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Population Genetics of Texas Spiny Softshell Turtles (*Apalone spinifera emoryi*) Under Various Anthropogenic Pressures in Two Distinct Regions of Their Range in Texas

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ABSTRACT. – Using 8 polymorphic microsatellite loci, we explored genetic variability in Texas spiny softshell turtles (*Apalone spinifera emoryi*) in the region of Big Bend National Park (BBNP) and the Lower Rio Grande Valley (LRGV), which are located in western and southern regions, respectively, of the distribution of the subspecies in Texas. The presence of multiple anthropogenic stressors, such as river flow alterations, human population expansion, and direct harvest, motivated us to evaluate whether genetic consequences of these stressors have become apparent in this species. A low but significant level of genetic differentiation was detectable between these 2 regions. There was also detectable isolation by distance among the turtles in LRGV but not among turtles in BBNP, possibly because the LRGV localities were discontinuous ponds, whereas the BBNP localities were continuously joined stretches of the Rio Grande. We detected no evidence of a recent population bottleneck in BBNP or the LRGV. However, turtles are generally long-lived and, because harvest activity peaked in the 1990s, it is likely that detecting harvest-related changes would be challenging. Continuous long-term sampling is necessary to evaluate the genetic consequences of anthropogenic pressures.

KEY WORDS. – Reptilia; Testudines; freshwater turtles; population genetics; commercial harvest; habitat alteration; microsatellites

Direct and indirect anthropogenic pressures on natural resources, such as overharvest and habitat fragmentation, can contribute to declines in population size and genetic diversity (Schaberg et al. 2008; Escalona et al. 2009; Hansen et al. 2009; Pinsky and Palumbi 2014). Reductions in population size due to overharvesting and habitat fragmentation can reduce migration rates, and loss of gene flow can affect genetic diversity within the larger geographic area (Allendorf et al. 2008). However, these effects can be masked or even off-set if a population is part of a wider geographical area connected by migration (Daley 1992; Vucetich and Waite 2000; Consuegra et al. 2005). Understanding genetic diversity of populations under anthropogenic pressures is essential in implementing proper conservation management strategies, such as harvest regimes, and in establishing buffer zones and travel corridors (Kuo and Janzen 2004; Schwartz et al. 2007).

Freshwater turtles are threatened by human exploitation and habitat degradation (Klemens 2000; Moll and Moll 2004) and molecular tools are increasingly used to detect the effects of these pressures on wild populations (e.g., Hauswaldt and Glenn 2005; Alacs et al. 2007; Vargas-Ramírez et al. 2007; McGaugh 2012). Microsatellite markers are often used to evaluate genetic differentiation, hybridization, and migration rates between populations (Spinks and Shaffer 2005; Escalona et al. 2009; Vandewege et al. 2012). For example, studies of turtles have detected low levels of heterozygosity in wood turtle (*Glyptemys insculpta*) populations (Fridgen et al. 2013), population bottlenecks in ornate box turtle (*Terrapene ornata*) populations (Kuo and Janzen 2004), limited dispersal in alligator snapping turtles (*Macrochelys temminckii*; Echelle et al. 2010) and Blanding's turtles (*Emydoidea blandingii*; Mockford et al. 2007), and recent reduction in population size of the giant Amazon river turtle (*Podocnemis expansa*; Pearse et al. 2006). However, evidence of low heterozygosity or bottlenecks in turtle populations resulting from exploitation or habitat degradation has been difficult to detect in some turtle species (e.g., Escalona et al. 2009; Bennett et al. 2010; Willoughby et al. 2013). This difficulty has been attributed to long generation times and late maturity associated with chelonian life histories (Willoughby et al. 2013). Therefore, short- and long-term studies are important for detecting the true effect of population disturbance on long-lived species (Kuo and Janzen 2004).

The spiny softshell turtle (*Apalone spinifera*) is one of several freshwater turtle species in Texas that are under continuous direct and indirect anthropogenic pressures. Four subspecies are recognized in the state: Texas spiny softshell (*Apalone spinifera emoryi*), Guadalupe spiny softshell (*Apalone spinifera guadalupensis*), pallid spiny softshell (*Apalone spinifera pallida*), and western

spiny softshell (*Apalone spinifera hartwegi*; Dixon 2013). Individuals regularly live up to 25 yrs (Snider and Bowler 1992). In Texas, the Texas spiny softshell turtle ranges from El Paso County in western Texas to the Lower Rio Grande Valley (LRGV) of southern Texas (Dixon 2013). Although it is primarily a riverine species, it often inhabits lakes, ponds, canals, and irrigation ditches (Ernst and Lovich 2009). Like other softshell turtles of North America, Texas spiny softshells are highly aquatic and sensitive to desiccation (Weisrock and Janzen 2000; Ernst and Lovich 2009). Therefore, migration between populations is restricted primarily to aquatic dispersal routes (Weisrock and Janzen 2000).

