

BUMBLE BEE SPECIES DISTRIBUTION AND GENETIC STRUCTURE OF *BOMBUS*
VAGANS IN THE SOUTHERN APPALACHIANS

A Thesis
By
ERIC SCOTT RAYFIELD

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APPROVED BY:

Jennifer C. Geib, Ph.D.
Chairperson, Thesis Committee

Michael Osbourn, Ph. D.
Member, Thesis Committee

Michael Gangloff, Ph. D.
Member, Thesis Committee

Zack E. Murrell, Ph.D.
Chairperson, Department of Biology

Michael J. McKenzie, Ph.D
Dean, Cratis D. Williams School of Graduate Studies

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Abstract

BUMBLE BEE SPECIES DISTRIBUTION AND GENETIC STRUCTURE OF *BOMBUS VAGANS* IN THE SOUTHERN APPALACHIANS

Eric Scott Rayfield
B.S., Lees-McRae College
M.S., Appalachian State University

Chairperson: Dr. Jennifer C. Geib

Bumble bees (*Bombus* spp.) have faced significant declines worldwide in the past 60 years due to human driven climate change, disease, habitat loss, and intensive farming practices. Twelve species of bumble bee in North America are currently listed as vulnerable. More of the 46 North American species could face declines, but with little baseline data and routine monitoring of populations, their fate is uncertain. With decreased population numbers, bumble bees are susceptible to reduced fitness through loss of genetic diversity. If bumble bees maintain a high degree of dispersal among populations, they may be able to maintain sufficient genetic diversity and persist in a changing climate and survive anthropogenic stressors. This study evaluated the relative abundance and distribution of *Bombus* species along a 900 km MegaTransect crossing three National parks in the southern Appalachians: Shenandoah National Park, Blue Ridge Parkway, and Great Smoky Mountains National Park. Additionally, this project investigated the population genetics and dispersal preferences of *Bombus vagans*, a locally abundant bumble bee that is native to eastern US and high elevations in the southern

Appalachians. I coordinated citizen science volunteers to collect bumble bee specimens from 390 roadside habitats within the national parks. I generated species distribution maps based on presence data using Maximum Entropy modeling software. I then used microsatellite markers to examine population genetic structure of *Bombus vagans* and used Maxent and Circuitscape geospatial modeling programs to elucidate the role that landscape features play in population genetic structure through isolation by distance and isolation by resistance.

I identified ten *Bombus* species among the 3700 bumble bees sampled at three parks, but species diversity was heavily skewed by one dominant species, *Bombus impatiens*. Two species of concern, *Bombus affinis* and *Bombus terricola*, were not present in any of the sites sampled. *Bombus vagans* genetic analysis indicated some levels of inbreeding within a few populations but overall genetic diversity was stable. Models including both isolation by resistance and isolation by distance described more genetic variation among *Bombus vagans* populations than isolation by distance alone, suggesting that gene flow follows a nonlinear pattern. Interestingly, one population was highly genetically distant from all the rest, suggesting a potential cryptic species. The best fitting Circuitscape models indicated a habitat dispersal preference of high solar radiation, habitat openness, and imperviousness. Maps of modeled dispersal pathways seemed to follow rural roadways. These patterns indicate that *Bombus vagans*, and potentially other bumble bees, may be using roadways to maintain population connectivity. Conservation management strategies should be directed towards creating more roadside pollinator habitat by planting native gardens and decreasing mowing frequency.

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Table of Contents

Abstract.....	iv
Acknowledgments	vi
Foreword.....	iv
Chapter 1: Bumble Bee Species Distribution in the Southern Appalachians	1
Introduction.....	1
Methods and Study System	3
Results	7
Discussion	9
Figure Legends.....	16
Figures.....	19
Chapter 2: Genetic Structure and Dispersal Patterns of <i>Bombus vagans</i> in the Southern Appalachians	37
Introduction.....	37
Methods.....	43
Results	54
Discussion	59
Figure Legends.....	72
Figures.....	74
Appendix.....	90
References Cited	106
Vita	118

Foreword

This work was completed in collaboration with the National Park Service and United States Geological Survey in order to gain a better understanding of southern Appalachian bumble bee population genetic status and species distribution. The format and references follow that of the Journal of Conservation Genetics. This thesis includes two chapters that are each a stand-alone report.

Chapter 1

Bumble Bee Species Distribution in the Southern Appalachians

Introduction

Insect pollinators play a critical role in maintaining plant communities, they are responsible for 90% of pollination among angiosperm plants (Ollerton et al. 2011), with bees being among the most efficient of pollinators (Roubik et al. 1995). Bees provide a valuable ecosystem function of pollinating a large majority of plants that support food chains, a number of codependent species of rare plants, as well as providing pollination to human-planted crops. One of the most common and notable groups of bees are bumblebees of the genus *Bombus*.

Bumble bee decline

Bumblebees are widespread and abundant, however several species of bumble bees have declined in recent years (Cameron et al. 2011; Colla et al. 2012). In North America, twelve *Bombus* species are listed as vulnerable, endangered or critically endangered by the International Union for Conservation of Nature's (IUCN) Red List of Threatened Species (IUCN 2021). Native bee decline is attributed to a number of anthropogenic ecosystem changes including pesticides, introduced invasive species, diseases, and habitat loss or fragmentation (Goulson et al. 2015; Winfree et al. 2015). Bumblebees, in particular, are more susceptible to population loss due to their low effective population size and monoandrous mating system (Darvill et al. 2006). Their need for diverse available habitats at different phases of their life cycle (e.g. nesting and overwintering) adds to their vulnerability (Colla & Packer 2008). In addition, because pollinator

conservation historically focused on the non-native European honeybee *Apis mellifera*, some species may have declined without notice, potentially complicating efforts to mitigate declines (Wilson et al. 2017).

Monitoring of native bumble bee populations has increased over the last decade (Geib et al. 2015b), though data remains deficient for a preponderance of all native bee species (IUCN 2021). Overwhelming data do show that some North American *Bombus* species have faced drastic declines. Once locally abundant across the Midwest and eastern US, including the Southern Appalachian Mountains, *Bombus affinis* and *B. terricola* have been absent from recent surveys conducted throughout their southern range (Grixti et al. 2009; Cameron et al. 2011). *B. terricola* is a federal Species of Concern and *B. affinis* was listed as endangered by the USFWS in 2018, because populations have declined or become extirpated from across a large part of their historical range (Hatfield et al. 2015a; Hatfield et al. 2015b). Declines of species like *B. affinis* and *B. terricola* may have seemed sudden, however, that perception is likely attributable to the lack of baseline and routine monitoring data in the US (Council 2007).

Growing awareness of worldwide bumble bee declines and range contractions has prompted growth in monitoring efforts and renewed interest in studying the natural history of pollinator species. I coordinated a citizen science inventory of bee populations in the Southern Appalachian Mountains to evaluate the presence and distribution of bumble bee species. Pollinator populations in this region have been historically under-sampled and inventory studies are needed to understand future population trends. The project, dubbed the Blue Ridge Bumblebee Megatransect (BRBBM) was initiated as a collaboration between the National Park

Service, US Geological Survey, and Appalachian State University. The specific aims were to sample bumble bee specimens across the 900 km Megatransect for later identification and assessment of community composition as part of the All-Taxa Bioinventory (ATBI) of the region, model the likely distribution of each species based on occurrence data, and compare diversity indices among sites with different management strategies. In addition to documenting the most common species in the region, I hoped to find remaining pockets of *B. affinis* and *B. terricola*, whose historical ranges included the Southern Appalachians.

Methods and Study System

Bombus samples for this study were collected by citizen science volunteers in 2015, in the southern Appalachian Mountains of western North Carolina and Virginia along the Blue Ridge Parkway, Great Smoky Mountains National Park, and Shenandoah National Park. Collection sites were on roadside high elevation meadows along the 900 km study area (BRBBM, Fig 1).

Study system

Bumble bees were selected as focal taxa for the inventory, due to their importance as keystone pollinators in most terrestrial ecosystem as well as their relatively larger size and ease of netting compared to most other bee taxa. Worldwide, there are approximately 250 species of Bumble bee (*Bombus*) and are generally confined to the Holarctic (Williams 1994). Within the eastern United States there are 21 species of *Bombus* (Colla et al. 2011). Bumble bees are a

generalist pollinator, foraging on a wide variety of floral resources. A number of wild plant communities rely predominately or exclusively on bumble bee pollination (Waser et al. 1996) and many agricultural practices benefit from wild bumble bee pollination (Goulson 2003).

Of the 21 eastern bumble bees, B. affinis (federally listed as endangered in 2018) and *B. terricola* (listed as vulnerable by IUCN) are known to have experienced significant population declines and range contractions. They are both northern species that are confined to only high elevations in the south. *B. affinis* and *B. terricola*, along with *B. vagans*, *B. bimaculatus*, *B. ternarius*, and *B. perplexus* are the earliest emerging of the eastern US bumble bees. They are often associated with forested areas where they can take advantage of early spring ephemeral floral resources (Colla & Dumesht 2010). These other early-emerging species could be vulnerable to continuing land use change and suffer the same fate as *B. affinis* and *B. terricola*. Early emerging species may be particularly affected due to climate change causing a disruption in their emergent time and put them out of sync with their floral resources emergent times. (Memmott et al. 2007) Increased monitoring efforts need to be implemented so that the eventual decline of other potentially vulnerable species can be detected with ample time to respond.

Volunteer recruitment, site assignments and training

Citizen science volunteers implemented the collections at the roadway sites. Citizen science is an effective tool to gather large amounts of data with limited resources and is also a helpful for educating the public about the scientific process and conservation efforts. It gives the

public a sense of ownership with the scientific process and enhances scientific literacy (Bonney et al. 2016). Volunteers were recruited by word of mouth, email list serves, and a publication on entomologytoday.org. Volunteers chose their collection sites along the Megatransect based on proximity to their location. Gaps in site assignment were filled by volunteers and project coordinators willing to travel from their home location. I created a training video to teach volunteers how to collect bumble bees, record collection data, and process the collected samples. I also conducted in person workshops to demonstrate bee collection. Site assignment, training video, collection status, and collection data were organized using handsontheland.org, a resource that was provided by The National Environmental Education Foundation (NEEF) to coordinate citizen science projects.

Sampling methods

Sampling took place in July-August 2015 when workers were most abundant. Alternate mile markers ($N = 390$) along the entire Megatransect were selected as collection sites. Sampling occurred no more than $\frac{1}{4}$ mile distance from the mile marker to ensure collections were taken from the predetermined coordinates.

At each site, volunteers actively collected for approximately 10 minutes by netting any observed bumble bees. Bees were lethally sampled by placing net in a soapy water solution for 5 minutes and then transferring bees to Whirlpaks containing 95% ethanol to preserve DNA. Available evidence suggests that lethal sampling of foraging workers has no detrimental effects

to a colony or brood rearing and does not affect bee communities in terms of abundance, richness, evenness, or functional group composition (Gezon et al. 2015).

Volunteers delivered preserved specimens to pre-established drop points along the parkway (e.g., visitor centers). Collected samples were then stored at the Appalachian State University Biology Department until they could be washed, dried, pin-mounted and identified to species level. Identifications were made using methods described in (Williams et al. 2014). Identifications were verified by Sam Droege at USGS Patuxent Wildlife Research center.

Measures quantified and statistical analyses

I quantified mean catch rate of bumble bees (bees/person/hour for all species pooled) during sampling as a proxy for mean bee abundance at the national parks, relative abundance of individual bumble bee species present at each national park, distribution of each species among the three parks, and relative abundance of short, medium and long-tongued bee species among samples collected. Tongue length is an important trait for pollinators that plays a role in determining their interactions with floral host species (Inouye 1978) and thus niche partitioning to reduce competition among the members of the pollinator community.

I calculated biodiversity measures for each site and for each park using the Shannon Wiener index (Shannon 1948; Spellerberg & Fedor 2003) which is the most powerful of diversity indices because it accounts for species richness, evenness, and dominance. (Heip & Engels 1974) The formula used to calculate Shannon H is:

$$H = -\sum_{i=1}^n p_i \ln p_i$$

Statistical analyses were conducted using R software (version 4.1.0). I used Analysis of Variance (ANOVA) to test for differences in mean collection rate and mean diversity per site (milepost) among the three National Parks. Non parametric Spearman correlations were performed on data to test correlations between elevation, species diversity, and bee abundance.

Environmental niche modeling

I acquired multiple georeferenced environmental variables that were believed to a priori significance to *Bombus* habitat preference. These included, digital elevation model (DEM), land cover (land use) (USGS 2014), solar radiation, canopy cover, wind intensity, and impervious surfaces. All bioclimatic and landscape variables were clipped to the Megatransect study area extent using ArcMap 10.3.1 (ESRI, Redmond CA). Each layer was projected into the WGS 1984 geographic coordinate system and converted to the same dimensions and cell size to allow for cross analysis. All environmental raster layers were exported to ASCII format using the ArcMap toolbox. A total of 652 presence records of the 10 species of *Bombus* were used in the Environmental Niche Model analyses. A breakdown of the occurrences of each species can be seen in Table 1.

MaxEnt: maximum entropy modeling

I created habitat suitability maps using environmental niche models (ENM) which uses the principle of maximum entropy as implemented in the program MaxEnt v 3.3.3 (Phillips et al. 2009; Elith et al. 2010);. MaxEnt uses presence-only locality data and background points randomly sampled from the study area to estimate the species distribution that is closest to uniform (i.e., maximizes entropy), given information on the environmental conditions of the study area. Multi-variate models were ran with all Bioclimatic variables together to determine which covariates contributed most to species presence. Landscape variables were ran with a single run of all covariates to determine the model that best estimated environmental suitability for each *Bombus* species. I then ran each species individually with their top-performing variables to create an accurate ENM. Model variables that performed best for each species and included in the final analysis are as follows: *B. auricomis*: imperviousness and solar radiation, *B. bimaculatus*: elevation and solar radiation, *B. citrinus*: solar radiation and wind, *B. fernaldae*: Elevation and canopy, *B. fervidus*: imperviousness and solar radiation, *B. griseocollis*: imperviousness and solar radiation, *B. impatiens*: canopy, elevation, and wind, *B. perplexus*: elevation and imperviousness, *B. sandersoni*: canopy and elevation, *B. vagans*: Elevation, canopy, land use, wind. (Table 1)

The threshold value for training presence was set to the 25 percentile (i.e., the value above which the model classifies correctly 75% of the training locations) for defining *Bombus* presences. All models were run to generate a logistic output for 5000 iterations. All other settings remained default unless otherwise noted (Phillips et al. 2009).

Sampling biases limit the generalizability of a model because sampling is not conducted randomly due to certain areas having more accessibility for sampling (i.e., roads, fields, etc.). This causes the model to give more probability of presence to these locations. This makes it difficult to differentiate the difference between a species' favorable habitat and an observer's favorable sampling terrain. (Merow et al. 2013). When sampling bias is accounted for, the null hypothesis states that individuals have only been observed in particular locations because those were the places that were sampled (i.e., individuals are uniformly distributed in geographic space) (Merow et al. 2013). To account for sampling bias, MaxEnt can limit where background points are selected from by applying a bias file. This provides MaxEnt with a background file that has the same bias as the presence locations (Young et al. 2011). Since most of the Megatransect collected *Bombus* samples from the roadside, my bias file consisted of a raster shapefile that included the roadway of the Blue Ridge Parkway, Skyline Drive, and Highway 441 in the Great Smoky Mountains

Results

A total of 3703 bumble bees were collected in 388 sites. 573 were collected within Shenandoah National Park (SHEN), 2434 were collected with the Blue Ridge Parkway (BRP), 696 were collected within the Great Smoky Mountains National Park (GSM). Sampling effort was not equal among the three parks. SHEN had 38 sites, GSM had 75 sites, and BRP had 275 sites. Bee abundance was standardized by calculating bees per person per hour of collection.

SHEN had the highest abundance with an average of 90 bees per person per hour, BRP had 48, and GSM had 27 (ANOVA $F_{2, 386} = 15.14796$ $P < 0.001$; Figure 2).

A total of 10 species of *Bombus* were represented in the specimens collected, including *B. auricomis*, *B. bimaculatus*, *B. citrinus*, *B. fernaldae*, *B. fervidus*, *B. griseocollis*, *B. impatiens*, *B. perplexus*, *B. sandersoni*, and *B. vagans* (Figure 2). The most common species by far was *B. impatiens* which made up 63% of all bees collected, followed by *B. vagans* with 15%. Most species were found in all three parks. *B. griseocollis* and *B. fervidus* were only found in BRP and SHEN while *B. fernaldae* was only found in BRP and GSM (Figure 3) No specimens of *B. affinis* or *B. terricola* were collected at any site. Almost all species were represented in the samples collected from at least two of three parks, except *B. auricomus*, which was found only on the Blue Ridge Parkway (Figure 4). The vast majority of collected bees were “medium-tongued” bees which include *B. impatiens*, *B. bimaculatus*, *B. griseocollis*, *B. perplexus*, and *B. vagans*. “Long-tongued” species that were collected include *B. fervidus* and *B. auricomis*. “Short-tongued” species that were collected include *B. sandersoni*. Parasitic species that were collected include *B. fernaldae* and *B. citrinis* (Figure 5).

Shannon-Wiener index (H') calculations based on total species collected in each park suggested that biodiversity was highest at sites along the Blue Ridge Parkway $H=0.918$, 1.235, and 1.166 for SHEN, BRP, and GSM, respectively; Figure 6A). However, mean H' calculations per site within each park suggested that diversity was higher at both Shenandoah National Park and the Blue Ridge Parkway (ANOVA $F_{2, 389} = 6.954147$, $P = 0.001$; Figure 6B).

Spearman correlations show that there is a significant positive correlation between elevation and Shannon diversity ($Rho = 0.33, P < 0.001$) (Figure 7), as well as a significant positive correlation between bee abundance and elevation ($Rho = 0.31, P < 0.001$). Species abundance significantly correlated with elevation with a positive relationship for *B. bimaculatus*, *B. fernaldae*, *B. impatiens*, *B. sandersoni*, *B. vagans*, and a negative relationship for *B. griseocollis* (Figure 8).

A total of 652 presence records among 10 species were used to train the Maxent program to create Environmental Niche Models (ENM). Maxent models show a wide variation in variables that describes species presence. The top two performing variables across all species were elevation and solar radiation (Table 1). Maps produced from this produced from ENMs showed clustered favorable habitat that varied for each species. *Bombus impatiens* had the most widespread habitat probability compared to the other species which tended to show preference towards higher elevation habitat (Figures 9-18).

Discussion

I coordinated a citizen science inventory of bumble bees (*Bombus* species) along a 900 km transect and determined the relative abundance, species distribution, and general habitat preferences of 10 *Bombus* species in the southern Appalachians. I identified: a) a regional geographic distribution of 10 *Bombus* species in the southern Appalachians; b) *Bombus* biodiversity of three major National Parks; c) and confirmed that two conservation priority

Bombus species, *B. terricola* and *B. affinis*, eluded a major *Bombus* targeted sampling effort, further validating previous claims of habitat contraction (Grixti et al. 2009; Cameron et al. 2011)

This large targeted sampling effort provides greater understanding of the status of *Bombus* in the southern Appalachians. A project such as this can help fill the gaps in native pollinator research. Compared to other key taxa, bees have relatively fewer baseline data (Council 2007). These data will be useful to land managers when designing and implementing conservation strategies for pollinators.

Shenandoah had the highest abundance (90 bees collected per person per hour) of the three parks. This could be due to the management practices in that park, as each park had their own mowing schedule for the road margins. Some volunteers noted anecdotally that Shenandoah appeared to have more flowers and was subject to less frequent mowing. Too frequent mowing can reduce flowering rates and result in loss of habitat for bumble bees. However, there were fewer citizen volunteers in Shenandoah and more experienced bee samplers, which may also have also biased abundance estimates.

Comparing biodiversity measures indicates that the Blue Ridge Parkway had the highest diversity, but that is likely due to its large size and because it traverses through more habitat gradients than Great Smoky Mountains National Park and Shenandoah National Park. Per site measures of biodiversity indicated that higher elevations had higher biodiversity, which aligns with previous studies that demonstrated that increased biodiversity is driven by habitat heterogeneity (Tews et al. 2004). The biodiversity measure used in this study was the Shannon-Wiener index, which a basic measure of species richness and evenness. However, each site had

varying sampling effort based on sampler experience and collection timing, which could provide an incomplete picture of biodiversity in my analysis. Further analysis using rarefaction and extrapolation techniques can provide a standardized measure of biodiversity based on sampling effort (Chao et al. 2014).

One of the drawbacks of citizen science projects is that many volunteers are inexperienced in data collection while others might be proficient. Therefore, abundance data can be difficult to compare. Biased collection data can still be useful as long as conclusions aren't drawn from abundance data. However, species diversity and relative abundance can be useful (Dickinson et al. 2010). The roadside sampling locations also introduce potential bias. The project was designed with ease of access to sampling sites by utilizing roadside habitat as collection sites. Roads can be home to a high diversity of pollinators because roadsides tend to be rich in meadow plants that are preferred by *Bombus* spp. (Saarinen et al. 2005). However, with no sites located further (> 0.5 km) from the road, this project may have only captured a limited number of species; though sampling bias should be minimized because the three park roadways that this study examined covered a broad range of habitat types.

One of the alarming trends of this study was the overwhelmingly unequal species representation. *B. impatiens* made up more than half of all specimens collected. They were found throughout the elevation gradient and all of the habitat types throughout the study area. It is clear that *B. impatiens* is very adaptive to many habitat types and may begin, or has already begun, to displace other more specialized species of *Bombus*. Climate change can cause high elevation habitats to become warmer and more suitable to lowland species. An upward shift of a lowland

species can create more competition for high elevation specialists (Miller-Struttmann et al. 2015). The numerical dominance of *B. impatiens* in most habitats indicates that it is likely capable of outcompeting other species in a changing climate and ecosystem.

Environmental Niche Models (ENM) produced high resolution maps of species occurrence probabilities for the southern Appalachians. As demonstrated in the other analyses in this project, *B. impatiens* had the highest probability of occurrence throughout the entire study area, compared to other species. These maps could be of importance to identify areas of key habitat for each species during conservation management. Additionally, they could be used to focus survey efforts to in future studies. ENMs have also been used to model species dispersal paths using least cost analysis (McRae & Shah 2009).

I found no *B. affinis* or *B. terricola* specimens among the 3700 individual bees collected during the BRBBM. This further confirms that these species are extirpated from the southern Appalachians. Future studies should determine whether other species are following a similar trajectory while conserving as much habitat and as many dispersal paths as possible. (Jacobson et al. 2018) examined the population declines of a number of species of bumble bees, concluding that *B. affinis* and *B. terricola* had been locally extirpated from most of their former range. Other species such as *B. vagans* began to show similar range contractions and replacement with more common species such as *B. impatiens*. Here in the Southern Appalachians, *B. vagans*, *B. sandersoni*, *B. perplexus*, have the potential for decline in the same way as *B. affinis* and *B. terricola* due to their similar habitat preferences and ecological niches as well as the potential of being replaced by competitors such as *B. impatiens*. A more in-depth analysis of high elevation

Bombus habitat requirements, nesting requirements, dispersal preferences, and population genetics would be advantageous to help managers preserve populations from local extirpation.

Table 1 Maxent ENM variable results by species

Species	# Training samples	Training AUC	Canopy		Elevation		Imperviousness		Landuse		Solar radiation		Wind	
			contribution	importance	contribution	importance	contribution	importance	contribution	importance	contribution	importance	contribution	importance
<i>Bombus auricomis</i>	2	0.9991	0	0	0	0	2.5899	51.942	76.5629	0	11.6294	44.3837	9.2178	3.6743
<i>Bombus bimaculatus</i>	94	0.9876	5.8772	9.3312	7.9227	21.9142	3.8307	8.5726	27.3945	1.7845	8.0226	50.5645	46.9523	7.8331
<i>Bombus citrinus</i>	26	0.9972	4.6502	5.8773	0.2516	3.5657	0.7	4.4356	46.6993	0.1317	5.1394	57.007	42.5595	28.9826
<i>Bombus fernaldae</i>	11	0.9993	0.3507	7.3952	77.0445	92.5785	0	0	22.6049	0.0263	0	0	0	0
<i>Bombus fervidus</i>	6	0.9984	10.1205	1.9267	0	0	8.845	51.0132	60.5957	0.406	3.3403	35.5548	17.0984	11.0993
<i>Bombus griseocollis</i>	58	0.9933	3.4283	2.0789	4.0074	2.5337	7.382	41.5377	35.2168	4.84	7.2724	38.1157	42.693	10.8939
<i>Bombus impatiens</i>	237	0.97	12.0656	33.6673	7.7794	24.1067	2.0552	4.7141	31.8352	5.1367	2.7892	4.2733	43.4754	28.1019
<i>Bombus perplexus</i>	23	0.9982	7.6719	12.1391	5.0421	25.4528	9.6671	39.6275	42.8617	2.0918	1.1066	7.567	33.6507	13.1218
<i>Bombus sandersoni</i>	52	0.9921	9.9684	14.6168	18.6629	63.4233	0.9199	1.4609	28.5743	5.085	1.5738	7.9129	40.3007	7.5011
<i>Bombus vagans</i>	143	0.9819	5.7373	6.0076	26.9132	80.1859	2.7369	3.1754	24.4224	4.7049	1.153	0.2731	39.0373	5.6531

Table 2 Spearman correlation (ρ) of species abundance by elevation

Species	ρ	p value
<i>B. auricomis</i>	-0.040	0.430
<i>B. bimaculatus</i>	0.156	0.002
<i>B. citrinus</i>	-0.019	0.712
<i>B. fernaldae</i>	0.250	0.000
<i>B. fervidus</i>	-0.032	0.535
<i>B. griseocollis</i>	-0.136	0.007
<i>B. impatiens</i>	0.112	0.027
<i>B. perplexus</i>	0.081	0.111
<i>B. sandersoni</i>	0.345	0.000
<i>B. vagans</i>	0.530	0.000

Figure Legends

Figure 1 *Bombus* collection sites for the Blue Ridge Bumble Bee Megatransect. Green markers indicate collection sites.

