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CONSERVATION GENETICS OF THE LARGEST CLUSTER OF FEDERALLY THREATENED GOPHER TORTOISE (*GOPHERUS POLYPHEMUS*) COLONIES WITH IMPLICATIONS FOR SPECIES MANAGEMENT

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Abstract: We conducted a genetic study of the largest cluster of US federally threatened Gopher Tortoise (*Gopherus polyphemus*) colonies. Our objectives were to (1) identify genetic variation within and among colonies across the landscape; (2) determine which factors are important in affecting genetic variation, including land use, habitat quality, and population size; and (3) determine whether genetic partitioning among populations exists and how this relates to (a) geographic distance between sites, (b) Gopher Tortoise natural history and spatial ecology, and (c) land-use history. We studied genetic variability of nine microsatellite DNA loci for 340 adult tortoises from 34 colonies separated by 1.3–45.1 km across a 56,000-ha military installation. Overall genetic variation was low across the landscape and within colonies. Observed heterozygosity (H_O) of tortoise colonies was 49% and allelic richness was 52% of that found in populations located in the eastern portion of the species distribution where habitat is naturally more continuous. Our single colony with highest genetic variation had H_O that was 57% and allelic richness that was 60% of eastern colonies. Genetic variation was greatest in sites with suitable habitat. We found weak to no genetic structure across the 45-km landscape (F_{ST} = 0.031; D_{ST} = 0.006) and evidence for only one genetic group (K). Although landscape reconfiguration to create sites for military activity has redistributed tortoise colonies and home ranges, we concluded that weak population structure is natural across our study area. Comparison to similar results from a cluster of connected eastern colonies suggests this is a general characteristic of tortoises across large, continuous landscapes and that populations are composed of multiple colonies across the landscape and are naturally large in spatial extent. To alleviate the tortoise–human land use conflict on Camp Shelby, Mississippi, USA and to ensure these created areas continue to benefit tortoises in the long term, maintenance of forest habitat surrounding these created open areas is required. We recommend managing tortoises at Camp Shelby as one unit.

Key words: Conservation genetics; *Gopherus polyphemus*; Habitat quality; Land-use history; Management; Population structure

The relationship between the distribution of organisms across multiple spatial scales and how this relates to structure of genetic variation is a fundamental concept in evolution and has major implications for conservation. However, genetic variation in an organism might not be partitioned across the landscape as predicted based on life history, spatial ecology, and landscape configuration (Manel et al., 2003; McCoy et al., 2010; Storfer et al., 2007). In some cases, genetic differentiation might be greater than expected due to subtle land-use changes or barriers that fragment populations (Booth et al., 2009), differences between dispersal estimates based on mark–recapture data and actual gene flow (Cabe et al., 2007), or incorrect assumptions of panmixia based on spatial distribution of individuals (Elmer et al., 2007). Therefore, to accurately interpret spatial genetic structure, one must not only examine spatial ecology and movement behavior of focal species (Chaput-Bardy et al., 2008; Darvill et al., 2009; Lowe et al., 2008), but also land-use legacy, because genetic data have the signature of historical events and processes (Jordan et al., 2009; Measey and Tolley, 2009). The sensitivity of molecular markers and genetic diversity measures and the ability to detect genetic

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discontinuities on a continuous landscape will depend on spatial ecology of study species, temporal scale, and statistical approaches (Anderson et al., 2010; Landguth et al., 2010; McCoy et al., 2010; Murphy et al., 2008).

Current population size and distribution of populations across the landscape may indicate future persistence of a species. However, these contemporary data may not be accurate estimators of current status or indicators of long-term persistence probability, because historic events, which are not always obvious from examination of populations, can have major impacts on probability of future persistence (Hoelzel et al., 2002; Measey and Tolley, 2009). For example, if population size and geographic extent were diminished in the past and followed by recovery, this may not be obvious based on current abundance and distribution (Hoelzel et al., 2002; Johnson and Dunn, 2006; Jordan et al., 2009). Therefore, the ability of a species to persist in the future and our ability to predict probability of persistence depend on extrinsic and intrinsic factors that impacted populations in the past and continue to do so (Johnson and Dunn, 2006; Measey and Tolley, 2009).

One such extrinsic factor is anthropogenic landscape alteration (Allentoft et al., 2009; Richter et al., 2009; Westemeier et al., 1998). Natural landscapes continue to be altered as humans encroach upon them. Consequently, as the severity of habitat loss, alteration, and fragmentation intensifies, populations of many nonhuman organisms that were once large and connected by dispersal become subdivided, reduced in size, and typically confined to discrete, small habitat patches (Allentoft et al., 2009; Darvill et al., 2009; Honnay et al., 2007; Richter et al., 2009). Because these alterations may affect a species' population growth and sustainability, this may result in a species becoming threatened or endangered (Fujii and Forstner, 2010; Honnay et al., 2007; Measey and Tolley, 2009).

