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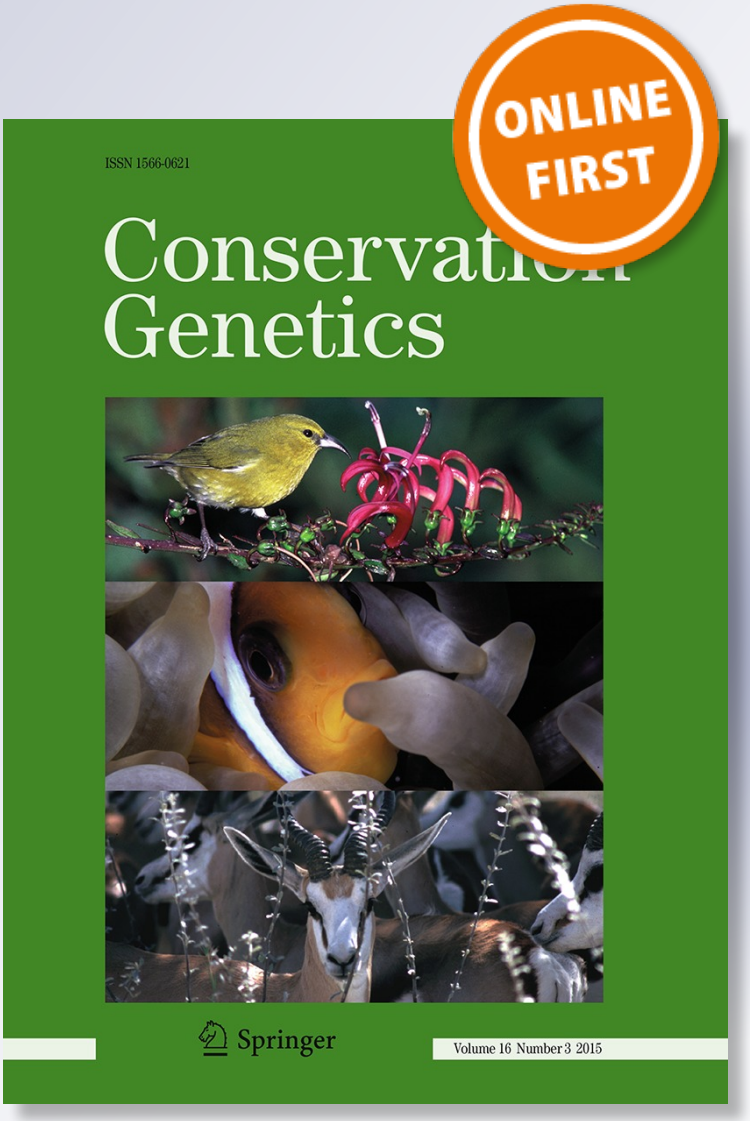

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RESEARCH ARTICLE

Population genetics of the diamondback terrapin, *Malaclemys terrapin*, in Louisiana

Charlotte Petre[1] · Will Selman[2] · Brian Kreiser[1] · Steven H. Pearson[3] · Jon J. Wiebe[3]

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Abstract Previous population genetic studies of diamondback terrapins (*Malaclemys terrapin*) have typically focused on either the entire range or relatively small spatial scales. The Louisiana coastline contains vast salt marshes suitable for terrapins; however, two major freshwater inputs (Atchafalaya River and Mississippi River Deltas) break up the seemingly contiguous habitat and may isolate populations of terrapins. To determine population genetic structure and connectivity of terrapins occupying Louisiana marshes, we collected 573 individuals from 26 study sites across the Louisiana coastline. Twelve microsatellite loci were used to evaluate population structure using standard genetic and spatially explicit approaches. Patterns of gene flow were examined via model testing, including those to determine if freshwater inputs serve as barriers to movement. We also assessed levels of genetic diversity, inferred the historical demography and estimated effective population sizes across our sampling. While we did not detect significant population structure, we found that terrapins are not panmictic, and demonstrated a pattern of isolation by distance along the Louisiana coastline. Genetic diversity in this study was comparable to the Atlantic coast, but was higher than other sites within the Gulf of Mexico. Though terrapins in eastern Louisiana were historically harvested and apparently experienced a genetic bottleneck, this is not reflected in estimates of effective population sizes. Although, there was no strong genetic structuring across Louisiana, historical differences and patterns of habitat loss suggest that it may be necessary to develop separate management strategies for the western and eastern portions of the state.