Texas spiny softshell turtles are harvested for commercial purposes in the LRGV (Ceballos and Fitzgerald 2004; Brown et al. 2012). In 1999, ~ 9045 Texas spiny softshell turtles were collected from Cameron and Hidalgo counties of the LRGV, with this single species from just these 2 southern Texas counties comprising 56% of overall reported harvest across the state (Ceballos and Fitzgerald 2004). In addition, the LRGV experienced significant increases in human populations between 1976 and 2006, contributing further to pressures on wild populations by habitat alteration and agricultural land expansion (Brown et al. 2012; Mali et al. 2013b). The western portion of the subspecies' range is considered an undisturbed refuge area, which contrasts with the LRGV. Commercial harvest reports for the region are very low and the majority of the subspecies' distribution in this region falls within federal- and state-regulated stretches of the Rio Grande (Big Bend Ranch State Park, Big Bend National Park [BBNP], Black Gap Wildlife Management Area, etc.). In western Texas, the Rio Grande plays an important role in the ecosystem because it is often the only available water source (Jackson 2010). However, the Rio Grande has been facing in-stream flow reduction for a century, which can degrade freshwater turtle habitat and negatively impact populations (Bailey et al. 2008; Jackson 2010). In the present study, we sought to assess the genetic diversity of softshell turtles in the harvested LRGV region, in comparison with the unharvested region of BBNP, to 1) determine whether the turtles from the two regions are genetically distinct, 2) estimate migration rates (i.e., gene flow) between the regions, and 3) determine whether there are signs of reduced genetic diversity.

METHODS

Sampling Sites. — Texas spiny softshell turtles were collected between 2008 and 2013, during a statewide freshwater turtle assessment project (Mali et al. 2011). We used 76-cm-diameter single-throated, single-opening hoop nets with 2.5-cm-square mesh to capture turtles (Mali et al. 2014). Traps were baited predominantly with canned sardines and other fish-based bait (Mali et al. 2013a, 2013b). We collected tissue samples from 6 and 4 sites in Cameron and Hidalgo counties of the LRGV, respectively. Two of these sites were located directly along the Rio Grande and were approximately 153 river-km apart (Fig. 1). Four of the 10 sites in the LRGV were considered sanctuary sites (i.e., Santa Ana National Wildlife Refuge, Southmost Preserve, The Edinburg Scenic Wetlands and World Birding Center, and Frontera Audubon), whereas the other 6 sites were not sanctuaries and most likely had been harvested prior to the modification of Texas commercial turtle-harvest regulations (Texas Parks and Wildlife Department 2007; Brown et al. 2011). In western Texas, we sampled 4 sites along the Rio Grande, 1 site in Black Gap Wildlife Management Area, and the other 3 sites in BBNP, with the 2 most isolated sites in the latter being approximately 185 river-km apart.

Tissue Collection. — Samples were collected by clipping a small soft-tissue sample from a posterior portion of the carapace and storing it in 1 ml of 95% EtOH or by extracting 50–100 µl of blood stored in 1 ml of lysis buffer (0.012 g of Tris, 0.037 g of EDTA, and 0.01 g of SDS per ml of ddH_2O). All samples were then stored at −80°C until DNA extraction.

Laboratory Methodology. — We extracted DNA using a Qiagen DNeasy Blood & Tissue kit following manufacturer protocols. We tested 11 microsatellite loci developed specifically for spiny softshell turtles (Davy et al. 2012). Primers were synthetized using a specific M13 tag, with a 5′ M13 tail added to the forward primer and 5′ pig tail on the reverse primer (Davy et al. 2012). Polymerase chain reaction (PCR) followed protocols described in King and Julian (2004). Briefly, there was an initial incubation at 94°C for 2 min followed by 35 cycles of 45 sec at 94°C, followed by annealing temperatures that differed from Davy et al. (2012) and varied by primer (Table 1), and then 72°C for 1 min. To optimize the proper binding of the M13 tag, a second cycle was added at the end of the protocol that consisted of an initial denaturation period at 94°C for 30 sec, followed by an annealing period at 53°C for 45 sec, and finally an extension period at 72°C for 1 min. The PCR protocol finished with an extension period at 72°C for 5 min (Schuelke 2000; King and Julian 2004). Amplifications were performed using a Peltier Thermal Cycler PTC-200 (MJ Research, Inc). To maximize the number of runs set for the sequencer, we ordered different dyes for the M13 tag (FAM, VIC, NED, and PET), which allowed us to label different primers and consequently stack 4 different primers in 1 plate. Samples were run on a 3500xL Genetic Analyzer with a LIZ internal size standard (Applied Biosystems®). Microsatellite fragment sizes were estimated with Gene Mapper software (Applied Biosystems).

