005-kansas-land-cover-patterns-level-iv/>).

^bDigital Elevation Model (USGS; <http://viewer.nationalmap.gov/basic/?basemap=b1&category=ned,nedsrc&title=3DEP%20View>).

^cDerived from Digital Elevation Model (USGS).

^dSoil Survey Geographic Data Base (National Resource Conservation Service, <http://websoilsurvey.sc.egov.usda.gov/App/WebSoilSurvey.aspx>).

grassland habitat as outlined in Ganser and Wisely (2013). Briefly, we trapped mosquitoes weekly for a total of nine weeks (May–August, 2011) using CDC CO₂ miniature light traps (J.W. Hock, Gainesville, FL). Light traps incorporated two attractants: a small light bulb (< 25 watts) and carbon dioxide released via sublimation of dry ice. Traps were placed at a height of ~1.5 m within a study area that included prairie-chicken nesting habitat to determine presence and absence of mosquito species across the landscape. Adults were identified to species level using dichotomous keys (Pratt and Stojanovich 1961). Vectors in the genus *Culex* were the most abundant genus during the peak of grassland bird nesting season (Ganser and Wisely 2013). Identified *Culex* spp. (*Cx. tarsalis*, *Cx. salinarius*, *Cx. pipiens*, and *Cx. restuans*) have been recognized as potential vectors of malarial parasites (Janovy 1966, Kazlauskienė et al. 2013, Valkiūnas et al. 2015) and arboviruses (Bolling et al. 2005, Turell et al. 2005, Flock 2012).

Prevalence of malarial parasites

Genomic DNA was extracted from blood samples using Qiagen DNeasy Blood and Tissue Extraction Kits according to the manufacturer’s protocol (Qiagen Inc, Valencia, CA). We detected circulating haemosporidia in blood samples of prairie-chicken using screening primer sets 343F/496R that amplify a 154 bp RNA-coding segment on the mitochondrial DNA of *Haemoproteus* and *Plasmodium*, and 213F/372R for a 160 bp segment encoding the XmnI restriction site of *Haemoproteus*, *Plasmodium*, and *Leucocytozoon* (Fallon et al. 2003, Beadell and Fleischer 2005). Both primer sets amplified a region outside the AT-rich region of cytochrome *b* that has been shown to be difficult to amplify (Fallon et al. 2003). Positive controls from a reference strain of *Plasmodium relictum* and negative controls based on sterile distilled water were amplified under identical conditions as target samples. PCR products were visualized via gel electrophoresis.

We used three sets of primer pairs 413F/1162R, 413F/926R, and 543F/926R to amplify a segment of the parasites cytochrome *b* gene for all samples determined to be infected by PCR screening (Ricklefs et al. 2005). We purified the amplified product using QIAquick PCR Purification kits according to the manufacturer’s protocol (Qiagen Inc, Valencia, CA). Sequencing was carried out using an ABI 3730 Automated Sequencer (Applied Biosystems Inc, Foster City, CA). Sequences were edited and aligned in BIOEDIT (Hall 1999) and are available through GenBank (accession numbers KU949763, KU949764). For identification of parasite genus we compared them with sequences in Genbank and the MalAvi database (Bensch et al. 2009).

Spatial analyses of *Culex* occurrence

We modeled the probability of *Culex* spp. occurrence across the study area as predicted by environmental variables using machine-learning approaches implemented in Program Maxent 3.2.1 (Phillips et al. 2006). We limited our predictive model to a matrix of grassland habitat excluding cropland habitats because prairie-chicken nests were mainly located in native grasslands (McNew et al. 2013, 2014) and we were interested in modeling vectors in habitat available to nesting prairie-chickens rather than all land cover types available to mosquitos. Environmental variables used in this model were previously found to correlate with the distribution of *Culex* spp. in this study area and included measures of topography, soil composition, and proximity to non-grassland landcover types (Table 1). NDVI and climatic variables did not show spatial variation on the study area and were excluded from the model. GIS map layers, from which all variable data were derived, were standardized to a spatial resolution of 50m x 50m pixel size to conserve fine-scale spatial information in ArcGIS 10.2.2 (ESRI, Redlands, CA). To avoid over-parameterization of the distribution model, we excluded variables that were highly correlated to one another (Pearson’s correlation coefficient > 0.80) (Hijmans 2012).