Keywords Gene flow · Microsatellites · Isolation by distance · Landscape genetics

Introduction

The distribution of Diamondback terrapins (*Malaclemys terrapin*) ranges from Cape Cod, Massachusetts to Corpus Christi, Texas; an isolated population also persist on the island of Bermuda (Ernst and Lovich 2009). Terrapins have historically been divided into seven subspecies based on morphological differences observed throughout their range (Ernst and Lovich 2009). However, genetic studies using mitochondrial DNA and microsatellite loci have failed to find support for all subspecies designations although there is population structure across its range (Lamb and Avise 1992; Hauswaldt and Glenn 2005; Hart et al. 2014). Genetic studies of terrapins have also been conducted on smaller spatial scales in various parts of their range. On the Atlantic coast, Hauswaldt and Glenn (2005) and Sheridan et al. (2010) found no genetic structure within Charleston Harbor and Barnegat Bay, respectively. Similarly, Coleman (2011) and Glenos (2013) found no evidence of genetic differentiation across sample sites within Mobile Bay (Alabama) and Galveston Bay (Texas), respectively. Most recently, Drabeck et al. (2014) failed to detect genetic structure in eastern Louisiana, although they included only

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31 individuals from southeastern Louisiana marshes (i.e., east of the Atchafalaya River).

Terrapins face threats at every life history stage and the number of threats impacting current populations continues to grow. Nests are depredated by human-subsidized populations of raccoons, armadillos, and foxes (Roosenburg 1990; Gibbons et al. 2001). Throughout their range, salt marsh habitat is being lost due to coastal erosion, marsh subsidence, and development. Additional anthropogenic disturbances include the fragmentation of the salt marsh by causeways (Brennessel 2006) and the installment and maintenance of channels. Mortality has been attributed to the invasive fire ant (*Solenopsis invicta*; Munscher et al. 2012), roadside mortality (Wood and Herlands 1997), crab pots including abandoned "ghost" crab pots (Roosenburg et al. 1997), and both commercial and recreational harvesting in portions of its range.

Historically, reports of terrapins being harvested for their meat date back to the 1700s (Hart and Lee 2006). During the height of terrapin consumption, Maryland was the center for harvesting, farming (including captive breeding), and distributing terrapins. As early as 1902, the United States Federal Bureau of Fisheries established the "artificial propagation program" to begin research on restocking, commercial exploitation, and experimental cross breeding (Hay 1917; Coker 1920; Hart and Lee 2006). During this time, terrapins from Louisiana and North Carolina were harvested and exported to Maryland to supplement their diminishing local stocks. The terrapin harvest industry collapsed due to prohibition (i.e., sherry was an important ingredient in turtle soup), collapse in terrapin populations, and the Great Depression (Brennessel 2006). Since then, a few states have prohibited the possession or take of terrapins, while others have a variety of statutes that regulate commercial/recreational take. For example, within Louisiana, there are regulations for both personal and commercial fishing for terrapins (Louisiana Department of Wildlife and Fisheries 2014). Because most of the human population within Louisiana is located in southeastern Louisiana, the proximity of southeastern Louisiana marshes to these populated areas (i.e., New Orleans) has also likely influenced historical harvest in the region.

Louisiana has approximately 653,000 hectares of brackish or saline marshes (Sasser et al. 2008), making Louisiana the state with the largest amount of potential habitat for terrapins throughout their range (Selman et al. 2014). However, the habitat is not necessarily homogenous; the western and eastern portions of Louisiana are separated by the expansive freshwater inputs at the mouths of the Atchafalaya and Mississippi Rivers. Major freshwater inputs like the Atchafalaya Delta and Mississippi River Delta (MRD) may isolate populations by creating a barrier to gene flow, thus ultimately leading to the formation of genetically distinct groups. Further, salt marsh habitat is being lost at high rates due to coastal erosion in Louisiana (Couvillion et al. 2011), and this may limit connectivity between populations or lead to local extirpation of populations due to habitat loss.

The limitations of previous studies conducted on Louisiana terrapins have restricted their focus in terms of spatial coverage and sample sizes. Thus, the inferences made by these studies are limited in order to determine the demographic history of these populations and the extent of genetic connectivity across the landscape. One goal of this study was to extensively sample terrapins across coastal Louisiana in order to determine if genetic structure exists, and if so, what landscape features along the Louisiana coastline (e.g., large freshwater inputs) may act as barriers to gene flow. We also wanted to examine the demographic history of this region, particularly testing for genetic bottlenecks and estimating effective population sizes. The output of this research can be applied to the development of wildlife management strategies, which will become increasingly important considering the high rate of coastal wetlands loss in the state (Couvillion et al. 2011).