Population Genetic Analyses. — We used MICRO-CHECKER 2.2.3 (Van Oosterhout et al. 2004) to test for genotyping errors due to presence of null alleles or large allele drop out. Genotypic data were analyzed using

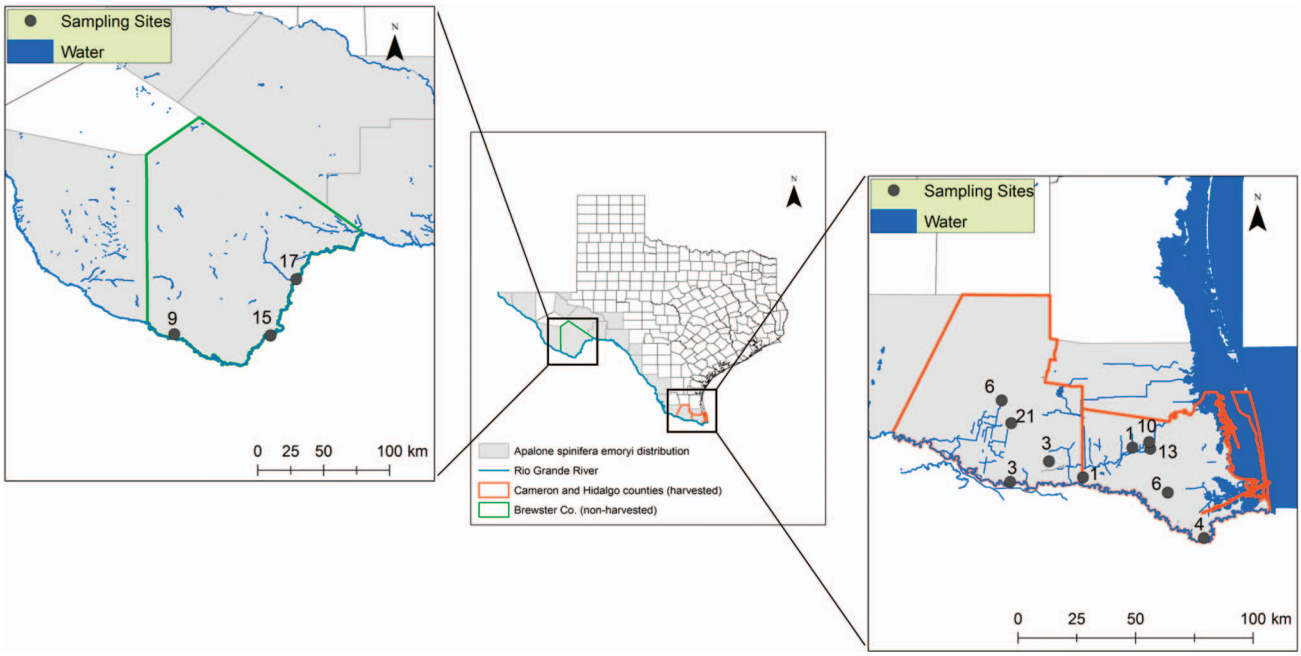

Figure 1. Locations of 10 sampling sites in the Lower Rio Grande Valley (LRGV; southern) region and 4 sampling sites in Big Bend National Park (BBNP; western) region of Texas. The number next to each sampling site represents the number of samples per site. Two sites in BBNP region ($n = 7$ and $n = 8$) were only ~ 65 m apart and are combined on this map (combined $n = 15$). (Color version is available online.)

