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Isolation by distance, local adaptation, and fortuitous coincidence of geo-political boundaries with spatial-genetic clusters in southern Bog Turtles

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ABSTRACT

Conservation strategies are often implemented within the jurisdiction of an administrative unit, such as a state or federal agency; however, boundaries between these units may or may not reflect biologically meaningful distinctions. Population genomic data provide a useful way to objectively assess whether boundaries of administrative units coincide with natural population structure, as well as compare future management scenarios within and among said units. Here we used 2658 SNPs generated by a triple-digest reduced representation library preparation method from 171 individuals to determine if genetic population structure of Bog Turtles corresponds with political boundaries. We also estimated genetic diversity within populations pertinent to setting management priorities and tested for genetic signatures consistent with local adaptation as a preliminary step to assess translocation risk. We found that genetic differentiation among populations was strongly predicted by geographic distance. Fortuitously, the patchy distribution of remaining Bog Turtle sites results in spatial-genetic clusters that do correspond with state boundaries. We observed low genetic diversity within populations and several instances where the census size exceeded our estimates of effective population size. Lastly, we detected 20 outlier loci consistent with signatures of local adaptation, suggesting that outbreeding depression may be a risk in some translocation options. Our approach allowed us to improve population parameter estimates for the federally threatened Bog Turtle to address key recovery plan objectives, some of which had not been addressed previously.
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1. Introduction

Conservation is inherently a crisis discipline, requiring quick solutions with limited time and resources. Unfortunately, we are often slow to take action either because we have not yet recognized a need for intervention or because we are uncertain as to how we should intervene. As a consequence, management intervention is often triggered long after substantial declines become apparent (Lindenmayer et al., 2013). For monitoring programs to be effective for species conservation, information should be gathered under the umbrella of explicit objectives linked to criteria that trigger pre-planned management interventions. But what information is necessary to develop these objectives, and how can the information be acquired quickly

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with limited money and personnel? Generally speaking, conserving imperiled species requires information pertinent to three key aspects of management: (1) delegating regions of the species' distributions to appropriate agencies and personnel, (2) prioritizing populations for dissemination of financial resources and management effort, and (3) strategizing what, when, and how interventions should be implemented.

Delegation of populations to administrative units must, by necessity, recognize political boundaries such as country, state, county, or property lines. However, management can be problematic if dictated solely by political boundaries, which may or may not reflect biological reality. Individual populations, including those within the same administrative unit, may be influenced by unique evolutionary and ecological processes and thus require different management strategies (Bernard et al., 2009). Furthermore, inter-agency cooperation may be necessary if populations in different administrative units are connected. The key is to identify unique management units—distinct populations or sets of populations with significant divergence of allele frequencies, which reflect current population structure (Moritz, 1994).

Once management units are identified, prioritizing management efforts within and among units is essential, particularly when we cannot realistically conserve all populations simultaneously or at least cannot do so sufficiently. One approach for prioritization includes comparing genetic measures of diversity and distinctiveness between populations and between proposed management strategies. Given the decreased ability of populations with low genetic diversity to adapt to future environmental change, populations with low genetic diversity might be at high risk of extirpation (Frankham, 2005). High extirpation risk may require more intensive and immediate management action to remedy; however, if these populations also harbor distinct evolutionary histories as suggested by relatively high levels of genetic distinctiveness, they may be worth the effort (e.g. Ciofi et al., 1999). Once priorities are set, managers can also use these genetic data to objectively compare proposed strategies. For example, low genetic diversity (in combination with lower fitness) within a population might generate concern for inbreeding depression and warrant a management strategy to increase diversity, like translocations—any movement of a species from one location to another (Schwartz et al., 2012). Selection of a suitable source (i.e. donor) population requires assessing the genetic distinctiveness of both the donor and recipient population. High genetic distinctiveness (in combination with signs of local adaptation) may caution against such a strategy due to increased risk of outbreeding depression (Rhymer and Simberloff, 1996).

Assessment of management unit distinctions and prioritization of management efforts within and among units are needed for many imperiled species. We focus on North America's smallest turtle, the Bog Turtle (*Glyptemys muhlenbergii*). The Bog Turtle was listed as a federally threated species in their northern range under the Endangered Species Act due to an estimated 50% decline within a 20-year period (USFWS 2001). This listing mandated a Species Recovery Plan to guide conservation and management of extant populations in New York, Connecticut, Massachusetts, New Jersey, Pennsylvania, Delaware, and Maryland. However, 400 km south of the southernmost northern population are additional Bog Turtle populations in the Appalachian Mountains of southern Virginia, North Carolina, Tennessee, and Georgia that are classified as "Similar in Appearance", which prohibits the take of Bog Turtles from southern populations but does not mandate an additional or inclusive Species Recovery Plan. However, all southern states have classified Bog Turtles as either threatened or endangered. Unfortunately, many of the populations in the southern region were not discovered until they were in the midst of their decline and were expected to go extinct within the next 50–100 years in the absence of effective management action (Klemens, 1991). Administrative units have been established in the southern region, but whether these administrative units are biologically meaningful needs to be confirmed. Furthermore, managers are facing a dilemma between the number of populations that require active management and limited resources in which to accomplish said management; thus, they need pertinent information to help them set priorities. Lastly, since many populations are extremely small and isolated, translocation has been proposed as a potential strategy to mitigate the impact of low genetic diversity, but managers are cautious about implementing the strategy given the potential for outbreeding depression; thus, a region-wide genetic assessment would help them identify the least risky translocation options.