Materials and methods

Sample collection and molecular techniques

Terrapin samples were collected from 26 locales across coastal Louisiana (Sabine Lake in the west to Lake Eugenie in the east; Fig. 1). Terrapins were collected either by hand or with fyke nets set into salt marsh tidal creeks (Selman and Baccigalopi 2012; Selman et al. 2014). Tissue samples (tail tips) were collected and preserved in 95 % ethanol. The sex and geographic location for each sample were recorded. Genomic DNA was extracted from the tissue samples using Qiagen DNeasy extraction kit reagents (Qiagen Inc., Valencia, California, USA) and Econospin spin columns (Epoch Life Science, Inc., Fort Bend County, Texas). Each individual was genotyped at 13 microsatellite loci that were developed for *M. terrapin* (*TerpSH1, TerpSH2,* and *TerpSH7;* Hauswaldt and Glenn 2005), *Glyptemys muhlenbergi* (*GmuB08, GmuD87, GmuD90, GmuD93, GmuD121, GmuD51, GmuD28, GmuD62,* and *GmuD21*; King and Julian 2004), or *Carretta carretta* (*Cc7*; FitzSimmons et al. 1995). Loci were chosen based on an initial estimate of polymorphism within a subset of the total samples and their ability to multiplex with other loci. Polymerase chain reactions (PCR) were performed in 12.5 μL reactions containing 100-200 ng of DNA, 50 mM KCl, 10 mM Tris–HCl (pH 8.3), 2.0 mM $MgCl_2$, 0.6 mM dNTPs, 0.1875 units of *Taq* DNA polymerase (New

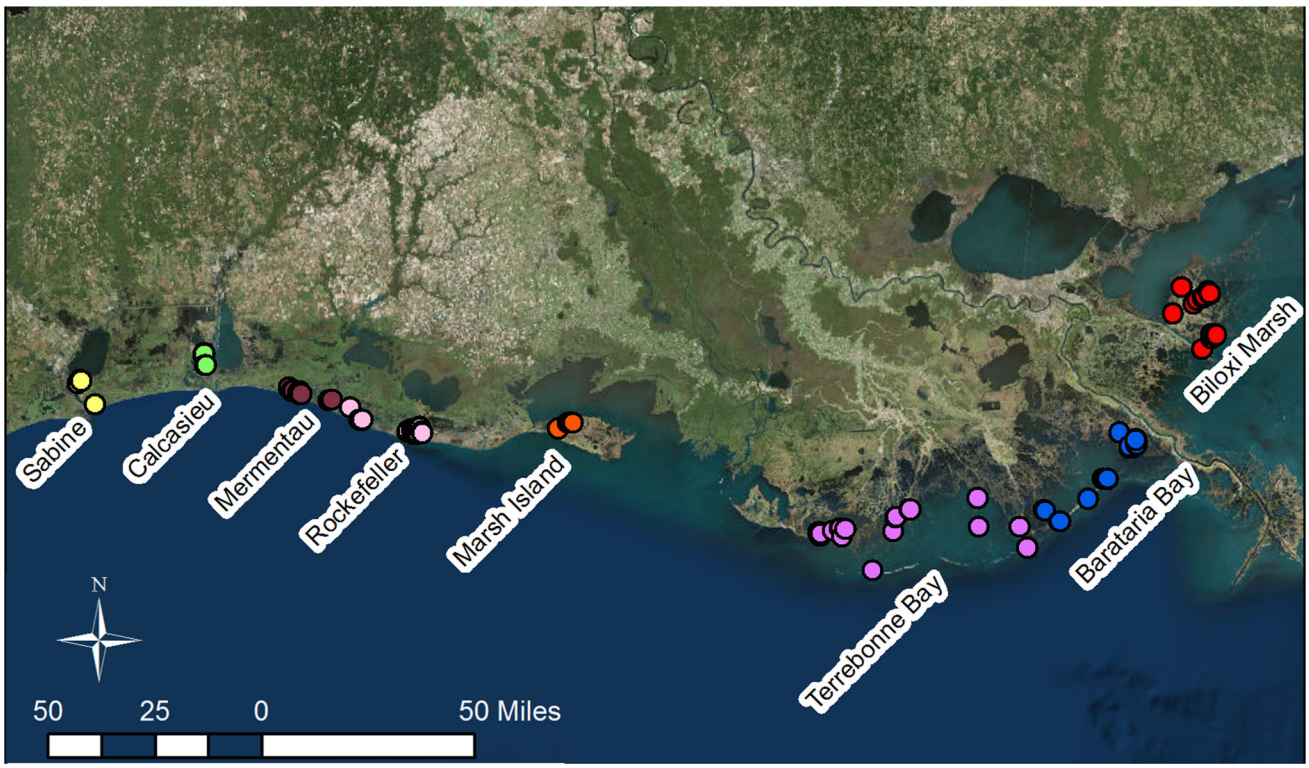

Fig. 1 Sample collection sites of *Malaclemys terrapin* in coastal Louisiana and how they were assigned to a priori groups (source of map ESRI)