STRUCTURE v.2.3.4 (Pritchard et al. 2000) to assign individuals to potential subgroups (K), without using any prior population information. We initiated 3 independent runs of $K = 1$–3 with 100,000 Markov chain Monte Carlo repetitions after a 10,000-run burn-in. STRUCTURE parameters were set to default values and the option "correlated allele frequencies" and the admixture model were utilized. For each value of K, the posterior probability was calculated using the estimated log-likelihood of K to select the optimal K (Evanno et al. 2005). The number of clusters was chosen based on inflection in the rate of change in log probability of successive K values (ΔK; Evanno et al. 2005) implemented in Structure Harvester (Earl and von Holdt 2012). We used ARLEQUIN v.3.0 (Excoffier et al. 2005) to calculate allelic range and richness, observed heterozygosity, and expected heterozygosity, to test for Hardy-Weinberg equilibrium (HWE), and calculate F_{ST} between turtles in the LRGV and BBNP populations. To detect significant departure from HWE for a particular locus, ARLEQUIN follows the procedure explained in Guo and Thompson (1992), which is analogous to Fisher's exact test on a 2-by-2 contingency table, but extended to a triangular contingency table of arbitrary size. Number of steps in the Markov chain was set to 1,000,000, with number of dememorization steps set to 100,000. The p-value of the test is the proportion of the visited tables having a probability smaller than or equal to the observed contingency table (Excoffier et al. 2005). Additionally, we performed sequential Bonferroni tests for HWE estimates across loci (Rice 1989). To explore the distribution of genetic variation graphically, we used the 2D module of GENETIX v.4.04 (Belkhir et al. 2001) to conduct Factorial Correspondence Analysis (FCA), and we used BayesAss 3.0.3 (Wilson and Rannala 2003) to estimate recent levels of gene flow (past 2 generations) between the 2 regions. Wilson and Rannala (2003) suggested that the most accurate results are obtained when the acceptance rates of proposed changes for migration rate (m), allele frequencies (p), and inbreeding coefficient (F) are between 20% and 60%. We performed 10 independent replicate runs of the algorithm, which produced the following acceptance rates: $m = 0.54$, $p = 0.60$, and $F = 0.70$. We then increased the proposal step size from 0.10 (default) to 0.30 for the mixing parameters associated with proposed moves of the inbreeding coefficient, produced an inbreeding coefficient of 0.39, which is within the accepted range. To determine

Table 1. The 11 microsatellite loci developed by Davy et al. (2012), their repeat motif, and the annealing temperatures (°C) used by Davy et al. and in the current study to amplify the loci.

Locus	Repeat motif	Annealing temp. (Davy et al. 2012)	Annealing temp. (current study)
As07	AGAT	52	60
As12	ATGGT	52	56
As13	CTTT	60	54
As14	GATT	58	56
As15	GTTT	54	60
As18	GTTT	58	58
AsB07	AAC	61	56
AsB08	AAC	58	58
AsB09	ATC	58	58
AsB12	AAT	58	58
AsB14	AAT	58	58

significance between genetic and geographical distances of samples across the landscape, we conducted Mantel tests (Mantel 1967) within each region. We used Alleles in Space (Miller 2005), which allows the analyses of interindividual pattern of genetic and geographical variation. Geographic distances were represented as the straight-line distances between pairs of sites and calculated using ArcView v.10.2.2 (ESRI). Although some sites were located directly along the Rio Grande (3 sites in BBNP and 2 sites in the LRGV), we were conservative in choosing straight-line distances between all pairs of sites in order to keep the methods consistent throughout analyses while incorporating the minimum dispersal connectivity among sites.

We tested for population bottlenecks in the 2 regions using BOTTLENECK v.1.2.02 to examine the deviation from mutation-drift equilibrium (Cornuet and Luikart 1996). In BOTTLENECK, we ran the 2-phase model because it is the most appropriate model for microsatellite data (Di Rienzo et al. 1994). As suggested by Piry et al. (1999), the parameters were set to 95% single-step mutations and variance among multiple steps of 12. However, to test the robustness of the data, we conducted additional tests with single-step mutations varying from 70% to 90% and variance among multiple steps varying from 2 to 30. We used the Wilcoxon test and mode-shift graphical method to assess any recent population bottleneck. In populations that experienced recent population bottlenecks, allelic diversity is reduced more quickly than heterozygosity (Nei et al. 1975). Thus, if observed heterozygosity is larger than the expected heterozygosity at mutation-drift equilibrium, it is likely that a population experienced reduction of its effective size and the Wilcoxon test will exhibit significant heterozygosity excess (Cornuet and Luikart 1996). The mode-shift method classifies alleles in 10 frequency classes. In a population that has not undergone a bottleneck, alleles with low frequencies are most abundant and the distribution follows a normal L-shape (Luikart and Cornuet 1998).

RESULTS

We collected 109 individual tissue samples: 68 from the LRGV and 41 from BBNP. All 11 microsatellite primers developed by Davy et al. (2012) successfully amplified. Three loci (As12, AsB09, and AsB12) were monomorphic across all populations and were excluded from further analyses. The average observed number of alleles was 10.25 in turtles of the LRGV and 6.75 in turtles from BBNP (Table 2). The differences in allelic richness per locus between regions were subtle, except for locus As15, which showed the greatest overall diversity ($k = 24$) in the LRGV. The mean observed heterozygosity was 0.66 in turtles from LRGV and 0.63 in turtles from BBNP; the mean expected heterozygosity was 0.68 for the LRGV and 0.60 for BBNP. Two of the 8 loci (As07 and As18) showed significantly lower observed heterozygosity in BBNP, whereas there was no significant deviation from HWE in the LRGV. However, none of the p-values were significant after sequential Bonferroni correction was applied. MICRO-CHECKER found evidence of homozygote excess in 2 loci in BBNP (As07 and As18), suggesting the presence of null alleles. There was no evidence of null alleles in the LRGV and neither region showed evidence of large allele dropouts.