We used genomic techniques to collect data for thousands of single nucleotide polymorphisms (SNPs) to gather information pertinent to three key questions related to genetic diversity and genetic distinctiveness of Bog Turtles: (1) does population structure correspond with the political boundaries of the administrative units?; (2) what are the genetic diversity and effective population sizes for each population?; and (3) are there genetic signatures consistent with local adaptation? Answers to each of these questions are necessary to delineate management units and coordinate efforts between administrative units, prioritize populations for allocation of conservation effort, and inform potential management interventions that increase the probability of Bog Turtles persisting in the future.

2. Methods

2.1. Study system

The Bog Turtle (*Glyptemys muhlenbergii*) is a semi-aquatic turtle in the family Emydidae. Individuals are easily identified by the yellow-orange blotches on each side of their necks. Typically, females lay an average of 3 eggs per year, which hatch within 2–3 months; offspring take about 6–12 years to become sexually mature and have a maximum carapace length of 11.5 cm (USFWS 2001). Bog Turtles are habitat specialists, living in spring-fed bogs, which in the southern region often consist of sphagnum moss, various sedges and grasses, and shrubs. Considering these life history characteristics, Bog Turtles are

particularly vulnerable to anthropogenic activities, such as alteration of fire regimes, development, ditching and draining of wetlands, and introduction of exotic species that reduce nesting and basking habitat (USFWS 2001).

Although anthropogenic habitat destruction has likely contributed to the fragmentation within the northern and southern regions, it remains unclear whether anthropogenic impacts are the primary cause of the 400 km gap between these regions. Bog Turtles are hypothesized to have evolved in wet prairie habitats west of the Appalachians (600,000 YBP) and expanded eastward during inter-glacial periods within the Pleistocene epoch, but were confined to the lower Susquehanna and southern Appalachia during glacial periods, isolating the two regions (Holman, 1977). Additionally, it is possible that Bog Turtles used the southern region as refugia during glacial periods and redistributed northward during each inter-glacial period, as was the case for other reptiles in the area during the Pleistocene (Auffenberg and Milstead, 1965). Alternatively, the northern and southern regions may have been in genetic contact until colonial times, becoming increasingly isolated as wetlands of the Shenandoah Valley were destroyed during the Civil War in 1861–1865 (Ernst and Lovich, 2009).

Regardless of what caused the north-south divide in the Bog Turtle range, today, the northern region (locations in New York, Connecticut, Massachusetts, New Jersey, Pennsylvania, Delaware, and Maryland) is designated as a Distinct Population Segment (DPS). Distinct Population Segments are classified by the United States Fish and Wildlife Service based on discreteness (marked separation from other populations of the same taxon), significance (evidence that loss would result in a significant gap in the range of the taxon and/or genetically distinction), and status (based on the ESA standards for listing). Although southern Bog Turtle populations do not have the same federal listing status as the northern DPS, all southern states list the Bog Turtle as an imperiled species and manage extant populations accordingly. Most conservation decisions pertaining to southern populations are managed independently by each state in conjunction with state allocated funds, with a few exceptions. These exceptions include the National Park Service which manages populations along the Blue Ridge Parkway in Virginia and North Carolina, the United States Fish and Wildlife Service which oversees the management of the species as a whole, and Project Bog Turtle (PBT), a conservation initiative of the North Carolina Herpetological Society comprised of federal, state, academic, and non-academic (e.g. zoo) representatives that meet once a year to discuss the status of Bog Turtles in the south and allocate general resources among state partners.