England BioLabs), 0.3 μM of M13 tailed forward primer, 0.3 μM reverse primer, 0.1 μM of M13 labeled primer (LI-COR), and water to the final volume. PCR cycling conditions for primers developed for *G. muhlenbergi* were performed as follows: initial denaturing step at 94 °C for 2 min followed by 35 cycles of denaturing for 45 s at 94 °C, primer annealing for 45 s at 56–60 °C, and elongation for 2 min at 72 °C, with a final 10 min elongation step at 72 °C. PCR cycling conditions for primers developed for *C. carretta* or *M. terrapin* were performed using touchdown PCR as follows: initial denaturing step at 94 °C for 2 min followed by 15 cycles of denaturing for 30 s at 94 °C, primer annealing for 30 s at 65 °C decreasing by 0.5 °C every cycle, and elongation for 1 min at 72 °C, followed by 15 cycles of denaturing for 30 s at 94 °C, primer annealing for 30 s at 56 °C, and elongation for 1 min at 72 °C, with a final 10 min elongation step at 72 °C. Microsatellite alleles were visualized on acrylamide gels using a LI-COR 4300 DNA Analysis system, and gel images were scored using Gene ImagIR v3.55 (LI-COR Biosciences, Lincoln, Nebraska, USA) or scored visually.

Genetic analyses

Traditional population genetic analyses require the a priori delineation of individuals into some set of groups. Rather than treat each site as distinct we pooled them into eight geographically explicit groups (Fig. 1) based on their location relative to river drainages (Sabine, Calcasieu, Mermentau, Biloxi Marsh), Wildlife refuges (Rockefeller and Marsh Island), and major bays (Terrebonne and Barataria). Within the Chenier plain (sites west of the Atchafalaya) suitable habitat for terrapins is predominately associated with river inlets. These inlet habitats are largely only accessible through the Gulf of Mexico, which terrapins are not likely to traverse. For some analyses, we clustered the 8 groups into 3 regions (east, central, and west) defined by major freshwater inputs of the Atchafalaya and Mississippi rivers. Tests of Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) within each group were conducted in GENEPOP v3.4 (Raymond and Rousset 1995). ML-Null (Kalinowski and Taper 2006) was used to determine if null alleles were present. GenAlEx v6.5 (Peakall and Smouse 2006) was used to calculate number of alleles per locus (Na) as well as observed (H_O) and expected (H_E) heterozygosity. Allelic richness (A_R) was calculated to account for differences in sampling effort among groups with FSTAT v2.9.3.1 (Goudet 2001). FSTAT was also used to calculate and test the significance of θ, Weir and Cockerham's (1984) unbiased estimator of F_{ST}. An analysis of molecular variance (AMOVA) was performed using ARLEQUIN v3.5 (Excoffier and Lischer 2010) at two different grouping levels:

the 8 a priori defined geographical groups and 3 larger regions. Measures of genetic diversity were also compared among groups using an ANOVA when assumptions of a normal distribution and equal variances were met. Otherwise, we used the non-parametric Kruskal–Wallis ranked sums test. All statistical tests were performed with JMP v7.0.1(SAS Institute Inc. 2007).

The number of genetically distinct groups was estimated using the Bayesian approach implemented by STRUCTURE v2.3.4 (Pritchard et al. 2000; Hubisz et al. 2009). Values of K were tested from 1 to 12 using the admixture model with correlated allele frequencies among groups and population location was used as a prior. Twenty replicates for each value of K were performed with a burn-in of 5,000,000 generations followed by a subsequent 500,000 generations. The best estimate of K was determined by first examining the probability scores for each value of K and comparing this with the method of Evanno et al. (2005; ΔK) as calculated by Structure Harvester v6.92 (Earl and von Holdt 2012).

The combination of genetic marker data, geospatial data and statistical methods has developed into the field of landscape genetics (Manel et al. 2003; Storfer et al. 2006). Spatially explicit programs, such as TESS (Chen et al. 2007) and GENELAND (Guillot et al. 2005), were specifically designed to evaluate the role landscape features play in shaping population structure by simultaneously using genotypic and geospatial data. Both GENELAND and TESS take somewhat different approaches in using a Bayesian clustering method to define population structure by simultaneously considering geospatial coordinates and multilocus genotype data without the use of a priori groups. We used both programs in order to evaluate the congruence of the results. We used TESS v2.3.1 to test values of K from 2 to 12 using the admixture model. Twenty replicates for each value of K were performed with a burn-in of 100,000 generations followed by a subsequent 50,000 generations with admixture. The best estimate of K was determined by examining the probability scores for each value of K and then viewing the hard clustering analysis tessellation. GENELAND v4.0.4 was run under the advanced model to accommodate a sample size greater than 300 individuals. Values of K were tested from 1 to 5 with separate runs either using correlated or uncorrelated allele frequencies. Each simulation was run with 100,000 iterations with 1,000 thinnings, and post processing was completed using 100 points and 150 points with a burn-in of 25. Runs were performed without and with uncertainty (0.05) in the coordinates. Including uncertainty within the coordinate is appropriate for organisms in which they are expected to disperse from the site of capture.

