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Spotted Turtle (*Clemmys guttata*) Population Genetics in the Southeastern United States

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ABSTRACT. – **With populations declining across their geographic range, the spotted turtle (*Clemmys guttata*) is currently a species of conservation concern. Though broadly distributed, the species is particularly enigmatic at its southern periphery, and many aspects of its ecology and population biology in this portion of the range have either just recently been described or are currently unknown. One of the current knowledge gaps is a robust assessment of the population genetics of the southern populations. We collected tissue samples from 204 spotted turtles from 5 sites across South Carolina, Georgia, and Florida and used 11 microsatellite loci to investigate the genetic diversity and population structure in these populations. We found that southern populations exhibited low, but significant, population differentiation (mean $F_{ST} = 0.062$) and each site clustered as its own genetic group. Genetic diversity across sites was comparable to estimates reported for northern populations. Net effective population sizes were generally robust and no populations showed indication of recent bottlenecks. Our results suggest that populations inhabiting relatively intact environments do not appear to face immediate threats from past loss of genetic diversity. However, continued monitoring, both demographic and genetic, of this long-lived species is an important management goal to insure that continued global changes do not threaten population viability.**

KEY WORDS. – **conservation genetics; Emydidae; *Clemmys*; effective population size**

Biodiversity is a term used to encompass the diversity of species, the genetic variation they possess, and the ecosystems that they form. Currently, the world is experiencing rapid declines in the number of species at rates unprecedented in human history (Gugerli et al. 2008; Ceballos et al. 2015; Cafaro et al. 2022). The loss of genetic variation has been demonstrated to have serious implications on the persistence of species (Spielman et al. 2004; Gugerli et al. 2008; Allentoft and O'Brien 2010) as genetic diversity is an important component for the long-term survival and adaptive potential of wild populations (Forest et al. 2007). The field of conservation genetics arose, in part, to evaluate, address, and mitigate these issues (Hedrick 2001). In particular, conservation genetics incorporates tools from the field of population genetics to quantify levels of genetic variation and characterize geographic patterns of genetic structure that may arise from nonrandom mating, population bottlenecks, physical barriers between populations (including habitat fragmentation), and other limitations to gene flow.

The southeastern United States is a global biodiversity hotspot (Noss et al. 2015) that includes a diverse turtle assemblage (Buhlmann et al. 2009). However, turtles are among the most imperiled vertebrate groups on the planet (Lovich et al. 2018). Among their major threats are habitat fragmentation, degradation, and alteration as well as their collection from the wild (Moll and Moll 2004; Beaudry et al. 2008; Buchanan et al. 2019a; Howell and Seigel 2019), all of which can lead to population declines. Because turtles exhibit a life history characterized by delayed sexual maturity coupled with high adult survivorship (Congdon et al. 1994), turtle populations can be especially sensitive to the loss of even a small number of adults (Howell et al. 2019). Smaller turtle populations are more susceptible to the loss of genetic diversity due to genetic drift, and in extreme cases, bottleneck effects (Luikart et al. 1998; Kuo and Janzen 2004). Because the loss of genetic diversity can affect the long-term survival of a species (Lande and Shannon 1996; Lai et al 2019), an important

aspect of turtle conservation should be an evaluation of both demographic and genetic viability.

One turtle species that has experienced range-wide declines in recent years is the spotted turtle (*Clemmys guttata*). Spotted turtles are small freshwater turtles with a geographic distribution that ranges across most of the eastern United States, from Florida to Maine, and stretches into parts of the Midwest, the Great Lakes region, and southeastern Canada (Ernst and Lovich 2009). Like many turtle species, spotted turtle populations face a myriad of threats, particularly in the form of habitat destruction and fragmentation (Beaudry et al. 2008; Buchanan et al. 2019a, 2019b; Howell and Seigel 2019), increased predation from human-subsidized predators (Browne and Hecnar 2007), and collection for the pet trade (Buhlmann and Gibbons 1997; Meylan 2006). Relatively high risk of extinction has been documented even in populations inhabiting pristine environments (Enneson and Litzgus 2009) and anthropogenic disturbances can increase the risks of population declines (Beaudry et al. 2008; Howell and Seigel 2019). Because of the many threats that spotted turtles face, the species was petitioned for federal listing under the US Endangered Species Act in 2012. The US Fish and Wildlife Service (2015) suggested that there is information enough to indicate the species may warrant federal listing. The species is already listed as Endangered on the International Union for Conservation of Nature Red List (van Dijk 2011) and as Endangered in Canada (Browne and Hecnar 2007). Range-wide status assessments for the species are currently underway in the US portion of the range, including widespread survey work, genetic analyses, and population modeling, for the forthcoming listing determination scheduled for 2023.