2.2. Sampling

We obtained tissue samples from a total of 209 Bog Turtles from 30 sites spanning all four southern states where Bog Turtles are known to occur (Fig. 1): 13 sites in Georgia (N = 66 turtles), 4 in North Carolina (N = 53 turtles), 4 in Tennessee (N = 35 turtles), and 9 in Virginia (N = 55 turtles). Unfortunately, many of the sampled populations are estimated to have fewer than 20 individuals, and given how cryptic Bog Turtles are in their densely vegetated habitat, typically only a subset of turtles were sampled (N = 1–31 turtles depending on the population). Collaborators collected many of the samples used in this study (see Acknowledgments) during the 2014–2015 field seasons. Others were collected approximately 10 years ago for microsatellite development (King and Julian, 2004). The remaining samples (all Tennessee samples and most Virginia samples) were obtained using a variety of sampling techniques, including visual surveys, probing, muddling (i.e., probing through mud and tussocks using hands), and trapping (Somers, 2000; Whitlock, 2002). Tissue samples were obtained from a 0.5 cm tail clip or full toenail clip and preserved in 95% ethanol and stored at −20 °C until DNA extraction (Hughe, 2010). This sampling protocol was approved by the IACUC at the University of Tennessee [2436-0316].

2.3. Laboratory and post-sequencing procedures

We extracted DNA from tissue samples using the DNeasy Blood and Tissue Kit (Qiagen Corporation, Valencia, CA). Prior to library preparation, DNA quantity and quality were assessed using a fluorometer to quantify the amount of DNA and gel electrophoresis to confirm extracted DNA was not degraded. Samples were then digested using three enzymes (ClaI, MspI, and BamHI-HF) as part of a triple-digest restriction site associated DNA sequencing (3RAD) library preparation protocol (Glenn et al., 2017). This procedure outperforms the more commonly used double-digest RADseq by reducing chimeras, increasing adapter ligation efficiency, and minimizing adapter dimers while simultaneously requiring less input DNA and improving sequencing efficiency through the use of variable length quadruple-index tags. The generated RADseq libraries were then pooled relative to their DNA concentration and 450–550 bp fragments were isolated using a Pippin Prep system (Sage Science Corporation, Beverly, MA), pooled with samples from unrelated projects, and sequenced for approximately 2 million reads per individual on an Illumina NextSeq PE150 run at the Georgia Genomics Facility.

Prior to quality control, filtering, and assembly with the software pipeline ipyrad (Eaton et al., 2010, http://ipyrad.readthedocs.io/; Eaton and Overcast, https://github.com/dereneaton/ipyrad), inner barcodes were trimmed. All ipyrad default parameter settings were used, with the following exceptions: the minimum depth at which majority rule base calls are made was set to 6, the cluster threshold was set to 0.907, the maximum number of unique alleles allowed in individual consensus reads after accounting for sequencing errors was set to 2, the minimum number of samples that must have data at a given locus for it to be retained was set to 6, the maximum number of SNPs allowed per final locus was set to 20 (10 for each read in paired locus), and the maximum proportion of shared polymorphic sites in a locus was set to 0.25 (which limits the number of heterozygous bases in common between two individuals for a given locus, rejecting loci likely to be paralogs). Subsequent filtering within the R software environment (R Development Core Team, Version 3.3.2) was necessary to confirm

Fig. 1. Bog Turtle sampling locations in the southern portion of their distribution. The gray shaded region represents the distribution of Bog Turtles in the southern region of the United States (Stratmann et al., 2016). Only the sites with sufficient sampling and sequence quality (i.e., used in data analyses) are shown (11 populations), with the exception of three North Carolina sites (represented by "?") for which geographic coordinates were not provided due to concern that the information could be intercepted by poachers. Unique site codes are shown next to their corresponding site, but the names have been omitted to protect the identity of sites.

that all loci with more than 2 alleles were removed and all loci had a minimum minor allele frequency of 0.05. Finally, we used VCFtools to identify and remove loci out of Hardy-Weinberg equilibrium (Danecek et al., 2011).

A total of 171 turtles and 2658 loci remained after extraction, library preparation, sequencing, and quality control and filtering (Georgia = 47 individuals, North Carolina = 50 individuals, Tennessee = 32 individuals, Virginia = 42 individuals). We used these remaining individuals and loci (or a subset when mentioned) for subsequent analyses.

2.4. Data analysis

To determine whether the southern Bog Turtle management units based on political boundaries (i.e., states) are biologically useful and to provide PBT with information to aid in allocation of general resources, we assessed patterns of genetic structure using a Bayesian algorithm in STRUCTURE (Version 2.3.2.1; Pritchard et al., 2000). This algorithm infers the proportion of ancestry from each cluster, for an assumed number of clusters (K) from individual multilocus genotypes. The default settings were used, including an admixture model without a priori knowledge of geographic location. To determine the most likely number of clusters, we conducted a series of analyses for five independent iterations of K = 1–10, using a burn-in period of 10 000 repetitions and Markov Chain Monte Carlo (MCMC) of 10 000 repetitions. We examined these results using STRUCTURE HARVESTER (Earl and vonHoldt, 2012). If state borders reflect genetic structuring of populations, ancestral proportions of individuals within the same state should be similar, where the greatest proportion of their genetic data correspond to the same genetic cluster and when K = 4 all individuals are clearly resolved by the state they reside in.