A Mantel test was conducted to determine if genetic similarity is related to geographic distance (i.e., isolation by distance). Geographic distance was calculated as the distance between centroids of the eight groups traced from Google maps, and the genetic distance used pairwise F_{ST} values. The two matrices were then analyzed using the Isolation by Distance Web Service v3.23 (Jensen et al. 2005). We also performed a spatial autocorrelation analysis (Smouse and Peakall 1999) as implemented by GenAlEx to compare the genetic similarity among individuals at different intervals of distance. For this analysis we selected one individual from each unique trapping site ($n = 150$ individuals) in order to not exceed the computational capacity of the software. A total of four analyses were run with intervals of 10, 20, 40 or 80 km with the number of pairwise comparisons for a given interval ranging from 26 to 4003 (average = 591). Significance testing was performed by 1000 random permutations of the data.

Seven models of gene flow was tested using Migrate-n v3.6.4; (Beerli and Felsenstein 1999) to estimate the marginal likelihood of each and then the models were ranked by their Bayes factor (Beerli and Palczewski 2010). The first two models represented the null scenarios of either a single panmictic population or full migration (i.e., gene flow is possible among all groups). The third model represented a stepping stone pattern of gene flow, with more migration expected with neighbor populations rather than more distant populations. Three models were designed with a stepping stone pattern of movement among adjacent sites and to reflect a barrier to movement imposed by the either the Atchafalaya basin, Mississippi River or both rivers. The last model was simulated full migration among sites bisected by the Atchafalaya basin. Models were designed based on the linear nature of the marsh habitat, the presence of potential freshwater barriers, dispersal ability, and the known tendency of terrapins to demonstrate high site fidelity within tidal creeks (Gibbons et al. 2001; Sheridan et al. 2010). For each analysis we used the Brownian mutation model, which is appropriate for microsatellite loci. The starting genealogy was taken from an Unweighted Pair Group Method with Arithmetic Mean (UPGMA) tree and initial theta and M values were derived from the F_{ST} calculation. Priors for theta were kept as uniform with minimum, maximum and delta values set to 0.01, 100.0, and 9.99, respectively. Static heating was applied to four independent chains using temperature settings of 1.0, 1.5, 3.0 and 1,000,000.0. A total of 500,000 steps were run, recorded every 100 generations, of which 10,000 were discarded as the burn-in. Stationarity for each parameter was assessed by determining if the effective sample size was > 1000 and there was a unimodal posterior distribution.

We characterized the demographic history of these groups by calculating effective population size (N_e) and testing for genetic bottleneck using two methods. We

estimated N_e for each group using NeEstimator v. 2.01 (Do et al. 2014), which uses a bias correction (Waples 2006) of the LD method (Hill 1981). During a genetic bottleneck, allelic diversity is lost faster than population heterozygosity, which produces an excess of heterozygosity relative to the observed number of alleles (Cornuet and Luikart 1997). We tested for genetic bottlenecks using BOTTLENECK (Cornuet et al. 1999) and the *M* ratio test (Garza and Williamson 2001). BOTTLENECK was used to test for a significant excess of heterozygosity in each of the eight groups under the two-phase mutation model, which is an improvement upon the stepwise mutation model in that it allows for larger jumps in mutation sizes. The *M* ratio test (Garza and Williamson 2001) was used to compare the number of alleles with their size distribution to look for evidence of a bottleneck. We used *M* ratio analysis parameters as suggested by the authors where θ was 10, 90 % of the mutations were single step, and the mean size of larger mutations was 3.5. The critical value of *M* for each site was identified from the 95 % threshold of 10,000 simulations of an equilibrium population.

Results

A total of 573 individuals from across the Louisiana coastline were collected from 26 sites. These sites were clustered into eight groups listed from west to east: Sabine (n = 8), Calcasieu (n = 46), Mermentau (n = 89), Rockefeller (n = 141), Marsh Island (n = 13), Terrebonne Bay (n = 108), Barataria Bay (n = 229), and Biloxi Marsh (n = 75). Sampled individuals were primarily adults with a sex ratio of 1.4 females to 1 male. Eight individuals were eliminated prior to analysis due to missing data at four or more loci. Null alleles were detected in *GmuD21* and therefore, this locus was excluded from further analysis. The remaining 12 microsatellite loci had 2-19 alleles per locus with observed heterozygosity ranging from 0.308 to 1.00 (mean = 0.730, SE ± 0.015) and expected heterozygosity ranging from 0.320 to 0.909 (mean = 0.746, SE ± 0.012) per locus. After a sequential Bonferroni correction (Rice 1989), no loci deviated significantly from HWE nor was there evidence of LD. Average values for genetic diversity measures were uniform across groups, and there were no significant difference in both H_O and H_E, but number of alleles was significantly different among groups (Table 1). For the latter, allelic diversity was significantly lower at the two sites with small sample sizes (Sabine and Marsh Island; $F_{(7,88)} = 2.857$, $p = 0.0098$). After excluding these two sites, allelic richness was not significantly different among groups; $F_{(5,71)} = 0.230$, $p = 0.948$.