Despite the species' broad geographic distribution, until recently most studies of the spotted turtle have been conducted in the northern half of its range. For instance, studies of reproductive biology (Ernst 1970; Litzgus and Brooks 1998), ecology (Litzgus and Brooks 2000; Litzgus et al. 1999; Ernst 1976), habitat use (Beaudry et al. 2009; Rasmussen and Litzgus 2010), and population genetics (Davy and Murphy 2014; Anthonysamy et al. 2018; Buchanan et al. 2019b) have been conducted in populations in the northern or midwestern United States and southern Canada. The work conducted by Litzgus and Mousseau (2003, 2004a, 2004b) at a site in South Carolina is the one notable exception. Recently, we have described several aspects of spotted turtle ecology using data from 2 Georgia populations and opportunistic observations from Florida (Chandler et al. 2019, 2020, 2022). However, there remain important gaps in our understanding of the ecology and status of the spotted turtle in the southern portion of its range. Perhaps most notable is the absence of an evaluation of the genetic diversity of turtles in this region.

Spotted turtles inhabit a variety of wetland types, typically characterized by shallow water depths and abundant vegetation (Milam and Melvin 2001; Rasmussen and Litzgus 2010). Some of these habitats are ephemeral and spotted turtles, especially in the southern portion of their range, are known to spend long periods on land when wetlands dry (Litzgus and Brooks 2000; Rowe et al. 2013; Chandler et al. 2020). Historically, populations may have inherently experienced some levels of isolation due to the ephemeral, discontinuous nature of wetland habitats, but these systems have become increasingly isolated over time as wetlands have been drained for development or converted to other uses and an increasingly dense road network has bisected the landscape (Buchanan et al. 2019a). The loss and fragmentation by roads of these small wetlands may reduce the probability of successful dispersal of turtles on the landscape (Gibbs 1993; Carter et al. 2000).

Despite the general conservation concern for spotted turtles, few genetic studies have been conducted (Parker and Whiteman 1993; Davy and Murphy 2014; Anthonysamy et al. 2018; Buchanan et al. 2019b; Scoville 2019). Here, we present the first assessment of population genetics in spotted turtles in the southern portion of their range. Our goals were to 1) evaluate the population genetic structure of the spotted turtle at focal sites in Florida, Georgia, and South Carolina; 2) compare metrics of genetic diversity among sites as well as to other published studies from northern portions of the species' range; and 3) characterize the demographic history of the southern populations by testing them for historic bottleneck events and estimating effective population sizes. We used the same microsatellite loci as those used to investigate the population genetics of the spotted turtle in southern Canada (Davy and Murphy 2014) and some of the same loci as Buchanan et al. (2019b). By using the same microsatellite loci, we can draw inferences on the genetic diversity of the species at the southern and northern aspects of its range. The data presented here increase our understanding of the genetic health of southern populations and will aid in future conservation and management of these populations.

