

MAPPING THE SPREAD OF THE PATHOGEN *RICKETTSIA PARKERI* THROUGH LANDSCAPE GENETIC ANALYSIS OF THE PRIMARY VECTOR *AMBLYOMMA MACULATUM*

Sara Benham

Old Dominion University

Advisor: Holly Gaff

Abstract

The Gulf Coast tick, *Amblyomma maculatum*, has expanded into the mid-Atlantic region in recent decades from its historic range along the United States Gulf Coast region. The Gulf Coast tick is a vector of the human pathogen *Rickettsia parkeri*, the causative agent of *R. parkeri* rickettsiosis. Gulf Coast tick populations are associated with open habitat types and areas with recent disturbance. Habitat connectivity likely contributes to movement of ticks on hosts, and the establishment of new populations in the region. From 2017-2020, we collected 980 adult Gulf Coast ticks from Virginia and North Carolina. Here we investigate the relationship between tick populations and remotely-sensed environmental variables that can be used to determine habitat suitability and, in turn, habitat connectivity that may permit the further spread of this tick vector. Variables that have significant correlations with Gulf Coast tick populations include National Land Cover Database land cover classes, the normalized difference vegetation index, climate seasonality and the minimum temperature of the coldest month. This work will contribute to development of a habitat suitability map that will enable us to explore the relationship between habitat connectivity and population genetics, and thus to identify hypothetical corridors and barriers to further range expansion.

Introduction

The burden of tick-borne diseases in the United States is a serious and increasing threat to public health (Sonenshine 2018). Much focus has been placed on medical and veterinary responses to tick-borne disease, yet there is a critical need to understand more about the environmental drivers that can act to increase the tick-borne disease (TBD) burden, including a better understanding of how geographic range expansions can occur and increase the risk of exposure to pathogens. Models that forecast mosquito-borne illness risks (e.g., Barker 2019) offer an example of applied, collaborative approaches to using remote-sensing and other types of technologies to address vector-borne diseases. A similar approach that incorporates environmental data with tick and pathogen surveillance can be useful to understand TBD risks using methods and spatio-temporal scales appropriate for ticks. The basic premise of paper is that environment can affect both tick and host populations, thus influencing the opportunities for infections to become established and spread. A greater understanding of the environmental variables associated with tick populations can improve surveillance and control programs.

The Gulf Coast tick, *Amblyomma maculatum*, is a vector of the pathogen *Rickettsia parkeri*, the causative agent of a disease called *R. parkeri* rickettsiosis in humans (Paddock et al. 2004). Spotted fever rickettsiosis (SFR) is a diagnostic term that refers to disease caused by several *Rickettsia* spp. pathogens. *Rickettsia parkeri* causes a

less severe disease than Rocky Mountain Spotted Fever (RMSF), caused by *R. rickettsii* (Paddock et al. 2008). Overall SFR's have a case fatality rate of between 5-10% (CDC, 2019). Incidence of SFR has been increasing overall with higher incidences in states that lie in the southeastern and central United States. The regions with high human burden of SFR generally overlaps with the *A. maculatum* range in many areas, an ecological indication that *A. maculatum* are currently contributing to the disease burden in the region and may further amplify disease risk in the future. In fact, cases of *R. parkeri* rickettsiosis identified outside of the documented *A. maculatum* range in 2014 (Herrick et al. 2016) prompted further investigation and ultimately new collection records for *Amblyomma maculatum* ticks from Arizona and New Mexico (Allerdice et al. 2017; Hecht et al. 2020). These additional collections strengthen the connection between the emergence of *R. parkeri* rickettsiosis and the presence of *A. maculatum* ticks.

Amblyomma maculatum populations are expanding north from the species' historic range about 100-240 km from the Gulf Coast (Teel et al. 2010). This trend of range expansion has been accompanied by a remarkable pattern of higher *R. parkeri* prevalence in the ticks collected in newly established populations along the northeastern margins of this range, specifically in the mid-Atlantic region (Wright et al. 2011, Paddock and Goddard 2015). Although it is not clear this pattern will continue, it has been consistently observed since 2010 (Wright et al. 2011, Nadolny et al. 2014). Investigating the spatial and environmental context of this range expansion as well as the population dynamics of the tick vector is one way to begin exploring how geography influences

R. parkeri infections in ticks. To do this, we will begin by exploring readily available, remotely-sensed variables that are commonly used in habitat suitability mapping. Identifying meaningful variables that can be derived from remotely-sensed, multispectral images is the first step in developing a habitat suitability map to map habitat connectivity. This advances our broader efforts to compare hypothetical habitat connectivity with genetic connectivity, calculated using tick mitochondrial haplotypes.