Figure 2 Mean bumble bees caught per hour at the three national parks in the Southern Appalachians. SHEN, BRP and GSM are Shenandoah National Park, Blue Ridge Parkway, and Great Smoky Mountains, respectively. Error bars represent standard error. Bars with different letters are significantly different from one another (ANOVA $F = 15.14796$ $P < 0.001$).

Figure 3 Relative abundance of bumble bee species present at each national park. SHEN, BRP and GSM are Shenandoah National Park, Blue Ridge Parkway, and Great Smoky Mountains, respectively.

Figure 4 Distribution of each species among the three national parks. SHEN, BRP and GSM are Shenandoah National Park, Blue Ridge Parkway, and Great Smoky Mountains, respectively.

Figure 5 Ecological niches of *Bombus* collected from each national park. Bars show relative abundance of short, long and medium-tongue length species among samples collected. SHEN, BRP and GSM are Shenandoah National Park, Blue Ridge Parkway, and Great Smoky Mountains, respectively.

Figure 6 Bumble bee biodiversity at the three national parks in the Southern Appalachians as determined by Shannon's Diversity Index. A) Whole site diversity based on total species collected per park, and B) diversity per site (milepost) within the three parks. SHEN, BRP and GSM are Shenandoah National Park, Blue Ridge Parkway, and Great Smoky Mountains,

respectively. Error bars represent standard error. Bars with different letters are significantly different from one another (ANOVA $F = 6.954147$, $P = 0.001$).

Figure 7 Spearman correlation between per site Shannon diversity and site elevation ($Rho = 0.329$ $P < 0.001$)

Figure 8. Spearman Correlation between species occurrences and elevation of sampling site. Asterisk indicates correlation is significant ($P < 0.05$)

Figure 9 *B. vagans* Maxent environmental niche model probability of occurrence. Blue indicates a low likelihood of occurrence. Red indicates a high likelihood of occurrence.

Figure 10 *B. auricomis* Maxent environmental niche model probability of occurrence. Blue indicates a low likelihood of occurrence. Red indicates a high likelihood of occurrence.

Figure 11 *B. bimaculatus* Maxent environmental niche model probability of occurrence. Blue indicates a low likelihood of occurrence. Red indicates a high likelihood of occurrence.

Figure 12 *B. citrinus* Maxent environmental niche model probability of occurrence. Blue indicates a low likelihood of occurrence. Red indicates a high likelihood of occurrence.

Figure 13 *B. fernaldae* Maxent environmental niche model probability of occurrence. Blue indicates a low likelihood of occurrence. Red indicates a high likelihood of occurrence.

Figure 14 *B. fervidus* Maxent environmental niche model probability of occurrence. Blue indicates a low likelihood of occurrence. Red indicates a high likelihood of occurrence.

Figure 15 *B. griseocollis* Maxent environmental niche model probability of occurrence. Blue indicates a low likelihood of occurrence. Red indicates a high likelihood of occurrence.

Figure 16 *B. impatiens* Maxent environmental niche model probability of occurrence. Blue indicates a low likelihood of occurrence. Red indicates a high likelihood of occurrence

Figure 17 *B. perplexus* Maxent environmental niche model probability of occurrence. Blue indicates a low likelihood of occurrence. Red indicates a high likelihood of occurrence.

Figure 18 *B. sandersoni* Maxent environmental niche model probability of occurrence. Blue indicates a low likelihood of occurrence. Red indicates a high likelihood of occurrence.

Figures

Figure 1



Figure 2

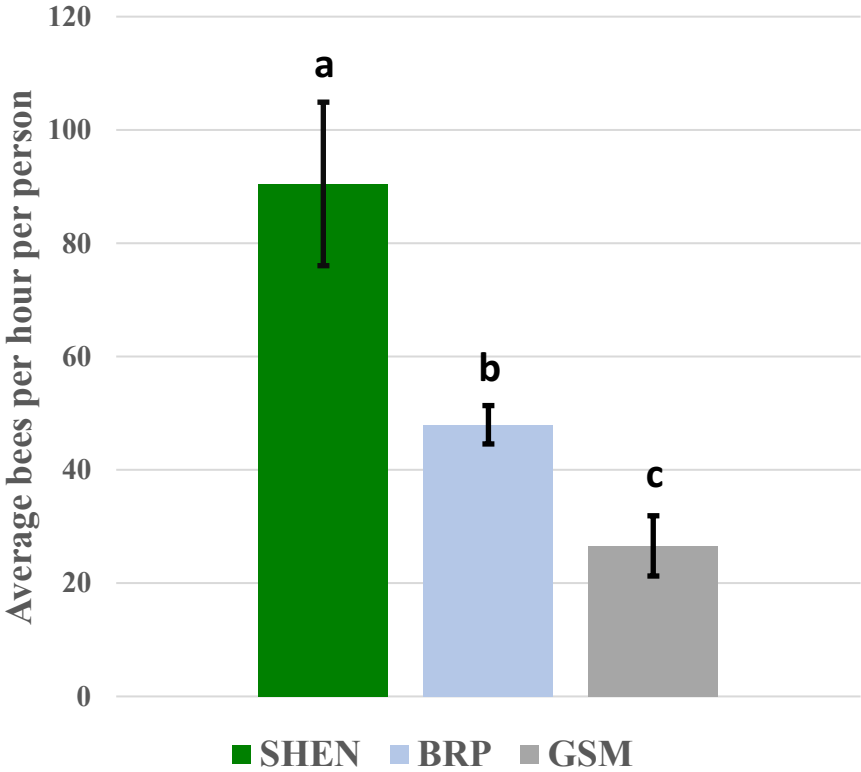


Figure 3

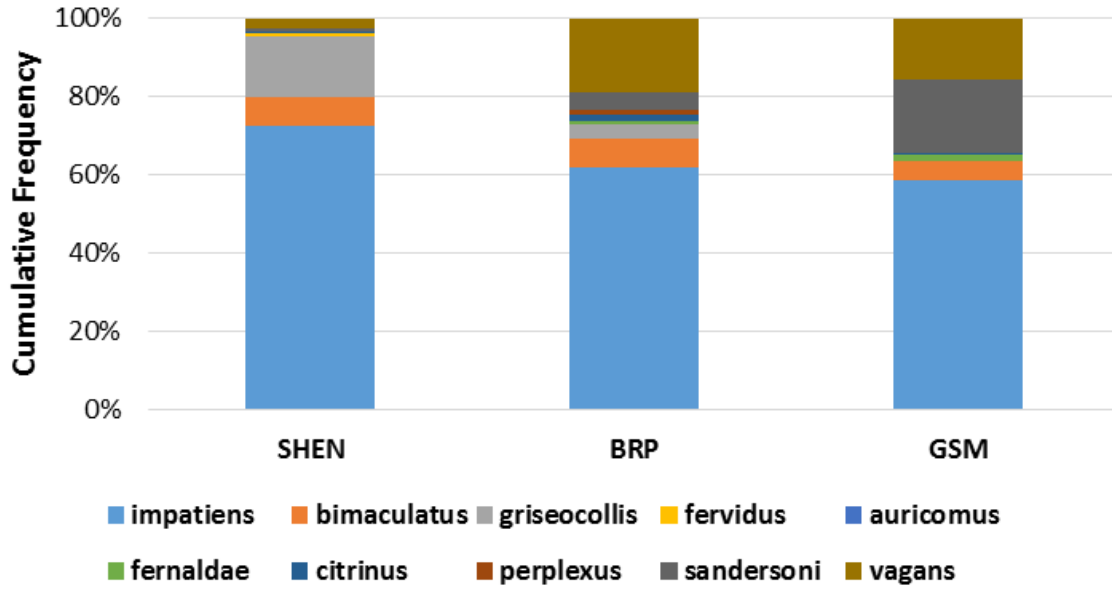


Figure 4

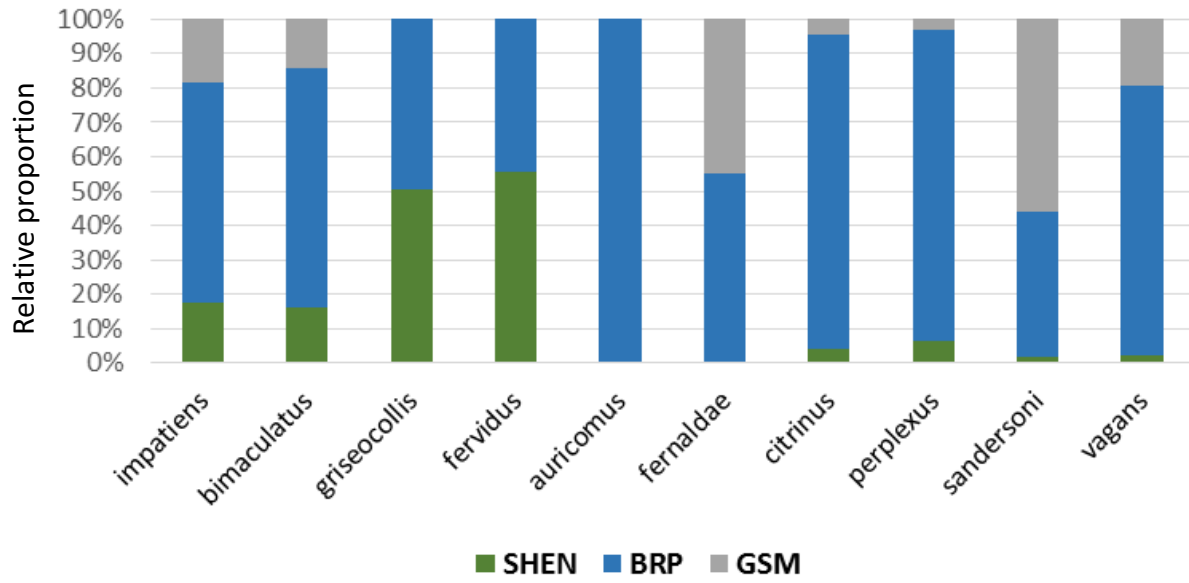


Figure 5

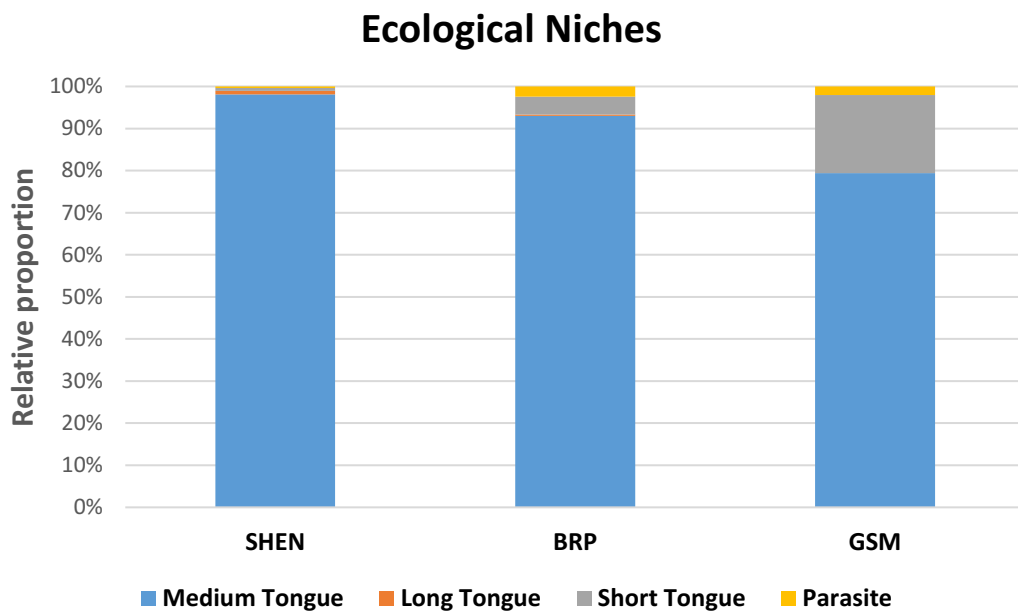


Figure 6a)

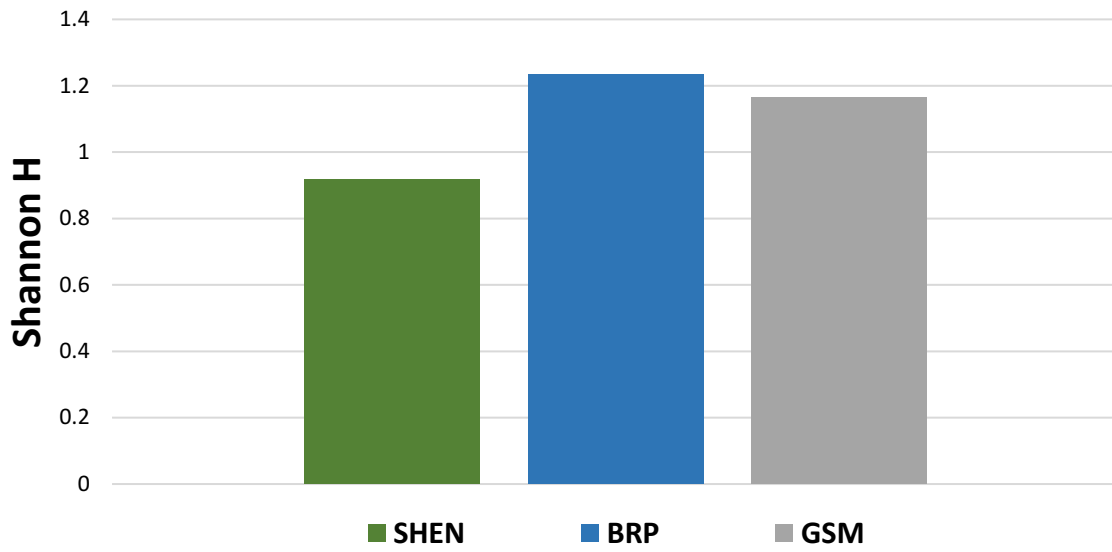


Figure 6b)

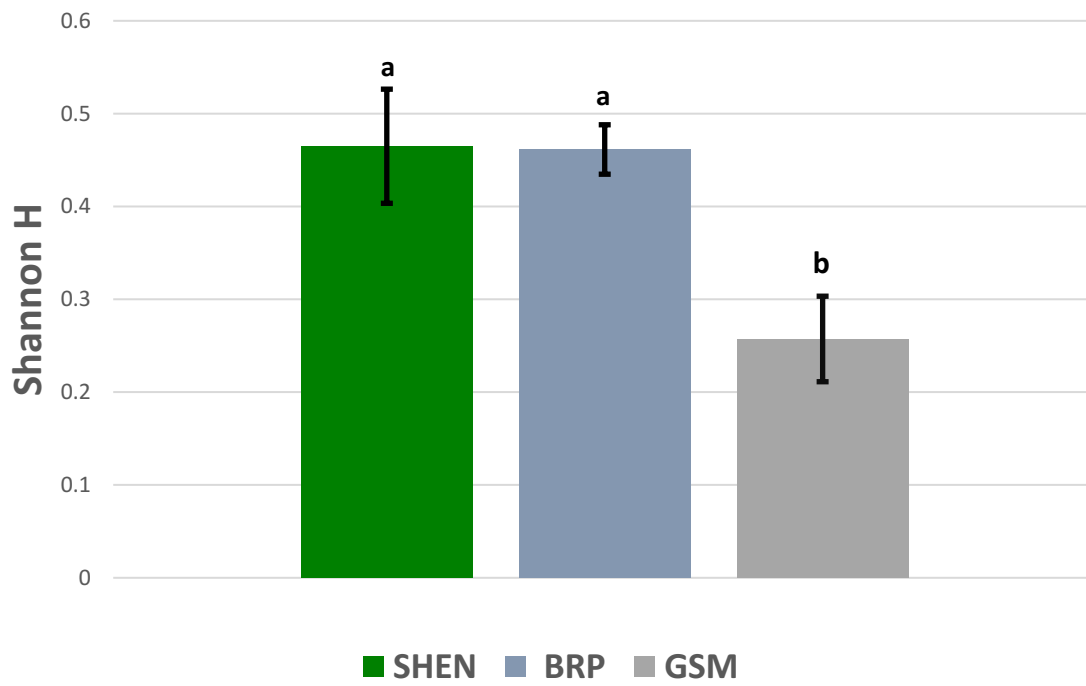


Figure 7

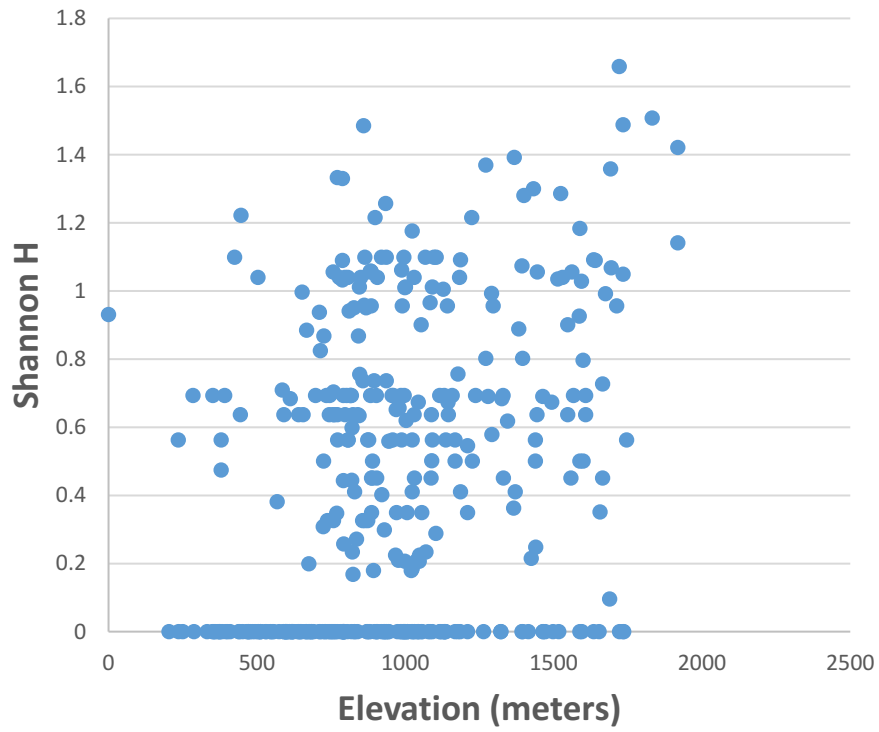


Figure 8

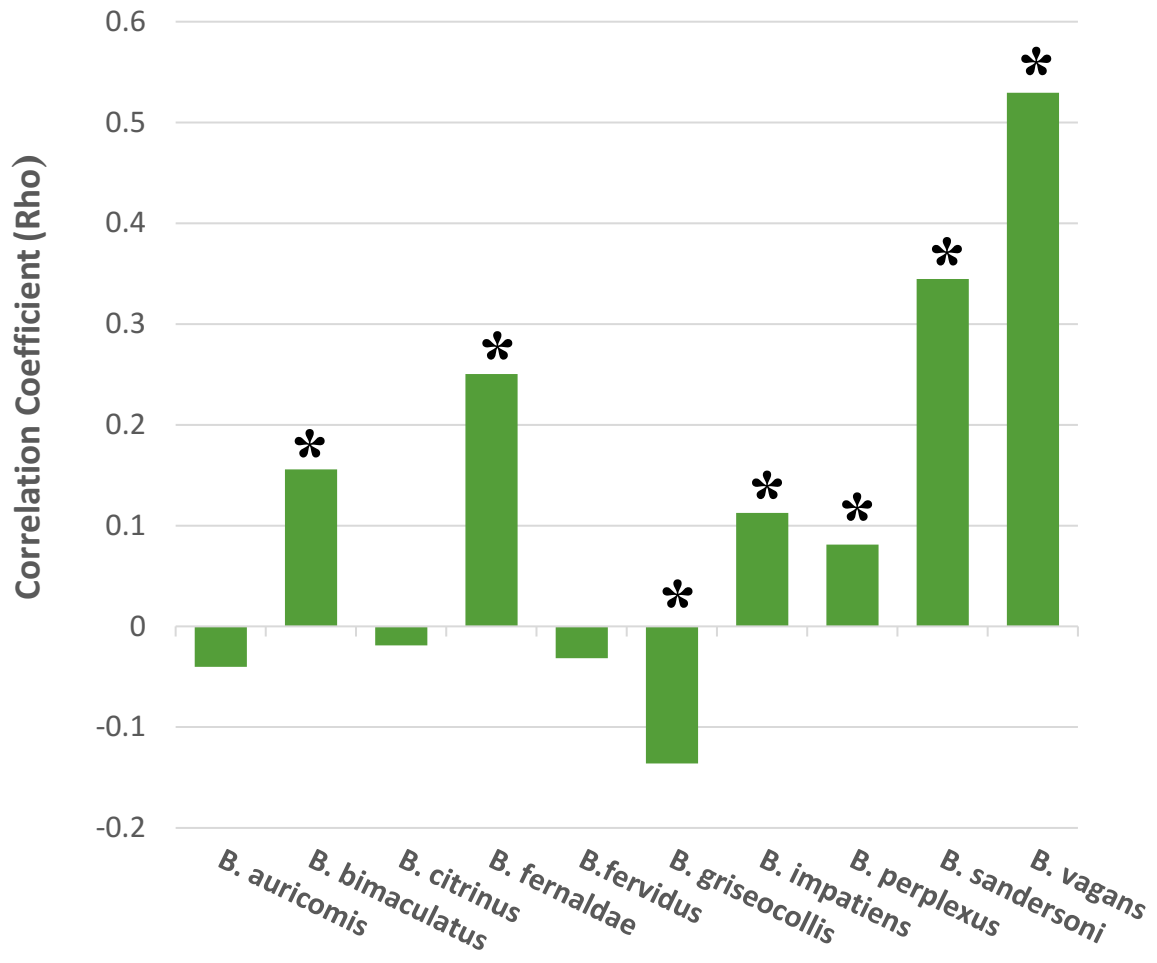


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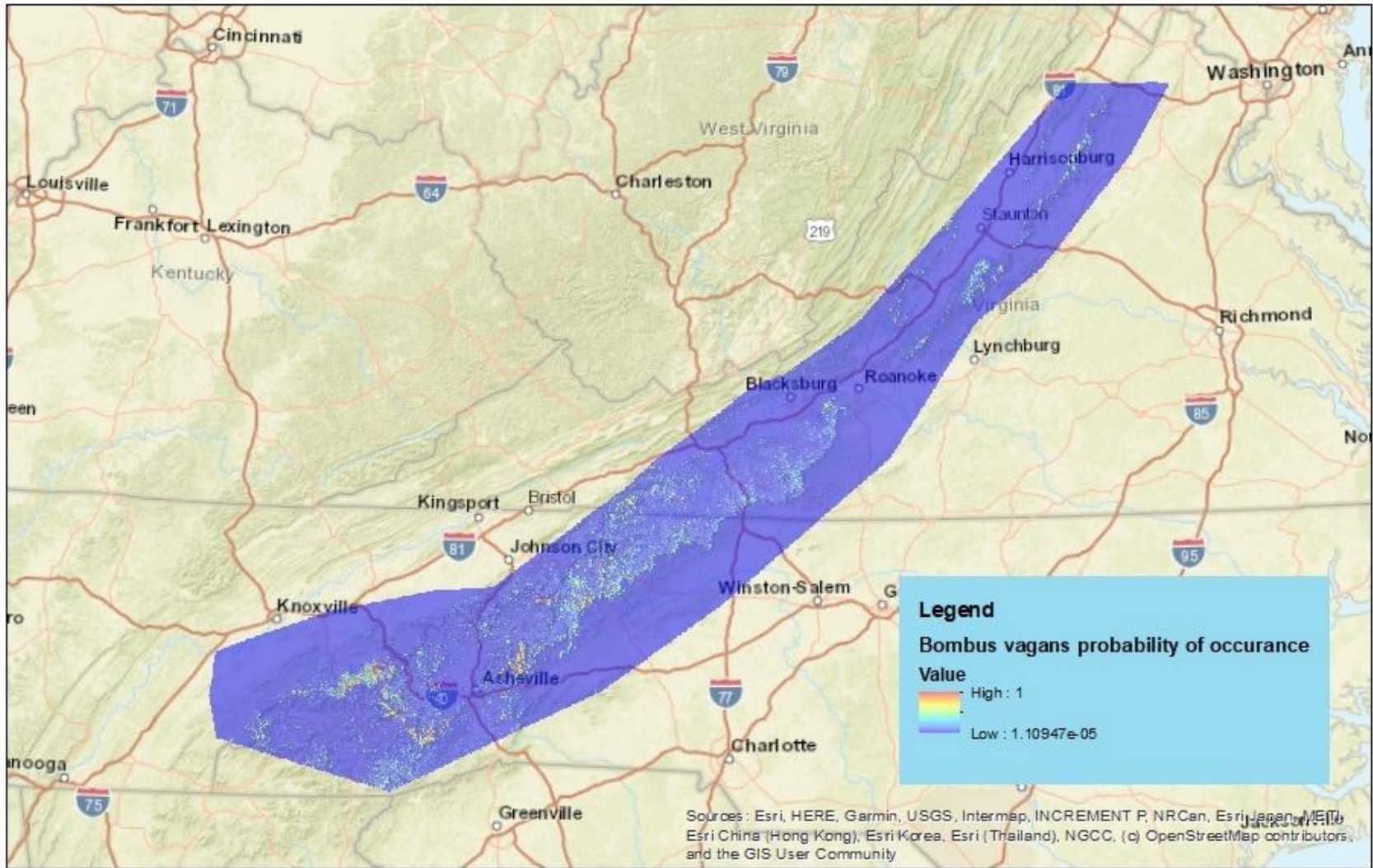