METHODS

Study Sites. — We collected spotted turtle genetic samples from individuals at sites in Florida, Georgia, and South Carolina (Fig. 1). We have withheld specific location information throughout because of collecting concerns in this species. In Georgia, we studied turtles at 2 sites that were approximately 145 km apart. Spotted turtles were typically located in shallow wetlands that were adjacent to flowing streams or rivers. These wetlands were often ephemeral in nature and consisted of both naturally occurring and manmade wetlands that were interspersed within the surrounding uplands, creating a discontinuous wetland complex at both sites (see details in Chandler et al. 2019, 2020). In Florida, we studied turtles at 2 sites located approximately 50 km apart and 250 km south of the nearest Georgia site, both consisting of shallow water pools with thick detritus-muck soils embedded in large swamp and floodplain complexes fed

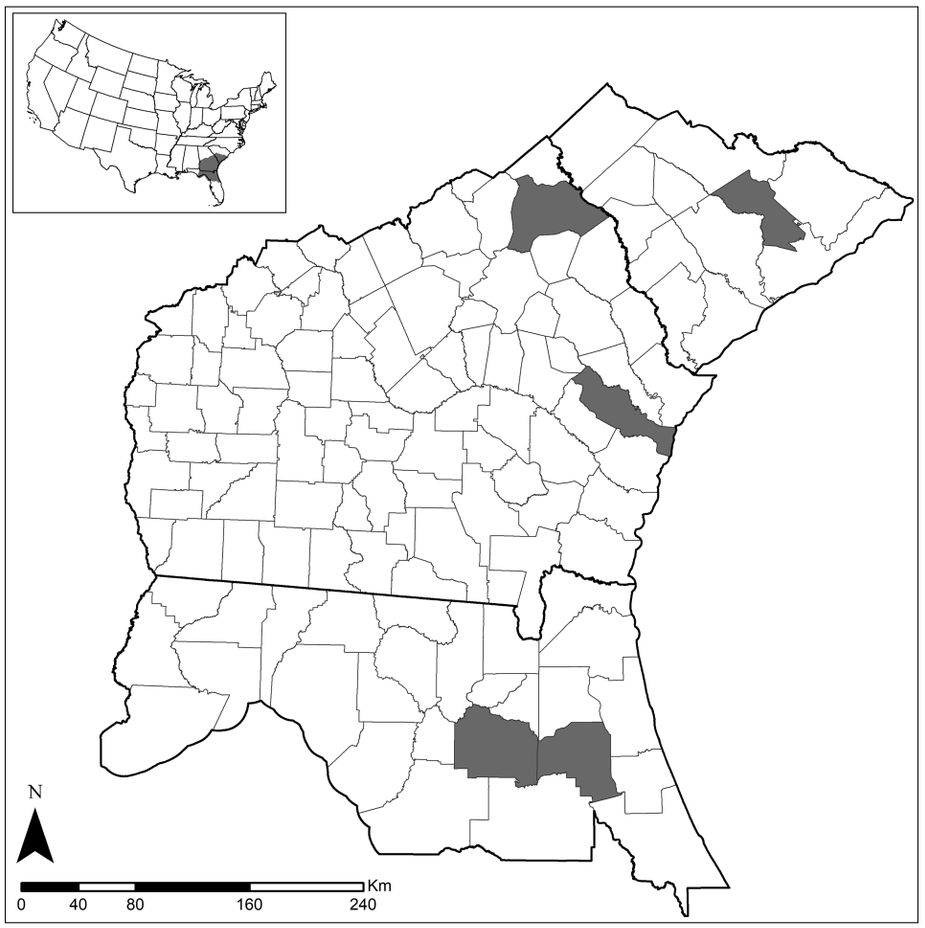

Figure 1. Counties where spotted turtle (*Clemmys guttata*) genetic samples were collected from 2014 to 2019.

and drained by small, low-gradient streams (see details in Chandler et al. 2022). In South Carolina, we sampled turtles at a single site (located approximately 160 km from the nearest Georgia site) that consisted of a large wetland complex interspersed around several flowing creeks. This site contained larger areas of open and flowing water than the other sites that were sampled during this study.

Sample Collection. — We collected tissue samples for genetic analyses as part of ongoing research and monitoring projects across these 5 study sites from 2014 to 2019 (not every site was sampled in every year). Spotted turtles were captured in the spring (typically February–May) of each year using a combination of aquatic traps baited with sardines and visual encounter surveys. Survey effort varied across years, but we generally attempted to capture as many turtles as possible in each season over a 1- to 3-wk period. Once captured, we processed all turtles, recording morphometric data, sex, and shell notch code for future identification. We collected a single tissue sample from each captured individual that primarily consisted of a small tail tip. In rare cases where a tail tip could not be collected, we collected either a small section of webbing from between the toes or shell shavings from the carapace. We placed all tissue samples in 95% ethanol and stored them in the freezer prior to genetic analyses.