Considering the physical distribution of sampled sites, in which sites from the same state tend to be geographically clustered, we also tested for isolation by distance (i.e., proportional increase in genetic distance as geographic distance between population increases). Geographic coordinates were provided by participating state agencies, with the exception of North Carolina which feared that the information might be intercepted by poachers; thus, North Carolina populations were excluded from this analysis. Genetic differentiation between pairs of populations (pairwise F_{ST}) was calculated using the R package 'diveRsity' (Keean et al., 2013; R Development Core Team 2016). The significance of differentiation was assessed

through the calculation of 95% confidence intervals using a bias corrected bootstrapping method with 1000 bootstraps. The estimated pairwise F_{ST} values were transformed ($\frac{F_{ST}}{1-F_{ST}}$) prior to running a Mantel test (9999 permutations) on the geographic distance and genetic distance matrices. All population-level calculations in this study included only the 11 populations with greater than five sampled individuals.

For use in the prioritization of populations for conservation initiatives, we calculated the genetic diversity and modeled the effective population size of sampled populations. For each population, the distribution of genetic diversity across loci and global genetic diversity (i.e., 'expected heterozygosity') was calculated using the basicStat function in the R package 'diveRsity' (R Development Core Team 2016). We used the linkage disequilibrium model with random mating in NeEstimator (Version 2; Do et al., 2014) to estimate contemporary effective population sizes (N_e) from the genetic data for each population with at least 6 sampled individuals. Parametric 95% confidence intervals were determined based on the chi-square approximation (Waples, 1989). Estimated effective population sizes were compared with estimated census sizes provided by state partners if available.

Lastly, we conducted an F_{ST} outlier analysis, to detect statistical signatures consistent with patterns of local adaptation (i.e., potentially greater outbreeding depression risk), using a Bayesian approach implemented in BAYESCAN (Version 2.1; Foll and Gaggiotti, 2008). BAYESCAN uses logistic regression to decompose F_{ST} coefficients into a locus-specific component (alpha) shared by all populations and a population-specific component (beta) shared by all loci. Loci potentially under selection are identified as those showing an atypical pattern of variability compared to the rest of the genome, i.e., those with a high posterior probability (q) of having a non-zero locus specific component (alpha). Positive values of alpha indicate loci potentially affected by divergent selection and negative values indicate loci potentially affected by balancing selection. Following suggestions made by Foll and Gaggiotti (2008), we used a prior odds of 10, a false discovery rate of 0.05, and chain parameters: 600 000 iterations with a thinning interval of 50 and 10 pilot runs of length 100 000 with a burn-in of 100 000. Model convergence was confirmed using Geweke's convergence diagnostic and Heidelberg Welch's convergence diagnostic and we verified non-correlated sampled parameters.

3. Results

We obtained a total of 296 857 917 PE150 reads for 195 individual turtles from 18 sites located across four states (Virginia, North Carolina, Tennessee, and Georgia). After filtering for a minimum depth and minimum number of samples per locus of six, in ipyrad, we obtained 29 081 'unlinked' SNPs (only one SNP used per paired-end read). Using R, we further filtered this dataset by removing all loci with more than 50% missing data (16 297 loci), then all individuals with more than 50% missing data (12 turtles), then loci with more than 2 alleles (59 loci), and then loci with a minor allele frequency of less than 0.05 (9489 loci). Finally, we identified and removed a set of 560 putative loci that were all highly correlated with each other (within state linkage disequilibrium greater than 0.5, and predominantly at the end of the ipyrad output, suggesting a systematic error in designating them as distinct loci). The final dataset consisted of 2658 markers across 171 turtles from a total of 11 populations.

Bootstrap 95% confidence limits for global F_{ST} were 0.2073 and 0.2343 (point estimate 0.2204), so we can reject the null hypothesis of no population structure. The Bayesian clustering plot generated using output from STRUCTURE clearly showed clustering of individual turtles by their state of origin (Fig. 2). The most likely number of clusters based on the Evanno method was $K = 2$, which distinguishes individuals from Georgia from other southern states. As we increased the number of clusters, the ancestral proportions for individuals from the same state were similar and clustered together, with the exception of individuals from one North Carolina site that formed a genetic cluster distinct from other North Carolina individuals, and tended to cluster with sites from Virginia when $K = 3$ and 4. Given our lack of locality information for North Carolina, it is possible that this site is geographically close to the Virginia border.

In general, the appearance of clustering by state is likely a result of isolation by distance. There was a strong and significant positive correlation between geographic distance and the genetic distance between sites (Fig. 3; Mantel test; $r = 0.916$, $p = 0.0002$). Moreover, there was no evidence of genetic similarity within vs. between states after taking geographic distance into account (Partial Mantel test; $r = 0.009$, $p = 0.968$). Thus, differences in genetic distance between pairs of populations increased as expected given the geographic distance between the populations.