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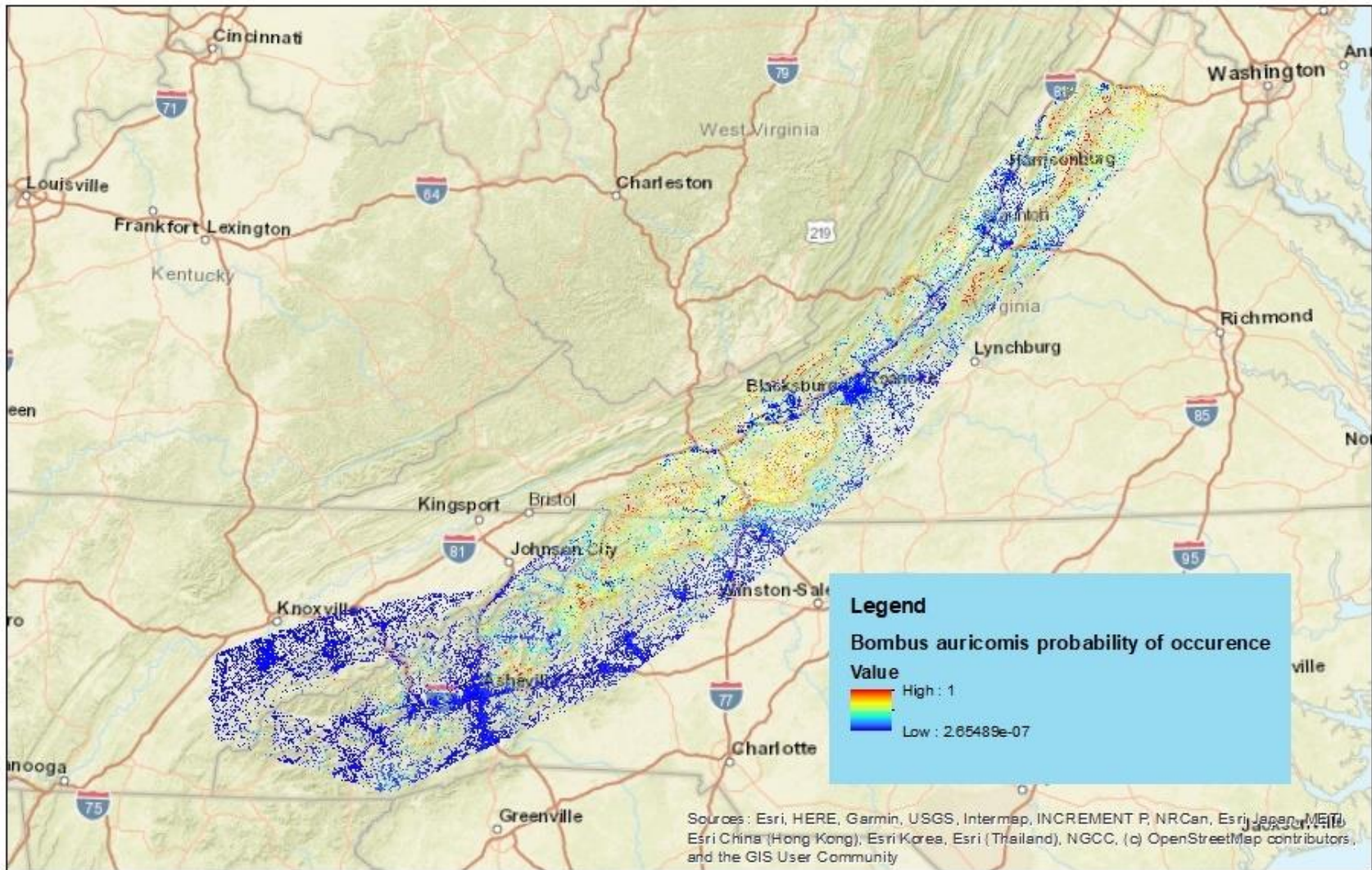


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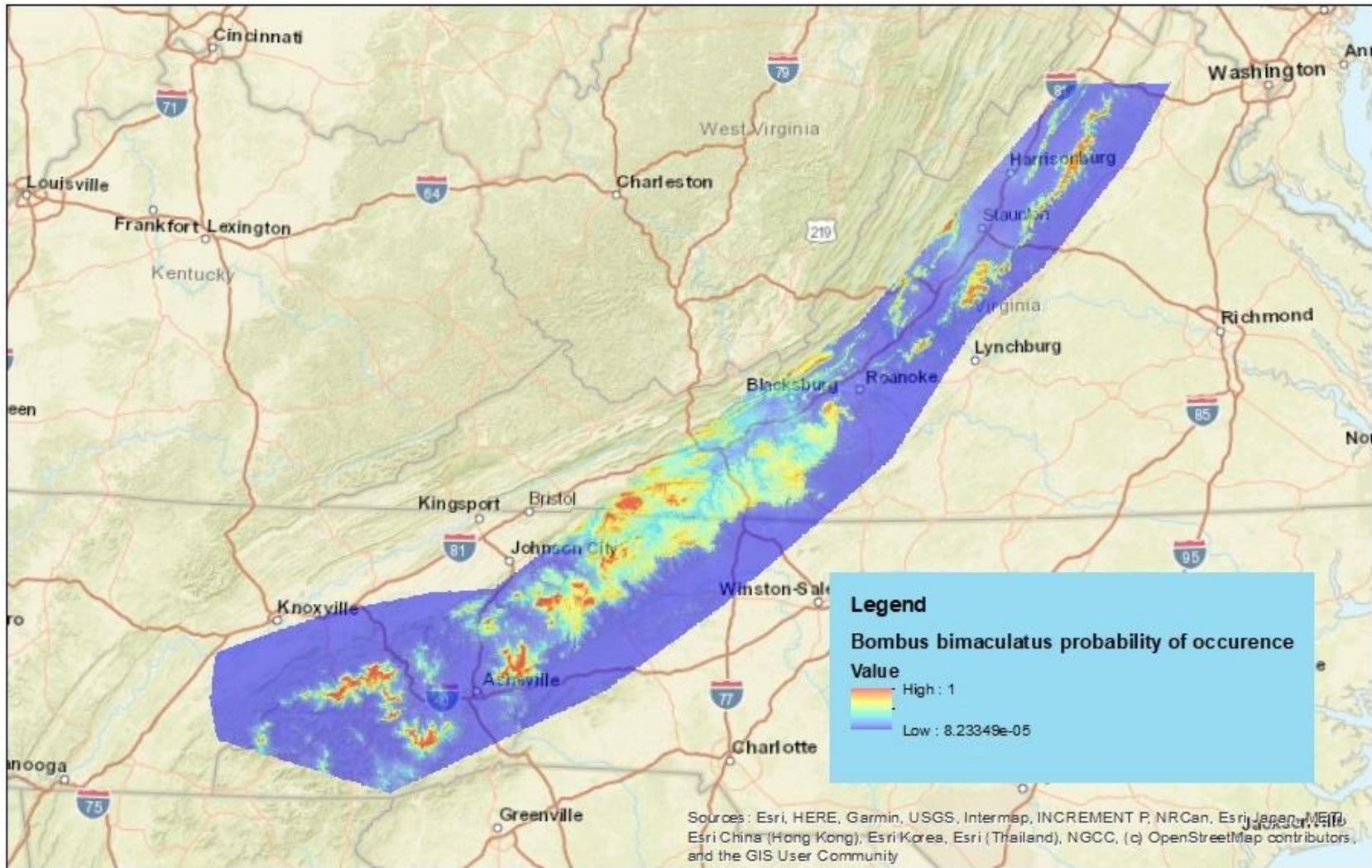


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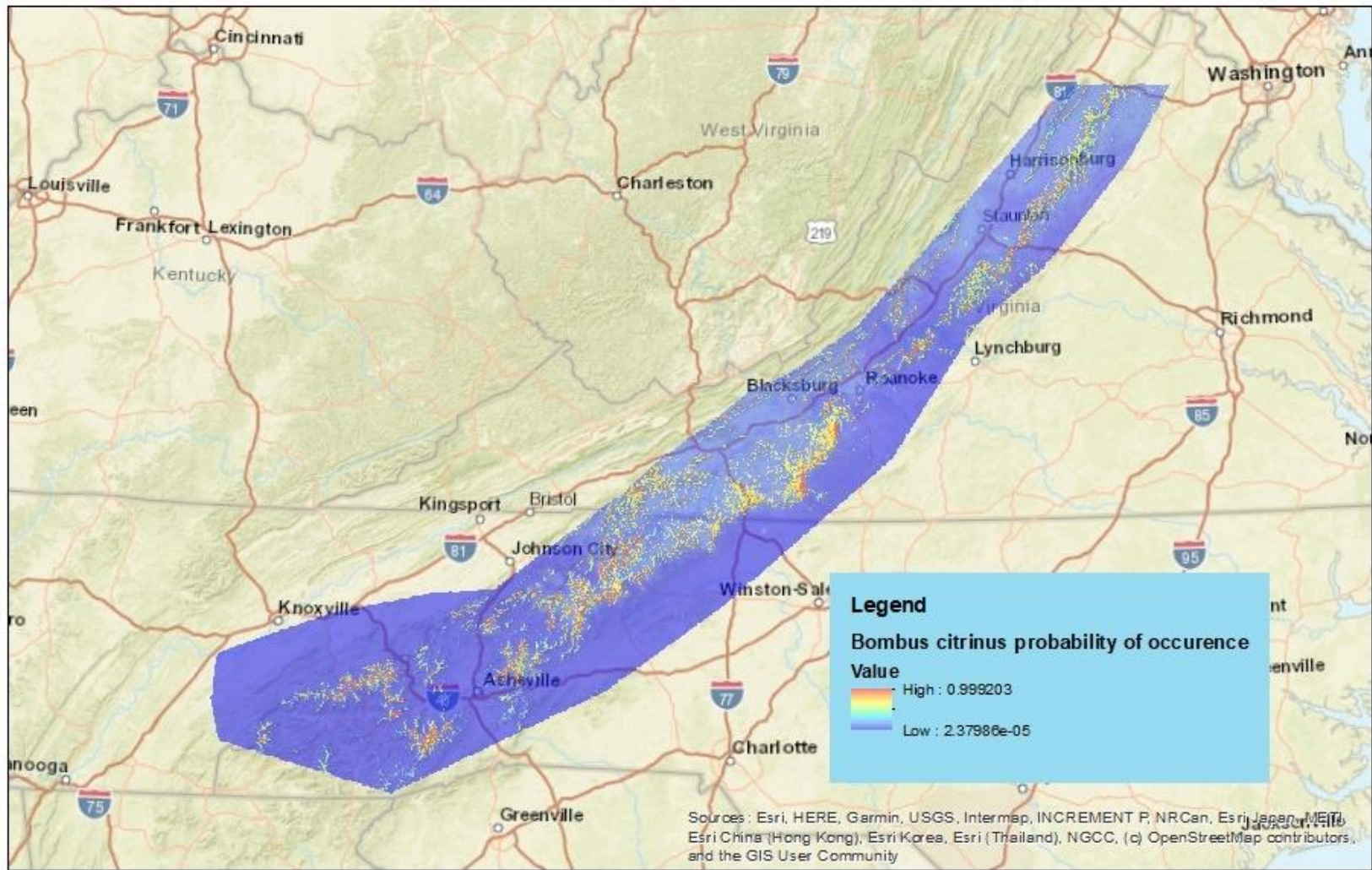