Molecular Methods. — Total genomic DNA was extracted from tissue samples or blood samples with a DNeasy Tissue Kit (QIAGEN Inc). A polymerase chain reaction was used to amplify the 11 microsatellite loci (*GmuD79*, *GmuD121*, *GmuD107*, *GmuA19*, *GmuD55*, *GmuD87*, *GmuD88*, *GmuB08*, *GmuD16*, *GmuD21*, *GmuD114*) identified by King and Julian (2004) following the cycling parameters of Davy and Murphy (2014), who used these same loci to characterize genetic structure in *C. guttata* populations near the species' northernmost distribution. Amplifications were conducted in a total volume of 12.5 µl using 7.76 µl of dH_2O, 1.25 µl of 10× standard *Taq* (Mg-free) buffer (New England Biolabs), 0.75 µl of 2 mM dNTPs, 0.75 µl of 25 mM $MgCl_2$, 0.25 units of *Taq* polymerase (New England Biolabs), 0.4 µl of 10 mM M13 tailed forward (Boutin-Ganache et al. 2001) and reverse primers, 0.09 µl of 1 µM labeled M-13 primer (LI-COR Co), and 20–50 ng of DNA template. Microsatellite alleles were visualized on a polyacrylamide gel using a LI-COR 4300 DNA analyzer. Alleles were sized using GeneProfiler ver. 4.05 (LI-COR Co). Each gel included internal standards as well as turtles of known

genotypes to facilitate accurate genotyping. Those turtles missing $> 20\%$ of their microsatellite genotype data were excluded from analyses.

Each locus was tested for Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) using the *genepop* package in R v. 3.6.1 (Rousset 2008; Rousset et al. 2020). A sequential Bonferroni correction was applied to the alpha value to account for multiple comparisons (Rice 1989). Loci were checked for null alleles and large-allele dropout using micro-checker v. 2.2.3 (Oosterhout et al. 2004). We used GenAlEx v. 6.503 (Peakall and Smouse 2006) to calculate observed (H_O) and expected (H_E) levels of heterozygosity. Allelic richness (A_R) and private allelic richness (PA_R) were calculated by rarefaction in HP-Rare v. 1.0 (Kalinowski 2004, 2005) to account for unequal sample sizes across sites. The inbreeding coefficient (F_{IS}) for each site was calculated using the package "hierfstat" (Goudet 2005) with 95% confidence intervals determined by 10,000 bootstrap replicates. We used analysis of variance (ANOVA) or Wilcoxon Rank Sum tests, if ANOVA assumptions of normality and equal variances were not met, to evaluate whether there were significant differences in allelic richness and levels of heterozygosity across southern US populations, as well as between southern US and Ontario populations, using metrics published in Davy and Murphy (2014). All analyses were performed in the "stats" package built into R v. 3.6.3 (R Core Team 2022) with an alpha value of 0.05. We used FSTAT v. 2.9.3 to calculate pairwise fixation index (F_{ST}) values for sites (θ, the unbiased estimator of F_{ST}; Weir and Cockerham 1984), with significance testing using 10,000 permutations of the data and a Bonferroni correction for multiple comparisons. An analysis of molecular variance (AMOVA; Excoffier et al. 1992) was used to examine how genetic variation was distributed within and among the 5 sites. This analysis was performed with Arlequin 3.5 (Excoffier and Lischer 2010), and statistical significance was determined by bootstrapping with 16,000 permutations.