Populations of threatened and endangered species are often primarily or solely located on federal, state, and nongovernmental protected lands; therefore, the stability and persistence of many species rely on continued protection of these lands (Tazik and Martin, 2002). Although the lands are protected, lack of habitat management and habitat degradation on preserves has detrimentally impacted such populations (McCoy et al., 2006; Parrish et al., 2003). As adjacent habitat is lost or altered for human land use, it becomes increasingly important for government-owned and other protected lands to be managed for threatened and endangered species with cooperation from local conservationists (Liu et al., 2001; Maehr, 1990; McCoy et al., 2006; Tazik and Martin, 2002). This underscores the importance of further investigation into management of threatened and endangered species. Management must be implemented that focuses on local populations and allows for movement between them. These within- and among-population dynamics maintain genetic and species diversity across regional scales (Zamudio and Wieczorek, 2007).

Gopher Tortoises (*Gopherus polyphemus*) are ideal candidates for such studies. The species is distributed along the southeastern US coastal plain from Louisiana to South Carolina, and is of particular interest because it is listed as federally threatened in the western portion of its range from Louisiana to the Mobile and Tombigbee rivers in Alabama (USFWS, 1987). Gopher Tortoises are also of conservation interest because they are a keystone species in the Longleaf Pine (*Pinus palustris*) ecosystem of the southeastern USA, and the burrows they excavate may contain the richest species diversity of all North American animal burrows (>360 species recorded; Eisenberg, 1983; Jackson and Milstrey, 1989). Burrows serve as hiding places, nesting sites, or overwintering dens for obligate and facultative commensals, many of which are legally protected (Guyer et al., 1996; Hansen, 1963; Schwartz and Karl, 2005). The excavated soil mounds outside the burrows may also serve as foci of botanical biodiversity and increase the overall floral diversity of the forest understory (Kaczor and Hartnett, 1990).

Populations of Gopher Tortoises are declining because of habitat fragmentation, loss and alteration, predation by imported fire ants, humans and other mammals, and upper respiratory tract disease (Brown et al., 2002; Diemer, 1986; Diemer Berish et al., 2000;

Epperson and Heise, 2003; McCoy et al., 2006; Wilson and Mushinsky, 1997). Even in Florida, where tortoise numbers are highest, habitat that supports virtually all tortoise populations has been reduced by >80% (Mushinsky et al., 2006). Much of the remaining habitat is isolated within an unsuitable intervening matrix of human development and other land uses (Auffenberg and Franz, 1982; McCoy et al., 2006; Mushinsky et al., 2006). Tortoise populations on protected lands in Florida have declined over the past 20 yr, presumably because habitat was not managed (McCoy et al., 2006).

Tortoises in the western portion of the distribution have been more severely impacted because suitable habitat is naturally more diffuse, and patches of suitable habitat tend to be smaller and separated by greater distances as compared to the eastern portion (Epperson and Heise, 2003; Mushinsky et al., 2006). Consequently, populations in the western part of the range tend to be smaller and separated by greater distances across the landscape, which renders them more susceptible to landscape alterations, habitat loss, and fragmentation. Despite this, the majority of biological studies have focused on the eastern portion of the range (but see Ennen et al., 2010; Epperson and Heise, 2003; Smith et al., 1997; Yager et al., 2007). Most population-genetic studies of Gopher Tortoises are restricted to eastern populations (Moon et al., 2006; Schwartz and Karl, 2005, 2008; Sinclair et al., 2010). Only one landscape-level population genetic study of Gopher Tortoises has been conducted, which focused on four interconnected populations at the Kennedy Space Center, Florida (Sinclair et al., 2010). Multiple landscape-level studies of western North American tortoises exist (e.g., Fujii and Forstner, 2010; Murphy et al., 2007). In the only study of western populations of Gopher Tortoises, Ennen et al. (2010) found low genetic variability in three geographically separated populations in the federally protected portion of the range, but we do not know how general this finding is for threatened populations of Gopher Tortoises.

To increase our understanding of landscape-level genetics in Gopher Tortoises and of genetic variability of populations in the federally threatened portion of the range, we conducted landscape-level genetic studies across 34 Gopher Tortoise colonies at Camp Shelby Joint Forces Training Center, Mississippi (hereafter, referred to as Camp Shelby). This facility is a military installation with the largest cluster of Gopher Tortoise colonies in the western portion of the geographic range, where the species is listed as federally threatened. We applied US Fish and Wildlife Service (1990) definition of colony as "three or more active adult burrows within 300 feet of each other, or any combination of active adult and active hatchling/subadult burrows within 100 yards of each other." Our objectives were to (1) identify genetic variation within and among colonies across the landscape; (2) identify factors that influence genetic variation, including land use, habitat quality, and population size; and (3) determine whether genetic partitioning among populations exists and how this relates to (a) geographic distance among sites, (b) Gopher Tortoise natural history and spatial ecology, and (c) land-use history. We predicted that genetic variation in colonies from the western portion of the geographic distribution would be less than that of colonies from the eastern portion of the distribution, primarily because of differences among these regions in landscape configuration (i.e., natural amount and distribution of suitable habitat). Additionally, based on intrinsic factors (longevity, generation time, site fidelity, effects of habitat preference on population density, and spatial ecology) and extrinsic factors (current landscape configuration and land-use history), we predicted that sites with higher quality habitat would support more tortoises, which will result in greater population genetic variation. Finally, we predicted that there would be discernable genetic structuring across the landscape, although the scale at which this would occur was difficult to predict.