Figure 13

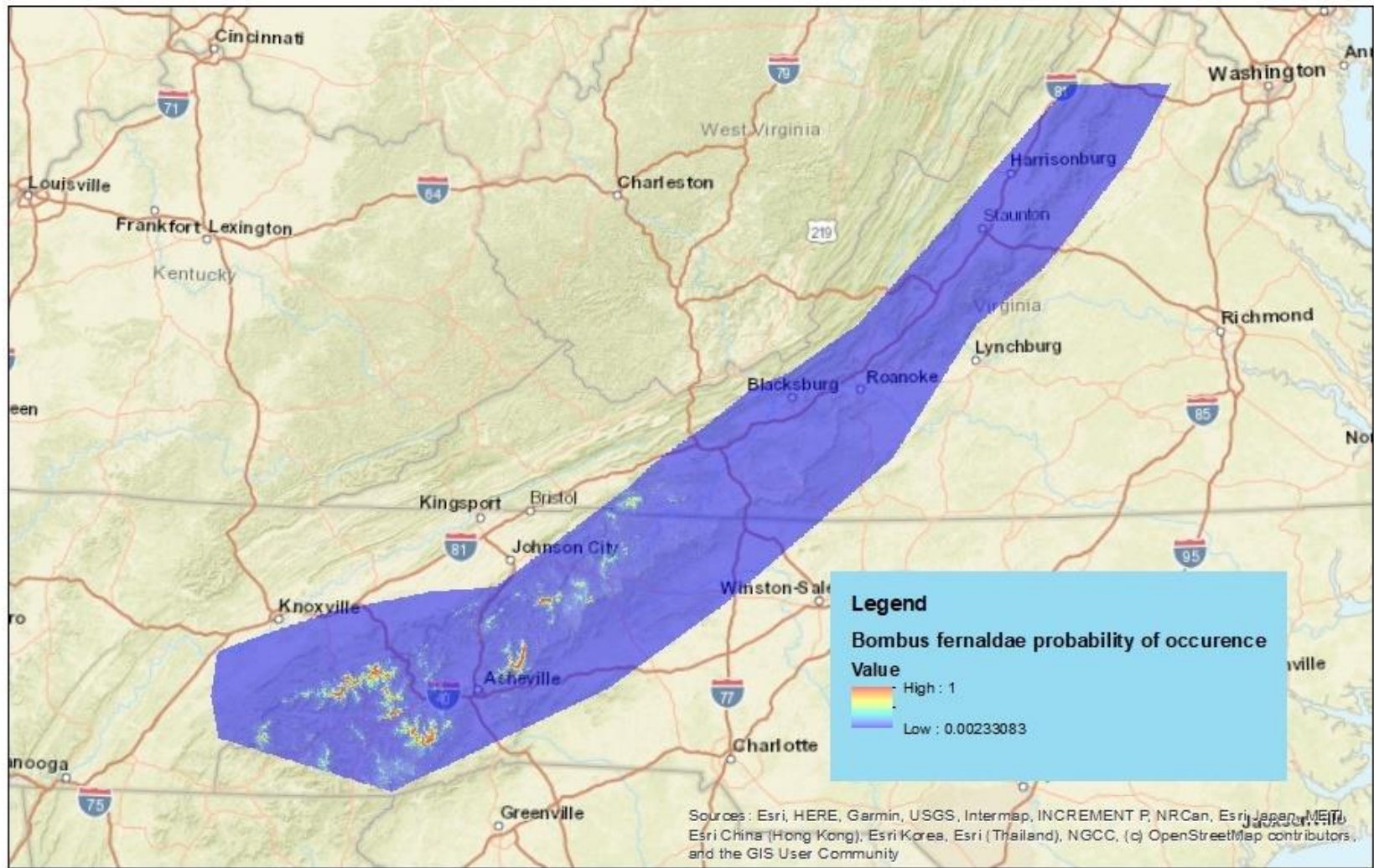


Figure 14

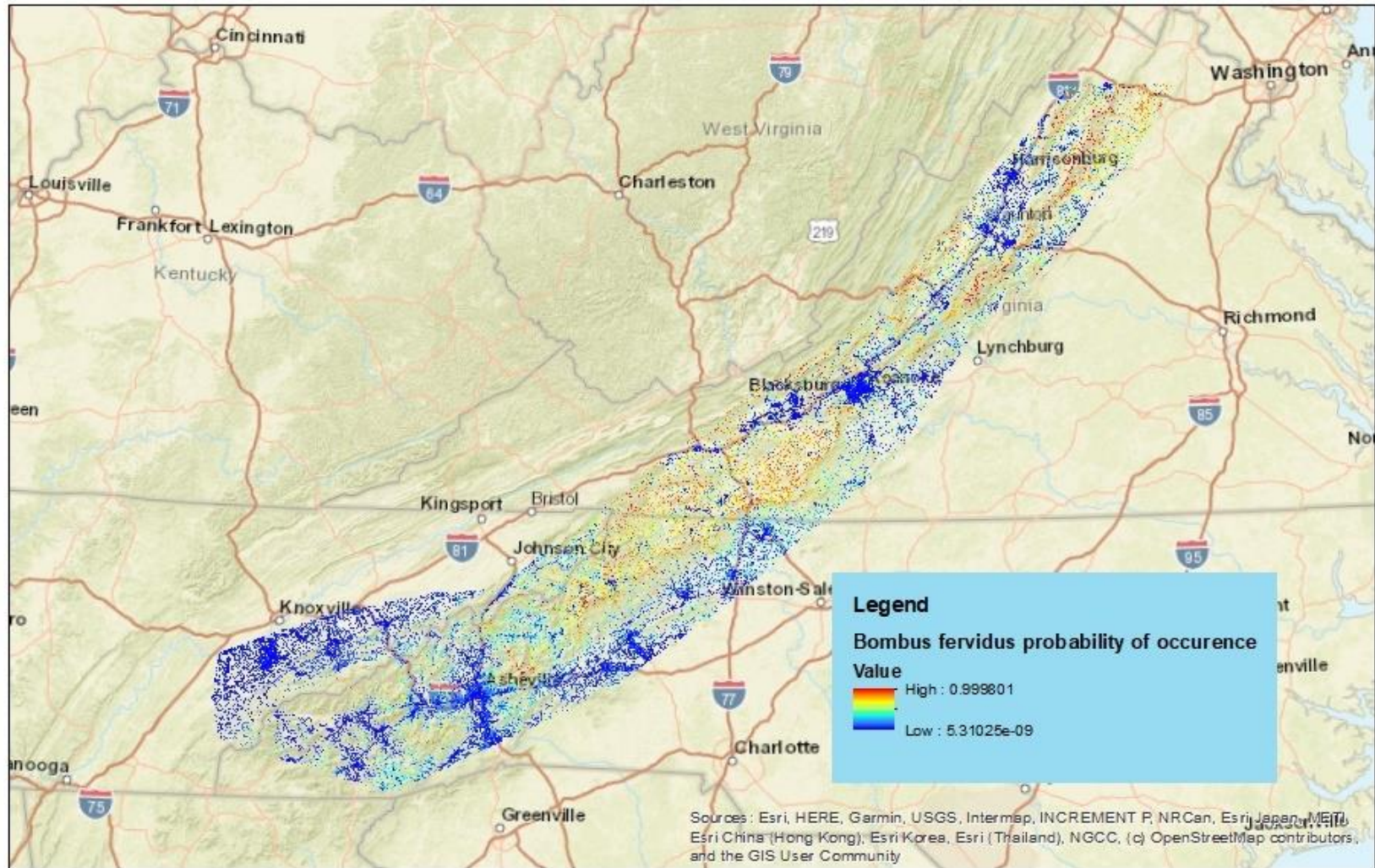


Figure 15

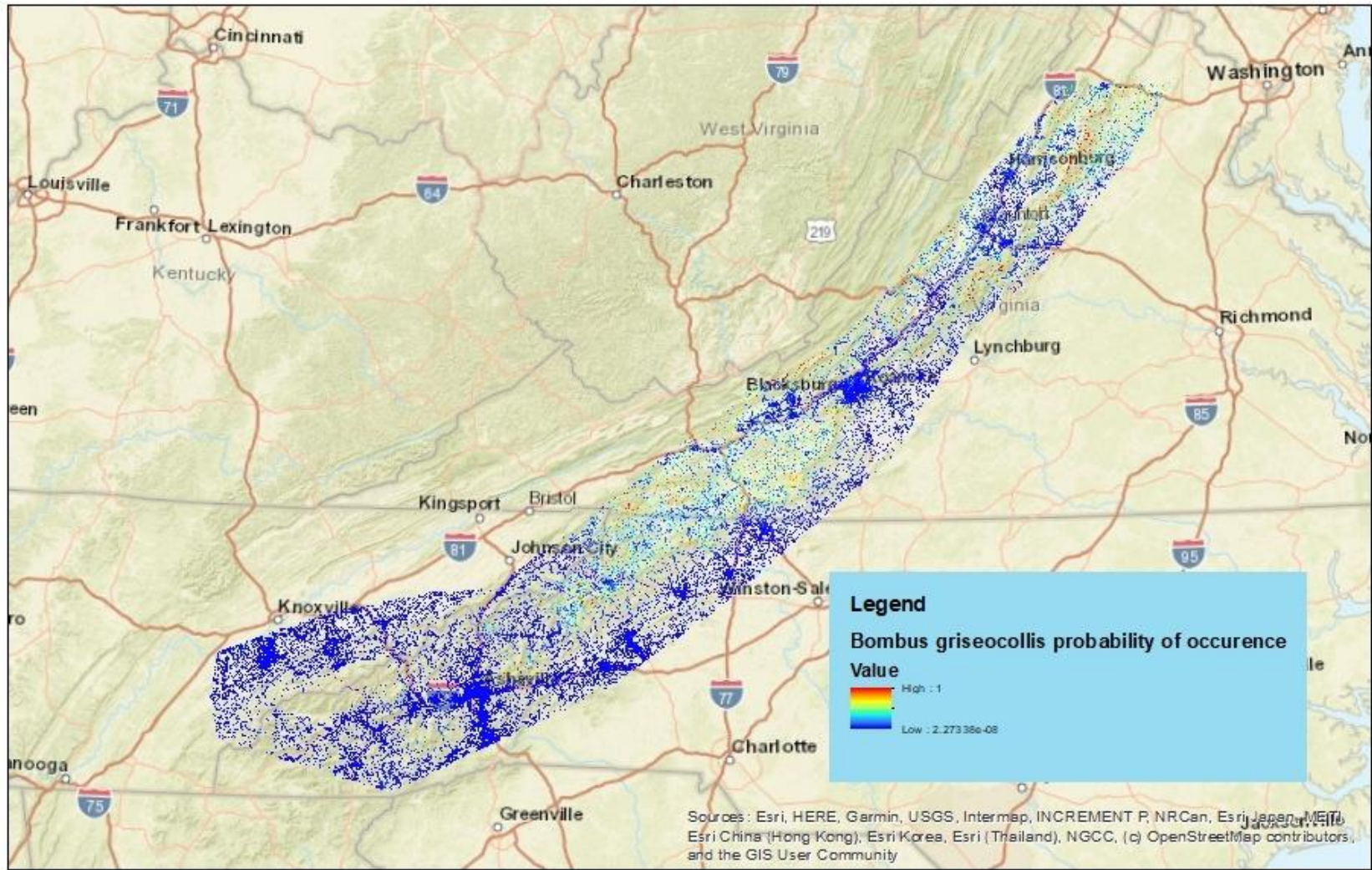


Figure 16

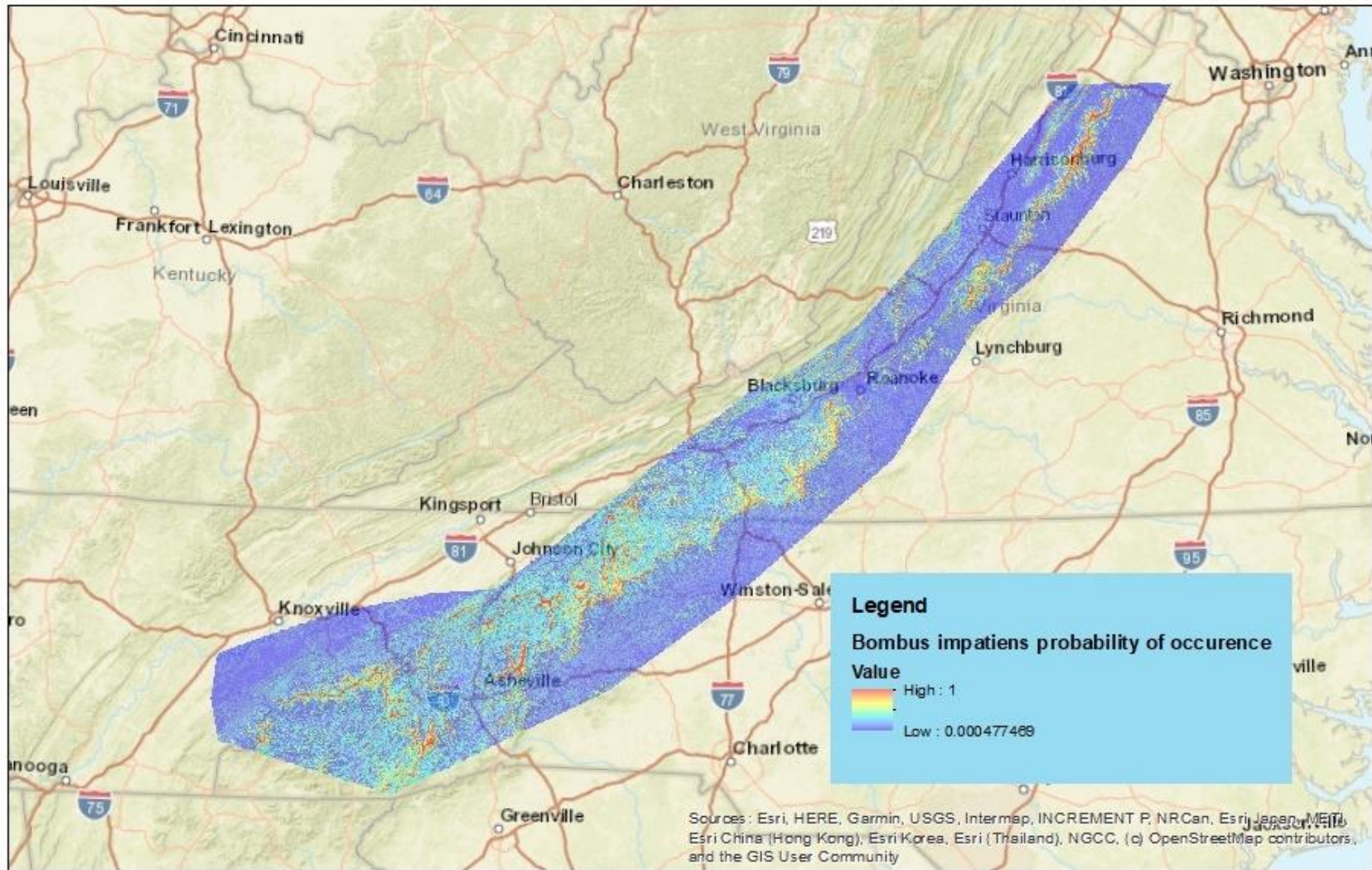


Figure 17

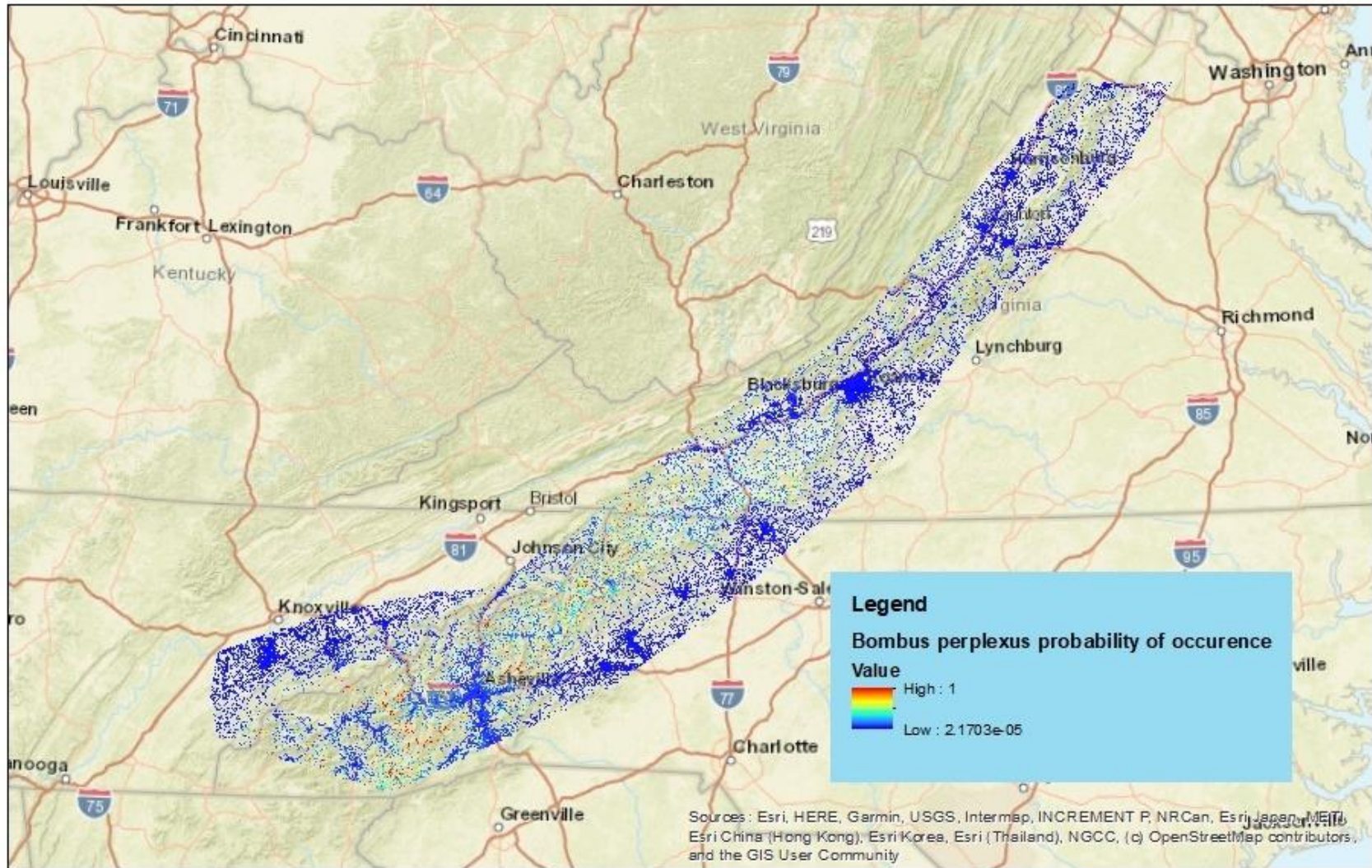
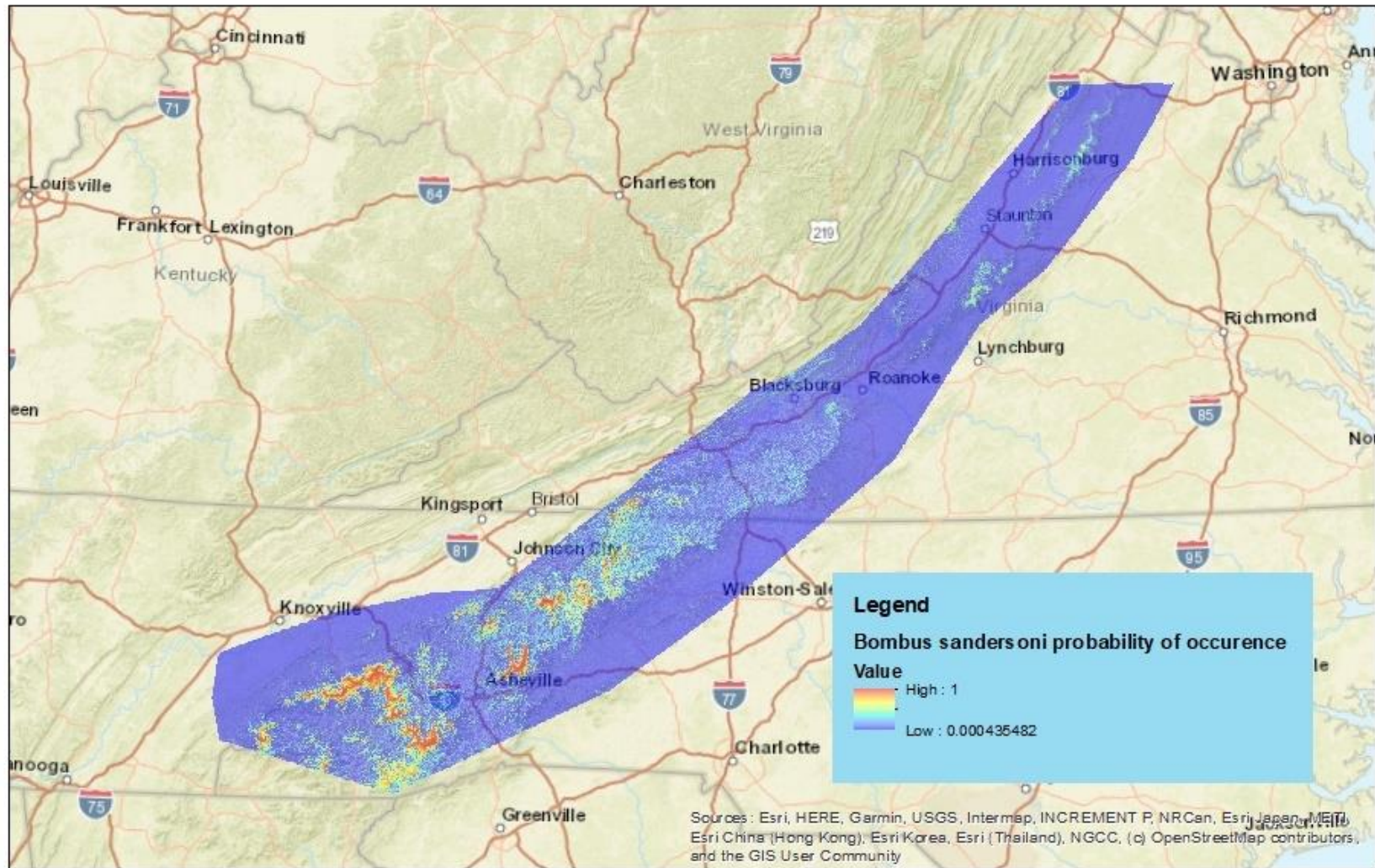


Figure 18



Chapter 2

Genetic Structure and Dispersal Patterns of *Bombus vagans* in the Southern Appalachians

Introduction

Insect pollinators play a critical role in maintaining plant communities. They are responsible for 90% of pollination among angiosperm plants (Ollerton et al. 2011), with bees being among the most efficient of pollinators (Roubik et al. 1995). Bees provide a valuable ecosystem function of pollinating a large majority of plants that support food chains, a number of codependent species of rare plants, as well as providing pollination to human-planted crops. Bumble bees in the genus *Bombus* (Hymenoptera: *Apidae*; *Bombinae*) are one of the most common and notable groups of bees.

Bumble bee decline

Bumble bees are widespread and abundant, however certain species of bumble bees have been in decline in recent years (Cameron et al. 2011; Colla et al. 2012). Native bee decline is likely attributed to a number of anthropogenic ecosystem changes including pesticides, introduced invasive species, diseases, and habitat loss or fragmentation (Goulson et al. 2015; Winfree et al. 2015). Bumblebees, in particular, are more susceptible to population loss due to their low effective population size and monoandrous mating system (Darvill et al. 2006).

Bumble bees form social colonies characterized by low genetic diversity; a single queen produces many cohorts of non-breeding female workers and one cohort of breeding males and females at the end of the summer. Sex determination is haplodiploid, where female offspring (workers and new queens) develop from fertilized eggs, giving the offspring diploid chromosomes, while the males develop from unfertilized eggs, making them haploid. Haploid males produce sperm that are identical to their own DNA, which decreases the amount of genetic diversity in their offspring; even diploid female offspring resulting from monandrous queen matings are 75% related to each other (Goulson 2010).

Dispersal

Dispersal across the landscape is critical for the maintenance of genetic diversity in bumblebees (and many other species) and functions to prevent inbreeding which enables them to recover from population fluctuations (Jha & Kremen 2013). Bumble bee dispersal generally occurs during three life cycle phases, 1) newborn drones at the end of the summer leave the nest to find mates, 2) newborn queens leave the nest to find overwintering habitat, and 3) when overwintered new queens emerge from hibernation (diapause) to locate nests. Due to their small size and long range of flight, it has been difficult to physically track bumblebees during their yearly dispersal events, so little is known about their preferred dispersal routes or dispersal capabilities. A few studies have implemented small radio trackers to examine dispersal across very small scale distances (Hagen et al. 2011). A more feasible way to indirectly determine

dispersal paths is by genetic sampling and modeling gene flow. However, relatively few studies have examined landscape genetics among bumblebees. Goulson et al. (2011) examined island biogeography and gene flow among bumble bees in the Scottish Isles; others studied bumble bee gene flow in agricultural areas (Dreier et al. 2014), or in other manipulated landscapes (Goverde et al. 2002; Carvell et al. 2012). Lozier et al. (2011), Lozier et al. (2013), Whitley (2018), and Christmas et al. (2021) examined mountain biogeography and landscape genetics in the Rocky Mountains. Findings from these studies conclude that bumble bees exhibit a wide range of dispersal distances, from as little as 1 km in agricultural settings to 30 km in an island setting. It is clear that certain landscapes are more conducive to dispersal while others are more restrictive. The most complex landscapes seem to present the most frequent/challenging dispersal barriers for bumble bees, but more studies are needed to better understand these patterns.

Biogeography and dispersal in mountain ecosystems

Mountaintop biogeography closely resembles island biogeography, in that mountaintops are physically separated from other mountaintops by lowland valleys or alternate ecosystems. As climate change causes an increase in temperatures, the higher elevations become more habitable for lowland species which can, through competition, force high elevation specialists further up into higher elevations and cause the island effect to become more exaggerated (Freeman et al. 2018). Complex montane landscape may limit dispersal (Lozier et al. 2011). Increased isolation at high elevations can lead to an overall decrease in genetic diversity and have an impact on

adaptability. This could potentially lead to population decline and local extinction, as habitat fragmentation and the resulting genetic consequences are one of the major contributors to bumble decline (Cameron et al. 2011). However, studies of landscape genetics and population connectivity in high elevation bumble bees have been situated almost exclusively in populations of the US Intermountain West. My study investigates gene flow in high elevation landscapes of the Southern Appalachians, a region that has been under-studied in prior studies of bumble bee landscape ecology and genetics.

Metapopulations and dispersal

Questions of biogeography and dispersal across landscapes evoke the concept of metapopulation dynamics. A metapopulation is a conglomeration of numerous population patches with various levels of interconnectivity. Evaluating the metapopulation dynamics of a species is a key step in evaluating the fate of their long-term population stability (Gliddon & Goudet 1994). The ability of a species to disperse and maintain active gene flow between habitat patches will determine the resilience of a population (Hanski & Gilpin 1991). Stochastic events apply pressure to subpopulations by introducing genetic bottlenecks that could cause genetic drift. However, if subpopulations have sufficient interconnectivity, the consequences of such bottlenecks can be lessened by recovering lost genetic diversity from stable subpopulations.

Conservation strategies for a potentially declining species should take into account their dispersal ability in a fragmented habitat. A common dilemma among conservation biologists is

whether to preserve several small patches of habitat or a single large tract of habitat. If the species can sufficiently disperse among heterogeneous habitat patches, the “several small” approach would be the most impactful by preserving the maximum amount of breeding habitat. If the species requires specialized habitat for dispersal, then the “single large” approach would be most appropriate to preserve breeding and dispersal habitat (Hanski & Gilpin 1991).

An alternative approach to habitat conservation is to employ a hybrid model by identifying and preserving dispersal corridors that connect habitat patches (Bennett 1998, 2003). It can be difficult to identify the exact dispersal habitat that a species prefers, especially for small animals that cannot be physically tracked. An indirect approach to identifying dispersal routes involves geographic modeling using least-cost analysis. This method analyzes landscape features that are hypothesized to limit or promote dispersal for a given species based on their habitat preferences. Least-cost models can be further optimized by incorporating spatially explicit genetic relatedness data (Epps et al. 2007). Once corridors have been successfully identified, habitat can be preserved or be altered through targeted management efforts to benefit dispersal of the species of interest.

To drive bumble bee conservation, population genetics studies should be at the forefront of conservation efforts. Species with the potential to face population declines from fragmented habitat, are especially good candidates for population genetics studies and dispersal analysis. The focal species for my study is the native bumble bee *B. vagans*. It may show an exaggerated population genetic structure because it has narrowly defined habitat preferences. It is confined to only the high elevation mountain habitats in the southern US (Colla et al. 2011), which is more

likely to limit dispersal due to their complex topography. No other studies have examined population genetics in this species.

The main objectives of this study were to 1) determine the genetic structure of *B. vagans* in the southern Appalachians using microsatellite markers and 2) to evaluate *B. vagans* ability to disperse between subpopulations by modeling habitat corridors using landscape dispersal models that have been optimized by their correlation with genetic data.

It is predicted that if *B. vagans*' dispersal between subpopulations was being limited due to landscape barriers to gene flow, then individual populations would show low genetic diversity and heterozygosity while also showing genetic drift between populations. I hypothesized that *B. vagans* populations would exhibit signs of metapopulation dynamics by existing within numerous subpopulations of favorable habitat surrounded by stretches of unfavorable habitat. This pattern would manifest in a positive association of Isolation by Distance, wherein populations that are geographically closer have high genetic relatedness. Additionally, I would expect to see positive associations of Isolation by Resistance, wherein populations that are connected by corridors of suitable dispersal habitat would be more genetically similar. In a homogeneous landscape, geographic distance would be a reliable predictor of genetic relatedness. However, my study area is made up of heterogeneous habitat. Therefore, Isolation by Resistance models should account for more variation in genetic relatedness than Isolation by Distance models alone.

Methods

Bombus vagans samples for this study were collected in 2015, in the southern Appalachian Mountains in western North Carolina and Virginia along the Blue Ridge Parkway, Great Smoky Mountains National Park, and Shenandoah National Park. Collection sites were on roadside high elevation meadows along the 900 km study area, which was dubbed the Blue Ridge Bumble Bee Megatransect, a large scale citizen science based monitoring project aimed at determining species ranges and abundances along roadsides of National Parks as part of the All-Taxa Bioinventory (ATBI).

My intended target species *Bombus affinis* and *B. terricola* were lacking any from collected specimens in the Megatransect survey efforts; therefore, *B. vagans* was chosen as the focal species because it shares similar habitat to *B. affinis* and *B. terricola*, and *B. vagans* may suffer the same fate of drastic decline and genetic isolation. *Bombus vagans* was also among the most numerous species collected in the Blue Ridge Bumble Bee Megatransect, which provided many specimens to perform genetic analysis on to study the population genetic structure.

Study System

The focal species *Bombus vagans* is a relatively common bumble bee in northeastern US. Its range includes northern US and southern Canada. The range of *B. vagans* extends south to North Carolina and Tennessee but it is confined to only high elevation sites with a northern climate. *B. vagans* shares high elevation habitat in eastern US with a number of other

bumblebees such as *B. perplexus*, *B. sandersoni*, *B. affinis*, *B. terricola*, *B. citrinus*, *B. fernaldae*. All of these species occur sporadically at high elevations in the south but are more abundant at any elevation in the north (Colla et al., 2011).

Sampling Methodology

Sampling took place in July-August 2015 when workers were most abundant. Surveys took place at multiple sites in the southern Appalachians located in Great Smoky Mountains National Park, and the Blue Ridge Parkway in western North Carolina and eastern NC. At each site, bees were collected for approximately 10 minutes by netting any observed bumble bees. Captured bees were stored in 95% ethanol to preserve DNA. Specimens were later dried, pin-mounted and identified to species level. Identifications were made using methods described in Williams et al. (2014). Identifications were verified by Sam Droege at USGS Patuxent Wildlife Research center. Sites that had produced at least 10 individuals of *B. vagans* were chosen to be included in the genetics project (Figure 1).

Molecular methodology

DNA Extraction

DNA was extracted from bee tissue using the MoBio Ultraclean DNA Isolation Kit (MoBio Labs Inc.). The tissue for the DNA extraction was obtained from either abdominal or thorax muscle. DNA was then tested for quality and concentration using a spectrophotometer to

ensure a concentration above 20 ng/ μ L. All DNA samples were diluted to a standard 20 ng/ μ L concentration before being used in PCR. DNA samples were also tested on an agarose gel to ensure proper fragment size ranges by traveling through gel matrix.

PCR

Polymerase chain reactions (PCR) were performed to amplify microsatellite markers using the following primers: B10, B96, B119, B124, BTERN01, BTERN02, BL11, BL13, BT10, and BT28 as used in (Geib et al. 2015a) and described in (Estoup et al. 1995; Estoup et al. 1996; Reber Funk et al. 2006). Multiplex PCR were carried out on samples and run for combinations of the loci B124(FAM)-BL11(PET)-BL13(PET)-BTERN01(VIC)-BT10(NED) and B96(PET)-B119(VIC)-BTERN02(NED)-B10(FAM)-BT28(VIC) (fluorescent markers indicated in parentheses).

PCR reactions were 10 μ l in volume and consisted of 1 μ l of template DNA, approximately 340 μ l of UV-treated and reverse osmosis water, 220 μ l of Promega 5X Buffer, 61.6 μ l of MgCl₂, 66 μ l of dNTP's, approximately 270 μ l of combined reverse and forward primers, 22 μ l of Bovine Serum, and 8.8 μ l of Promega Flexi GoTaq® polymerase (Figure A11). Samples were denatured at 95°C for 7 minutes, followed by thirty 90-second cycles consisting of a denaturing step at 95°C for 30 seconds, an annealing step at 54°C for 30 seconds, and an extension step at 72°C for a further 30 seconds. This was then followed by a final extension step at 72°C for 1 hour, based on optimization trials (sensu Geib et al. 2015). Two control samples

were placed on each plate of 96 samples to ensure that all plates were behaving the same. These were the same two individual bees and remained constant on all plates sequenced. As an additional quality control measure, the complete PCR product was run on an agarose gel to ensure PCR bands separate properly and that the reaction produced a product that can be visualized on the DNA sequencer (Fragment Analyzer by Advanced Analytical Technologies.).

A master mix of 1000 uL of HiDi Formamide with 100 ul of Genscan LIZ 500 size standard ladder was made and 10 ul of that mixture was added to each of the 96 wells on a semi-skirted plate, then 3 ul of the complete PCR product was added to each well and then heated to 95°C for 3 minutes and then chilled on ice before being shipped overnight to the University of Georgia Genomics and Bioinformatics Lab (Athens, GA) before the run and to be visualized on a capillary DNA sequencer (Fragment Analyzer by Advanced Analytical Technologies.)

Genetic data analysis

Genotyping microsatellites

GeneMapper® 4.0 (Applied Biosystems, 2005) was utilized to import and analyze raw fragment files produced from sequencing. LIZ ladder peaks were checked for each individual to ensure all the appropriate base pair lengths. Microsatellite peaks were manually scored for each locus and individual and binned into discrete classes. Any individuals that failed to amplify were excluded from analysis. Marker BT10 failed to amplify for a majority of the individuals and was excluded from analysis.

Identification and removal of sibling workers

Sibling workers were identified and removed from further analyses using Colony v. 2.0 (Jones & Jinliang 2010). This ensures that genetic similarity between individuals was not overestimated by pseudo-replication of siblings. To assign sibship or parent-offspring relationships, Colony uses maximum likelihood methods. This is one of the most reliable methods for assigning sibship in bumble bees (Lepais et al. 2010), because full sisters share 75% of genes by descent (Goulson et al. 2011). Model parameters are as follows: I assumed lack of inbreeding, as no inbreeding data is available for bee within these populations. I assumed “male monogamy” and “female monogamy,” which is the most conservative approach and most bumble bee females tend to only mate with one male (Schmid-Hempel & Schmid-Hempel 2000) and male bees often lose their endophallus during mating, only allowing a single mating (Paxton 2005). “Without clone” was chosen because all offspring genotypes were assumed to come from distinctive individuals who were not clone mates. The genetic markers were specified as co-dominant, allelic dropout rate was set to 0.0000, and genotyping error was set to 0.0075. Most studies report an error rate between 0.5 and 1%, so 0.75% was selected for the error rate for all markers (Pompanon et al. 2005). All individuals were removed that had a probability of a full sibling dyad greater than 0.5. Care was taken to remove only one sibling from each sibling pair if in separate populations so that sample sizes for each population were not affected greatly. However, Population 13 was omitted due to the high number of siblings at that site.

Population genetics testing

Each collection site was treated as a discrete population for all population genetic analyses, except for sites 14 and 15. These sites were combined due to their very close proximity to each other. Hardy-Weinberg Equilibrium (HWE) and linkage disequilibrium (LD) tests were performed using the Markov chain method (dememorization = 1000, batches = 100, iterations per batch = 1000) using Genepopv 4.1.2 (Raymond & Rousset 1995). Tests of heterozygote excess and deficiency were performed for each locus and population as well as across all loci and populations using the Score U test in Genepop. Linkage disequilibrium was tested for each locus pair and population using log-likelihood ratio tests and probability in Genepop.

Population differentiation estimates

Allele frequencies, average alleles per locus, and the expected and observed numbers of homozygotes and heterozygotes were calculated for each population and locus in Genepop. Allelic richness and unbiased estimates of heterozygosity were estimated for each locus in Fstatv 2.9.3 (Goudet 1995). Genetic differentiation between populations were estimated with F_{ST} (Wright 1943), in Fstat. Pairwise F_{ST} were transformed to $F_{ST}/(1-F_{ST})$ with Genepop (Weir & Cockerham 1984). F_{IS} (inbreeding coefficient) was estimated for each locus and population in Fstat.

Genetic clustering

Bayesian genetic clustering approach was used to determine genetic population structure using Structure v 2.3.4 (Pritchard et al. 2000). Admixture and Correlated Allele Frequency models were used. Multiple runs were performed with K varying from 1 to 4, with six runs for each K value using 10,000 burn in periods and 50,000 Markov Chain Monte Carlo (MCMC) repetitions (Falush et al. 2003). I used Structure Harvester to determine K (Earl & vonHoldt 2012) using the mean of estimated Ln probability of data (LnP(D)) (Evanno et al. 2005). To obtain final Structure results, an additional long run with final K of 2 was performed using 50,000 burn in periods and 100,000 MCMC.

Estimating isolation by distance

I assessed the correlation between geographical distance and genetic differentiation (expressed as $F_{ST}/(1-F_{ST})$) with a nonparametric Spearman Rank Correlation Coefficient implemented in SPSS (IBM Corp 2019) and as described in Legendre and Fortin (2010).

Environmental niche modeling

I acquired multiple georeferenced environmental variables that were believed to a priori significance to *B. vagans* habitat preference. These included 19 bioclimatic variables known as WorldClim V1.4 (Hijmans et al. 2005), digital elevation model (DEM), land cover (land use) (USGS 2014), solar radiation, canopy cover, wind intensity, and impervious surfaces.

All bioclimatic and landscape variables were clipped to the Megatransect study area extent using ArcMap 10.3.1 (ESRI, Redmond CA). Each layer was projected into the WGS 1984 geographic coordinate system and converted to the same dimensions and cell size to allow for cross analysis. All environmental raster layers were exported to ASCII format using the ArcMap toolbox. Highly positively correlated variables were removed in order to avoid any bias in the contribution of each variable (Alvarado-Serrano & Knowles 2014). I used SDM toolbox add on for ArcMap to remove correlated variables (Brown 2014).