We observed low, but variable genetic diversity for each of the eleven populations (H_e range = 0.155–0.219) and several instances where the effective population size was estimated to be substantially lower than the assumed census size (estimated by local experts), and in some cases lower than our sample size (Table 1). We noted two populations where the effective population size was estimated to be substantially less than the census size: Site 3 in Georgia ($N_e = 3$, $N = 20$) and Site 8 in Virginia ($N_e = 6$, $N = 28$). Site 6 in North Carolina also had an estimated effective population size less than the census size ($N_e = 26$, $N = 31$), but the two values were relatively close. Although we did not have an accurate census size estimate for Site 6, we did have samples from 31 turtles which likely represents far fewer turtles than then the actual census size, so the number of turtles we sampled exceeded the effective population size we estimated. Although we could not estimate effective population size for all populations, several additional populations had low genetic diversity, which could correspond to low effective population sizes.

F_{ST} outlier analysis in BAYESCAN was consistent with some degree of local adaptation. We detected 20 outlier loci, 19 of which were consistent with diversifying selection and 1 consistent with balancing selection (Fig. 4). Excluding these outliers did not change our estimates of F_{ST} and N_e to two decimal places.

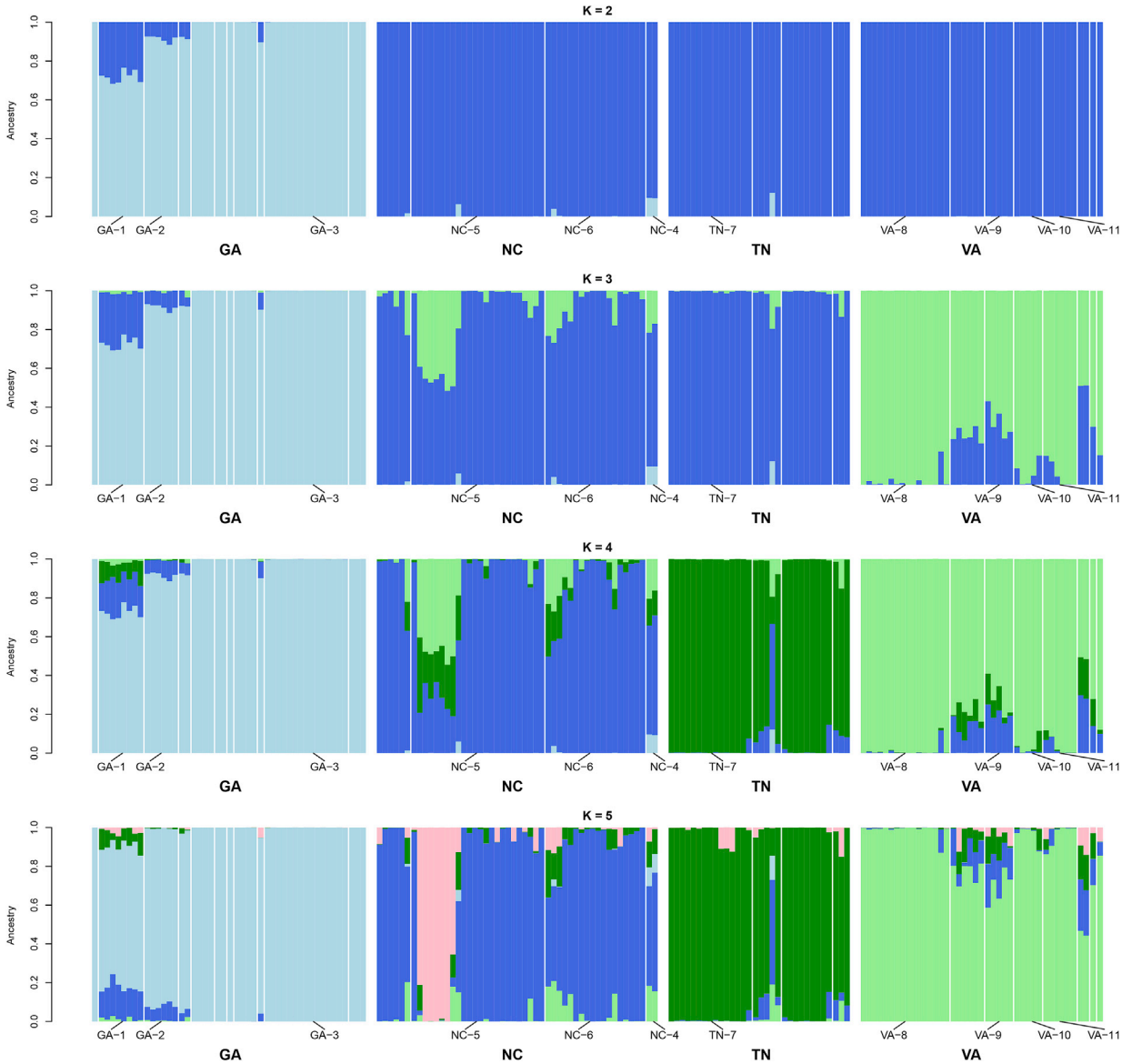

Fig. 2. Ancestry proportions of Bog Turtle individuals, sampled in four states (GA Georgia, NC, North Carolina, TN Tennessee, and VA Virginia), to population clusters determined with the software STRUCTURE. The genetic data are fit to four different models, a two-cluster model, three-cluster, four-cluster, and five-cluster (K = 2–5). Each vertical colored bar represents an individual turtle; note that the ancestry proportion for some individuals = 1, demonstrating confident assignment to a single cluster. Each color corresponds to a distinct genetic cluster. Thin spaces separate individual sites, and thicker spaces separate states. Only the 11 locations used in subsequent analyses (e.g., F_{ST}, and N_e) are labeled with their corresponding site code (see Fig. 1). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

4. Discussion