Based on the STRUCTURE analysis, the highest likelihood was observed for $K = 2$, with the STRUCTURE plot identifying general differences between BBNP and the LRGV (Fig. 2). In congruence with the STRUCTURE results, a pairwise comparison showed low but significant genetic differentiation between the 2 regions ($F_{ST} = 0.03327$, $p < 0.0001$) and the FCA plots exhibited overlapping but different sets of points for the BBNP and LRGV samples (Fig. 3). The separation on axis 1 explained 4.39% of the observed variation, whereas axis 2 explained 4.29% of observed variation. Individuals from BBNP formed a relatively compact cluster in comparison with the LRGV individuals, which showed a greater scatter. BayesAss analyses showed that within the BBNP region, proportion of turtles that were considered migrants from the LRGV was 0.278 (SD = 0.019) per generation. Within the LRGV region, proportion of turtles that were considered migrants from the BBNP region was 0.154 (SD = 0.020) per generation.

The distances between the two geographic regions ranged from 566 to 718 km, with distances among sampling sites within the LRGV ranging from 3 to 97 km and distances among sampling sites within BBNP ranging from 46 to 91 km. Two sites in western Texas were only 65 m apart: one site had evident river connectivity and the other site was a disconnected pond. However, we treated these 2 locations as a single site with an assumption that softshell turtles often travel short overland distances in undisturbed regions. Minimum distance between the sites in the LRGV was 3 km overland. Although some freshwater turtle species are known to travel overland distances of up to several kilometers (Buhlmann and Gibbons 2001), North American softshell turtles are particularly sensitive to desiccation because of their high rates of water exchange (Stone and Iverson 1999), and therefore have restricted overland movement (Plummer et al. 1997). Therefore, we treated sites at ≥ 3-km distance as separate entities. The Mantel tests suggested a significant positive correlation between genetic and geographic distance within the LRGV region ($r = 0.17$, $p = 0.001$) but not within BBNP ($r = 0.05$, $p = 0.17$).

The Wilcoxon test implemented in BOTTLENECK did not show significant heterozygosity excess ($p = 0.19$) for turtles in the LRGV. Subsequent iterations also provide nonsignificant results, with p-values ranging from 0.37 to 0.98. There was nonsignificant heterozygosity excess ($p = 0.47$) for BBNP, with additional iterations also showing nonsignificant results (p ranging from 0.32

Table 2. Measurements of microsatellite genetic diversity, number of alleles (*k*), allelic range, observed heterozygosity (H_o), expected heterozygosity (H_e), and corresponding *p*-values, in 2 distinct regions of the range of the Texas spiny softshell turtle (*Apalone spinifera emoryi*) in Texas.

	Lower Rio Grande Valley Region					Big Bend National Park Region				
Locus	*k*	Range	H_o	H_e	*p*	*k*	Range	H_o	H_e	*p*
As07	17	64	0.86765	0.90937	0.27403	14	56	0.80000	0.91930	0.02291
As13	11	44	0.89552	0.84110	0.26971	11	48	0.78049	0.74375	0.90079
As15	24	120	0.94118	0.93911	0.43203	13	88	0.82927	0.89190	0.30448
As18	8	28	0.41176	0.40915	0.62576	3	8	0.21951	0.31768	0.03098
AsB07	4	9	0.25000	0.32048	0.06330	2	3	0.05405	0.05331	1.00000
AsB08	4	12	0.60294	0.53519	0.35958	3	9	0.77500	0.67373	0.55682
AsB14	8	24	0.66176	0.73214	0.13339	4	15	0.78049	0.58446	0.03819
As14	6	24	0.66154	0.72177	0.33011	4	16	0.78049	0.58446	0.03832
Mean	10.250	40.625	0.66154	0.67604		6.750	30.375	0.62741	0.59608	
SD	6.985	36.625	0.24220	0.23103		5.007	30.326	0.30653	0.29086	

to 0.47). Exclusion of the 2 loci that had evidence of null alleles and deviated from HWE (As07 and As18) showed a constant nonsignificant heterozygosity excess ($p = 0.5$) across all runs. Heterozygosity deficiency was not detectable in either region ($p > 0.05$). Mode-shift graphical representation shows the normal L-shaped form of allele frequency spectra for both regions (Fig. 4), further suggesting that neither region has undergone a recent population bottleneck.