The correlation threshold was to $\theta > 0.70$ and one variable in each pair of correlated variables was retained. All occurrence data for analysis was limited to specimens collected from 2015 Bumble Bee Megatransect (Rayfield et al. 2015).

A total of 168 presence records of *B. vagans* were used in the Environmental Niche Model analyses.

Spatial Modeling

I created habitat suitability maps using environmental niche models (ENM) which uses the principle of maximum entropy as implemented in the program MaxEnt v 3.3.3 (Phillips et al. 2009; Elith et al. 2010). MaxEnt uses presence-only locality data and background points randomly sampled from the study area to estimate the species distribution that is closest to uniform (i.e., maximizes entropy), given information on the environmental conditions of the study area. Multivariate models were ran with all Bioclimatic variables together to determine

which covariates contributed most to species presence. Landscape variables were ran with various combinations of covariates to determine the model that best estimated environmental suitability for *B. vagans*. I developed 2 multivariate ENM's, after removing correlated variables, that modeled the effects of all bioclimatic variables, all landscape variables on *B. vagans* distribution. I developed 4 ENM's that modeled the synergistic effects of various combinations of landscape variables. Finally, I developed one univariate ENM on the Landuse variable because its values were categorical form and not compatible with subsequent resistance modeling.

The threshold value for training presence was set to the 25 percentile (i.e., the value above which the model classifies correctly 75% of the training locations) for defining *B. vagans* presences. All models were run to generate a logistic output for 5000 iterations and averaged over 10 sub-sampled replicates. A randomized 25 test individuals were used to test the model's efficacy and provide a corrected measure of model accuracy. All other settings remained default unless otherwise noted (Phillips et al. 2009).

Sampling biases limit the generalizability of a model because sampling is not conducted randomly due to certain areas having more accessibility for sampling (i.e., roads, fields, etc.). This causes the model to give more probability of presence to these locations. This makes it difficult to differentiate the difference between a species' favorable habitat and an observer's favorable sampling terrain. (Merow et al. 2013). When sampling bias is accounted for, the null hypothesis states that individuals have only been observed in particular locations because those were the places that were sampled (i.e., individuals are uniformly distributed in geographic

space, Merow et al. 2013). To account for sampling bias, MaxEnt can limit where background points are selected from by applying a bias file. This provides MaxEnt with a background file that has the same bias as the presence locations (Young et al. 2011). Since most of the Megatransect collected *B. vagans* samples from the roadside, my bias file consisted of a raster shapefile that included the roadway of the Blue Ridge Parkway, Skyline Drive in Shenandoah National Park, and Highway 441 in the Great Smoky Mountains National Park.

Modeling dispersal pathways and resistance to dispersal

To model potential dispersal pathways based on geographic variables, I used Circuitscape v4.0, which uses algorithms electronic circuit theory to create resistance and conductance surfaces (McRae 2006). To calculate dispersal paths between two points, Circuitscape models all possible pathways for dispersal across a landscape simultaneously and predicts the most likely paths using random walk probabilities (Spear et al. 2010). Connectivity between population patches increase as the number of connected pathways increase. These pathways in which individuals disperse are modeled by hypothetical electricity flows between two circuits (McRae 2006). The Circuitscape model assigns resistance values to each pixel on a raster map of a theorized relevant landscape parameter. The most easily-traveled landscape features receive low resistance values and the most difficult to navigate terrain receive high resistance values (McRae 2006). After assigning resistance values, Circuitscape calculates pairwise resistances between sites and create maps of current flow and voltage across a landscape (McRae 2006). The resulting pairwise resistance values can be modeled with genetic distance to elucidate the most

influential landscape variables to gene flow. The associated maps provide a visualization of corridors of dispersal, which can pinpoint the most important areas for maintaining habitat connectivity.

Estimating Isolation by Resistance

Patterns of isolation by resistance (IBR) were examined by coupling the habitat suitability models produced in MaxEnt with circuit theory approach implemented in Circuitscape. The various models were first input into MaxEnt to receive an output raster of habitat suitability, which then was input into Circuitscape and labeled as a conducting variable that facilitates dispersal. Raw variables were also modeled in Circuitscape without the first step of being modeled in Maxent. Parameters for used for Circuitscape included pairwise mode with the four-neighbor cell connection scheme for all models. The subsequent pairwise resistance distances were then correlated with genetic distances ($F_{ST}/1-F_{ST}$) to test for isolation by resistance (IBR) using non parametric Spearman Correlation rather than the traditionally used Linear regression and Mantel test, (Legendre & Fortin 2010) as implemented in SPSS (IBM Corp 2019).

Results

Out of 135 megatranssect sites where *Bombus vagans* was discovered, a total of 17 sites had at least 10 individual females and were incorporated into the genetic study. A total of 267 individuals were genotyped for 10 microsatellite loci. Thirty-five sister pairs were detected with sibling analysis, assuming female and male monogamy, the most likely scenario for *B. vagans*. After removing sibling workers a total of 232 individuals were left to conduct the subsequent genetic analyses (Table A6).

Population genetic testing

The total number of alleles detected per polymorphic locus ranged from 3 (locus BT28) to 48 (locus BL11) and the mean number of alleles per locus ranged from 1.5 ± 0.29 (BT28) to 15.64 ± 2.35 (BL11) (Table 1). Population genetics testing Hardy-Weinberg (HW) expectations were rejected in 21 out of 171 loci/population combinations (Table A7). The alleles that differed significantly from HW were clustered in loci B119 and Btern02. Populations 12 and 17 had considerably more cases that were significantly different from HW expectations compared to other populations. Heterozygote deficiency was significant in 19 out of 171 cases. Alleles exhibiting significant differences from HW expectations were clustered in locus B119 but did not exhibit any clustering by population. Heterozygote excess was significant in 1 out of 171 cases in population 10 on locus BL13. Global Heterozygote deficiency was significant in 8 out of 17 populations and 4 out of 8 loci (Table A7). Log-likelihood ratio tests detected a highly

significant linkage disequilibrium (LD) for 2 out of 36 locus pairs: BL13-BTERN02, B119-BTERN02 (Table A7).

LD was found in 12 of 612 comparisons among the analyzed loci and populations when tested with the log-likelihood ratio statistic (Table A7). Populations 4, 12 and 17 had 2, 3, and 2 of the significant cases, respectively.

Population differentiation estimates

The overall level of expected heterozygosity (HE) was relatively low (overall HE: 0.61). The overall level of observed heterozygosity (HO) was lower than expected (overall HO: 0.56). The inbreeding coefficient (F_{IS}) varied among populations, with overall F_{IS} = 0.067 (Table 2). F_{IS} estimates for each locus and population were positive in 76 out of 170 cases (Table 2). Genetic variation was relatively low, with overall F_{ST} = 0.066. Pairwise comparisons of F_{ST} between populations ranged from -0.012 to 0.37 (Table 3). Populations 15 and 10 were the most genetically dissimilar (pairwise F_{ST} 0.37), followed by 15 and 9 (F_{ST} = 0.36) and 15 and 8 (F_{ST} = 0.36). Populations 14 and 17 were the most genetically similar (F_{ST} = -0.012), followed by 8 and 16 (F_{ST} = -0.007) and 5 and 11 (F_{ST} = -0.006). Per locus estimates of HE ranged from 0.094 (locus BT28) to 0.95 (locus BL11) (overall HE = 0.61) (Table 4). Observed heterozygosity ranged from 0.086 (locus BT28) to 0.934 (locus BL13), with overall H_O = 0.569 (Table 4). Total gene diversity (H_T) ranged from 0.12 (locus BT28) to 0.957 (locus BL11), with overall H_T = 0.653

(Table 4). Per locus F_{IS} estimates ranged from -0.064 (locus BL13) to 0.449 (locus BTERN02). F_{ST} estimates ranged from 0.008 (BL11) to 0.318 (locus B119) (Table 4).

Genetic clustering

Of the six repeated analyses for each of the possible number of genetic groups ($K = 1-4$), the most likely number of populations was $K=2$ using $\text{LnP}(D)$ estimates and $K=2$ using delta K estimates. The bar charts for $K = 2$ show that populations 12 and 15 form a distinct cluster while the remaining populations form a separate cluster (Fig. 2). The red genetic cluster is only represented by 4 of the 17 populations, of which, only two were predominately red and were located in the southwestern portion of the study area in the Great Smoky Mountains National Park. (Fig. 2). The green genetic cluster is dominant cluster in 13 of 17 populations and was widespread across the study area ranging from the southwestern portion of the Blue Ridge Parkway, all the way to the northernmost population near Boone, NC (Figure 2).

Estimating Isolation by Distance

Distances between populations ranged from 363-188028 m (Table 3). There was a significant correlation of isolation by distance (IBD) when correlating pairwise Euclidean distance with pairwise F_{ST} between populations (Spearman $Rho = 0.015$ ($P = 0.041$, Table 3; Fig. 4).

MaxEnt environmental niche modeling

Average AUC for the environmental niche models was 0.73. The model with the highest AUC (0.81) was M2 which included the three variables canopy cover, elevation, and land-use (Table. 5). A map of model M2 shows that *Bombus vagans* probability of occurrence is confined to mostly mountain tops or high elevation habitats (Figure 3). The model that included all non-climate variables (M1, Table 5) demonstrated that land-use had the strongest influence on the model (percent contribution =83%), followed by elevation (percent contribution =12%). The only univariate model created with Maxent was land-use because of its significance in contributing to the non-climate model. This univariate model revealed that land-cover codes 31 (Barren Land) and 24 (High intensity) had the highest ability to predict *B. vagans* presence. The model that included climate variables (M6, Table 5) demonstrated that BIO04 (Temperature seasonality) had the strongest influence on the model (percent contribution =48%), followed by BIO15 (Precipitation Seasonality, percent contribution =15.7%), and BIO8 (Mean Temperature of Wettest Quarter percent contribution 14.8%).

Estimating Isolation by Resistance (IBR)

There were a number of IBR correlations that were indicative of gene flow among regions. However, a few had an even stronger correlation than the null hypothesis of genetic distance by geographic distance. The models with the highest correlation with genetic data were the All landscape variables (non-climate) model (Rho =0.307, Figure 5), wind (Rho= 0.156,

Figure 6), solar (Rho= 0.152, Figure 7) and imperviousness (Rho= 0.249, Figure 8), all of which were significant predictors of genetic variation (Table 5). The models with the lowest correlations with genetic data were the canopy/land-use (Rho= -0.042), land-use only (Rho = 0.029), and canopy/elevation/land-use (Rho= 0.041).

Potential Dispersal Pathways and Landscape Connectivity

The model that correlated most strongly with genetic distance was the M1-all landscape variables multivariate model. The Circuitscape current map reveals dispersal paths that appear to allow some connectivity among sites (Figure 9). There seems to be the most paths in the central area of the study site where more sites are clustered. Sites in the Great Smoky Mountains had fewer among-site dispersal paths when compared to those on the Blue Ridge Parkway. Upon closer inspection of the dispersal paths, they seem to follow most roadways or cleared lands and to avoid dense forests that may act as barriers to dispersal. The two sites in the northeast corner of the study area seem to have some dispersal pathways with the rest of the sites but the paths get weaker as they approach Asheville, NC which may also act as a dispersal barrier. The M6 model using bioclimatic variables showed a high degree of connectivity with few corridors and did not significantly correlate with genetic distance (Figure 10). The M9 elevation model did not improve the genetic distance correlation compared to geographic distance alone and it showed a low amount of resistance with no corridors (Figure 11). M12 imperviousness model showed a similar dispersal pattern to M1, many narrow corridors connecting populations (Figure 12). The

M7 landuse model had no significant correlation with genetic distance and showed a moderate amount of connectivity with many wide corridors connecting populations (Figure 13).

Discussion

I genetically sampled various populations of *B. vagans* and determined how genetic structure was influenced by the heterogeneous landscape of the southern Appalachians. Specifically, I tested the hypotheses that (i) geographically isolated populations that are separated by unfavorable habitat will have reduced genetic diversity and higher levels of inbreeding than populations that are in close proximity and are connected by suitable habitat; (ii) populations will show a trend of isolation by distance; (iii) models of isolation by resistance would describe more variation of genetic relatedness than models of isolation by distance alone. I examined the population genetic structure among *B. vagans* subpopulations using microsatellite markers; generated a regional geographic distribution of *B. vagans* in the southern Appalachians using maximum entropy environmental niche modeling; and identified factors associated with landscape-scale pathways of and barriers to *B. vagans* gene flow and dispersal. Results of this study may help managers understand the role of landscape-scale factors in predicting effects Bumble bee habitat use, dispersal and population genetic structure in the Southern Appalachian Mountains.

Population Genetics

Only a small portion of cases (12 %) analyzed deviated from Hardy Weinberg (HW) equilibrium. This suggests that the majority of the populations were maintaining genetic diversity. Of the cases that were significant, most were clustered within locus B119 and Btern02. This could potentially indicate a genotyping error, especially for B119 because a similar pattern was revealed by the heterozygote deficiency analysis for that locus. Significant HW cases were also clustered around populations 12 and 17. This pattern could indicate that those two populations are undergoing genetic drift causing their overall genetic diversity to decrease. Inbreeding coefficient was significant in almost half of the cases, which is likely due to a significant global heterozygote deficiency in more than half of all populations. Overall observed heterozygosity was much lower than was expected, which could indicate reduced gene flow among populations. Bumblebees in particular are more susceptible to inbreeding due to their monoandrous mating system and low effective population sizes (Darvill et al. 2006). Bumble bee colonies contain 200 or more non-breeding workers and only one reproductive queen, which is responsible for producing all the offspring. This means that even though there are numerous individuals, the effective population size is much smaller and populations may actually have limited genetic diversity and may be more vulnerable to stochastic effects (Dreier et al. 2014).

Population Differentiation Estimates

Overall F_{ST} was remarkably low (0.066) compared with other bumble bee population genetics studies (Koch 2015; Whitley 2018). Overall low genetic distance indicates sufficient gene flow across the landscape. Structure analysis only identified two distinctive genetic groups.

One genetic group (green) dominated all but one population which was found at population 15 (red) (Figure 2). Within the green genetic group, populations seemed to follow a general trend of isolation by distance. However, pairwise F_{ST} comparisons show a very high genetic distance (0.18-0.37) between population 15 and all other populations, even though it is geographically close to many of the other sites. Structure analysis also supports this pattern of isolation at population 15. This is an interesting pattern and may indicate a cryptic species within *B. vagans*. Numerous studies have identified cryptic species among *Bombus* species that live sympatrically (Murray et al. 2008; Williams et al. 2012; Williams et al. 2015; Christmas et al. 2021). However, no other studies have identified a cryptic species in the southeastern US. Cryptic species can occur within *Bombus* due to certain species having nearly indistinguishable phenotypes. Color patterns have converged among several *Bombus* species as a form of Mullerian mimicry to warn predators that they provide a venomous sting (Williams 2007). Determining the species delineation threshold of genetic differentiation is somewhat arbitrary due to the flexible concept of a “species”. However, a rule of thumb to follow is that the putative cryptic species should have an average of ten times greater genetic distance as the average genetic distance of the overall population variation of the alternate species (Hebert et al. 2004). Site 15 had an average pairwise F_{ST} of 0.32 while all the other sites had an average pairwise F_{ST} of 0.01. This comparison is more than ten times greater. This indicates that the samples collected at population 15 most likely were a cryptic species that closely resembles *B. vagans* or potential subspecies of *B. vagans*. A consecutive study with modern genomic techniques and targeted phenotype analysis as implemented in Christmas et al. (2021), would provide a more conclusive answer to the status of a cryptic species complex within *B. vagans*. Of the remaining sites, isolation by

distance (IBD) was correlated but not very strongly ($R = 0.15$, $P = 0.04$). Sites as close as 363-1779 m (e.g., Sites 12 and 16, 10 and 17) were less genetically related ($F_{ST} = 0.03$, $F_{ST} = 0.06$), while sites as far as 41237-41878m from one another (e.g., Sites 9 and 16, 3 and 18) were more genetically related ($F_{ST} = 0.0002$ and $F_{ST} = 0.001$). These examples demonstrate how much variation in the population genetic structure model that is not accounted for by the isolation by distance. Within a complex landscape, such as the Appalachian Mountains, isolation by distance is not sufficient to describe much of the variation in genetic structure, but is more likely influenced by the heterogeneity of the microgeography and microclimates within the landscape (Goulson et al. 2011; Jha & Kremen 2013; Lozier et al. 2013). Lozier et al. (2013) found that a montane species, *B. bifarius*, had dispersal limited by large desert valleys between mountains. High elevation habitat populations had more genetic differentiation than those found at lower elevation and more homogeneous habitats due to the narrower and more convoluted suitable dispersal paths that connected sites at high elevations (Lozier et al. 2013). Due to the high degree of habitat complexity and landscape heterogeneity in montane landscapes, isolation by distance models do not sufficiently predict gene flow patterns and Isolation by Resistance (IBR) models may provide better resolution.

Bombus vagans is typically found at higher elevations in the southern Appalachians. All individuals that were collected in my study were found at elevations of 600-1900 m. Because elevation plays an important role in predicting *B. vagans* occurrence, it was the first parameter that I included in models of gene flow. Additionally, I created a number of environmental niche models (ENM) that predicted *B. vagans* distribution, and elevation was always the top contributing landscape variable in these models. Surprisingly, however, elevation performed

much more poorly in IBR models of genetic distance than did geographic distance ($R = 0.11$, $p = 0.08$).

Other studies witnessed similar results with elevation not enhancing the IBR relationship more than IBD (Goulson et al. 2011; Jha & Kremen 2013; Bartlett et al. 2016). Other landscape factors that have been utilized in IBR modeling include ocean bathymetry (Goulson et al. 2011), ocean area (Darvill et al. 2010; Jha & Kremen 2013), impervious cover, and land use (Jha & Kremen 2013; Jha 2015). These various landscape factors can improve models of IBR.

Of all the landscape features I examined, I hypothesized that road surfaces or impervious surfaces would have inhibitory effect to gene flow on *B. vagans*. Interestingly, I observed the inverse. The IBR model M12- Imperviousness was the second best performing IBR model. Model M1- multivariate model with all landscape variables seemed to also favor roads and open areas within the Circuitscape modeling software (Figure 15a-c). This result is likely attributed to the meadow habitat that highways typically provide on the verges of the roadway. One study found that Lepidopteran species diversity was correlated with road verge width, especially if the road was through a mature forest; roads through urban areas or agricultural areas were less diverse (Saarinen et al. 2005). Consistent with this, I found that current maps for *B. vagans* dispersal paths were very strong on roads within dense forest and weaker on roads in urban areas. Since much of the southern Appalachians are made up of dense forest, road verges may act as open highways of dispersal for *B. vagans*.

My methods may include a potential bias for road preferences. The ENM of land-use favors Barren Land and High Intensity. Roads fit these classifications and while a bias file was used within the Maxent modeling program, this result may still may be experiencing sampling

bias since most of the Megatranssect sampling took place on road surfaces. However, the model M12-Imperviousness was modeled directly into Circuitscape and was not created using an ENM or any occurrence data, yet M12 still had a significant correlation with genetic distance and pathways seemed to follow roadways.

In addition to landscape variables, I included bioclimatic variables into my ENM and IBR models. I made only one model of climate variables (M6) using software to remove autocorrelated variables. The covariates that had the strongest influence on the climate ENM were BIO04 (Temperature seasonality), BIO15 (Precipitation Seasonality) and BIO8, (Mean Temperature of Wettest Quarter). Both BIO04 and BIO15 are measures of variance of temperature and precipitation. The amount of variation of temperature increases in as elevation increases (Ohmura 2012), which indicates another measure of *B. vagans* preferring habitat in high elevations. It is unclear how climate change might affect *B. vagans* since it seems to prefer a more variable climate. However multiple studies have documented effects of climate change on *Bombus*. Some species have begun evolving to adapt to changes in plant food source abundance (Miller-Struttman et al. 2015), others have experienced range contractions in response to a warming climate (Martins et al. 2015; Biella et al. 2017). Although the climate ENM did not perform very well, it did have a nearly significant correlation in the IBR model.

The strongest performing model for IBR was M1 multivariate ENM including land-use, wind, canopy, elevation, imperviousness, and solar radiation. This model performed much better than geographic distance alone, describing twice as much variation the genetic distance between populations. This outlines the synergistic effect of modeling ENM and IBR using multiple

biologically appropriate variables. Each variable on its own didn't perform exceptionally, but combined they were able to create a viable model to study dispersal paths.

IBR models that had a correlation equal to or greater than geographic distance include, M1-all non-climate variables, M12-imperviousness, M10-wind, M11-solar radiation. More variable combinations may provide an even more accurate models to describe *B. vagans* dispersal paths in future studies. Additionally, incorporating more accounts and less-biased occurrence data into the ENM may give a more accurate habitat preference model, and in result make a stronger IBR model. Furthermore, most of my populations for genetic analysis had low sample sizes, which may be skewing results. Future studies with increased sample sizes would provide more powerful analyses.

B. vagans seems to be a stable species for the moment and has numerous populations, but it could potentially suffer the same fate as *B. affinis* and *B. terricola* (Jacobson et al. 2018), which have been extirpated from most of their range despite being some of the most common species at one time (Cameron et al. 2011). Further elucidating *B. vagans* habitat and dispersal preferences should be a priority for conservationists. The low observed heterozygosity and high rates of inbreeding that I observed are alarming and could indicate that *B. vagans* may begin to decline due to reduced genetic diversity. My study proposes that roadways may be an important dispersal path for *B. vagans* in the southern Appalachians. Roadway pollinator management practice as discussed in Wojcik and Buchmann (2012) and Hopwood et al. (2015) may benefit the dispersal pathways for *B. vagans* and increase genetic diversity. Future studies may examine the interesting case of a potential cryptic species that I observed. Invisible biodiversity is even more in danger of being lost, and identifying cryptic species can drive conservation efforts even

further. The southern Appalachians are a very diverse ecosystem, preserving its bumble bees is of utmost importance because of their pollination services that support the diverse food webs of wild habitats or the vital agricultural plants that feed the world.

Table 1 Number of alleles sampled per population and locus for *Bombus vagans*. Averages reported with 95% Confidence interval.

Number of Alleles Sampled											
Locus	B124	BL13	BT28	BTERN01	B10	B119	B96	BL11	Btern02	Total	Average
pop1	10	11	1	3	11	2	7	12	1	58	6.44 ± 3.06
pop2	9	10	2	2	12	3	10	17	4	69	7.66 ± 3.4
pop3	10	12	1	2	16	3	9	20	3	76	8.44 ± 4.3
pop4	8	10	1	2	12	3	9	15	3	63	7 ± 3.23
pop5	12	13	1	4	10	2	8	16	2	68	7.56 ± 3.61
pop6	12	10	1	3	10	2	7	14	2	61	6.78 ± 3.214
pop7	7	7	1	4	11	1	7	10	1	49	5.44 ± 2.53
pop8	9	9	1	2	12	1	13	15	2	64	5.64 ± 3.68
pop9	10	13	3	4	17	1	9	14	2	73	8.11 ± 3.81
pop10	12	10	2	3	14	1	12	20	2	76	8.44 ± 4.38
pop11	9	11	2	2	12	1	11	9	2	59	6.55 ± 3.04
pop12	10	11	2	6	10	3	12	17	6	77	8.55 ± 3.10
pop15	5	4	2	6	3	2	10	21	11	64	7.11 ± 4.00
pop16	15	15	2	5	18	2	14	29	2	102	11.33 ± 6.0
pop17	9	11	2	6	9	2	11	15	7	72	8 ± 2.7
pop18	8	9	2	3	10	3	9	10	3	57	6.3 ± 2.2
pop19	11	7	1	3	12	1	11	12	2	60	6.66 ± 3.22
All	26	26	3	10	32	4	25	48	15	189	21 ± 9.48
Average	9.76 ± 1.08	10.17 ± 1.22	1.5 ± 0.29	3.5 ± 0.69	11.70 ± 1.63	1.9 ± 0.39	9.9 ± 1.00	15.64 ± 2.35	3.24 ± 1.22	74.27 ± 5.62	

Table 2 Inbreeding rate (F_{IS}) per population and locus for *Bombus vagans*. NA indicates that no comparison could be made.

Fis Per population										
	B124	BL13	BT28	BTERN0	B10	B119	B96	BL11	Btern02	All
pop1	0.345	-0.104	NA	0.065	0.024	1	-0.08	0.041	NA	0.086
pop2	-0.148	-0.13	0	-0.043	-0.102	1	0.022	-0.043	0.852	0.109
pop3	-0.074	0.04	NA	-0.077	0.018	1	0.011	0.042	1	0.102
pop4	0	0.008	NA	-0.143	-0.075	1	-0.108	0.099	0.84	0.179
pop5	0.016	-0.078	NA	-0.086	0.154	1	0.013	-0.052	0.436	0.058
pop6	0.059	-0.016	NA	0.448	-0.091	1	-0.231	-0.036	1	0.067
pop7	-0.151	0.231	NA	0	-0.077	NA	0.167	-0.063	NA	0.019
pop8	-0.158	-0.1	NA	-0.176	-0.01	NA	-0.068	0.159	-0.053	-0.041
pop9	0.053	-0.14	-0.014	-0.115	-0.062	NA	0.035	-0.038	-0.091	-0.038
pop10	-0.027	-0.208	0	-0.036	0.151	NA	-0.019	0.216	-0.086	0.022
pop11	-0.091	-0.067	-0.067	-0.143	0.066	NA	-0.099	0.407	0	0.029
pop12	-0.026	-0.095	0.429	0.346	0.2	1	0.029	-0.028	0.342	0.182
pop15	-0.059	0.072	0.021	0.107	-0.03	1	-0.043	0.02	-0.064	0.018
pop16	0.078	-0.055	0	0.004	-0.055	1	0.07	0.038	0.791	0.054
pop17	-0.125	-0.098	-0.059	0.433	0.192	1	-0.071	-0.029	0.327	0.127
pop18	-0.066	-0.125	0	-0.184	0.127	1	-0.006	-0.098	1	0.114
pop19	0	-0.246	NA	-0.301	0.158	NA	0	0.006	0	-0.033

Table 3 Pairwise F_{ST} per population across all loci below diagonal and pairwise Euclidean distance above diagonal for *Bombus vagans*.