From a single data set, we acquired a wide variety of information pertinent to the management of the federally threatened Bog Turtle in the southern region, a portion of the range that, thus far, has not receive the same federal mandates and protections as their northern counterparts. Specifically, (1) we found that in general, population structure did correspond with the political boundaries of the administrative units, (2) we showed that genetic diversity and effective population size estimates varied among populations, and (3) we found genetic signatures consistent with local adaptation.

Given that our data suggest that the current administrative units are, for the most part, biologically meaningful, the next step is to prioritize populations within each unit for management and allocate resources accordingly. The more secure investment would be to delegate management resources to populations most likely to persist—those populations with higher genetic diversity and effective population sizes. However, arguments could be made for delegating management resources to

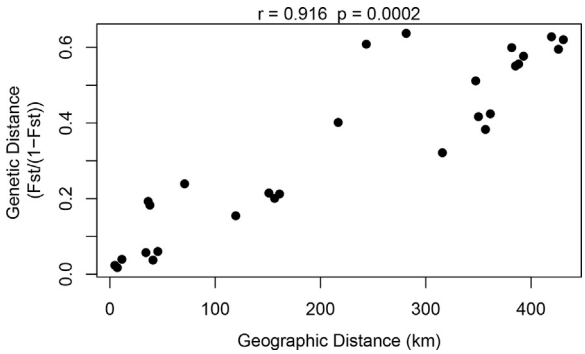

Fig. 3. Isolation by distance analysis for Bog Turtle populations in southern Distinct Population Segment. Correlation of genetic distance (transformed pairwise F_{ST} values) and geographic distance (distance among centralized point for each site). Three North Carolina sites were excluded from this analyses as geographic coordinates were not available.

Table 1
Gene diversity (H_e), effective population size (N_e), assumed census size (N), and pairwise F_{ST} values for eleven Bog Turtle populations in the four United States (GA Georgia, NC North Carolina, TN Tennessee, VA Virginia).

	Site	GA - 1	GA - 2	GA - 3	NC - 4	NC - 5	NC - 6	TN - 7	VA - 8	VA - 9	VA - 10	VA - 11
H_e[a]		0.219	0.195	0.200	0.219	0.155	0.218	0.186	0.201	0.209	0.197	0.194
N_e[b]		11 (10.7, 11.6)		3 (3.0, 3.1)		18 (16.2, 21.3)	26 (26.0, 26.7)		6 (6.1, 6.2)			
N		9	8	20			31	30	28	20	15	20
F_{ST}	GA - 1	0.00										
	GA - 2	0.16	0.00									
	GA - 3	0.19	0.15	0.00								
	NC - 4	0.25	0.33	0.36	0.00							
	NC - 5	0.35	0.45	0.44	0.20	0.00						
	NC - 6	0.27	0.33	0.36	**0.00**	0.19	0.00					
	TN - 7	**0.29**	0.38	0.39	**0.11**	0.26	**0.11**	0.00				
	VA - 8	0.30	0.37	0.38	0.13	0.21	0.13	0.18	0.00			
	VA - 9	0.24	0.34	0.36	**0.08**	0.21	**0.10**	**0.13**	**0.06**	0.00		
	VA - 10	0.28	0.36	0.37	**0.12**	0.22	0.13	**0.17**	**0.02**	**0.04**	0.00	
	VA - 11	0.29	0.37	0.39	0.13	0.23	0.13	**0.18**	**0.04**	**0.05**	**0.02**	0.00

F_{ST} values shown in bold were not statistically significant based on 95% confidence intervals.
[a] Gene diversity or "expected heterozygosity".
[b] Numbers in parentheses represent 95% confidence intervals and N_e estimates were only obtained for sites where at least 6 individuals were sampled.