Pairwise F_{ST} values were small (Table 2) with values ranging from −0.0174 to 0.0105. Only nine of the 28 pairwise F_{ST} values were significantly different from zero, with all nine comparisons associated with the Calcasieu and Mermentau sites. In both of the models tested by the AMOVA, most of the variation was found within individuals (three regions, 98.65 %, $p = 0.033$; eight groups 98.66 % $p = 0.025$). The amount of variation partitioned among groups in both models was very small (0.16 % and 0.35 %, respectively), but this was significant in the 3-region model ($p = 0.026$).

The highest likelihood score from the STRUCTURE run was for a K of 1 (average lnL = −25187.1; SD = 0.2). Similarly, both TESS and GENELAND failed to detect any evidence of population structure across the range of our samples. However, despite the lack of strong genetic differentiation, the other analyses suggest that Louisiana terrapins are not one panmictic group. The Mantel test of geographic and genetic distances demonstrated a significant, although weak, positive correlation ($p = 0.0370$, r = 0.395; Fig. 2). These results were congruent with the outcome of the model testing using Migrate-n. The "stepping stone" pattern had the highest probability (marginal likelihood: −66076.06; $p = 1.0$) of any of the seven models (Table 3). For the stepping stone analysis we also compared the mutation scaled migration rates (M) among sites (Fig. 3). These rates were relatively uniform between sites east of the Atchafalaya River. Across the Atchafalaya and for the two western most sites, there was a strong westward bias in migration rates between adjacent sites. Genetic correlations among individuals in the first distance class were significantly positive when the spatial autocorrelation analysis was run with 10, 20 and 40 km intervals and marginally significant ($p = 0.057$) with 80 km intervals. No other distance classes were significantly different from zero.

BOTTLENECK detected significant excesses of heterozygotes at Barataria Bay ($p = 0.0017$) and Biloxi Marsh ($p = 0.0031$), but *M* ratio failed to detect any genetic bottlenecks. Non-negative estimates of effective population size were found for five of the eight groups ranging from 194 for Marsh Island to 39,168 for Terrebonne Bay (Table 4). Negative estimates of N_e are reported by the software as infinity and may be the result of limited sampling; however we can still examine the lower bounds of the 95 % confidence intervals (Waples and Do 2010). The largest values of N_e and highest lower limits of the confidence interval were consistently found in the eastern portion of Louisiana. West of the Atchafalaya, the groups had broadly overlapping confidence intervals for N_e, although Rockefeller did have the largest estimated value.

Table 1 Measures of genetic diversity for *Malaclemys terrapin* groups and regions across Louisiana. Average values across loci for the number of alleles (Na), allelic richness (A_R) and observed (H_O) and expected heterozygosities (H_E) are reported for each group along with the standard deviation

Group	Sample size n	Number of Alleles (Na)	Allelic Richness (A_R)	Observed Heterozygosity (H_o)	Expected Heterozygosity (H_E)
1. Sabine	8	5.769	N/A	0.760 ± 0.125	0.743 ± 0.204
2. Calcasieu	46	9.615	9.951 ± 3.390	0.772 ± 0.103	0.779 ± 0.215
3. Mermentau	89	9.615	8.942 ± 3.143	0.740 ± 0.103	0.772 ± 0.216
4. Rockefeller	141	10.385	9.184 ± 3.602	0.749 ± 0.116	0.778 ± 0.216
5.Marsh Island	13	6.308	N/A	0.765 ± 0.172	0.749 ± 0.207
6.Terrebonne Bay	108	9.615	9.076 ± 3.502	0.762 ± 0.131	0.781 ± 0.216
7. Barataria Bay	86	9.077	8.693 ± 3.734	0.750 ± 0.111	0.763 ± 0.212
8. Biloxi Marsh	75	9.000	8.581 ± 3.723	0.760 ± 0.142	0.765 ± 0.210
ANOVA or Kruskal-Wallace		$F_{(7,88)} = 2.857$ $p = 0.0098$	$F_{(5,71)} = 0.230$ $p = 0.948$	$X^2 = 1.518$ $df = 7$ $p = 0.982$	$F_{(7,88)} = 0.205$ $p = 0.9836$

The results of the significance testing for each diversity measure are also reported

Table 2 Pairwise F_{ST} values (below the diagonal) and geographic distances in kilometers (above the diagonal) among the eight groups of terrapins

Group	Sabine	Calcasieu	Mermentau	Rockefeller	Marsh Island	Terrebone Bay	Barataria Bay	Biloxi Marsh
Sabine		55.7	82.53	119.93	188.50	253.57	315.98	408.08
Calcasieu	−0.0045		27.33	64.25	132.82	218.97	260.3	352.4
Mermentau	−0.0065	**0.0047**		36.92	105.49	232.97	325.07	391.68
Rockefeller	−0.0073	**0.0067**	**0.0045**		68.57	196.05	288.15	354.76
Marsh Island	−0.0174	0.0036	−0.0026	0.0030		127.48	219.58	286.19
Terrebonne Bay	−0.0101	**0.0105**	**0.0087**	0.0012	0.0002		92.10	158.71
Barataria Bay	−0.0120	**0.0083**	**0.0081**	0.0026	0.0066	0.0027		66.61
Biloxi Marsh	−0.0128	**0.0066**	**0.0046**	0.0015	−0.0002	0.0013	−0.0002	