DISCUSSION

The use of genetic tools for evaluation of wildlife populations has become a common practice in conservation and wildlife management. Studies that are able to rely on previously designed microsatellite markers offer an inexpensive approach to gain insight about population diversity and overall population health that cannot always be achieved using field methods. They can be particularly useful in the evaluation of genetic diversity over time (Fridgen et al. 2013). The loss of genetic diversity can make populations vulnerable because of decreased fitness and increase the likelihood of extirpation or even extinction (Frankham 1998; Reed and Frankham 2003). Such information is useful to management agencies, because it addresses levels of threats to natural populations and facilitates development of protocols for species protection. Our study shows the utility of microsatellite data in assessing habitat connectivity and genetic diversity in the two regions of Texas spiny softshell distribution.

Freshwater turtles in Texas have experienced persistent anthropogenic pressures in the past century. Until 2007, Texas allowed unlimited harvest of all species not listed under Convention on International Trade in Endangered Species, which, in turn, resulted in significant levels of harvest of spiny softshell turtles, particularly contemporaneous to the collapse of Asian turtle populations harvested to supply meat markets in the 1990s (Ceballos and Fitzgerald 2004; Brown et al. 2011). Harvest was particularly prevalent in the LRGV (Ceballos and Fitzgerald 2004), where field studies reported a significant decline of Texas spiny softshells compared with historical data (Brown et al. 2012). Consequently, direct take from wild populations, coupled with the loss of connectivity due to habitat alteration can cause significant decrease of genetic diversity in depleted or isolated populations.

The pairwise comparison showed statistically significant differentiation between LRGV and BBNP turtles. The estimation of recent migration rates shows that majority of turtles (> 70%) within both regions consists of nonmigrants, with slightly more migrants moving from the LRGV to BBNP (28% migrants) than from BBNP to the LRGV (15% migrants) per generation. Therefore, the Rio Grande likely still serves as an important corridor for turtles between southern and western Texas. There was no significant isolation by distance in the sampled region of

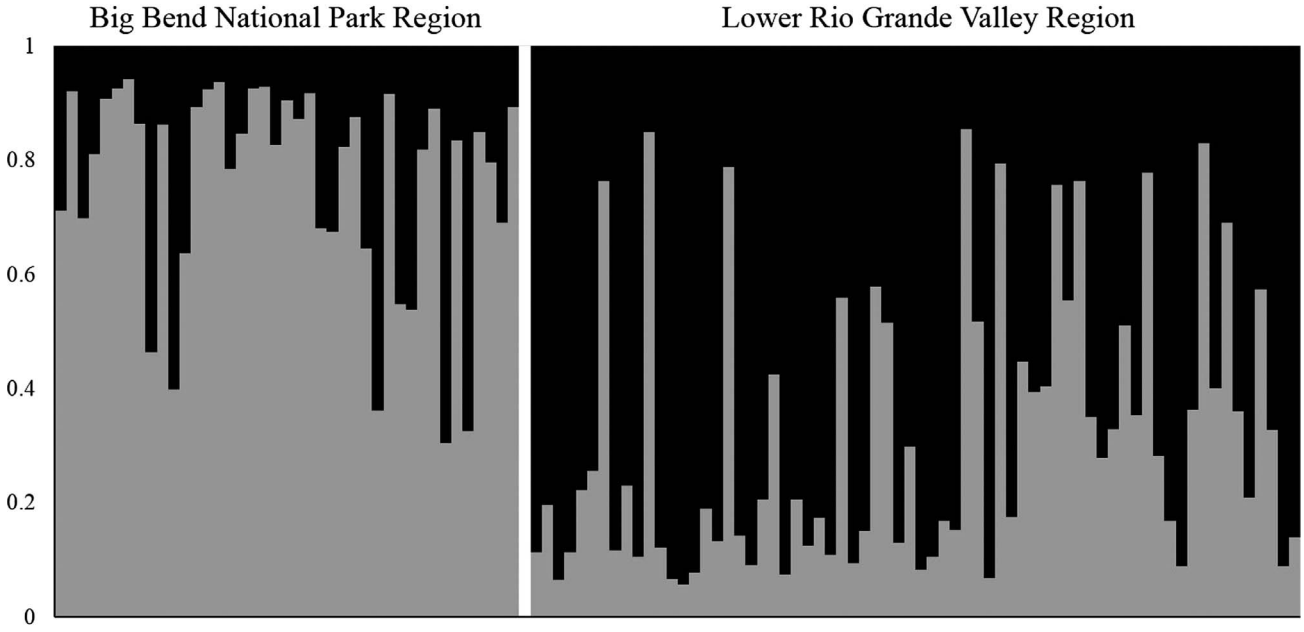

Figure 2. Estimated population structure generated by the program STRUCTURE, assuming 2 subpopulations ($K = 2$). Each individual is represented by a vertical line partitioned into 2 segments that represent the individual's probability of belonging to each of the 2 hypothetical populations. Vertical white line separates individuals into 2 distinct regions (western and southern Texas).