Materials and Methods

Study Sites

Our study area, Camp Shelby, is located in the De Soto National Forest 24 km south of Hattiesburg, Mississippi and was established in 1917. It is the largest state-owned and operated field-training site in the United States, ~56,000 ha. In 1956, the US National

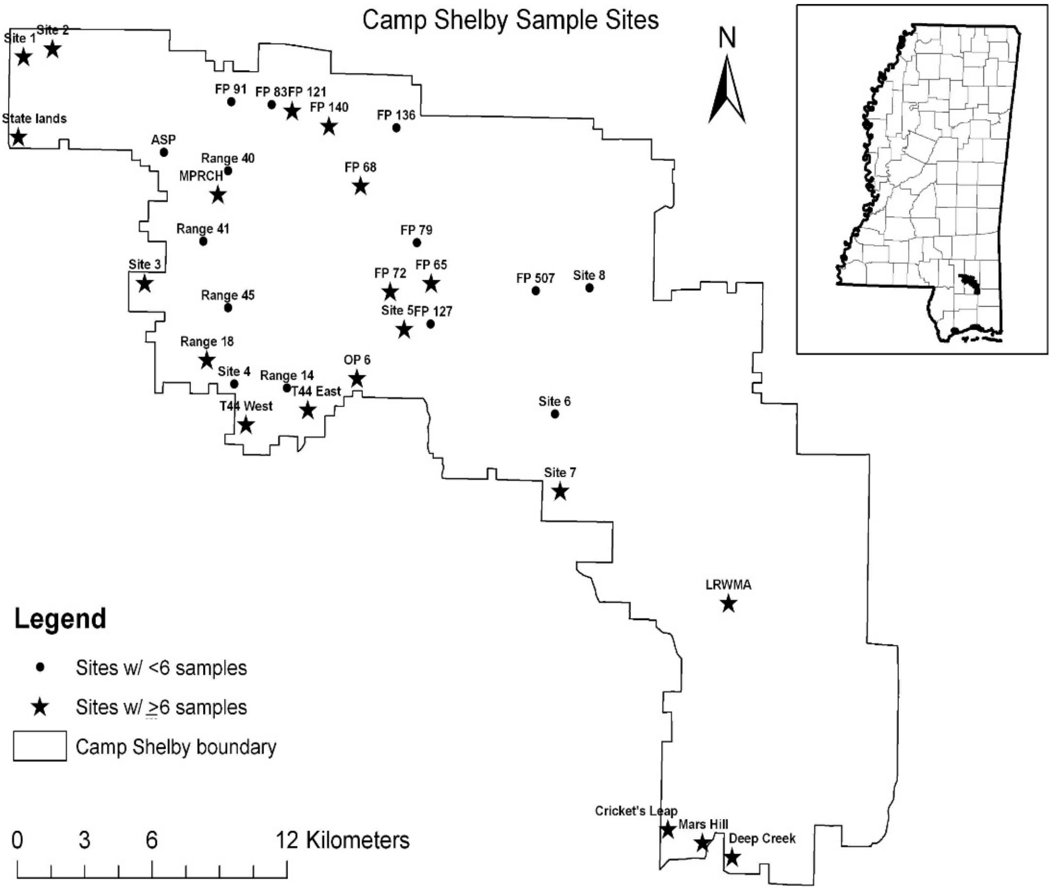

FIG. 1.—Distribution of Gopher Tortoises across the Camp Shelby Joint Forces Training Facility, Mississippi, USA, with study sites indicated by dots (where $n < 6$) and stars (where $n \geq 6$). Inset map of Mississippi indicates location of Camp Shelby.

Guard established Camp Shelby as a permanent training installation. Approximately 2000 Gopher Tortoises reside within the boundaries of Camp Shelby (M. Hinderliter, personal observation) and are clumped in distribution based on habitat suitability. Our study sites were selected where clumping of burrows into colonies occurred (Fig. 1). Many of these sites represent firing points and other land-use practice areas that were created within the past 20–40 yr (Mississippi Army National Guard records) by converting forest habitat to open-canopy, ruderal habitat. Many of these sites had a closed canopy, had few to no tortoises prior to habitat conversion, and were colonized by tortoises over the years following their creation.

At 34 colonies across Camp Shelby, tortoises were captured by hand or through use of live-traps that were placed at the entrance of occupied burrows. Immediately upon capture, blood samples were taken from each tortoise from the brachial vein using heparinized syringes. Approximately 50% of tortoises were sampled in 2006 as part of a larger study; the other blood samples were obtained from historical samples collected as long ago as the late 1990s during a long-term mark–release–recapture study. Colony size estimates for each site were obtained based on the number of tortoises observed at each site during a base-wide burrow camera survey conducted in 2004–2005 (M. Hinderliter, personal observation). No tortoises sampled at a site were ever documented at a different site according to tortoise mark–recapture records from 1998 to 2006, and we do not have any duplicate tortoise samples across years. Hence, we were able to treat all tortoise samples from the years as a single sample. In addition, because tortoises have long generation times and longevity, we feel that our samples are representative of our study sites.