populations most vulnerable to extinction, particularly those harboring unique genetic variation. In our case, the more secure investment would be to prioritize populations such as Site GA − 1, a population with higher genetic diversity and effective population size. In contrast, Site GA − 3, NC − 6, and VA − 8 are in more immediate need of management resources based on their relatively low genetic diversity and N_e estimates, but these investments may be more uncertain. Regardless, making management recommendations based on census size alone might be misleading given that effective population sizes (N_e) were often lower than the census sizes (N). The cause of the disparity between census size and effective population size, including instances where N_e was greater than N is unknown, but life history characteristics of Bog Turtles may be a contributing factor; specifically, age at maturity and lifespan (Waples et al., 2013). Based on our inability to estimate effective population sizes for all populations and our exclusion of seven sites from most analyses due to low sample sizes (with the exception of the population structure analysis in STRUCTURE), we would encourage conservation practitioners to continue taking genetic samples, especially from poorly sampled populations and collect demographic data to better estimate census sizes. These additional data would allow for the modeling of effective population sizes and to obtain more accurate and precise estimates of genetic diversity in addition to other population genetic parameters.

Once populations are prioritized for management, the most appropriate management approach(es) need to be implemented. One such approach that has already been proposed for Bog Turtles, is translocation. Translocation is often proposed as a strategy to restore connectivity and minimize inbreeding depression (Dupulus-Desormeaux et al., 2018), but translocation between populations locally adapted to different environments likely correspond to greater outbreeding depression risk, as locally adapted gene complexes would be broken up in admixed offspring, producing offspring maladapted to the present environment (as seen in laboratory crosses between different nematode strains, Dolgin et al., 2007, largemouth bass crosses in experimental ponds, Goldberg et al., 2005, and in third generation offspring partridge pea plants, Fenster and Galloway, 2000). In our case, genetic differentiation between populations was consistent with the geographic distance between populations; genetic differentiation was generally highest between populations from different states and lowest between populations from the same state. However, we did observe two exceptions in North Carolina, (1) between Site 4 and

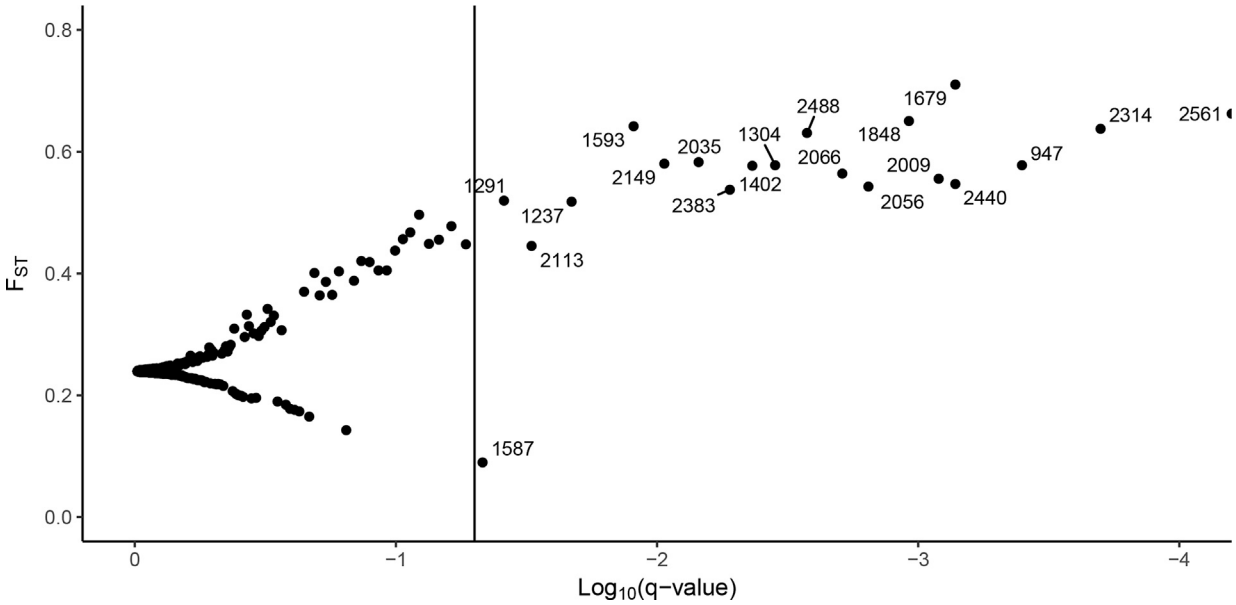

Fig. 4. F_{ST} outlier analysis of 2658 SNP markers in BAYESCAN 2.1. Pairwise F_{ST} values are plotted against the log10-transformed q-values (the minimum false discovery rate at which a locus becomes significant). Nineteen loci show greater genetic differentiation than expected under neutrality (FDR = 0.05, vertical line), consistent with diversifying selection. Locus 1587 shows less genetic differentiation than expected, consistent with balancing selection. Note that Locus 2561 has a $\log_{10}$(q-value) equal to negative infinity, but it is displayed here at the edge of the plotting window for the purpose of visualization.