We based our vector occurrence data on species lists generated for each trap during a nine-week trapping period. Because Maxent model predictions are sensitive to underlying biases in detection probability, we defined a vector as present at each of our 20 sampling sites if it was trapped at ≥ three times during our nine-week sampling period. The distribution model was generated using presence data from the traps and 10,000 randomly generated background points (pseudo-absence data). The fit of the model was iteratively improved over 1,000 training runs using a convergence threshold of 0.00001. To limit over-prediction, the probability distribution was modeled using only the empirically derived range of variables (i.e., no “clamping” was performed, Owens et al. 2012). We used a jackknife validation for small sample sizes, since our dataset of vector occurrence was limited to 20 sampling sites (Pearson et al. 2007). The output of the model produced a probability of presence for each pixel on the map of our study area.

Statistical analyses of parasite exposure and infection status

Inference of the predictive model of *Culex* spp. occurrence, which was based on one season of field data, may have been imprecise due to inter-annual variation in precipitation. We obtained weekly averages of precipitation from Weather

Table 2. Probabilities of *Culex* spp. occurrence at nests and randomly generated points and results from multi-response permutation procedure (MRPP).

Selection procedure	Sample size	Range	Mean	SD	MRPP	
					A	P
Nest	110	0.18-93.93	0.36	0.23		
MCP ^a	110	0.01-85.83	0.27	0.26	0.021	0.001
95% kernel ^b	110	0.04-94.83	0.27	0.26	0.026	0.001
50% kernel ^c	110	0.00-94.83	0.28	0.26	0.016	0.003

^aMinimum Convex Polygon. ^b95% estimate of core range of nest sites. ^c50% estimate of core range of nest sites.

Underground (www.wunderground.com) at the nearest weather station (Blosser Municipal Airport, Concordia, KS) for the 2007–2011 prairie-chicken nesting seasons to determine if there was temporal discord among years with precipitation as a major predictor of mosquito emergence. We compared differences in weekly averages of precipitation among years with a Kruskal-Wallis test in R 3.2.0 (R Development Core Team 2015) since the assumptions of ANOVA were not met (Quinn and Keough 2002). We did not find a significant difference in precipitation among years ($\chi^2 = 3.47$, $p = 0.48$). Hence, we proceeded to test model utility by (1) assessing the predicted occurrence of these vectors at known locations of nests of Greater Prairie-Chicken, (2) evaluating if shared habitat use between vectors and hosts was correlated with malarial infection status of Greater Prairie-Chicken. We assessed the potential for host exposure to vector-borne parasites by comparing the Maxent estimated probability of *Culex* occurrence at nests ($n = 110$) with randomly generated points in grassland habitat ($n = 110$). Sampling of random points was restricted to the core area of nesting sites, which was defined using three approaches: minimum convex polygon (MCP), and kernel estimates defining 95% or 50% of the core range based on nest site locations (Geospatial Modeling Environment 0.7.3 and ArcGIS 10.2.2; www.spatial ecology.com) (Table 2). We tested the distribution of vector probability for normality using Shapiro-Wilk tests in R 3.2.0 (R Development Core Team 2015). Neither nest locations ($W = 0.96$, $p < 0.01$) nor random locations (MCP: $W = 0.86$, $p < 0.01$; 95% kernel estimate: $W = 0.86$, $p < 0.01$; 50% kernel estimate $W = 0.89$, $p < 0.01$) followed a normal distribution; applied transformations did not improve the fit to a normal distribution. We opted to use multi-response permutation procedure (MRPP), a non-parametric procedure to test the differences between Maxent estimated probability of *Culex* occurrence at nests vs randomly generated grassland locations (R 3.2.0, McCune and Grace 2000).

To evaluate if shared habitat use between vectors and hosts was correlated with *Plasmodium* infections in prairie-chickens, we compared the modeled probability of *Culex* occurrence at nests of female prairie-chickens infected with malarial parasites ($n_{\text{positive}} = 7$) to nests of uninfected females ($n_{\text{negative}} = 103$). Because of unequal sample sizes, we used Monte Carlo resampling procedures implemented in R 3.2.0 (R Development Core Team 2015, Manly 2007) to simulate a representative sample of the probability of vector occurrence at nests of uninfected females. Nests of uninfected females were randomly resampled (10,000 iterations) in group sizes of seven individuals from the sampled distribution of 103 uninfected individuals. The 95% confidence limits of 10,000 iterations of resampling were used to evaluate if nests of infected females were associated with a higher probability of vector occurrence than nests of uninfected females.