Genetic Data Collection

Whole genomic DNA was isolated from all blood samples using a Qiagen DNEasy® kit and protocol. We genotyped individual tortoises using nine microsatellite loci developed by Schwartz et al. (2003). Polymerase Chain Reaction (PCR) conditions followed Schwartz et al. (2003). Tortoises were genotyped for each microsatellite DNA locus using an ABI 310 Genetic Analyzer (Applied Biosystems, Inc.) by pooling samples of PCRs for three loci per individual using different fluorescently labeled primers for each locus. Allele sizes were scored using Genescan version 3.2 (Applied Biosystems, Inc.). In total, 340 tortoises from 34 colonies were genotyped for 9 loci.

Population Genetic Analyses

Population genetic diversity measures were obtained and genetic analyses were performed using FSTAT version 2.9.3 (Goudet, 2001). Additionally, allelic richness (A_r) was estimated using rarefaction to standardize based on the size of the smallest sample size ($n = 6$; El Mousadik and Petit, 1996). Prior to conducting primary analyses, we examined the data for linkage equilibrium between all pairs of loci in each sample using a log-likelihood ratio *G*-statistic and tested for Hardy Weinberg equilibrium (HWE) within each sample using global tests with 10,000 randomizations. Alpha was adjusted using Bonferroni corrections. We found no linkage disequilibrium among the nine loci and one locus (GP 15) with significant departure from HWE. Therefore, where applicable, we performed genetic analyses including and excluding this locus. Results did not vary when the locus was included or not. We used genetic data collected by Schwartz and Karl (2008) and Sinclair et al. (2010) of populations from the eastern portion of the geographic distribution and Ennen et al. (2010) from the western portion to assist in the interpretation of our data.

We analyzed a subset of tortoise colonies for genetic signatures of recent population declines using BOTTLENECK Version 1.2.02 (Cornuet and Luikart, 1996; Piry et al., 1999). Colonies were selected that were located in natural areas (i.e., sites not created by the military) and with $n > 10$. For each locus, heterozygosity excess was evaluated by calculating H_E and then estimating heterozygosity expected at mutation-drift equilibrium (H_{EQ}), which is based on observed number of alleles and sample size. Populations that have not undergone a recent change in population size should have $H_E = H_{EQ}$ (Piry et al., 1999). H_{EQ} was estimated under three models of mutation: infinite alleles model (IAM), stepwise mutation model (SMM), and two-phase mutation model (TPM) based on 5000 iterations. The TPM was used with 95% single-step mutations (5% multistep) and a variance among multiple steps of 12% as recommended by Piry et al. (1999). A one-tailed Wilcoxon's signed-ranks test was used to determine whether $H_E > H_{EQ}$.

Habitat Analyses

We determined the relationship between genetic variation and habitat quality and population size. To do so, we defined habitat quality as follows. Suitable habitat was defined as either (1) cleared areas on Camp Shelby, such as firing points that are maintained as grass and short, ground-level vegetation (ruderal vegetation), or (2) forested areas where tree canopy <50% allowed sufficient light at ground level to support low-level vegetation such as grasses, herbs, and legumes (Mushinsky and McCoy, 1994; Wilson and Mushinsky, 1997). Unsuitable habitat was defined as either (1) areas with high percentage of shrubs, vines, and midstory vegetation, or (2) forested areas with tree canopy >50%, both of which reduce amount of sunlight reaching forest floor and inhibit growth of grasses, herbs, and legumes (Mushinsky and McCoy, 1994). We then compared genetic variation within and among colonies using A_r and H_E. To evaluate differences among

TABLE 1.—Demographic and genetic data for colonies on Camp Shelby Joint Forces Training Facility, Mississippi, USA, where $n \geq 6$ individuals. Mean ± 1 SE data are presented for each of expected heterozygosity (H_E) and allelic richness (A_r).

Colony name	Colony size	Sample size	Habitat quality	H_E	A_r
Cricket's Leap	8	8	Suitable	0.194	1.78
Deep Creek	15	11	Suitable	0.207	1.67
FP 121	20	20	Suitable	0.246	2.11
FP 140	15	15	Suitable	0.219	2.00
FP 507	7	6	Suitable	0.183	1.63
FP 65	7	8	Suitable	0.189	1.56
FP 68	17	17	Suitable	0.242	2.00
FP 72	15	15	Suitable	0.235	1.78
FP 79	9	6	Suitable	0.233	1.78
FP 91	6	6	Suitable	0.198	1.67
LRWMA	11	9	Suitable	0.163	2.00
Mars Hill	20	10	Suitable	0.191	1.78
MPRCH	40	40	Suitable	0.204	2.11
OP 6	25	12	Suitable	0.244	1.89
Range 18	20	7	Suitable	0.189	1.67
Range 45	35	6	Suitable	0.208	1.78
Site 1	25	21	Suitable	0.211	2.00
Site 2	7	7	Suitable	0.214	1.56
State Lands	25	19	Suitable	0.229	1.89
T 44 E	25	17	Suitable	0.199	2.11
T 44 W	30	20	Suitable	0.238	2.22
Site 3	10	7	Unsuitable	0.182	1.67
Site 5	7	7	Unsuitable	0.213	1.67
Site 6	6	6	Unsuitable	0.192	1.75
Site 7	14	9	Unsuitable	0.197	1.67
		$\bar{X}$ ± 1 SE unsuitable habitat		0.196 ± 0.006	1.69 ± 0.02
		$\bar{X}$ ± 1 SE suitable habitat		0.211 ± 0.005	1.85 ± 0.04