Site 5 and (2) between Site 5 and Site 6. These pairwise comparisons had higher F_{ST} values (0.20 and 0.19, respectively) than pairwise comparisons between these North Carolina populations and some Virginia populations (e.g. NC − 4 and VA − 9 = 0.08). So, while genetic structuring often corresponded to state boundaries, state borders alone did not always distinguish distinct genetic clusters. This information, in combination with the presence of 20 outlier loci, suggest that translocations between North Carolina Site 5 and another North Carolina population, e.g. Site 4, would be surprisingly riskier relative to translocations between North Carolina Site 4 and an out-of-state Virginia population, Site 9. The genetic "similarity" between the North Carolina populations and the Virginia population is likely a result of their close geographic proximity. However, without fitness data to assess whether or not inbreeding depression is occurring in prioritized populations and whether outbreeding depression has occurred in an existing translocated population (Dresser et al., 2017), all we can provide here is a preliminary and relative assessment of potential outbreeding depression risk. Moreover, non-genetic factors, such as disease (Cunningham, 1996) and site fidelity (e.g. Bell et al., 2005) should be considered when discussing the suitability of translocations to meet conservation objectives.

Our ability to capitalize on the recent advances in genomics likely make our estimates of genetic diversity and effective population size more informative than previous estimates based on allozyme, mitochondrial, or microsatellite markers (Allendorf, 2017). Furthermore, our study includes a more extensive sampling of the entire southern region by sampling populations from every state, including for the first time, populations from the state of Tennessee (as compared to Amato et al., 1997; Rosenbaum et al., 2007; Pittman et al., 2011; Shoemaker and Gibbs, 2013). The need for and value of such genetic data is not unique to the conservation of Bog Turtles, because populations of many species have experienced increased isolation associated with habitat loss and fragmentation. While alternative methods are available for designating management units, such as satellite or radio telemetry to determine the extent of inter-population dispersal (e.g. Mauritzen et al., 2002; Lovich et al., 1992), these data lack certainty in regards to effective dispersal (i.e., breeding between migrants and residents). Genetic data are also being increasingly used for conservation prioritization (e.g. Rieman and Allendorf, 2001; Taylor et al., 2010; Palkovacs et al., 2013; Yumnam et al., 2014). Such data provide a wider lens in which to assess past demographic fluctuations unobtainable with recent implementation of traditional field methods (e.g. historic bottlenecks) and infer future persistence in the context of climate change (Ramey et al., 2000 and St Clair and Howe, 2007, respectively). Our results have conservation implications similar to those from other studies of conservation genetics in turtles, highlighting the importance of shared evolutionary history in predicting conservation needs. First, isolation-by-distance, as we demonstrated here, is common across spatial scales (e.g., within the Berkshire-Taconic region of the Bog Turtle range, Shoemaker and Gibbs, 2013; range-wide in Eastern Box Turtles, Kimble et al., 2014). Furthermore, scientists have retroactively recognized how population isolation has contributed to high levels of inbreeding in Wood Turtles in Ontario, Canada (Fridgen et al., 2013) and regional genetic differences among Blanding's turtles in the St. Lawrence Valley (McClusky et al., 2016). Lastly, other studies have wisely sought to transform how isolated populations are managed based on genetic population structure rather than

political boundaries, such as the recognition of six discrete genetic clusters within a single evolutionary significant unit of Western Painted Turtles in British Columbia, Canada (Jensen et al., 2013).

Clearly, time is of the essence when it comes to matters of species listing and setting recovery criteria, yet the amount of information necessary to make an informed decision and set management objectives can make the task seem impossible. We have demonstrated how a single genomic data set can help agencies acquire information pertinent for delegating management units, prioritizing the use of limited management resources, and strategizing how best to manage species in decline. Specifically, this study addresses two objectives from the Bog Turtle Species Recovery Plan: describe genetic differentiation in the southern portion of the species range, and evaluate genetic consequences of translocation (USFWS 2001). Obviously, genetics is only one piece of the puzzle when making conservation decisions, but it is a particularly valuable one, considering the limited resources allocated to conserving the world's imperiled species.

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Data archiving

Raw sequence data are available from the NCBI SRA (BioProject ID PRJNA505048). A VCF file, analysis scripts, and select output files are available from Mendeley Data (doi:10.17632/3rnnc8hfvj.1).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gecco.2018.e00474.

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