We used STRUCTURE v 2.3.4 (Pritchard et al. 2000) to determine the number of discrete populations (K) of *Clemmys* across sites in South Carolina (1), Georgia (2), and Florida (2). We tested values of K from 1 to 6 using a model of admixed ancestry and assuming correlated allele frequencies between groups using population location as a prior (Hubisz et al. 2009). The program STRUCTURE is sensitive to uneven sampling across groups (Wang et al. 2017) and because our sampling across sites was uneven, we used a smaller alpha value than the default ($1/\sim K$, which we estimated as 0.5 for this analysis). For each value of K, we ran 20 replicates with 750,000 Markov chain Monte Carlo iterations and a burn-in set at 75,000. We selected the best value of K by examining the log-likelihood values for each K, the ΔK method (Evanno et al. 2005) and the estimators (MedMeaK, MaxMeaK, MedMedK, MaxMedK) described by Puechmaille (2016) as calculated by the online program StructureSelector (Li and Liu 2018). StructureSelector averaged the 20 runs for the best value of K using Clumpak (Kopelman et al. 2015) and we visualized the average ancestry coefficient (q) for each individual with Distruct v. 1.1 (Rosenberg 2004). Because the ΔK method detects the uppermost level of population structure (Evanno et al. 2005), we used a hierarchical approach and ran subsequent and separate STRUCTURE runs on groups identified in the original analysis using the same parameters (Vähä et al. 2007).

To evaluate relatedness or kinship of turtles at a site, we used the program COLONY v. 2.0 (Jones and Wang 2010). COLONY uses a maximum likelihood method to determine full- or half-sibling relationships based on the allele frequencies present at a given site and without knowledge of parental genotypes. The program identifies potential dyads of siblings or half-siblings along with an associated probability score. We used a threshold probability of 0.9 for both sibship and half-sibship assignments.

We used 2 methods to test for potential population bottlenecks at each site. The first method used the program BOTTLENECK (Piry et al. 1999). We tested for heterozygote excess (Cornuet and Luikart 1996) using the 2-phased model of mutation with parameters set to 95% single-step mutation and 5% multistep mutation, as recommended for studies with < 20 microsatellite loci (Piry et al. 1999). A Wilcoxon Rank Sum test evaluated the significance of the heterozygote excess. The second method was the M-ratio test (Garza and Williamson 2001), which compares the number of alleles with their size distribution. We used the recommended parameters suggested in Garza and Williamson (2001): a proportion of 1-step mutations of 90%, an average size of non–1-step mutations of 3.5, and a value of 10 for θ. The critical value of M was calculated from the 95% threshold of 10,000 simulations of an equilibrium population for each of the 5 sites. Lastly, we calculated the effective population size (N_e) for each site using NeEstimator v. 2.01. The program uses a bias correction (Waples 2006) of the linkage disequilibrium method (Hill 1981) with 95% confidence intervals estimated by jackknifing. Mating was assumed to be random and alleles with frequencies less than 0.02 were excluded from the analysis.

RESULTS

From 2014 to 2019, we collected tissue samples from 204 spotted turtles across 5sites in Georgia, Florida, and South Carolina. By state, the sample sizes were as follows: South Carolina (SC-1, $n = 15$), Georgia (GA-1, $n = 74$; GA-2, $n = 37$), and Florida (FL-1, $n = 58$; FL-2, $n = 20$). Only 1 locus (*Gmu21*) showed consistent deviations from HWE and LD and it was excluded from subsequent analyses. Two loci showed evidence of large-allele dropout, but not for all populations. Only 4 turtles were missing data for $> 20\%$ of their loci (2 from GA-1, and 1 from GA-2 and FL-2), thus all subsequent analyses were performed across the remaining 200 *C. guttata* at 10 microsatellite loci.

Table 1. Number of turtles sampled (n), alleles (N_a), allelic richness (A_R), private allelic richness expected heterozygosity (H_E), observed heterozygosity (H_O), and inbreeding coefficient (F_{IS}, with 95% confidence intervals [95% CIs]) averaged across loci for 200 spotted turtles (*Clemmys guttata*) genotyped at 10 microsatellite loci. Also reported are effective population size (N_e) estimates and 95% confidence intervals as calculated by NeEstimator (lowest allele frequency used = 0.02).