colonies, only those with $n \geq 6$ were included, which reduced the number of colonies to 25 (Table 1). We used these measures to evaluate the relationship between habitat quality and genetic variability using unpaired *t*-tests. Prior to statistical analyses, A_r was square-root transformed and H_E was arcsine–square-root transformed (Sokal and Rohlf, 1995). We also performed regression analysis to determine the relationship between population size and genetic diversity. Prior to regression analysis, population size was square-root transformed.

Spatial Genetics

We used two approaches to infer patterns of genetic differentiation across the landscape. F_{ST}-based analyses were performed using FSTAT version 2.9.3 (Goudet, 2001), including only colonies with $n \geq 6$ individuals, and Bayesian clustering analyses were performed, including all 340 individuals, using STRUCTURE 2.3.3 (Hubisz et al., 2009; Pritchard et al., 2000). To quantify genetic structuring across the landscape, we calculated D_{ST} (Nei, 1973) and F_{ST} (Weir and Cockerham, 1984) with 95% confidence intervals generated using bootstrap sampling. We tested for population differentiation using global tests not assuming HWE with 10,000 randomizations. Pairwise F_{ST} values were used to describe genetic differentiation between each pair of sample sites. We then evaluated the data to test an isolation-by-distance model to determine whether genetic similarity between populations can be explained by geographic distance between the populations using a Mantel test with 10,000 randomizations (Mantel, 1967).

We used a Bayesian approach implemented with Markov chain Monte Carlo (MCMC) algorithms to infer the number of genetic groups (K) across the landscape using Structure 2.3.3 (Hubisz et al., 2009; Pritchard et al., 2000). Because individuals in each of our clusters might be recent migrants from nearby populations and their genetic composition may have a signature of nearby demes, we used an admixture model with uniform prior on the degree of admixture and assumed correlated allele frequencies across clusters (Falush et al., 2003; Pritchard et al., 2000). Using sampling location as a prior potentially allows for detection of weak population structure without introducing a bias toward detecting nonexistent structure (Hubisz et al., 2009). We inferred K for six models: two pairs where sampling location was included as a prior in one model and not the other. Each pair of models differed by type of data included. One pair included all individuals from our 34 colonies, the second pair included the 26 sites where sample size was ≥6 individuals per site, and the third pair included 12 sites that naturally harbored Gopher Tortoises prior to human land-use change (see Discussion). The putative number of populations (K) was set as 1–34, 1–26, or 1–12, and for each value of K, we performed five replicates of model-fitting with an initial burn in of 200,000 iterations followed by 500,000 iterations in the MCMC. To determine the value of K that best fit our data for each model, we averaged log-likelihood values across our five replicates for each value of K and then calculated posterior probability for each K using Bayes' rule.

RESULTS

Within-colony Genetic Diversity and Habitat Analyses

Overall, genetic variation of Gopher Tortoises on Camp Shelby was low both installation-wide ($H_E = 0.220$; allelic richness = 27) and within the 25 colonies ($\bar{X}$ $H_E \pm 1$ SE = 0.209 ± 0.004, range = 0.163–0.246; $\bar{X}$ allelic richness ± 1 SE = 1.8 ± 0.04, range = 1.6–2.0; Table 1). Genetic variation was greater in colonies located in suitable habitat ($\bar{X}$ $H_E \pm 1$ SE = 0.211 ± 0.005; $\bar{X}$ allelic richness ± 1 SE = 1.9 ± 0.04; $\bar{X}$ estimated allelic richness ± 1 SE = 1.692 ± 0.023) than in colonies located in unsuitable habitat ($\bar{X}$ $H_E \pm 1$ SE = 0.196 ± 0.006; $\bar{X}$ allelic richness ± 1 SE = 1.7 ± 0.02; $\bar{X}$ estimated allelic richness ± 1 SE = 1.638 ± 0.038; Table 1). However, we found no significant relationship between habitat quality and genetic variability in the analysis of heterozygosity ($t_{23} = 1.3$, $P = 0.22$), allelic richness ($t_{23} = 1.66$, $P = 0.11$), and estimated allelic richness ($t_{23} = 1.0$, $P = 0.33$). Genetic variation was not explained by colony size for heterozygosity ($F_{1,23} = 3.2$, $P = 0.09$, $r^2 = 0.12$) but was for allelic richness ($F_{1,23} = 18.7$, $P < 0.001$, $r^2 = 0.45$). No recent population bottleneck events were detected in any colony through analysis of heterozygote excess under the IAM, SMM, or TPM mutation models.