RESULTS

Prevalence of malarial parasites

We screened 110 female prairie-chickens for malarial parasites, seven of which (6.4%) tested positive by PCR. Prevalence across years was variable in this population. The highest prevalence (33.3%; $n_{\text{screened}} = 9$, $n_{\text{positive}} = 3$) was observed in 2007 and none of the tested females were positive for malarial parasites

in 2008 (0.0%; $n_{\text{screened}} = 19$, $n_{\text{positive}} = 0$). In 2009, 2010, and 2011 prevalence was highly variable with 11.1% ($n_{\text{screened}} = 27$, $n_{\text{positive}} = 3$), 0.0% ($n_{\text{screened}} = 28$, $n_{\text{positive}} = 0$), and 3.7% ($n_{\text{screened}} = 27$, $n_{\text{positive}} = 1$), respectively. Nests of infected females were widely separated by an average distance of 4.6 ± 3.9 km. We obtained sequences from five samples that tested positive for malarial parasites and distinguished parasite lineages based on ~380 nucleotides of cytochrome *b* sequences. We identified all sequences as belonging to the genus *Plasmodium*.

Probability map of *Culex* occurrence

The probability map of *Culex* occurrence was built with nine variables including aspect, curvature, elevation, slope, drainage classes, distance to roads, distance to water, Topographic Wetness Index, and distance to woodlands (Table 1). The model had an AUC of 0.86 indicating a high fit of the model to the input data. The prediction of the potential distribution of *Culex* spp. as measured by the Pearson's Jackknife method was significantly better than compared to random expectations ($p < 0.01$).

Shared habitat use between vector and host

Nest locations were associated with a significantly higher probability of *Culex* occurrence (0.36 ± 0.23 SD) than random points (MCP, 50% kernel estimate and 95% kernel estimate had probabilities of 0.27 ± 0.26 , 0.28 ± 0.26 , and 0.27 ± 0.26 , respectively) (Table 2). This positive association suggests that nesting prairie-chickens had a higher probability of encountering *Culex* mosquitoes while on the nest than at nearby sites within grassland patches.

We used a Monte Carlo resampling procedure to test for differences in the probability of vector occurrence between nests of infected vs uninfected females. We did not find evidence that infected females nested in habitats with a higher probability of vector presence (0.44 ± 0.34) than uninfected females (0.25 ± 0.23), but sample sizes were small. Despite the wide confidence intervals in the probability of vector occurrence at nests of uninfected females (0.23–0.52), three of seven nests of infected females nested in habitat that was associated with a > 0.58 probability of *Culex* occurrence.

DISCUSSION

We constructed a high-resolution species distribution model for *Culex* spp. as vectors of avian malaria within the grassland ecoregion of north-central Kansas. Our model indicated a high potential for nesting prairie-chickens to be exposed to disease vectors but failed to link exposure to malarial infection status. Despite a large sample of female prairie-chickens, parasite prevalence was low. Nonetheless, our model demonstrated how this approach may be used for future assessments of exposure to disease vectors in wildlife and the importance of fine-scale environmental variables in that assessment.

Using the interpolated probability of *Culex* occurrence generated from this model, we inferred that the potential of exposure to vector-borne parasites at nests of prairie-chickens was greater than other available grassland locations. The probability of vector occurrence was 50% greater at nests than elsewhere in grassland habitat, suggesting that *Culex* spp. and prairie-chickens

shared habitat associations and proximity. These associations should increase the probability of parasitism by *Culex* mosquitoes, hence increasing the potential for exposure to malarial parasites and other pathogens. Maxent response curves indicated a higher affinity of *Culex* spp. for valleys and grassland hilltops than slopes. Indeed, vector habitat predicted by our distribution model shared a common characteristic with previous habitat models and empirical data for prairie-chickens (Gregory et al. 2011, McNew et al. 2014); both *Culex* mosquitoes and nesting prairie-chickens were found more frequently on grassland hilltops than surrounding areas.

Nest site selection is a result of multiple factors. The variables include availability of food and cover as well as avoidance of anthropogenic structures, predators, and parasites (Matthews et al. 2013, McNew et al. 2013, Winder et al. 2014). Nesting prairie-chickens did not avoid habitats suitable for the transmission of vector-borne parasites. The lack of avoidance behavior and the low observed prevalence of malarial parasites indicated that other factors may exert a stronger proximate influence on decision-making. On this landscape, more than 90% of prairie-chicken mortality events were attributable to predators (Winder et al. 2014) and high predation rates may have driven the selection of nest sites even though it may expose prairie-chickens to an increased probability of parasitism. Conversely, host and vector habitat overlap may have been driven by vector attraction to the host. Prairie-chickens are large-bodied avian hosts and may have served as attractants for *Culex* mosquitoes and this factor may have driven the vector distribution during the nesting season.