F_{ST} values in bold were significant after sequential Bonferroni correction. Geographic distance was measured as the coastline distance between the centroid for each group

Discussion

Population structure

Significant population structure was not detected across the Louisiana coastline, even with sites that were up to 100 km apart (Marsh Island to Terrebonne Bay—across the Atchafalaya River). Using 12 highly polymorphic microsatellite loci in this study were comparable to the data collected in other population genetic studies of terrapins (Table 5). Similar to other studies with sites spanning small spatial scales, we observed low F_{ST} values (average $F_{ST} = 0.0004$) and detected no meaningful differentiation among groups across larger regions (Drabeck et al. 2014; Sheridan et al. 2010; Coleman 2011; Hauswaldt and Glenn 2005). Although strong genetic differentiation was absent, there was an interesting geographic pattern in the pairwise F_{ST} values, which were lower among eastern sites than among sites in the western part of Louisiana. This may be a result of differences in the salt marsh habitat structure across coastal Louisiana. In southeastern Louisiana, the salt marshes are more open due to coastal marsh fragmentation making an open habitat network, while suitable southwestern marshes are isolated from one another, with beaches/dunes occurring between tidal inlets/estuarine habitats making them less continuous; thus greater terrapin gene flow might be expected in southeastern Louisiana compared to southwestern Louisiana. Studies with a broader geographic focus also reported a general pattern of genetic homogeneity across large portions of the range (Hart et al. 2014; Coleman 2011; Hauswaldt and Glenn 2005). This study contrasts with the previous studies in that we intensively sampled across the Louisiana coastline and that we also employed analyses that explicitly considered this spatial coverage. Results of all of our analyses clearly demonstrated that the two major freshwater inputs into Louisiana salt marshes have not resulted in strong genetic differentiation. Our results confirm Drabek et al. (2014)

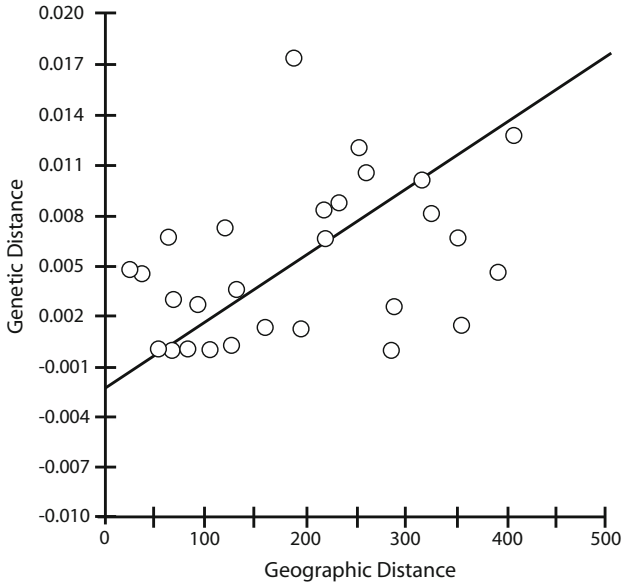

Fig. 2 Isolation by distance graph for *Malaclemys terrapin*. Correlation of genetic distance (pairwise F_{ST} values) and geographic distance (distance among centralized point for each group)

who found an absence of a genetic break created by the Mississippi River, although the inferences were made with a small sample size.

Isolation by distance and connectivity

Despite lack of strong genetic structuring, terrapins along the Louisiana coast are not panmictic, but rather the sites demonstrated a pattern of isolation by distance in both the Mantel test and the Migrate-n analyses. Although Migrate-n selected the stepping stone model as the most likely representation of gene flow, mutation scaled migration rates were not uniform or symmetrical between all groups. In particular, there was a much higher westward migration rate across the Atchafalaya River delta between Terrebonne Bay and Marsh Island. We see a similar asymmetry in migration rates among the westernmost three groups. Perhaps these patterns reflect movement from higher density to lower density areas, or it might be an artifact of small sample sizes for two of the groups in these comparisons where population densities are low (Marsh Island and Sabine). Further sampling in these regions would improve our estimates of migration among these groups. The extent of genetic connectivity among adjacent locales is also seen in the results of the spatial autocorrelation analysis in that significantly positive genetic correlations are seen among individuals up to 40 km apart.