Spatial Genetics

Based on F_{ST} and D_{ST}, we found weak to no population structuring of genetic variation across Camp Shelby when all sites were included ($F_{ST} = 0.025$; 95% confidence interval = 0.017–0.030; $D_{ST} = 0.006$) and when only sites that existed prior to land-use change were included ($F_{ST} = 0.031$; 95% confidence interval = 0.0–0.052; $D_{ST} = 0.005$). Colonies were separated by 1.3–45.1 km between pairs, and pairwise F_{ST} values ranged from 0 to 0.17. Genetic differences between colonies (i.e., pairwise F_{ST}) were not explained by geographic distances between them, as would be predicted by an isolation-by-distance model when all sites were included (Mantel test; $P = 0.07$; $r^2 = 0.01$) or when only natural sites were included (Mantel test; $P = 0.44$; $r^2 = 0.01$). Bayesian analyses corroborated results of F_{ST} analyses. Of the six STRUCTURE runs, five had K = 2 with posterior probabilities < 0.02, and the other had K = 2 with posterior probability = 0.178. Therefore, for each of our six models, including those with only natural sites, we inferred K = 1.

DISCUSSION

We found low genetic variability within colonies and across Camp Shelby, which is similar to the results of Ennen et al. (2010; Table 2). Genetic variability at Camp Shelby was less than eastern colonies. For example, average H_O of tortoise colonies at Camp Shelby was approximately half (49%) and allelic richness just over half (52%) that of eastern colonies (Table 2). The single colony of our study with highest genetic variation had H_O that was 57% and allelic richness that was 60% of eastern colonies. This is particularly alarming because (1) our study area is the largest cluster of colonies in the western portion of the species' distribution, (2) the landscape is composed primarily of large patches of suitable habitat, which is rare in

TABLE 2.—Genetic diversity ($\bar{X}$ ± 1 SE) comparison for Gopher Tortoises in the western portion of the geographic distribution (this study and Ennen et al., 2010) and in colonies from geographically separated areas in Georgia and Florida, USA (Schwartz and Karl, 2008) and in a geographically connected area at the Kennedy Space Center (KSC), Florida (Sinclair et al., 2010). Genetic data are averaged across colonies within each study and area.

Location (no. colonies)	Sample size	Allelic richness	Observed heterozygosity	Study
Western Range				
Camp Shelby (25)	12.4 ± 1.6	1.8 ± 0.0	0.209 ± 0.004	This study
Mississippi (4)	22.3 ± 7.4	1.9 ± 0.1	Not reported	Ennen et al. (2010)
Eastern Range				
Georgia (3)	17.0 ± 1.7	3.0 ± 0.5	0.420 ± 0.078	Schwartz and Karl (2008)
Florida (9)	16.6 ± 1.4	3.4 ± 0.2	0.438 ± 0.023	Schwartz and Karl (2008)
KSC, Florida (5)	19.2 ± 1.8	4.0 ± 0.2	0.417 ± 0.023	Sinclair et al. (2010)

the western portion of the species' range, and (3) low diversity may have significant impacts on tortoise fitness (Coltman et al., 1998; Hansson and Westerberg, 2002; Hansson et al., 2001).

We did not detect population bottlenecks in our study area, but genetic analyses may not detect actual population declines depending on the nature of the genetic data (sample size, number of loci, and allelic diversity of loci; Piry et al., 1999) and on biological processes occurring since the bottleneck occurred (e.g., gene flow among populations; Whitehouse and Harley, 2001). Tortoise populations have declined severely in both size and number (~80% reduction) over the past century, and these historic declines presumably resulted in population bottlenecks and decreased connectivity of populations (Auffenberg and Franz, 1982; McCoy et al., 2006; Mushinsky et al., 2006).

Tortoises in the western portion of the distribution would be more severely impacted by these historic events because suitable, Longleaf Pine habitat was more diffuse, and patches of suitable habitat were less extensive (Epperson and Heise, 2003; Landers et al., 1995; Mushinsky et al., 2006). Historically, western populations would have been smaller and separated by greater distances across the landscape as compared to eastern populations. Our lack of direct evidence for bottlenecks appears to be due to the low allelic diversity of our colonies or because of recent migrations of tortoises naturally and because of landscape reconfiguration (see below).

We predicted that we would find significantly greater genetic variability at sites with higher habitat quality because the number of tortoises in an area is positively related to habitat quality (Diemer, 1986; Hermann et al., 2002), and population genetic variation is similarly correlated to population size. Although we did not find a statistically significant relationship between habitat quality and genetic variability based on any diversity measures, we feel that the biological relationship is real. The availability of suitable habitat on Camp Shelby is not limiting, and the number of populations on unsuitable sites is low. Because of this we were only able to sample 5 unsuitable habitat sites of our total 34 sites, and only 4 of these had large enough size to include in the analyses of our 25 sites. This resulted in low statistical power to detect differences. This becomes more evident if we statistically evaluate the differences in population size on suitable versus unsuitable habitat sites. Mean population size ± 1 SE is 18.1 ± 2.1 on suitable and 9.25 ± 1.8 on unsuitable habitat sites. These differences are also not statistically significant ($t_{23} = 1.9$, $P = 0.08$) due to low power, but again the biological significance is obvious.