Site	n	N_a	A_R	PA_R	H_E	H_O	F_{IS}	95% CI	N_e	95% CI
SC-1	15	5.7	5.67	0.93	0.684	0.696	0.034	−0.12–0.17	∞	66.6–∞
GA-1	72	7	5.34	0.37	0.666	0.665	0.010	−0.06–0.07	100.2	52.3–352.9
GA-2	36	6.6	5.38	0.61	0.644	0.621	0.053	−0.003–0.11	122.6	34.9–∞
FL-1	20	4.9	4.65	0.19	0.622	0.610	0.049	−0.07–0.19	31.8	11.1–∞
FL-2	57	6.5	5.37	0.14	0.719	0.687	0.053	0.004–0.12	96.6	55.6–254.1

Genetic diversity metrics averaged across loci were similar across sites (Table 1 and Supplemental Table S1; S1; all supplemental material is available at http://dx.doi.org/10.2744/CCB-1560.1.s1). Mean allelic richness per site ranged generally between 5 and 6 alleles per site, except for the FL-1 site, which was the only site with an average allelic richness < 5. Mean private allelic richness was < 1 across all sites, but the South Carolina site tended to have more private alleles, on average, than other sites, while the Florida sites had fewer. Average levels of expected and observed heterozygosity across sites were more consistent, all ranging between 0.61 and 0.72. Average F_{IS} values were all close to 0 (from 0.034 to 0.053) with the 95% confidence intervals all overlapping 0 except for FL-2 (Table 1). None of the differences in average allelic richness, private allelic richness, or observed and expected heterozygosity across southern sites were significant (A_R, $F = 0.26$, $p = 0.902$; PA_R, $\chi^2 = 3.12$, $p = 0.538$; H_O, $F = 0.258$, $p = 0.903$; H_E, $F = 0.552$, $p = 0.699$), nor were they significantly different when compared with Canadian sites (A_R, $F = 0.23$, $p = 0.95$; H_O, $F = 0.428$, $p = 0.827$; H_E, $F = 0.941$, $p = 0.462$). Pairwise F_{ST} values for our populations ranged from 0.032 to 0.077 (mean = 0.063 ± 0.008 SD) and were all significantly different from 0 after adjusting for multiple comparisons. These values of F_{ST} tended to be higher between the 2 Florida sites and all other sites, with few exceptions (Table 2). The AMOVA revealed that most of the genetic variation was found within sites (94.6%), but a significant ($p < 0.0001$) amount represented differences among sites (5.4%).

The STRUCTURE analysis also revealed patterns of genetic structure among sites. A ΔK analysis initially found a K of 2 across all populations with site FL-2 falling out as a discrete population (Fig. 2 and Supplemental Fig. S1) but with a secondary peak at a ΔK of 5, suggesting further hierarchical structuring. For $\Delta K = 2$, the average ancestry score (q) for group 1 (sites SC-1 through FL-1) was 0.96 ± 0.03 SD, while the average q score for individuals in the FL-2 site was 0.8 ± 0.09 SD. The log-likelihood plot began to plateau at $K = 5$, which agreed with the secondary ΔK peak (Supplemental Fig. S1). The second STRUCTURE analysis, excluding the FL-2 population, identified each of the 4 remaining sites as representing their own genetic groups (Fig. 2). The average q scores for these groups were as follows: SC-1 = 0.90 ± 0.02 SD; GA-1 = 0.95 ± 0.02 SD; GA-2 = 0.76 ± 0.06 SD; FL-1 = 0.71 ± 0.17 SD. The Puechmaille (2016) estimators agreed with the selection of the best value of K.