western Texas. These results should be interpreted with caution—the analysis possibly failed to detect significant isolation by distance because of the low number of sampling locations. On the other hand, turtles along that stretch of the Rio Grande could be well-connected. Future studies would benefit from more sampling locations in western Texas to increase the power of the analysis. Sites in the LRGV showed signs of isolation by distance, possibly because of the nature of the sites. Although BBNP was sampled along the river, the vast majority of sites in the LRGV were composed of lentic water bodies, such as ponds and irrigation canals, which lent themselves to local population substructuring. Assuming that softshell turtles make overland movements less frequently and across shorter distances than other aquatic turtles, we expected to encounter isolation by distance patterns in this study, but doing so on the relatively small scale of $\sim$ 100 km was unexpected. Human population expansion in the

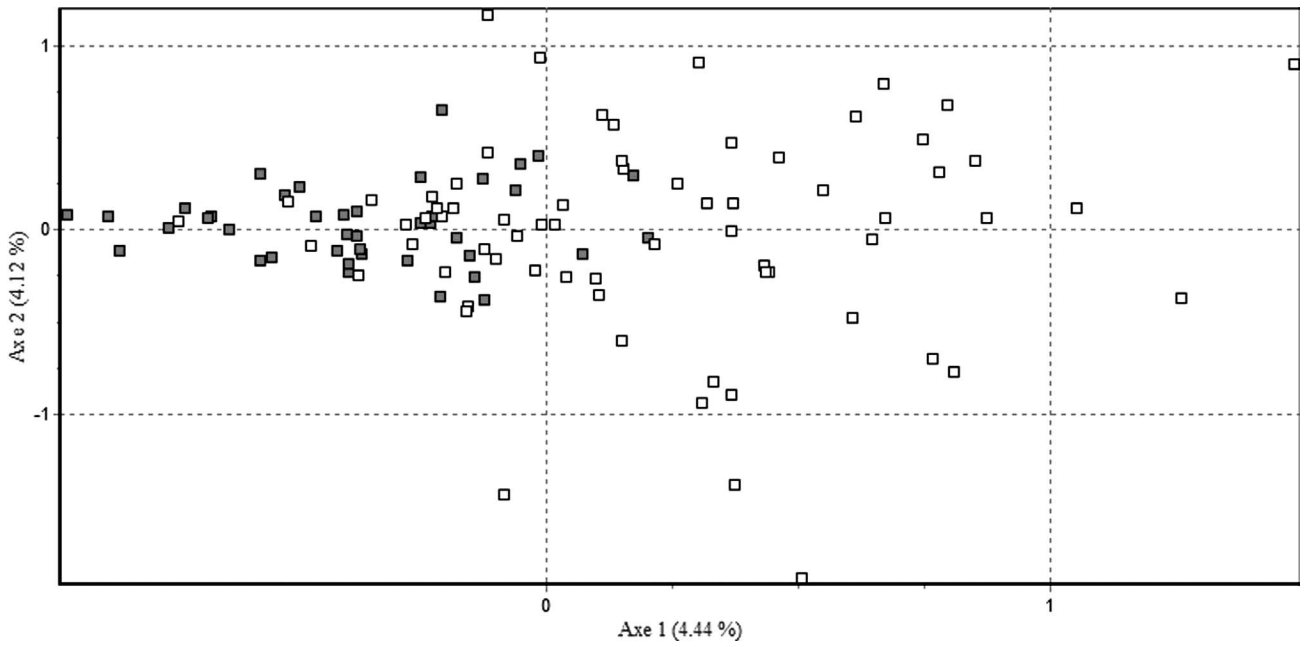

Figure 3. Graphic representation of Factorial Correspondence Analysis (FCA) created using 2D module of software GENETIX v.4.04 (Belkhir et al. 2001) on 109 individuals from the Big Bend National Park region in western Texas (filled squares) and the Lower Rio Grande Valley in southern Texas (white squares) for Texas spiny softshell turtles (*Apalone spinifera emoryi*).