We detected weak or no population structure; however, it is important to understand that weak population structure based on microsatellite DNA should not be dismissed as biologically irrelevant without attempting to include other data to assist interpretation of the genetic data (Postma et al., 2009). Additionally, lack of genetic signature of population structure does not exclude its existence. Therefore, we addressed three nonexclusive explanations for lack of, or weak, genetic signature of population structure: (1) rate of genetic exchange among colonies was sufficient to produce weak population structure;

(2) historic changes in land use and habitat at Camp Shelby resulted in reconfiguration of colonies and tortoise home ranges; and (3) genetic signature of population structure has not developed due to length of tortoise generation time.

Population structure results when discrete populations across a landscape have low migration rates among them. However, landscapes may change at a greater rate than the genetic structure of organisms, especially for long-lived organisms (Pavlacky et al., 2009). Understanding both Gopher Tortoise life history and the land-use history of Camp Shelby is crucial to interpreting the genetic data and drawing meaningful conclusions. Many of our study sites were created within the past 20–40 yr (Mississippi Army National Guard report) and had few to no tortoises prior to habitat conversion. From our F_{ST}-based and Bayesian analyses of all colonies and of only colonies that existed prior to habitat reconfiguration, we deduced that population structure is naturally weak or absent across the more than 45-km landscape of our study system. There was no support for more than one genetic group when all sites were included and when only natural sites were included. Because the recent reconfiguration of the landscape occurred within the past 1–2 generations of tortoises, population structure might potentially exist without a genetic signature.

Our finding of weak genetic structure was somewhat unexpected based on the extent of our study area, what we knew about tortoise spatial ecology (i.e., high site fidelity coupled with low dispersal between sites; Eubanks et al., 2003; McRae et al., 1981), evident population clustering, and current patchiness of habitat on Camp Shelby. Numerous similar genetic studies exist of *Gopherus* species in the western USA, and although life histories are similar, landscapes in the western USA are more resistant to dispersal (Edwards et al., 2004; Fujii and Forstner, 2010; Murphy et al., 2007). Sinclair et al. (2010), which is the only other genetic study of connected Gopher Tortoise populations, found the same level of structure ($F_{ST} = 0.030$; $K = 1$) as our study. Interpretation of these data requires understanding tortoise spatial ecology and demography.

Gopher Tortoise spatial ecology has been extensively studied across the geographic distribution of the species (Diemer, 1992; Eubanks et al., 2003; McLaughlin, 1990; McRae et al., 1981; Smith, 1995; Smith et al., 1997). Gopher Tortoises have been documented making movements of several hundred meters in just a few days to 2.6 km over 2 yr (Berry, 1986; Eubanks et al., 2003; McRae et al., 1981). Eubanks et al. (2003) inferred that dispersal was a rare event because only 3% of adult males made these long-distance moves. Similarly, one radio-telemetry study of tortoises at Camp Shelby found that 7% of 403 documented movements were >300 m and <1% were >1 km (C. Guyer, personal communication), while another radio-telemetry study on Camp Shelby documented only 1% of 1372 interburrow movements were >300 m (Yager et al., 2007). Additionally, Gopher Tortoise movement data were available on Camp Shelby via 15 yr of mark and recapture. Four recaptures were 2.5–3.0 km away from the initial capture site over a maximum capture interval of 12 yr.

Although most individual tortoises at Camp Shelby and at other sites throughout the range have high site fidelity and low dispersal rates between sites, there is infrequent dispersal of a small proportion of individuals. An adult tortoise making these types of movements, especially several in succession in the same direction, could potentially transfer its genes from one breeding population to another. These events might be considered rare in the context of 1–2-yr studies. However, because tortoises have a generation time of 31 yr (FFWCC, 2006; Miller, 2001), the occurrence of these annual events within a generation time is not rare. Biologically, small numbers of effective migrants (i.e., those that actually breed and produce offspring) can sustain enough gene flow among populations to prevent genetic divergence (Mills and Allendorf, 1996; Wang, 2004), even for species with strong philopatry (Alcaide et al., 2009; this study). Even if only a small number of individuals make periodic long-distance migratory or dispersal treks, one effective migrant per generation is sufficient to maintain similarity among populations of organisms (Mills and Allendorf, 1996; Wang, 2004). Given the long generation time of tortoises, this gene flow has fairly high probability of occurrence, but the spatial scale and degree of genetic differentiation will depend on the distribution of barriers to movement and amount and availability of suitable habitat over time. Based on genetic data, a general characteristic of Gopher Tortoises is that populations are large in size and spatial extent and have gene flow among multiple colonies across natural landscapes. Our results and those of Sinclair et al. (2010) suggest that spatial data may underestimate connectivity of colonies.