While predicted occurrence of *Culex* spp. was higher at nests than other grassland locations, we did not find evidence that infected females nested in habitats with a higher probability of vector presence than uninfected females. Despite the high predictive power of the vector distribution model based on grassland habitat variables (Table 1), we were unable to associate the increased probability of vector occurrence with infection status of female prairie-chickens. The lack of a relationship could simply be due to the fact that all prairie-chickens are at greater risk of vector parasitism because these birds serve as an attractant to *Culex*. The low variation in probability of occurrence at nest locations provides evidence for this interpretation.

An alternate explanation for the lack of correspondence between vector and pathogen occurrence was due to Type 2 error and we did not have sufficient power to detect a difference in the probability of vector occurrence among infected and uninfected hosts. Power was reduced by the small sample size of infected individuals. A retrospective power analysis (Steidl et al. 1997) indicated that our sample sizes were only sufficiently large to detect effect sizes greater than 25%. To detect differences in vector probability observed in this analysis, sample sizes with greater than 30 infected individuals would be required.

Another potential limitation of this portion of the analysis was the temporal disparity between parasite and vector sampling. Blood samples to determine infection status were acquired annually from late March to early May, which in some individuals was prior to nest site selection. As such, infection may have occurred during the nesting season of the previous year, although likely at the same site and the same host individual since nesting birds are philopatric to their nesting sites. In our study,

however, we confined our inference to determine if infected and uninfected females selected nesting sites based on the occurrence of mosquitoes.

Our vector distribution model revealed that variables related to the availability of permanent water sources (Distance water) and ephemeral water sources (TWI, Drainage class, and Curvature) were important in predicting the occurrence of *Culex* mosquitoes at fine spatial scales (Table 1). Topographic features were strong predictors of mosquito occurrence which overlapped with prairie-chicken nesting habitat. The affinity of mosquitoes for grassland hilltops is unusual, particularly since previous studies have found a negative correlation between elevation and mosquito abundance (Li et al. 2008, Schurich et al. 2014). This finding lends support to the idea that mosquitoes may be selecting habitats populated by nesting prairie-chickens. Mosquitoes may be able to exploit hilltops in our study area due to the shallow elevational gradient (474.39 ± 17.43 m) that decreases the stratification in mosquito distribution between valleys and hilltops.

A fundamental problem of using vector distribution models to address underlying mechanisms of disease transmission is the potentially poor fit between models of vector distribution and actual vector abundance on the landscape (Ostfeld et al. 2005, Eisen and Eisen 2011). Thus, a vector occurrence model can inform wildlife managers about which vertebrate species have overlapping distributions with disease vectors and have the potential to be exposed to parasites or pathogens, but the models may not be as useful for determining which individuals within the landscape are at a greater risk of exposure to vector parasitism and vector-borne diseases.

The complex agro-ecosystem of the north-central Great Plains represents an interface for the transmission of multiple avian diseases including avian malarial parasites as well as arboviruses. For arboviruses, managed rangelands create a complex transmission network among humans, livestock, and grassland nesting birds. The emergence of ornithophilic *Culex* spp. during the nesting season of grassland-nesting birds (Ganser and Wisely 2013), when females are exposed for extended periods of time, increases disease transmission, and results in peak parasitemia for birds (Valkiūnas 2005, Burkett-Cadena et al. 2011). Temporal synchrony coupled with spatial overlap in habitat between vectors and hosts makes the prairie ecosystem a potential source for the amplification of arboviruses and other mosquito-borne pathogens. The potential effects of vector-borne disease on the population viability of prairie-grouse (Peterson 2004, Adler et al. 2007) and the conservation status of many grassland bird species warrants a closer investigation of this system. It has been demonstrated that under current land management practices, population viability of many grassland nesting birds is imperiled (With et al. 2008) and that disease may contribute proximately to the extinction vortex that occurs when prairie-chicken populations become small, fragmented, and are subjected to stochastic processes (Westemeier et al. 1998).

The conservation status of many grassland bird species warrants a closer investigation of the potential effects of vector-borne diseases on the population viability of these species (Peterson 2004, Adler et al. 2007). Our findings that vectors and prairie-chickens share similar habitats indicates that nest choice may increase the probability of sympatric grassland birds being

parasitized by mosquitoes and their associated disease agents (Knopf 1996) and also supports the mechanism of transmission of avian malaria and other zoonotic pathogens, including arboviruses.

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