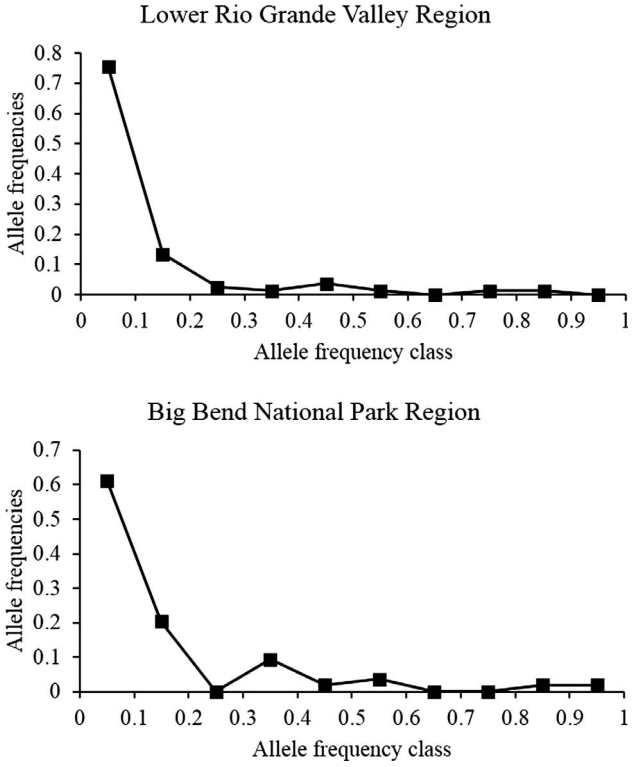

Figure 4. L-shaped mode-shift graph showing absence of bottlenecks in Texas spiny softshell turtles (*Apalone spinifera emoryi*) from 2 regions.

region and road development might also play a significant role in genetic isolation among water bodies in the future. On the other hand, it is possible that agricultural irrigation and flood control canals now provide important corridors that could offset the severity of habitat fragmentation in the LRGV and actually enable connectivity among sites.

We detected no reduction in the effective population size of Texas spiny softshell turtles in either the LRGV, where historical harvest levels had been high, or in BBNP, which had not been directly affected by harvesting. Because turtles have long generation times, detecting genetic changes and attributing them to recent events can be challenging (Bennett et al. 2010; Willoughby et al. 2013). A high-intensity softshell turtle harvest occurred in the 1990s in the LRGV (Ceballos and Fitzgerald 2004), with our sampling effort occurring 2 decades later. By conservative estimates, spiny softshell turtle longevity approaches 25 yrs (Snider and Bowler 1992) and it has been estimated that spiny softshells can live up to 50 yrs in the wild (Breckenridge 1955), meaning that at most 1 generation has passed since the harvest episode of the 1990s. Even though we could not detect a population decline at the genetic level, this does not necessarily indicate a healthy and stable population. We do not know how long the high-intensity harvest had been underway prior to 1990s, but we speculate that a combination of factors made the harvest of the 1990s a uniquely intense event for this population, making it possible that genetic changes are yet to be detected. Although Texas banned harvest from public water bodies in 2007 (Texas Parks and Wildlife Department 2007), we believe that this regulation offers limited levels of harvest management because many water bodies are privately owned in Texas (Brown et al. 2011). Therefore, maintaining viable populations and genetic diversity now depends solely on turtle dispersal from unharvested to harvested water bodies. Given softshell turtle sensitivity to desiccation (Ernst and Lovich 2009) and the already fragmented landscape in the region (Mali et al. 2013b), dispersal might not be sufficient if unlimited commercial harvest of softshell turtles from private water bodies continues in the LRGV.

Change in river flow rates in Texas can have significant effects on the environment and consequences for the flora and fauna. Before 1915, the Lower Rio Grande flow was virtually unimpeded (Bailey et al. 2008). The most drastic modification in Texas occurred with the construction of Amistad Dam in 1969 (US Department of Interior 1998). Continuous flood-control practices and the construction of dams, channels, and water diversions along the Rio Grande has placed the river on the list of top 10 most endangered rivers in America (American Rivers 2003). Untreated sewage inflows, runoff from agriculture and mining activities, and elevated levels of pollutants such as arsenic, mercury, DDT, etc., all contribute to declining water quality of the Rio Grande (US Department of Interior 1998). These factors are likely to affect softshell turtle populations along the Rio Grande. As presented in this study, the Rio Grande serves as an important corridor between Texas spiny softshell turtle populations of western and southern regions of their range. Pollution and flow reduction could not only affect population density, but could also decrease movement rates along the river. Such effects have already been observed in fish populations (Bestgen and Platania 1991). For species with long generation times, such as turtles, these subtle effects can accumulate over time (Fridgen et al. 2013). Therefore, continuous sampling and population assessment along the Rio Grande is necessary for species risk evaluation.

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