Population	1	2	3	4	5	6	7	8	9	10	11	14	15	16	17	18	19
1																	
2	0.0307																
3	0.0092	0.0095															
4	0.0426	-0.0053	0.0154														
5	0.007	0.0171	-0.0008	0.0151													
6	0.0038	0.0268	-0.0061	0.0118	-0.0056												
7	0.0199	0.018	0.0072	0.035	0.0071	0.0085											
8	0.0013	0.0284	0.0013	0.0346	-0.0013	0.0028	-0.0013										
9	0.0073	0.0317	0.0023	0.044	0.0056	0.0022	0.0099	0.0018									
10	0.0277	0.0366	0.0155	0.0512	0.0136	0.0233	0.0252	0.0097	0.0017								
11	0.0071	0.0337	-0.0011	0.0378	-0.006	0.0102	0.0063	-0.0053	0.0014	0.0097							
14	0.047	0.0355	0.0353	0.0032	0.0314	0.0265	0.046	0.0449	0.0492	0.0557	0.0361						
15	0.3583	0.3279	0.3454	0.2823	0.3439	0.3432	0.3587	0.3628	0.3651	0.3782	0.3542	0.1891					
16	0.0098	0.0262	-0.0015	0.0355	0.0024	0.0046	0.0051	-0.007	0.0003	0.0058	-0.0005	0.0398	0.3526				
17	0.0314	0.0412	0.0246	0.0153	0.0288	0.0233	0.0391	0.0349	0.0429	0.0633	0.0302	-0.012	0.2018	0.0373			
18	0.0221	0.0153	0.001	0.0039	0.0076	0.0156	0.0158	0.014	0.0254	0.0372	0.0139	0.0087	0.3117	0.0151	0.0248		
19	0.0124	0.0537	0.0092	0.0424	0.0066	0.0038	0.0207	0.0065	0.0055	0.0232	0.0183	0.0451	0.3695	0.0043	0.0468	0.0154	

Table 4 Estimates of gene diversities and differentiation by locus for *Bombus vagans*. H_o observed heterozygosity, H_E expected heterozygosity, H_T total gene diversity, F_{ST} Wright's fixation index, F_{IS} Wright's estimate of inbreeding coefficient.

Nei's estimation of heterozygosity

Loci Name	H_o	H_E	H_t	F_{ST}	F_{IS}
B124	0.891	0.875	0.909	0.037	-0.019
BL13	0.934	0.878	0.903	0.027	-0.064
BT28	0.086	0.094	0.12	0.217	0.082
BTERN01	0.377	0.399	0.454	0.12	0.055
B10	0.839	0.87	0.925	0.06	0.036
B119	0	0.218	0.32	0.318	1
B96	0.901	0.882	0.912	0.033	-0.022
BL11	0.913	0.95	0.957	0.008	0.038
Btern02	0.176	0.32	0.378	0.152	0.449
Overall	0.569	0.61	0.653	0.066	0.067

Table 5 Maxent environmental niche models for *Bombus vagans* showing model accuracy (AUC) values and Spearman correlation coefficients with significance values comparing the relationship between resistance distance and genetic differentiation (F_{ST}). One asterisk indicates significance at 0.05 and two asterisks indicate significance at 0.01

Model Name	Model Description	Circuitscape Analysis		Maxent Analysis
		Spearman Coefficient	Significance	Maxent Model Test AUC
	Geographic distance(Meters)	0.150*	0.041	-
M1	All Nonbioclim Variables (Maxent Model w/ Bias and 25% test)	0.307**	0.000	0.6199
M2	Canopy Elevation Land-use (Maxent Model w/ Bias and 25% test)	0.041	0.316	0.8057
M3	Canopy Elevation Wind (Maxent Model w/ Bias and 25% test)	0.047	0.293	0.7454
M4	Canopy Elevation (Maxent Model w/ Bias and 25% test)	0.049	0.287	0.7581
M5	Canopy Land-use (Maxent Model w/ Bias and 25% test)	-0.042	0.313	0.7872
M6	Bioclim Correlated Variables Removed (Maxent Model w/ Bias and 25% test)	0.120	0.083	0.7169
M7	Land-use Maxent (Maxent Model w/ Bias and 25% test)	0.029	0.369	0.7374
M8	Canopy Raw Circuitscape	0.058	0.251	-
M9	Elevation Raw Circuitscape	0.111	0.098	-
M10	Wind Raw Circuitscape	0.156*	0.034	-
M11	Solar Radiation Raw Circuitscape	0.152*	0.039	-
M12	Imperviousness Raw Circuitscape	0.249**	0.002	-

Figure Legends

Figure 1 Pink dots indicate survey sites for *Bombus vagans*. Green lines show boundaries of federal land. Pink, black, and yellow lines represent roadways.

Figure 2 Cluster analysis map for *Bombus vagans*. Genetic clustering results two prospective clusters. Each individual is represented by a thin vertical line divided into two colored segments (green and red).

Figure 3 Maxent environmental niche model M2 for *Bombus vagans*. Model generated using landscape variables: canopy cover, elevation, and land-use. Map raster shows likelihood of *Bombus vagans* occurrence on a 0 to 1 scale. Areas of blue represent low likelihood and areas of red indicate high likelihood of occurrence.

Figure 4 Isolation by distance Spearman correlation. $F_{ST}/1-F_{ST}$ by Euclidean Distance in Meters for *Bombus vagans*.

Figure 5 Isolation by resistance Spearman correlation Model M1 Spearman Correlation $F_{ST}/1-F_{ST}$ by resistance distance for *Bombus vagans*.

Figure 6 Isolation by resistance Spearman correlation Model M10 Spearman Correlation $F_{ST}/1-F_{ST}$ by wind resistance distance for *Bombus vagans*

Figure 7 Isolation by resistance Spearman correlation Model M11 Spearman Correlation $F_{ST}/1-F_{ST}$ by solar radiation resistance distance for *Bombus vagans*.

Figure 8 r^2 Isolation by resistance Spearman correlation Model M12 Spearman Correlation $F_{ST}/1-F_{ST}$ by imperviousness resistance distance for *Bombus vagans*.

Figure 9 Resistance model current map of M1 All landscape variables multivariate model. White areas indicate little to no travel, pink areas indicate low levels of travel, yellow indicates moderate levels of travel, and red indicates high levels of travel.

Figure 10 Resistance model current map of M6 Bioclim model. White areas indicate little to no travel, pink areas indicate low levels of travel, yellow indicates moderate levels of travel, and red indicates high levels of travel.

Figure 11 Resistance model current map of M9 Elevation univariate model. White areas indicate little to no travel, pink areas indicate low levels of travel, yellow indicates moderate levels of travel, and red indicates high levels of travel.

Figure 12 Resistance model current map of M12 Imperviousness univariate model. White areas indicate little to no travel, pink areas indicate low levels of travel, yellow indicates moderate levels of travel, and red indicates high levels of travel.

Figure 13 Resistance model current map of M7 Land-use univariate model. White areas indicate little to no travel, pink areas indicate low levels of travel, yellow indicates moderate levels of travel, and red indicates high levels of travel.

Figure 14a) M1 resistance model map showing southern portion only.

Figure 14b) M1 resistance model map showing southern portion overlaid with road map to compare dispersal paths with roadmap.

Figure 14c) Study sites in southern portion of study area with road map.