Three sites (GA-1, GA-2, and FL-2) met the recommended assumptions of BOTTLENECK (> 29 individuals at 10 loci; Piry et al. 1999). None of these 3 sites showed a significant excess of heterozygosity, suggesting that these populations have not undergone a bottleneck event. Furthermore, the M-ratio tests also did not detect evidence of historic bottleneck events with average M ratio values ranging from 0.76 (GA-2) to 0.91 (SC-1). Estimates of N_e ranged from 31.8 to 122.6 (Table 1). The South Carolina site yielded a negative estimate of N_e, which the software interprets as infinite. This result can stem from a large population size at a site or from a limited number of samples. When it does occur, the lower end of the 95% confidence interval can be used to approximate N_e (Waples and Do 2008). Only the 2 sites with the largest sample sizes (GA-1 and FL-2) produced bounded

Table 2. Pairwise fixation index (F_{ST}) derived from 10 microsatellite loci across 5 southeastern sites across 3 states. All values were significantly different from 0.

	SC-1	GA-1	GA-2	FL-1
SC-1	—	—	—	—
GA-1	0.045	—	—	—
GA-2	0.038	0.032	—	—
FL-1	0.073	0.077	0.041	—
FL-2	0.056	0.066	0.064	0.075

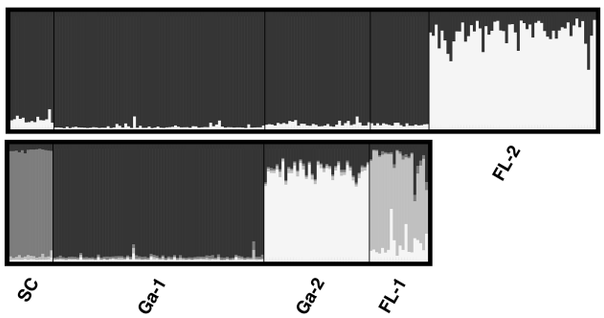

Figure 2. STRUCTURE bar plots of individual assignment probabilities for each inferred genetic cluster. Above shows high level of population structure at a K of 2, and below shows a K of 4 for remaining populations when FL-2 was excluded from analysis.

confidence intervals. Kinship analyses of each population did not detect any sibling or half-sibling dyads above the 0.9 probability threshold.

DISCUSSION

This study was the first population genetic assessment for spotted turtle populations in the southern portion of their range, including samples from Florida, Georgia, and South Carolina. Our results indicate that all 5 populations included in this study were significantly differentiated from one another and that heterozygosity was relatively high across all populations. Furthermore, sites lacked evidence for genetic bottlenecks and generally possessed similar estimates of N_e. These results are broadly similar to those from other studies of freshwater turtle species native to the eastern United States (Tessier et al. 2005; Mockford et al. 2007; Buchanan et al. 2019b; Liebgold et al. 2023).

The STRUCTURE analysis indicated an initial split of $K = 2$ populations, but subsequent analysis suggested that each population clusters as its own genetic group. These groups reflected moderate (although not insignificant) levels of population differentiation (average $F_{ST} = 0.063$; Wright 1965; Men et al. 2017). Among populations, the first major split in population structure was found between the FL-2 site and all other sites. Interestingly, the largest pairwise F_{ST} value for FL-2 was actually FL-1 (Table 2), suggesting that it may not be geographic proximity driving some differentiation among population and that there may be other cryptic historical barriers responsible for the genetic differentiation of these populations, as seen in genetic studies of other Florida taxa (Clark et al 1999; Gaillard et al. 2017).