This paper describes the use of Landsat 8 imagery to derive Normalized Difference Vegetation Index (NDVI) values from multiple field sites for which tick sampling data are available. The purpose of this is to identify whether NDVI values help to discriminate between transects where Gulf Coast ticks are present and sites where they are absent based on the vegetation density in the immediate area. The process of identifying environmental variables that can be mapped to create predictive suitability maps will help to contextualize genetic connectivity of the Gulf Coast tick, in addition to improving ongoing sampling efforts.

Materials and Methods

Sampling

Sample sites were determined through county-by-county sweeps across Virginia to identify sites with Gulf Coast tick presence. GCT collections began in April 2021 when the first ticks were starting to emerge for the season. Sampling continued bi-weekly at regularly sampled GCT sites, and at sites throughout the eastern Virginia coastal plain, focusing specifically where GCT had been collected in prior years to identify any new

populations. During the summer, we identified two new sites, from which we collected >20 ticks each. The total number of GCT collected in 2020 was 226 individuals from 10 sites. The sampling period continued from April through November, with no ticks collected by November. Overall, 980 GCT have been collected between 2017-2020 by the ODU Tick Team using both regular flagging and special sampling to increase GCT collections.

Data analysis

The first step of this work is to classify spatial points based on the presence and absence of *A. maculatum* populations based on collections from the 2017-2020 field seasons. Population presence is defined as at least two adult ticks collected from a single transect in one year. All spatial points were thinned to include one observation per 0.5km resolution. This resolution preserves nearby transects that have distinctly different vegetation and different tick species collected.

Counts of adult ticks by collection sample (identified by site, transect, year) were combined to create a species assemblage matrix. Due to data thinning and removal of sites from which environmental variables could not be extracted, 792 adult GCT were ultimately included in the statistical analysis. In addition, 4326 adult ticks from 5 additional species were included to study environmental effects on species assemblages.

Square root transformation was applied to the species matrix, and data were then standardized by sample to generate relative abundances. A Bray-Curtis (BC) rank order resemblance matrix was created

from the assemblage data for further analysis.

Multispectral Landsat 8 OLI/TIRS (USGS) imagery was downloaded, filtered for scenes containing < 25% cloud cover, and pre-processed by running the raster function Apparent Reflectance in ArcGIS Pro (ESRI Inc.) to correct for relative differences in spectral gain and biases between multiple raster scenes and time periods. The corrected scenes were used to calculate NDVI with the NDVI raster function (Table 1). Available NDVI rasters for July were mosaicked by year (2017-2020) using maximum values to combine four scenes including all spatial points (Table 1).

Table 1. Landsat 8 OLI/TIRS scenes available with < 25% cloud cover for years. The number of transects for tick sampling is indicated for each scene. Note, only years for which values could be obtained for all sites were included in analysis.

Row/Path	No. Transects	NDVI Years
14, 34	26	2017, 2018, 2019, 2020
14, 35	5	2017, 2019, 2020
15, 34	14	2018, 2019, 2020

An ecological matrix was created with each collection sample as a row, along with 19 Bioclim variables (Fick and Hijmans 2017), elevation extracted from the NOAA coastal digital elevation model (NOAA), and the NDVI values for 2019 and 2020.

The BEST routine in Primer 6 (Clarke and Gorely 2006) was run using the Biota-environment (BIOENV) option with the BC resemblance matrix as the biotic data and the ecological matrix with 24 variables as the environmental dataset. This procedure identifies the environmental variables that contribute the most to the species

assemblages observed. Best reports a global correlation statistic Rho and percent significance based on 99 permutations.

An analysis of similarity (ANOSIM) test was run on the root-transformed BC resemblance matrix using National Landcover Database 2017 land cover classes (Yang et al., 2018) as a factor describing each collection site.

All of these routines were repeated on a dataset with two tick species removed: the lonestar tick, *A. americanum* and unknown *Ixodes* sp. in order to detect the strength of associations between these species specifically and the environmental variables. The lonestar tick is present across many sites, therefore the rationale for removing this species is that stronger correlations may be seen in species like *Ixodes scapularis* and *A. maculatum*, which appear to have stronger habitat associations, based on field observations.