Management of Gopher Tortoises on Camp Shelby

An obvious requirement for conserving biodiversity is preservation of populations across the landscape. Equally important is the ability of individuals to respond to changing environments (Measey and Tolley, 2009). If a response involves adaptation, species may be limited by genetic potential if management practices do not include preservation of genetic variability (Le Rouzic and Carlborg, 2007). Several life-history characteristics of Gopher Tortoises, including limited home-range size, high site fidelity, high longevity, delayed age at maturity, and high degree of habitat specialization render it particularly vulnerable to rapid environmental change. Another characteristic that affects vulnerability to evolutionary change is amount of genetic diversity. Our data indicate that genetic diversity in Camp Shelby tortoises is low. There are at least three scenarios to account for this. One is that the genetic diversity in the western population in general, and at Camp Shelby in particular, has been affected by recent anthropogenic activities. Another is that a historically low level of genetic diversity is a natural phenomenon of the western Gopher Tortoise population that does not necessarily impact long-term sustainability. A third scenario is that the levels of genetic diversity, either at Camp Shelby or for the western population in general, were historically higher than at present, but have been reduced by factors other than recent anthropogenic activity. At present, we cannot distinguish among these scenarios. However, due to the fact that the western population of Gopher Tortoises is federally listed as threatened and because of the uncertainty in assessing the cause of the low genetic diversity, it would be prudent to apply the "precautionary principle" and assume that the evolutionary potential of Gopher Tortoises at Camp Shelby is low, which has implications for long-term population viability and response to environmental changes.

Population genetic diversity, although low, was positively related to habitat quality, as was colony size. Gopher Tortoises thrive in healthy, fire subclimax communities of Longleaf Pine forests with an understory that is primarily composed of nonwoody (herbaceous) plants. This type of community is maintained by frequently reoccurring growing-season fires that have a typical return interval of 5–10 yr. If Longleaf Pine trees are removed or if fires are suppressed, a thick oak forest with a sparsely vegetated understory will result (Varner et al., 2005), which is unfavorable habitat for tortoises.

When Longleaf Pine habitat is not maintained, species restricted to this habitat may decline and become isolated in patches of suitable habitat (e.g., Red-cockaded Woodpecker, *Picoides borealis* [Ross et al., 1997]; Dusky Gopher Frog, *Rana sevosa* [Richter and Jensen, 2005]), which results in inbreeding, lowered genetic variability, and lowered probability of persistence (Bouzat et al., 2009; Richter et al., 2003, 2009; Westemeier et al., 1998). Regional abundance and genetic diversity are dependent on both within-population and among-population dynamics (Zamudio and Wieczorek, 2007).

Tortoises thrive in open areas maintained by fire or anthropogenic activity because these areas provide forage material, as would a healthy forest understory (Yager et al., 2007). The habitat alterations on Camp Shelby, in which forest habitat was converted to open-canopy, ruderal habitat for military activities, appear to have benefitted Gopher Tortoises, based on population sizes and genetic diversity at these sites. However, preference for open areas maintained by fire or anthropogenic activity can result in tortoises congregating in relatively high densities (Mushinsky et al., 2003). Therefore, the fitness of tortoises on these sites may be highly dependent upon sufficient availability of resources in the area (Auffenberg and Franz, 1982; Diemer 1986;

Yager et al., 2007). If surrounding Longleaf Pine forest habitat is not managed with fire, there will be lower resource and habitat availability for tortoises. Additionally, ineffective management of surrounding forest will result in a landscape composed of highly suitable patches of open habitat isolated within a matrix of unsuitable habitat (McRae et al., 1981). This would lead to isolation of tortoise populations and could have a negative effect on long-term persistence.

Interpretation of our data in light of other studies suggests that tortoise populations are naturally large in spatial extent and are composed of multiple colonies across the landscape. Sound management practices across the geographic distribution should focus locally to maximize habitat and resource availability to support the maximum density of tortoises and at large spatial scales to maximize the number of colonies. Our data support one management unit across the landscape of Camp Shelby. The US military and US Forest Service should continue actively managing the habitat across Camp Shelby via controlled burning and centralization of human structures. To ensure that sites cleared for military activity continue to benefit tortoises both in the short term and in evolutionary time, maintenance of intervening forest habitat is requisite. With this approach to management, we predict that population dynamics within and among populations will continue similar to that of years prior to human settlement of the US southeastern coastal plain and that genetic variation will be maintained and have potential to increase.

An indirect benefit of maintaining surrounding forests is that tortoises may migrate into adjacent forest, and fewer tortoises would then occupy sites intended for military use, thus helping to alleviate conflict between tortoises and human land use (Yager et al., 2007). In addition to issues of human–tortoise interaction, the establishment of isolated patches of ruderal habitat (and subsequent increase of tortoise density to unnaturally high concentrations) may bring with it new risks, such as disease transmission or increased predation (Ashton and Ashton, 2008). Until we fully understand all the variables affecting tortoise persistence on the landscape, the most logical course of action is to try to maintain or return the habitat to its natural state; that is, large, connected patches of high-quality habitat.

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