The results of our study are comparable to analyses by Davy and Murphy (2014), who evaluated the population genetics of spotted turtles across a comparable spatial scale in southern Canada, as well as to Buchanan et al. (2019b) in Rhode Island. Davy and Murphy (2014) also recovered a $K = 2$ with additional substructure that was also not explained by site proximity or isolation, and Buchanan et al. (2019b) found evidence K from 1 to 3 depending on subset analysis. In our study, we used the same microsatellite loci (Davy and Murphy 2014), or many of the same loci (Buchanan et al. 2019b), which allows us to make comparisons between the southern and northern populations. In species that span a wide breadth of latitude, there is generally an inverse relationship in allelic diversity and latitude (Schmitt et al 2002; Schmitt 2007). However, when comparing our dataset to Davy and Murphy (2014) and Buchanan et al. (2019b), this pattern does not appear to apply to the spotted turtle. The overall mean for number of alleles across southern populations is 6.14, and when rarefied across populations, it ranges from 4.65 to 5.67 (Table 1). Davy and Murphy (2014) had similar allelic richness across regions (6.75 in southwestern Ontario and 6.11 in southeastern Ontario) and across sites (3.18–4.49 in southwestern Ontario and 3.34–4.1 in southeastern Ontario), while Buchanan et al. (2019b), who used additional loci, found an allelic richness of 8.59 in their study area, with a mean richness of 4.78–4.97 across sites. Typically for species that have undergone rapid, postglacial (i.e., post-Pleistocene) expansion, allelic diversity is higher at southern latitudes that served as refugia rather than at northern latitudes where the species has recently expanded (Schmitt et al. 2002; Schmitt 2007; Flight et al. 2012). This result could suggest high connectivity and high levels of gene flow during their postglacial expansion, or perhaps the core range or refugia of the species was located more centrally. Levels of heterozygosity, too, were similar between Canadian, Rhode Island, and southern populations. In southwestern Ontario populations, the mean expected and observed heterozygosities were 0.728 and 0.679, respectively; in southeastern Ontario populations the mean expected and observed heterozygosities were 0.707 and 0.718, respectively; and in Rhode Island mean expected and observed heterozygosities were 0.68 and 0.66, respectively. The mean expected and observed heterozygosities across southern populations were 0.667 and 0.656, respectively, which did not differ significantly from values published in Davy and Murphy (2014).

We were able to obtain estimates of effective population size (N_e) for 4 of the 5 sites. The lowest effective population size was for the FL-1 site, which is also the site with the lowest allelic richness when corrected for population size. This site is isolated in nature and although it is the longest monitored site in Florida, only 20 turtles have been detected despite intensive surveys. BOTTLENECK did detect a genetic bottleneck at this site, but this site did not meet the assumptions of the analysis (i.e., < 29 samples; Piry et al. 1999). Therefore, it is not yet possible to determine if the bottleneck is genuine or a consequence of limited sample size. Other sites produced more robust estimates of population size and did not exhibit signs of genetic bottlenecks (Table 1). Estimates of $N_e > 100$ are equivalent or larger than most other published estimates of total population size for spotted turtle populations across their range (Milam and Melvin 2001; Litzgus and Mousseau 2004a; Enneson and Litzgus 2009; Buchanan et al. 2019b; Howell and Seigel 2019). However, Davy and Murphy (2014) suggest cautious interpretation of these estimates in long-lived species, which can retain genetic variation even in the face of recent demographic declines (Kuo and Janzen 2004; Lippé et al. 2006; Ennen et al. 2011; Pittman et al. 2011). Thus, continued demographic monitoring of these populations that ultimately generates robust estimates of abundance or population growth rate and assess the effects of potential threats to population viability are an important management goal.

The spotted turtle is a species of high conservation concern throughout its range and our data indicate that the

southern populations exhibit similar levels of genetic differentiation and allelic richness as was found in other populations. The southern populations also are no exception to the threats faced by other spotted turtle populations range-wide. Fragmentation and isolation of populations are pernicious threats to the species and threaten to reduce levels of heterozygosity and increase inbreeding depression. These risks are exemplified in this study: site FL-1 is a small wetland complex isolated by roads with no obvious corridors for dispersal. The genetic data presented in the present study corroborate the survey data and suggest that this is a small population that may have experienced a recent genetic bottleneck. However, a limitation of the present study was the inability to directly compare the differentiation between northern and southern populations. Even across the sites in the southeastern United States, our analyses indicate each site is its own genetic group that is demographically and geographically independent of all others. These results are not too surprising given their geographic distance from one another.

The present study highlights the need for a comprehensive range-wide assessment of the population genetics of the spotted turtle. The genetic data presented herein provide a cursory rather than mechanistic understanding of the genetic variation present in southeastern spotted turtle populations. Furthermore, sampling for this study took place where turtles were known to occur. Future research should focus on systematic or a priori hypothesis-driven sampling to identify and genotype additional populations to provide a higher-resolution picture of the species' genetic health in the southeastern United States.

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