Figures

Figure 1

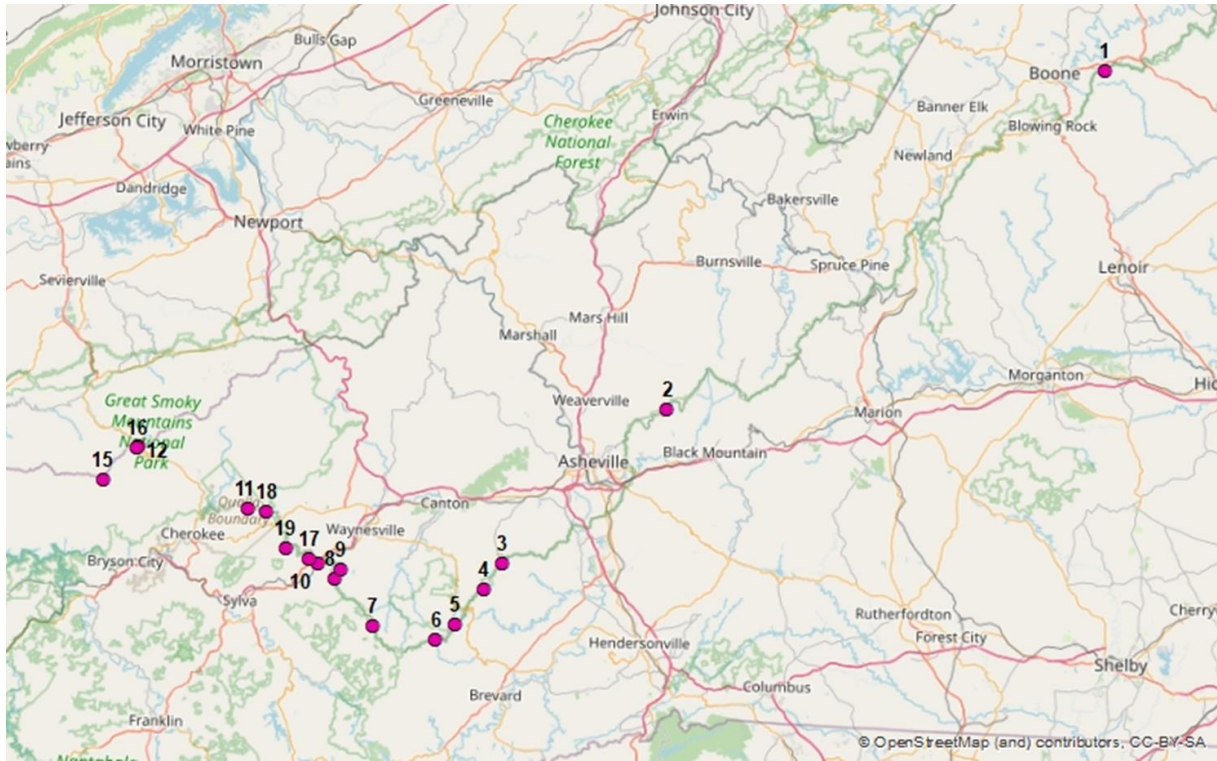


Figure 2

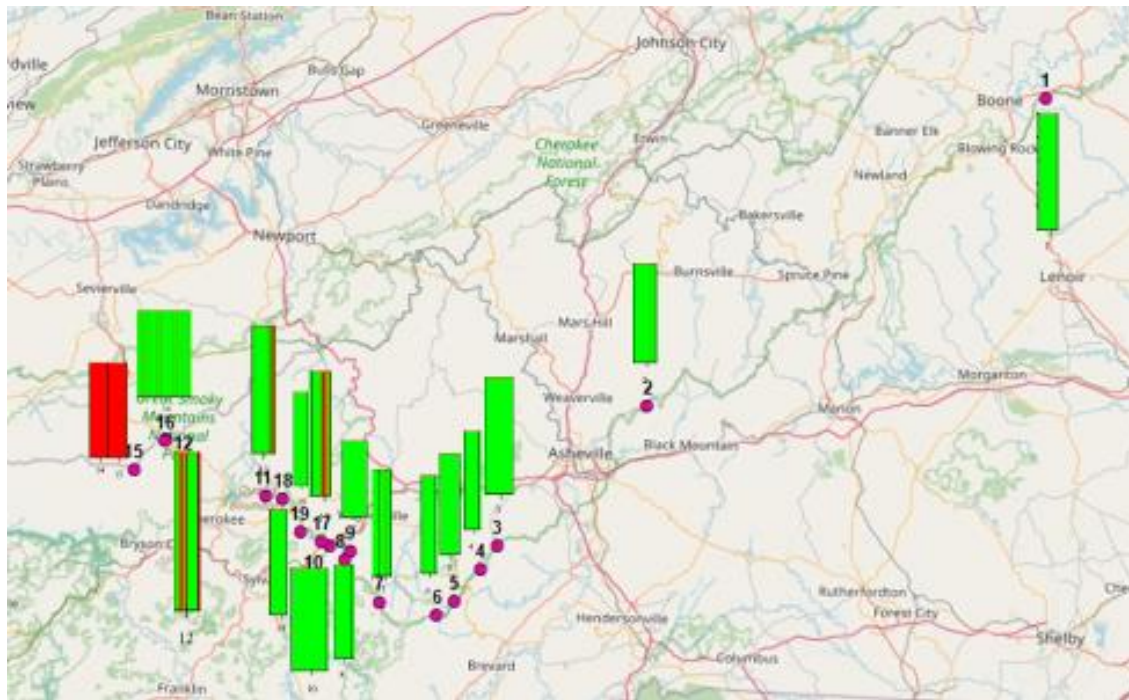


Figure 3

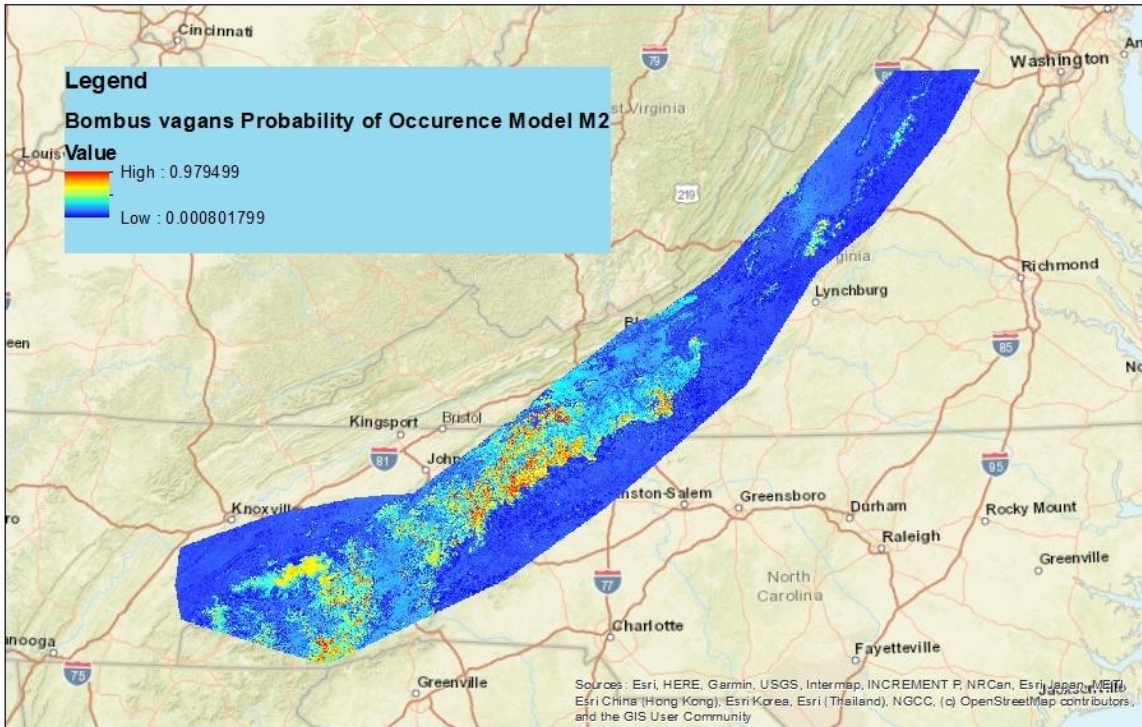


Figure 4

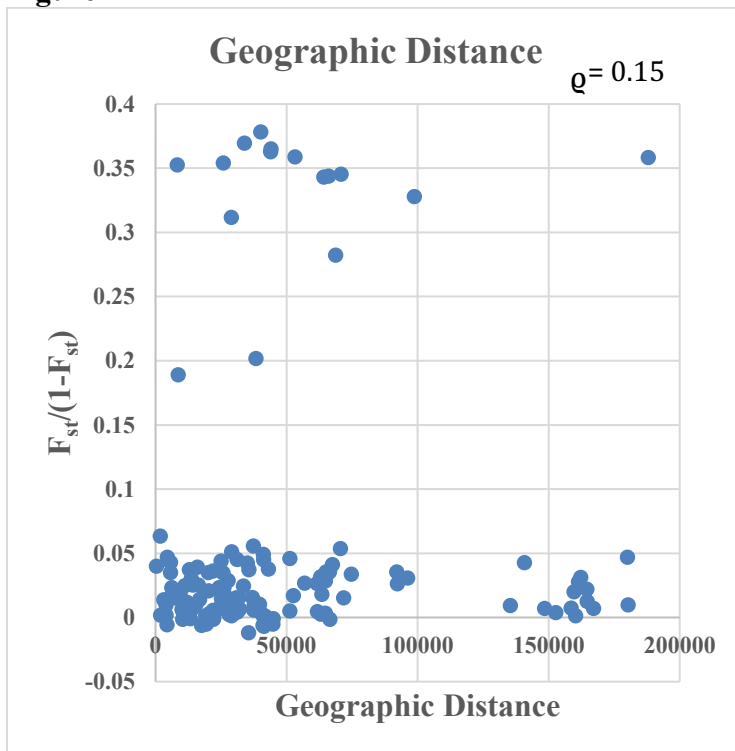


Figure 5

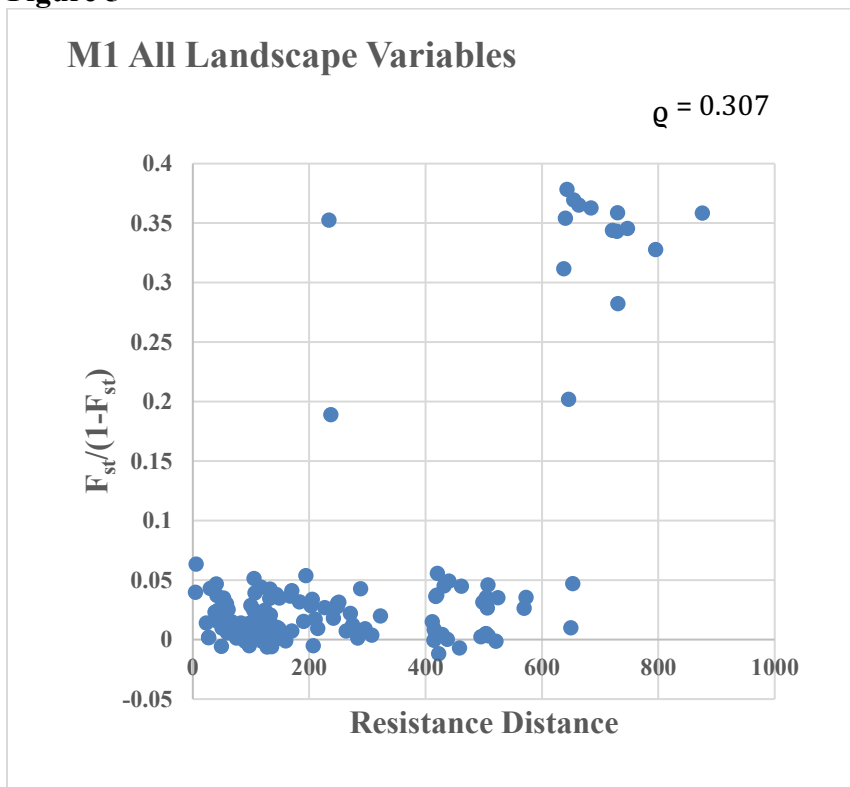


Figure 6

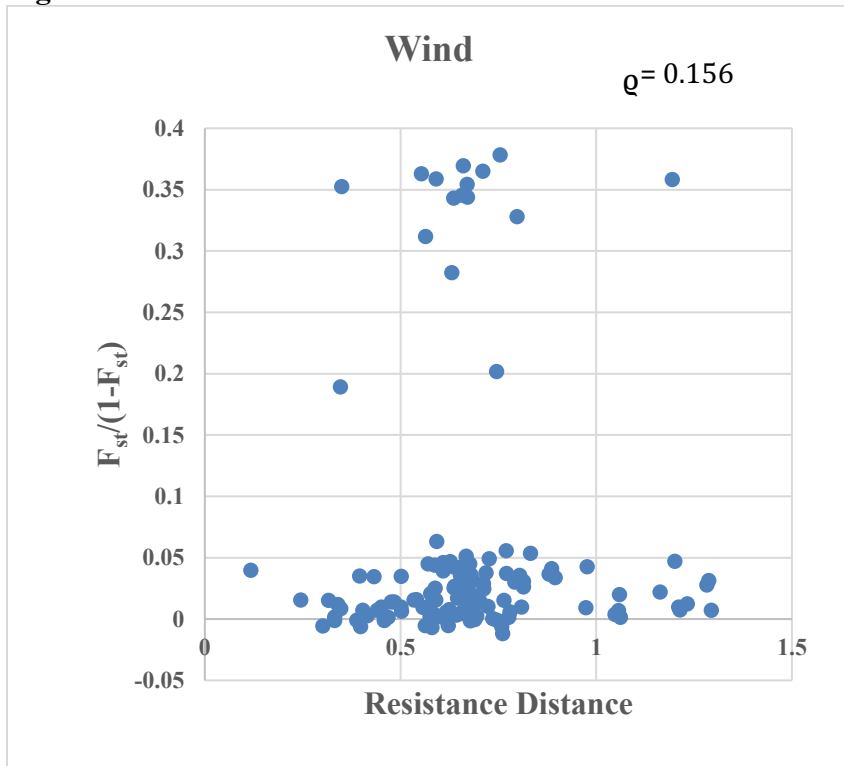


Figure 7

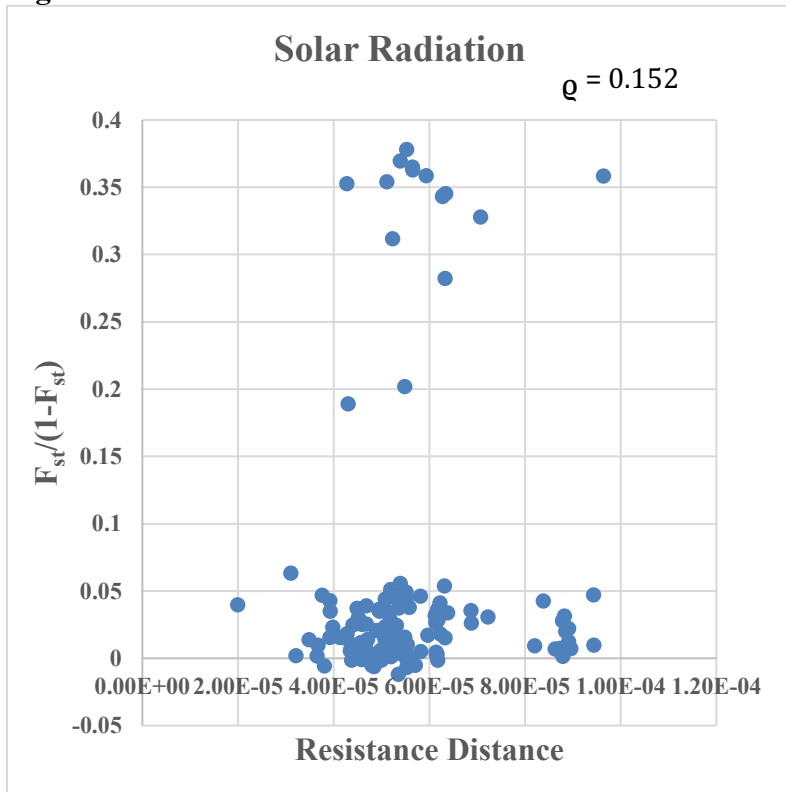


Figure 8

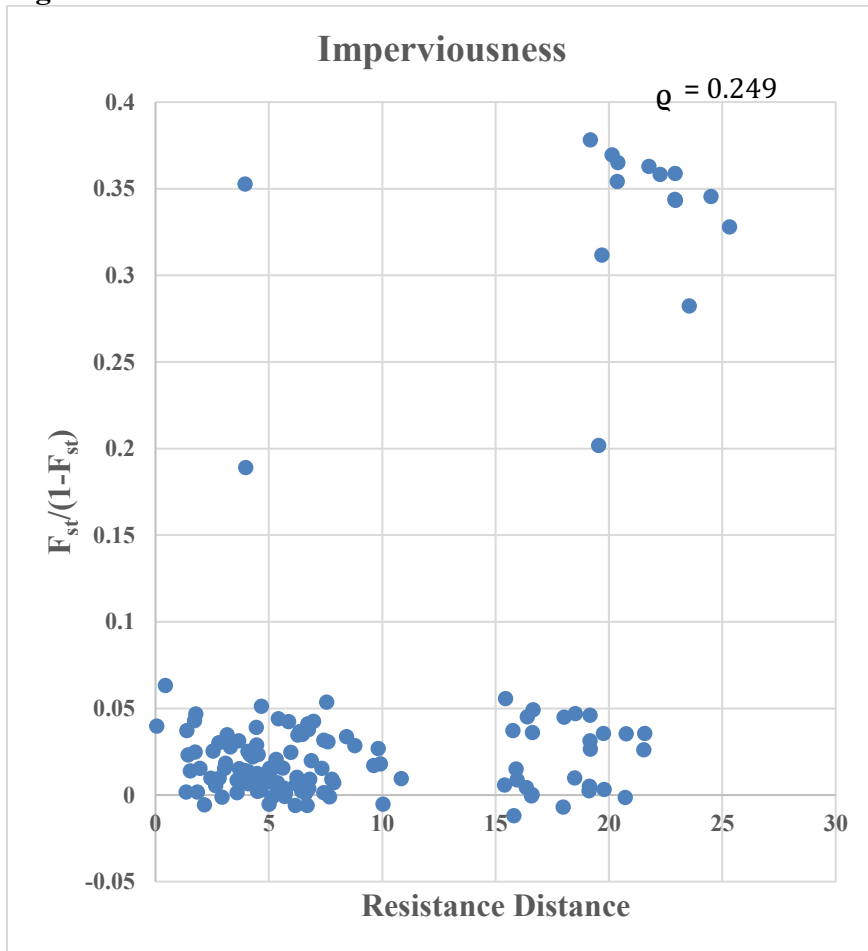


Figure 9

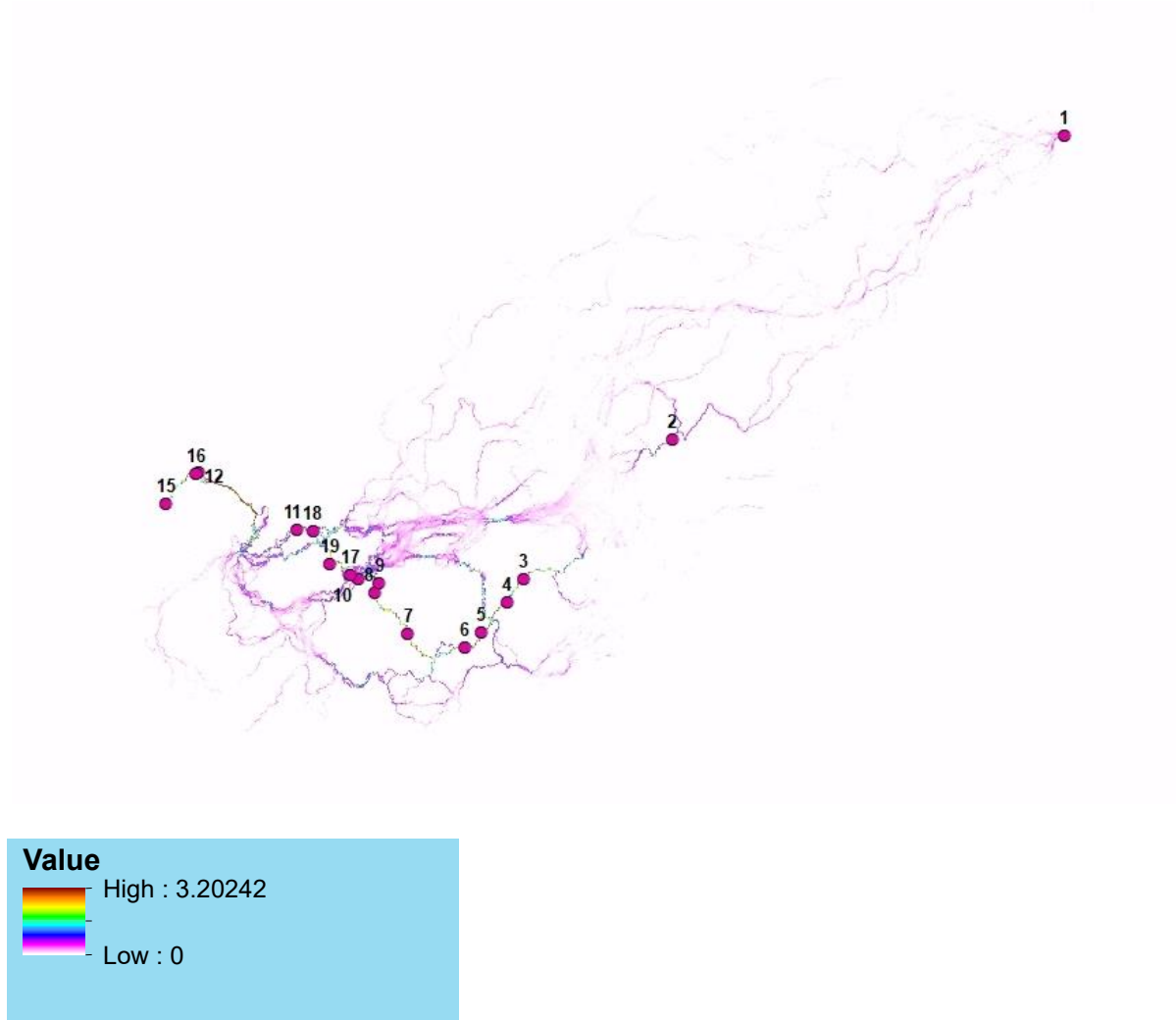


Figure 10

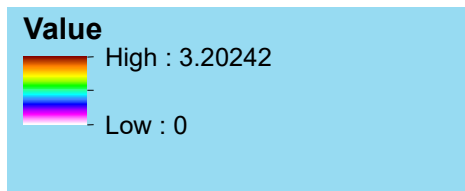
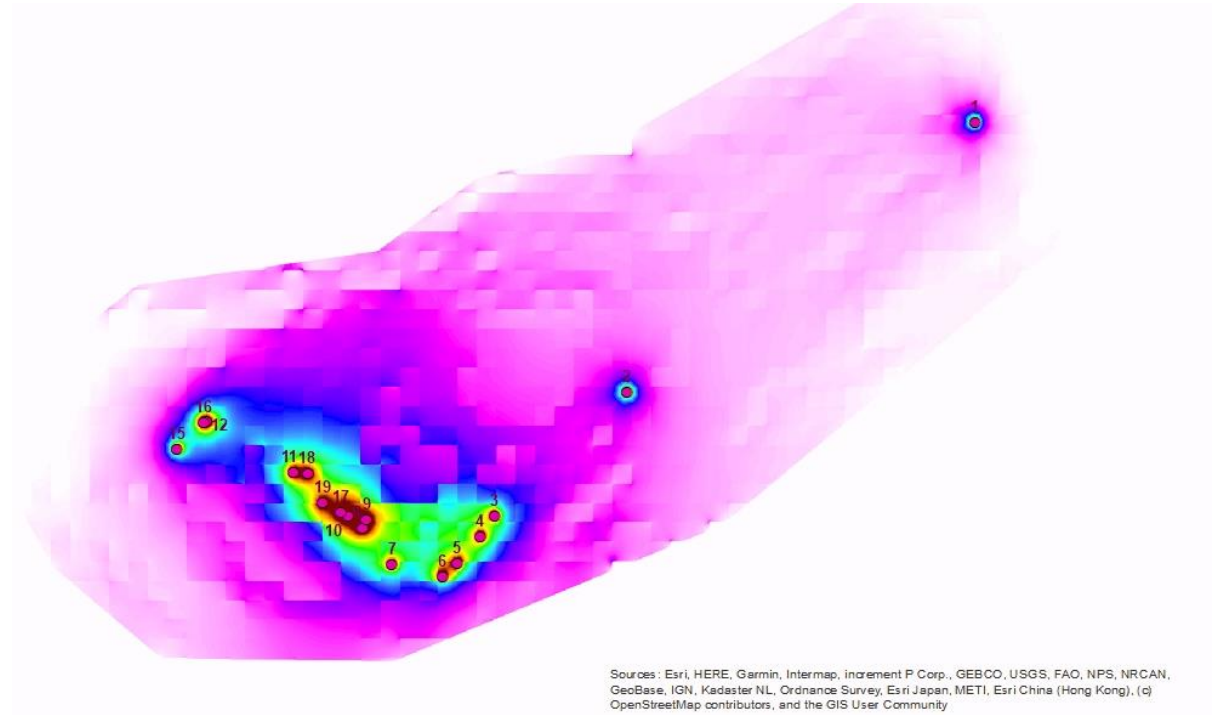


Figure 11

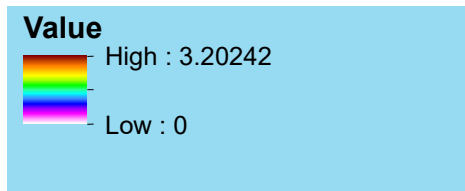
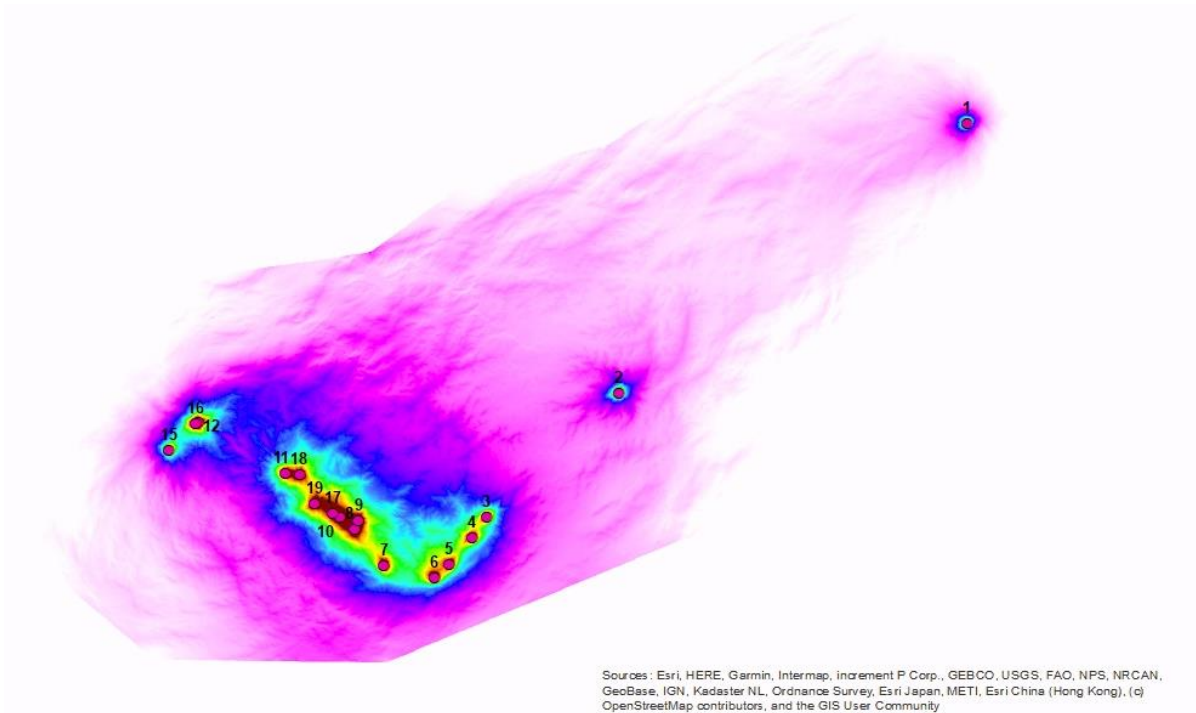


Figure 12

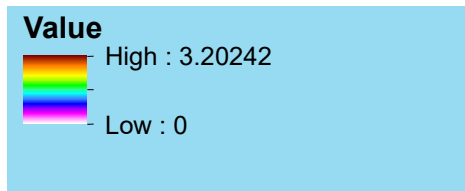
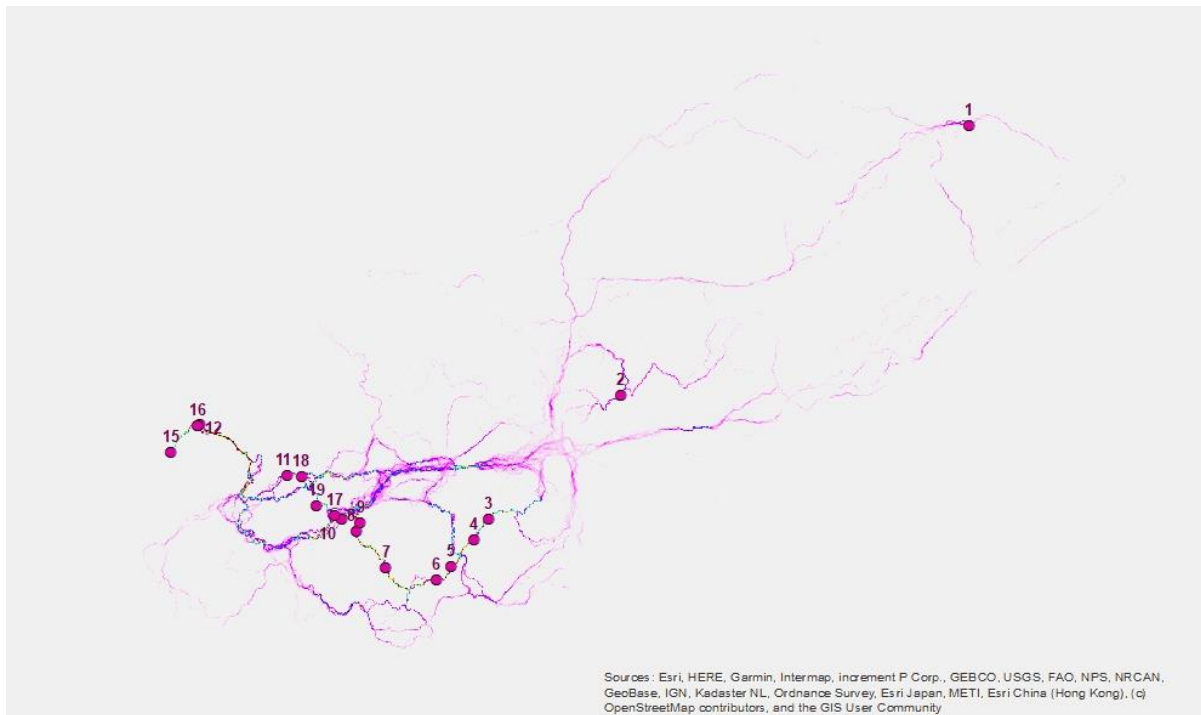


Figure 13

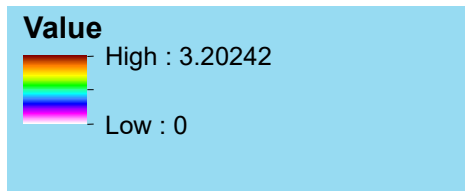
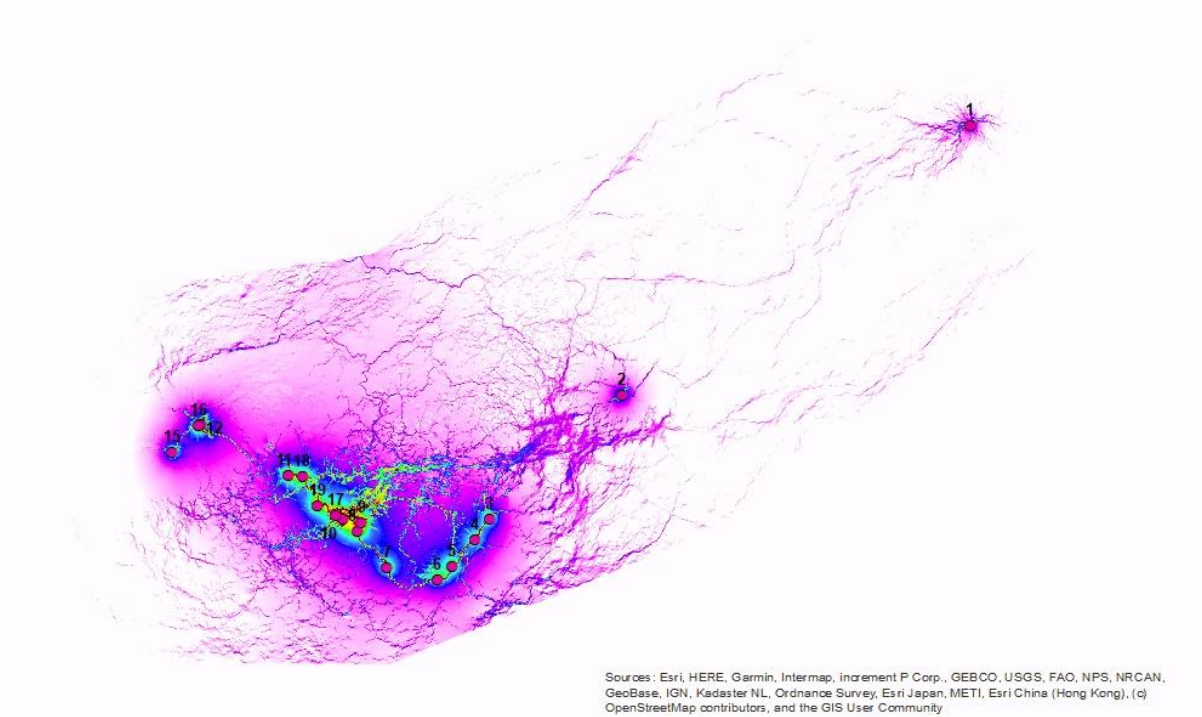


Figure 14a)



Figure 14b)

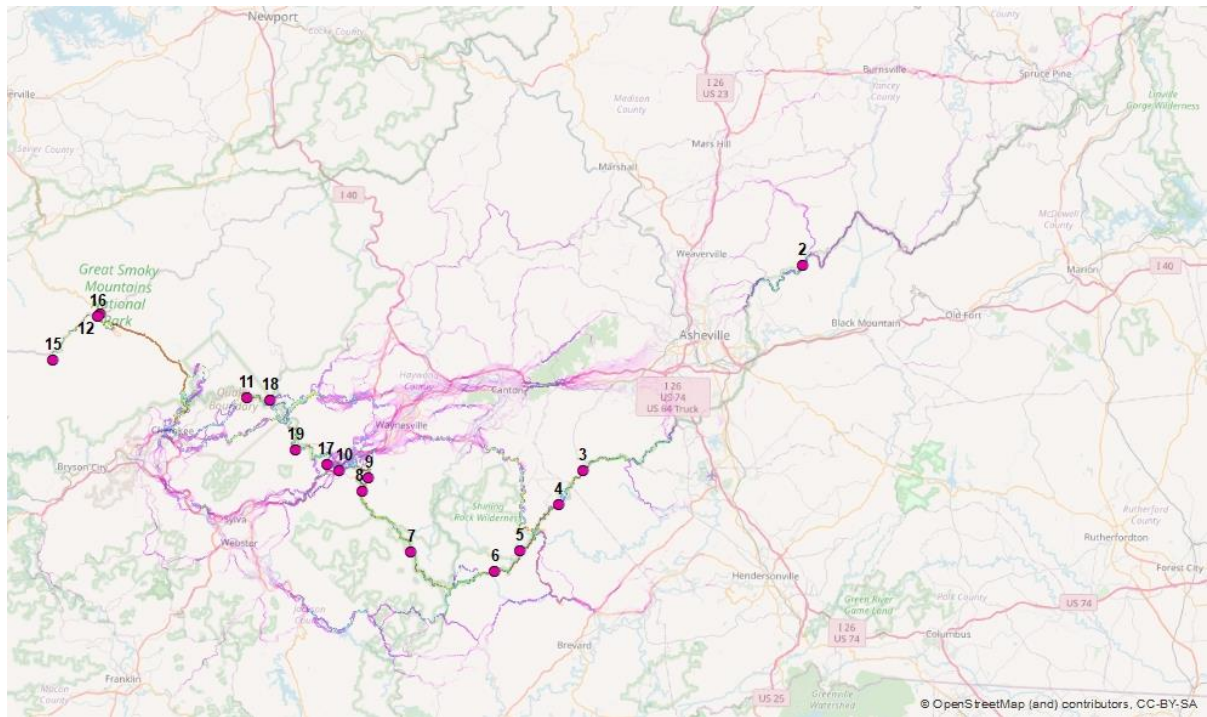
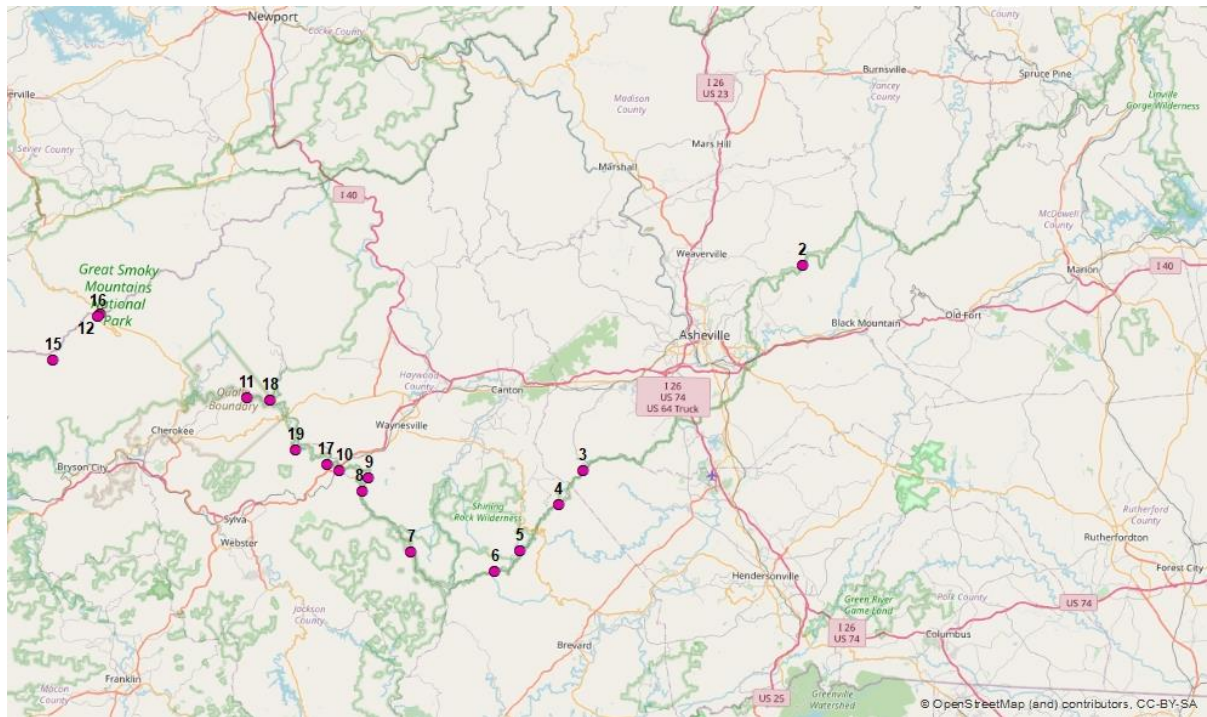


Figure 14c)



Appendix

Table A6 *Bombus vagans* Individuals used for population genetics. Includes site number and coordinates of sample in WGS 1984 projection.