Genetics

Tick samples were processed using a protocol described in Benham et al. (2021) to isolate target mitochondrial DNA fragments, amplify fragments using polymerase chain reaction (PCR), and sequence 217-218 nucleotide base pairs by Sanger Sequencing. Forward and reverse sequence reads were aligned in Geneious (Kearse et al. 2012). Genetic distance was calculated in Arlequin v. 3.5 (Excoffier and Lischer 2010) using a distance measure Φ_{ST} based on heterozygosity within and between populations. Distance values range from 0-1, with a distance of 0 indicating greater connectivity between populations, and a distance of 1 indicating complete isolation.

Results

Raster mosaics for 2019 and 2020 were used to derive NDVI values for all transects, since some data were missing for years 2017 and 2018. Forty-five unique transects were included in the analysis.

The BEST analysis showed the strongest environmental variables associated with the species assemblages were seasonality and 2019 NDVI values (Rho = 0.217, significance = 0.01). ANOSIM results revealed a significant (Rho = 0.129, significance = 0.005) association between land cover class and species assemblage.

When these same routines were run with a four-species assemblage, excluding lonestar ticks and unknown *Ixodes*, the land cover association increased slightly (Rho = 0.157, significance = 0.02), and the BEST analysis identified new variable combinations related to the reduced assemblages. The best variable combination included minimum temperature of the coldest months and quarters, maximum temperature of the wettest quarter, and 2019 NDVI (Rho = 0.255, significance = 0.01), followed by a combination of only two variables, 2019 NDVI and minimum temperature of the coldest month (Rho = 0.254, significance = 0.01).

Population genetic connectivity for ticks processed from 2017-2018 showed that Gulf Coast tick populations in the region are generally not well-connected, and most appear to be isolated from one another (Benham et al. 2021). Data from ticks collected from the same sites, plus several additional sites in 2019, however, revealed greater connectivity between populations, with only one site in North Carolina that appears to be genetically isolated from two nearby sites, but not genetically distinct from populations at three other sites (Table 2).

Table 2. Pairwise genetic distances by site measured by Φ_{ST} (below the diagonal), and corresponding p-values above the diagonal. Significant values are in bold.

	1	2	3	4	5	6
1	0	0.54	0.25	0.35	0.06	0
2	0.03	0	0.17	0.45	0.13	0.01
3	0.01	0.06	0	0.38	0.32	0.14
4	0.004	0.02	0.004	0	0.50	0.37
5	0.04	0.03	0.001	0.01	0	0.0
6	0.15	0.11	0.07	0.01	0.03	0

Discussion

Several environmental variables appear helpful for predicting habitat suitability of the Gulf Coast tick at the regional scale. The NDVI has the potential to be an especially useful predictor of suitable habitat because experts in the field already rely on local vegetation characteristics as indicators of possible GCT habitat. Further investigation of the strength and direction of this association need to be explored in order to use NDVI to generate well-supported suitability maps. Here we considered NDVI during July of two sequential years, among other variables, to understand which variables had the strongest associations with GCT populations. Going further with a temporal investigation of NDVI changes over years in relation to tick phenology can reveal the relationship between vegetation and tick populations. Canopy closure has been identified as a factor in GCT population decline and extirpation (Nadolny 2016). A temporal analysis using measures derived from Landsat 8 multispectral imagery, including NDVI, could also reveal key information about population responses to disturbance and canopy regrowth. Space-time cubes are

data structures that can be created for longer-term temporal analysis with multidimensional datasets, such as those that incorporate many different predictor variables. This work can be applied to identify new populations using spatial imagery and to better understand the spatial context of human infection risk posed by *R. parkeri* in Gulf Coast ticks.

Population genetic analysis has revealed inconsistent patterns of connectivity between populations from year to year. A general trend of temporal stability is apparent at sites where populations have persisted and populations are typically not well-connected (Nadolny et al. 2015; Benham et al. 2021). The latest genetic data presented here shows that populations might not be as isolated from each other as prior work has shown. Development of higher resolution genetic markers, such as single-nucleotide polymorphisms can improve the resolution of this genetic analysis and may help resolve questions about population connectivity.

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