<i>Bombus vagans</i> Individuals Used for Population Genetics			
Sample Number	Site	Coordinates	
001	1	36.2226	-81.5775
002	1	36.2226	-81.5775
003	1	36.2226	-81.5775
004	1	36.2226	-81.5775
005	1	36.2226	-81.5775
006	1	36.2226	-81.5775
007	1	36.2226	-81.5775
008	1	36.2226	-81.5775
009	1	36.2226	-81.5775
011	1	36.2226	-81.5775
012	2	35.6859	-82.4136
013	2	35.6859	-82.4136
014	2	35.6859	-82.4136
015	2	35.6859	-82.4136
016	2	35.6859	-82.4136
017	2	35.6859	-82.4136
018	2	35.6859	-82.4136
019	2	35.6859	-82.4136
021	2	35.6859	-82.4136
022	2	35.6859	-82.4136
023	2	35.6859	-82.4136
024	2	35.6859	-82.4136
025	2	35.6859	-82.4136
026	3	35.4398	-82.7261
027	3	35.4398	-82.7261
029	3	35.4398	-82.7261
030	3	35.4398	-82.7261
031	3	35.4398	-82.7261
032	3	35.4398	-82.7261
033	3	35.4398	-82.7261

034	3	35.4398	-82.7261
035	3	35.4398	-82.7261
036	3	35.4398	-82.7261
037	3	35.4398	-82.7261
038	3	35.4398	-82.7261
039	3	35.4398	-82.7261
040	3	35.4398	-82.7261
041	3	35.4398	-82.7261
042	4	35.3986	-82.7605
043	4	35.3986	-82.7605
044	4	35.3986	-82.7605
045	4	35.3986	-82.7605
046	4	35.3986	-82.7605
047	4	35.3986	-82.7605
048	4	35.3986	-82.7605
049	4	35.3986	-82.7605
050	4	35.3986	-82.7605
051	5	35.3444	-82.8144
052	5	35.3444	-82.8144
053	5	35.3444	-82.8144
054	5	35.3444	-82.8144
056	5	35.3444	-82.8144
057	5	35.3444	-82.8144
058	5	35.3444	-82.8144
060	5	35.3444	-82.8144
061	5	35.3444	-82.8144
062	5	35.3444	-82.8144
063	5	35.3444	-82.8144
064	5	35.3444	-82.8144
065	6	35.3185	-82.8500
066	6	35.3185	-82.8500
067	6	35.3185	-82.8500
068	6	35.3185	-82.8500
069	6	35.3185	-82.8500
070	6	35.3185	-82.8500
071	6	35.3185	-82.8500
072	6	35.3185	-82.8500
073	6	35.3185	-82.8500
082	7	35.3400	-82.9710

083	7	35.3400	-82.9710
084	7	35.3400	-82.9710
085	7	35.3400	-82.9710
086	7	35.3400	-82.9710
087	7	35.3400	-82.9710
088	7	35.3400	-82.9710
089	8	35.4109	-83.0441
090	8	35.4109	-83.0441
091	8	35.4109	-83.0441
092	8	35.4109	-83.0441
093	8	35.4109	-83.0441
095	8	35.4109	-83.0441
096	8	35.4109	-83.0441
097	8	35.4109	-83.0441
098	8	35.4109	-83.0441
099	8	35.4109	-83.0441
100	8	35.4109	-83.0441
101	9	35.4266	-83.0344
102	9	35.4266	-83.0344
103	9	35.4266	-83.0344
104	9	35.4266	-83.0344
105	9	35.4266	-83.0344
107	9	35.4266	-83.0344
108	9	35.4266	-83.0344
109	9	35.4266	-83.0344
110	9	35.4266	-83.0344
111	9	35.4266	-83.0344
112	9	35.4266	-83.0344
113	9	35.4266	-83.0344
114	9	35.4266	-83.0344
115	9	35.4266	-83.0344
116	9	35.4266	-83.0344
117	9	35.4266	-83.0344
118	9	35.4266	-83.0344
119	9	35.4266	-83.0344
120	9	35.4266	-83.0344
121	10	35.4339	-83.0777
126	10	35.4339	-83.0777
127	10	35.4339	-83.0777

128	10	35.4339	-83.0777
129	10	35.4339	-83.0777
130	10	35.4339	-83.0777
131	10	35.4339	-83.0777
132	10	35.4339	-83.0777
133	10	35.4339	-83.0777
134	10	35.4339	-83.0777
135	10	35.4339	-83.0777
136	10	35.4339	-83.0777
137	10	35.4339	-83.0777
139	10	35.4339	-83.0777
140	10	35.4339	-83.0777
141	10	35.4339	-83.0777
142	11	35.5176	-83.2129
143	11	35.5176	-83.2129
144	11	35.5176	-83.2129
145	11	35.5176	-83.2129
146	11	35.5176	-83.2129
147	11	35.5176	-83.2129
148	11	35.5176	-83.2129
151	11	35.5176	-83.2129
152	11	35.5176	-83.2129
153	12	35.6119	-83.4267
154	12	35.6119	-83.4267
156	12	35.6119	-83.4267
157	12	35.6119	-83.4267
158	12	35.6119	-83.4267
159	12	35.6119	-83.4267
160	12	35.6119	-83.4267
161	12	35.6119	-83.4267
162	12	35.6119	-83.4267
163	12	35.6119	-83.4267
165	12	35.6119	-83.4267
176	15	35.5571	-83.4938
177	15	35.5571	-83.4938
178	15	35.5571	-83.4938
179	15	35.5571	-83.4938
180	15	35.5571	-83.4938
181	15	35.5571	-83.4938

182	15	35.5571	-83.4938
183	15	35.5571	-83.4938
184	15	35.5571	-83.4938
185	15	35.5571	-83.4938
186	15	35.5571	-83.4938
187	15	35.5571	-83.4938
188	15	35.5571	-83.4938
189	15	35.5571	-83.4938
190	15	35.5571	-83.4938
191	15	35.5571	-83.4938
192	15	35.5571	-83.4938
193	15	35.5571	-83.4938
194	15	35.5571	-83.4938
195	15	35.5571	-83.4938
196	15	35.5571	-83.4938
197	15	35.5571	-83.4938
198	15	35.5571	-83.4938
199	15	35.5571	-83.4938
200	16	35.6099	-83.4299
204	16	35.6099	-83.4299
205	16	35.6099	-83.4299
206	16	35.6099	-83.4299
208	16	35.6099	-83.4299
209	16	35.6099	-83.4299
210	16	35.6099	-83.4299
211	16	35.6099	-83.4299
212	16	35.6099	-83.4299
213	16	35.6099	-83.4299
215	16	35.6099	-83.4299
216	16	35.6099	-83.4299
218	16	35.6099	-83.4299
219	16	35.6099	-83.4299
221	16	35.6099	-83.4299
222	16	35.6099	-83.4299
223	16	35.6099	-83.4299
224	16	35.6099	-83.4299
225	16	35.6099	-83.4299
226	16	35.6099	-83.4299
227	16	35.6099	-83.4299

228	16	35.6099	-83.4299
229	16	35.6099	-83.4299
230	16	35.6099	-83.4299
231	16	35.6099	-83.4299
232	16	35.6099	-83.4299
233	16	35.6099	-83.4299
234	16	35.6099	-83.4299
235	16	35.6099	-83.4299
236	16	35.6099	-83.4299
237	16	35.6099	-83.4299
238	16	35.6099	-83.4299
239	16	35.6099	-83.4299
240	16	35.6099	-83.4299
241	16	35.6099	-83.4299
242	16	35.6099	-83.4299
243	16	35.6099	-83.4299
244	17	35.4408	-83.0954
245	17	35.4408	-83.0954
246	17	35.4408	-83.0954
247	17	35.4408	-83.0954
248	17	35.4408	-83.0954
250	17	35.4408	-83.0954
251	17	35.4408	-83.0954
252	17	35.4408	-83.0954
253	17	35.4408	-83.0954
254	17	35.4408	-83.0954
255	18	35.5151	-83.1787
256	18	35.5151	-83.1787
257	18	35.5151	-83.1787
258	18	35.5151	-83.1787
259	18	35.5151	-83.1787
260	18	35.5151	-83.1787
261	18	35.5151	-83.1787
262	18	35.5151	-83.1787
263	18	35.5151	-83.1787
264	18	35.5151	-83.1787
265	19	35.4577	-83.1402
266	19	35.4577	-83.1402
267	19	35.4577	-83.1402

268	19	35.4577	-83.1402
269	19	35.4577	-83.1402
270	19	35.4577	-83.1402
271	19	35.4577	-83.1402
272	19	35.4577	-83.1402
273	19	35.4577	-83.1402
274	19	35.4577	-83.1402

Table A7

Tests for Hardy-Weinberg probability and heterozygote deficiency and excess by locus and population for *Bombus vagans*. Significant values are in bold. Dashes indicate no comparison could be made.

Locus	Population	<u>HW</u> <u>Probability</u>		<u>Excess F_{IS}</u> <u>estimates U</u> <u>test</u>		<u>Deficiency F_{IS}</u> <u>estimates U</u> <u>test</u>	
		P-val	S.E.	P-val	S.E.	P-val	S.E.
B124							
	1	0.0413	0.0102	1	0	0.0009	0.0007
	2	0.9745	0.0057	0.203	0.024	0.9716	0.0078
	3	0.9282	0.0099	0.3036	0.0237	0.9111	0.0146
	4	0.9235	0.0076	0.6416	0.0236	0.6736	0.0202
	5	0.8119	0.0224	0.7127	0.0303	0.5079	0.0301
	6	0.4887	0.0337	0.751	0.0335	0.4616	0.0376
	7	0.413	0.0161	0.4078	0.0174	1	0
	8	1	0	0.2004	0.0179	1	0
	9	0.772	0.0152	0.6038	0.0242	0.4102	0.0249
	10	0.4181	0.0283	0.3704	0.0297	0.7078	0.0271
	11	1	0	0.4392	0.0252	1	0
	12	0.152	0.017	0.7463	0.0242	0.3338	0.0269
	15	0.4722	0.0091	0.4422	0.0088	0.5633	0.0088
	16	0.2949	0.0267	0.867	0.0211	0.134	0.0213
	17	0.2473	0.0169	0.3091	0.024	1	0
	18	0.8845	0.0094	0.484	0.0218	0.8336	0.0139
	19	0.5593	0.029	0.7441	0.029	0.3014	0.0297
BL13							
	1	0.8396	0.0197	0.3767	0.0327	1	0
	2	0.993	0.0026	0.1926	0.0203	1	0
	3	0.6423	0.0259	0.7972	0.0245	0.3052	0.0283
	4	0.8623	0.0162	0.7532	0.0252	0.379	0.0295
	5	0.8112	0.0265	0.4461	0.037	1	0
	6	0.9278	0.0142	0.6267	0.0326	0.7912	0.0247
	7	0.2296	0.0131	0.9386	0.0083	0.1255	0.0102
	8	1	0	0.3797	0.0189	1	0

9	0.2804	0.0266	0.0704	0.0168	1	0
10	0.3071	0.0218	0.0098	0.0045	1	0
11	0.5544	0.0316	0.5649	0.0332	1	0
12	0.3998	0.0292	0.3862	0.0311	1	0
15	0.5679	-	0.7989	0.0054	0.2234	0.0056
16	0.8785	0.0214	0.1422	0.0243	0.8776	0.0233
17	0.8368	0.0207	0.4018	0.0342	1	0
18	0.5985	0.0228	0.3159	0.0212	1	0
19	0.8636	0.013	0.1112	0.0106	0.9947	0.0019

BT28

1	-	-	-	-	-	-
2	-	-	-	-	-	-
3	-	-	-	-	-	-
4	-	-	-	-	-	-
5	-	-	-	-	-	-
6	-	-	-	-	-	-
7	-	-	-	-	-	-
8	-	-	-	-	-	-
9	1	-	0.9743	0.0021	1	0
10	-	-	-	-	-	-
11	1	-	0.9408	0.0013	1	0
12	0.2782	-	0.9924	0.0005	0.2795	0.0021
15	1	-	0.691	0.0036	0.6238	0.0034
16	-	-	-	-	-	-
17	1	-	0.9476	0.0012	1	0
18	-	-	-	-	-	-
19	-	-	-	-	-	-

BTERN01

1	0.4799	-	0.7763	0.0045	0.4811	0.006
2	1	-	0.9595	0.001	1	0
3	1	-	0.897	0.0015	1	0
4	1	-	0.8225	0.0018	1	0
5	1	-	0.746	0.0098	1	0
6	0.1216	-	0.997	0.0005	0.1144	0.0033
7	0.6643	-	0.7155	0.0074	0.6731	0.0082
8	1	-	0.7252	0.002	1	0
9	1	-	0.505	0.0106	1	0
10	1	-	0.9178	0.0036	1	0
11	1	-	0.8229	0.0017	1	0

12	0.014	0.003	0.9546	0.0046	0.0695	0.0061
15	0.0522	0.0051	0.9755	0.0035	0.0281	0.0037
16	0.9023	0.0074	0.637	0.0156	0.5128	0.0151
17	0.0116	0.0035	0.9822	0.003	0.0197	0.0032
18	1	-	0.5269	0.0061	1	0
19	1	-	0.3472	0.0049	1	0

B10

1	0.0717	0.0126	0.8282	0.0229	0.5704	0.0322
2	0.7235	0.0259	0.3076	0.0321	1	0
3	0.3123	0.0352	0.9244	0.02	0.4047	0.0412
4	1	0	0.5704	0.0378	1	0
5	0.2865	0.0263	0.8209	0.0205	0.198	0.0209
6	0.7029	0.024	0.4778	0.0288	1	0
7	1	0	0.6292	0.0375	1	0
8	0.9142	0.0202	0.6342	0.0332	0.6885	0.0284
9	1	0	0.3048	0.0373	1	0
10	0.1641	0.0307	0.9307	0.0172	0.0795	0.0187
11	0.5265	0.0344	0.8084	0.0311	0.4276	0.0387
12	0.0058	0.003	0.7888	0.0232	0.3551	0.0279
15	1	-	0.935	0.0036	1	0
16	0.394	0.0368	0.0836	0.019	0.9472	0.0164
17	0.0196	0.007	0.7391	0.0204	0.2827	0.0226
18	0.1212	0.0173	0.8732	0.02	0.2043	0.0231
19	0.1662	0.0242	0.9742	0.0111	0.0887	0.0209

B119

1	0.0526	-	1	0	0.0533	0.0012
2	0	-	1	0	0	0
3	0.0013	-	1	0	0.0011	0.0004
4	0.0004	-	1	0	0.0005	0.0002
5	0.0435	-	1	0	0.0457	0.0012
6	0.0588	-	1	0	0.0577	0.0012
7	-	-	-	-	-	-
8	-	-	-	-	-	-
9	-	-	-	-	-	-
10	-	-	-	-	-	-
11	-	-	-	-	-	-
12	0.0001	-	1	0	0	0
15	0.0213	-	1	0	0.0219	0.0008
16	0.0006	-	1	0	0.0006	0.0001

17	0.0031	-		1	0	0.0027	0.0003
18	0.0006	-		1	0	0.0007	0.0002
19	-		-			-	

B96

1	0.0817	0.0091	0.75	0.0146	0.2686	0.0155
2	0.2328	0.0224	0.5564	0.0281	0.6418	0.027
3	0.1405	0.0168	0.923	0.0104	0.0826	0.0109
4	0.8231	0.0162	0.4674	0.0252	1	0
5	0.9673	0.0049	0.6617	0.0214	0.4577	0.0199
6	0.9185	0.0074	0.1194	0.0106	1	0
7	0.5594	0.0166	0.9073	0.0117	0.2098	0.0143
8	0.7046	0.0324	0.414	0.0386	1	0
9	0.5504	0.0188	0.671	0.0183	0.37	0.0177
10	0.4995	0.0272	0.4912	0.0333	0.7343	0.0273
11	0.7683	0.0238	0.3691	0.0329	1	0
12	0.7104	0.0285	0.7237	0.0305	0.4458	0.034
15	0.0201	0.0063	0.7599	0.0191	0.2441	0.0192
16	0.2665	0.0238	0.7312	0.0282	0.2729	0.0286
17	0.5896	0.0278	0.5214	0.032	1	0
18	0.9412	0.0074	0.5664	0.0232	0.6596	0.0227
19	0.3798	0.0295	0.7352	0.0292	0.729	0.0268

BL11

1	0.2013	0.0268	0.8143	0.0254	0.2809	0.0317
2	0.5518	0.0407	0.548	0.0451	1	0
3	0.3751	0.0436	0.8651	0.0302	0.1702	0.0333
4	0.1982	0.0359	1	0	0.1534	0.0317
5	0.5702	0.0429	0.5139	0.0427	1	0
6	1	0	0.7869	0.0349	1	0
7	1	0	0.6794	0.0274	1	0
8	0.0043	0.0043	0.9854	0.0082	0.0412	0.0141
9	0.9989	0.0007	0.3861	0.0352	0.694	0.0327
10	0.0208	0.0116	0.9968	0.0027	0.0032	0.0027
11	0.0187	0.0049	1	0	0.0013	0.0012
12	1	0	0.7156	0.0413	1	0
15	0.3563	0.0392	0.666	0.0433	0.5104	0.0445
16	0.4588	0.0456	0.9607	0.016	0.0641	0.0214
17	0.2869	0.0382	0.7828	0.0353	1	0
18	0.4214	0.0236	0.3743	0.0256	1	0
19	0.3579	0.0345	0.7562	0.0327	0.66	0.0344

Btern02

1	-		-		-	
2	0	-	1	0	0	0
3	0.0013	-	1	0	0.0015	0.0004
4	0.0012	-	1	0	0.0015	0.0003
5	0.2547	-	0.9935	0.0004	0.2528	0.0018
6	0.0588	-	1	0	0.0597	0.0012
7	-		-		-	
8	1	-	0.9524	0.0012	1	0
9	1	-	0.8399	0.0019	1	0
10	1	-	0.8478	0.0019	1	0
11	-		-		-	
12	0.0034	0.0016	0.9126	0.0073	0.1139	0.0087
15	0.8077	0.0164	0.2122	0.0197	0.9241	0.0114
16	0.0029	-	1	0	0.0032	0.0003
17	0.0111	0.0043	0.9421	0.0091	0.0741	0.0106
18	0.0031	-	1	0	0.0035	0.0007
19	-		-		-	

Table A8 Global Heterozygote Excess and Deficiency for all populations and loci for *Bombus vagans*. Significant values are in bold.

Population	Het Excess		Het Deficiency	
	P-val	S.E.	P-val	S.E.
1	0.9821	0.0062	0.0179	0.0062
2	0.8675	0.018	0.1326	0.018
3	0.9999	0.0001	0.0001	0.0001
4	0.9949	0.0015	0.0051	0.0015
5	0.8289	0.0196	0.1721	0.0196
6	0.9154	0.0106	0.0846	0.0106
7	0.7009	0.0182	0.315	0.0182
8	0.3268	0.0267	0.6928	0.0264
9	0.0959	0.0144	0.9052	0.0143
10	0.9754	0.0098	0.0246	0.0098
11	0.8344	0.0183	0.1876	0.02
12	0.9871	0.0036	0.0129	0.0036
15	0.912	0.0129	0.088	0.0129
16	0.9739	0.0077	0.0261	0.0077
17	0.7435	0.0222	0.2565	0.0222
18	0.9654	0.0059	0.0346	0.0059
19	0.6659	0.0282	0.335	0.0283
Locus				
B124	0.4823	0.0267	0.5177	0.0267
BL13	0.0011	0.0005	0.9989	0.0005
BT28	0.7693	0.0028	0.3049	0.0028
BTERN01	0.9512	0.0035	0.0488	0.0035
B10	0.6063	0.0311	0.3937	0.0311
B119	1	0	0	0
B96	0.6106	0.0261	0.3894	0.0261
BL11	0.9999	0.0001	0.0001	0.0001
Btern02	1	0	0	0

Table A9 Pairwise Locus Linkage Disequilibrium per population for *Bombus vagans*.

Significant values are bold. A dash indicates no comparison could be made.

Locus Pairs		Population																
Locus#1	Locus#2	1	2	3	4	5	6	7	8	9	10	11	12	15	16	17	18	19
B124	BL13	-	1	1	-	-	-	-	-	0.06193	0.41271	-	1	0.83268	1	1	1	1
B124	BT28	-	0.53703	-	-	-	-	-	0.97971	0.69001	-	-	0.37235	0.60921	0.68113	0.73204	0.80366	-
BL13	BT28	-	0.86058	-	-	-	-	-	-	1	1	1	0.24526	0.69104	0.44148	0.64245	0.60962	-
B124	BTERN01	-	0.84893	0.90535	-	-	1	-	1	0.31959	-	-	1	0.19994	0.84507	0.60558	1	1
BL13	BTERN01	0.32454	0.70838	1	-	0.41812	-	-	0.7519	0.52978	1	1	0.36315	0.44875	0.12634	0.2234	0.53798	0.89367
BT28	BTERN01	-	1	-	-	-	-	-	0.38756	1	1	1	0.03295	0.35268	1	0.21807	1	-
B124	B10	-	1	1	-	-	-	-	-	1	1	1	1	0.13597	1	0.22151	1	-
BL13	B10	1	1	1	-	-	-	-	-	1	1	1	1	0.68122	1	1	0.0319	-
BT28	B10	-	0.6793	-	-	-	-	-	-	0.8165	-	-	0.9064	0.52967	1	1	0.79449	-
BTERN01	B10	1	0.95048	0.67412	-	0.4262	1	-	-	0.48271	-	-	0.19191	0.43952	0.73597	1	0.3204	-
B124	B119	-	0.49663	0.56046	-	-	-	-	-	-	-	-	0.69387	1	0.4756	0.13508	0.47342	-
BL13	B119	0.79424	0.48778	0.74051	-	1	-	-	-	-	-	-	0.0241	1	0.97606	0.531	0.76384	-
BT28	B119	-	1	-	-	-	-	-	-	-	-	-	0.13174	0.49089	0.74837	0.08957	0.22646	-
BTERN01	B119	1	1	1	0.64781	1	1	-	-	-	-	-	0.88341	1	0.38279	0.30007	0.49229	-
B10	B119	0.60396	0.75759	0.73153	-	0.83902	0.78757	-	-	-	-	-	1	0.28732	1	1	1	1
B124	B96	-	1	1	-	-	-	-	1	1	1	1	1	0.20745	1	1	1	1
BL13	B96	1	1	1	-	1	-	-	1	1	1	1	1	0.96757	0.5428	0.63617	-	1
BT28	B96	-	0.53068	-	-	-	-	-	0.86954	0.63611	0.60099	-	-	0.96757	0.5428	0.63617	-	-
BTERN01	B96	0.54645	0.84943	0.42032	1	1	0.72499	-	0.49206	0.93352	0.98732	1	-	0.88536	0.98168	1	-	1
B10	B96	1	1	1	-	1	-	-	-	1	1	1	1	0.53625	0.05584	1	-	-
B119	B96	0.69156	0.90976	0.83807	0.35412	1	0.5478	-	-	-	-	-	-	0.34208	0.5375	-	-	-
B124	BL11	-	1	-	-	-	-	-	-	-	-	-	-	0.19991	1	1	1	1
BL13	BL11	1	1	-	-	1	-	-	-	-	-	-	-	0.25973	1	1	0.07472	1
BT28	BL11	-	0.86343	-	-	-	-	-	-	-	-	-	-	0.1216	0.93336	0.64305	0.60023	-
BTERN01	BL11	0.35939	0.70551	-	-	1	-	-	0.49661	-	-	-	-	0.24825	1	1	0.08029	1
B10	BL11	1	1	-	-	1	-	-	-	-	-	-	-	0.95662	0.15475	1	0.05039	-
B119	BL11	0.80105	0.44013	-	-	0.83694	-	-	-	-	-	-	-	0.82299	0.85983	0.51954	0.78586	-
B96	BL11	1	1	-	-	1	-	-	1	-	-	-	-	0.11021	1	1	1	1
B124	Btem02	-	0.46246	0.51168	-	-	-	-	-	0.40705	1	-	0.54848	0.4162	0.52785	0.80696	0.60739	0.80587
BL13	Btem02	-	0.4513	0.74384	-	1	-	-	0.73844	0.06251	0.77536	1	0.39002	0	0.9969	0.35437	1	0.49127
BT28	Btem02	-	1	-	-	-	-	-	-	1	1	1	0.05841	0.14112	1	1	1	1
BTERN01	Btem02	-	1	1	0.0247	-	1	-	1	0.29302	0.72352	0.33698	0.45086	0.11371	0.39592	0.02036	0.44546	1
B10	Btem02	-	0.74148	0.76096	-	-	-	-	-	1	1	1	0.55604	0.84935	0.21128	1	1	-
B119	Btem02	-	0.00058	0.01118	0.02337	-	-	-	-	-	-	-	0.00232	0.71169	0.00131	0.03408	0.06494	-
B96	Btem02	-	0.90332	0.83684	1	1	0.55169	-	1	0.90457	1	0.77923	-	0.27798	0.65855	0.35703	-	0.79961
BL11	Btem02	-	0.45488	-	-	0.5676	-	-	1	-	-	-	-	0.16713	0.80102	1	1	1

Table A10 Pairwise Linkage Disequilibrium across all populations for *Bombus vagans*. Locus pair comparison only. Significant values are in bold.

Pairwise Linkage Disequilibrium: Locus Pair Comparison Only		
Locus Pairs		P value
Locus 1	Locus 2	
B124	BL13	0.993675
B124	BT28	0.976532
BL13	BT28	0.98672
B124	BTERN01	0.998481
BL13	BTERN01	0.84667
BT28	BTERN01	0.738715
B124	B10	0.97316
BL13	B10	0.993899
BT28	B10	0.99887
BTERN01	B10	0.99523
B124	B119	0.538767
BL13	B119	0.998727
BT28	B119	0.374929
BTERN01	B119	0.873939
B10	B119	0.98278
B124	B96	0.999952
BL13	B96	0.999999
BT28	B96	0.975168
BTERN01	B96	0.999998
B10	B96	0.989984
B119	B96	0.979414
B124	BL11	0.993783
BL13	BL11	0.952238
BT28	BL11	0.767171
BTERN01	BL11	0.848519
B10	BL11	0.776904
B119	BL11	0.986839
B96	BL11	0.997993
B124	Btern02	0.951965
BL13	Btern02	<0.004290
BT28	Btern02	0.752464
BTERN01	Btern02	0.471822
B10	Btern02	0.994923
B119	Btern02	5.59E-09
B96	Btern02	0.999592
BL11	Btern02	0.978124

Table A11 PCR reaction recipe for multiplex A and B. Colors represent dye labeled primers.

	Plex A				Plex B		
	PCR	1 sample (μl)	Plate (μl)		PCR	1 sample (μl)	Plate (μl)
	Buffer 5x Promega	2	220		Buffer 5x Promega	2	220
	MgCl ₂ 25mM	0.56	61.6		MgCl ₂ 25mM	0.56	61.6
	dNTP	0.6	66		dNTP	0.6	66
	B124-1	0.4	44		B96-1	0.4	44
FAM-blue	B124-2	0.4	44	PET-red	B96-2	0.4	44
	B116	0.4	44		B119-1	0.2	22
VIC-green	B116	0.4	44	VIC-green	B119-2	0.2	22
	BL13-1	0.3	33		BL11	0.2	22
PET-red	BL13-2	0.3	33	PET-red	BL11	0.2	22
	Btern01-1	0.165	18.15		B10-1	0.3	33
VIC-green	Btern01-2	0.165	18.15	FAM-blue	B10-2	0.3	33
	BT10-1	0.165	11		BT28-1	0.1	11
NED-yellow	BT10-2	0.165	11	VIC-green	BT28-2	0.1	11
	BSA	0.2	22		BSA	0.2	22
	H ₂ O	2.7	311.3		H ₂ O	3.16	347.6
	Taq	0.08	8.8		Taq	0.08	8.8
	DNA	1	110		DNA	1	110

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Vita

Eric Scott Rayfield grew up in Cherryville, North Carolina and graduated Cherryville High School in 2009. He attended Lees-McRae College in Banner Elk, North Carolina for his undergraduate studies. In 2013, he graduated with a major in Wildlife Biology and a double minor in Wildlife Rehabilitation and Outdoor Adventure Studies. During his undergraduate career, he volunteered at the Lee-McRae wildlife rehabilitation center, caring for injured wildlife and training animals for environmental education, worked as an avian trainer and educator at the Carolina Raptor Center, and worked as a Rock Climbing and Backpacking instructor at Lees-McRae College Outdoor Programs. In 2014, he came to Appalachian State University and enrolled in a Master of Science program in the Biology Department and graduated in 2021. During his graduate studies, he worked as a field tech for pollination biology, worked as a naturalist on the Appalachian Trail, and worked as a contract biologist for the Conservation Trust for North Carolina. Eric currently works as a microbiologist for a pharmaceutical company and he plans to remain in western North Carolina and pursue a career in wildlife biology.