It is unclear how the connectivity of terrapins is maintained across major freshwater rivers such as the Atchafalaya and Mississippi. For example, the break in the salt marsh habitat produced by the Atchafalaya river delta (approximately 50 km; Sasser et al. 2008) is greater than the largest recorded movement of a terrapin in mark-recapture studies (Sheridan et al. 2010, 8508 m). Furthermore, terrapins (age >3) maintain high site fidelity during the non-breeding season (Sheridan et al. 2010; Gibbons et al. 2001). One possibility is that these "freshwater breaks" between salt marsh habitat favored by terrapins are not static, but their geographic extent fluctuates based on the dynamic nature of the freshwater inputs from the MRD. For example, the MRD has been subject to five active delta switches over the last 4600 years in southeastern Louisiana (Day et al. 2007). Thus, these "freshwater breaks" likely only act as short-term barriers to gene flow and not long-term barriers that would be detectable in our genetic analyses. Thus, the oscillation of the MRD and its impact on the distribution of salt marsh habitat appears to have maintained a level of genetic connectivity across the eastern marshes of Louisiana.

Historic harvesting and bottlenecks

Only one of the tests—the heterozygosity excess test—detected genetic bottlenecks. The two tests (BOTTLENECK and M ratio) we used are different analytical

Table 3 Description of migration models tested Migrate-n along with the marginal likelihood score

Model	Description of gene flow	Bezier approximation score (marginal likelihood)
Panmixia	One single population—null hypothesis	−337315
Full	From one site to any other site—null hypothesis	−70057
Stepping Stone*	From one site to any adjacent site	−66076
Bisected Full	From one site to any other site but not across the Atchafalaya River	−415679
Chenier vs Deltaic Stepping Stone	From one site to any adjacent site but not across the Atchafalaya River	−222828
MS River only Stepping Stone	From one site to any adjacent site but not across the Mississippi river	−357039
Both rivers Stepping Stone	From one site to any adjacent site but not across the Atchafalaya or Mississippi river	−506394

* Denotes the model of gene flow among the eight sites with the highest probability

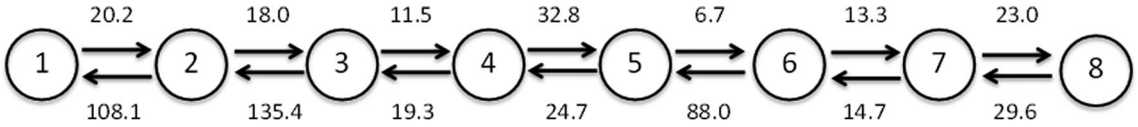

Fig. 3 Results of Migrate-n stepping stone pattern of *Malaclemys terrapin* among the eight groups from west to east. Numbers reference sample sites from Table 1. Mean mutation scaled migration rates are reported above respective *arrows*

Table 4 Effective population size for the eight groups and three of terrapins with the 95 % confidence intervals of those estimates

Sites	Ne	95 % Confidence interval
Sabine	∞	(214, ∞)
Calcasieu	∞	(221, ∞)
Mermentau	274	(157, 834)
Rockefeller	920	(378, ∞)
Marsh Island	194	(31, ∞)
Terrebonne Bay	39,168	(461, ∞)
Barataria Bay	2342	(341, ∞)
Biloxi Marsh	∞	(436,∞)

approaches and thus, they differ in their sensitivity to the presence of bottlenecks and violations in their underlying assumptions. In particular, the heterozygosity excess test appears to be more sensitive to demographic bottlenecks that are more recent and less severe (Williamson-Natesan 2005). The evidence of genetic bottlenecks within the Barataria Bay and Biloxi Marsh is similar to the results of a population bottleneck documented by Hart et al. (2014) from the same region and is also congruent with historical documentation of terrapins being harvested from these regions. Davis (1973) described some aspects of terrapin harvesting in Louisiana, particularly the Barataria region. For example, terrapin fishermen indicated a preference for female terrapins and frequently captured them on nesting shoals or in mud flats. Additionally, the historical importance of terrapins in coastal Louisiana has been preserved in the naming of coastal bayous and islands (e.g., Turtle Bayou, Terrebonne Parish and Turtle Pen Island, St. Bernard Parish). Although terrapins in eastern Louisiana were historically harvested and apparently experienced a genetic bottleneck, this is not reflected in estimates of effective population sizes, which were higher than any of the sites west of the Atchafalaya. Again differences in the salt marsh habitat structure along the coast could explain this result, particularly if habitat east of the Atchafalaya historically supported greater numbers of terrapins.

The absence of genetic bottlenecks in the western portion of Louisiana may reflect the smaller human population relative to the eastern Louisiana and a more restricted access to major portions of the salt marsh. For example, within the western part of Louisiana is Rockefeller Wildlife Refuge (RWR), which is owned and operated by Louisiana Department of Wildlife and Fisheries (LDWF). RWR was donated to the state in 1920 and within the deed of donation it made it a criminal offense to "destroy, kill, or pursue game, fish, and birds, fur bearing animals or terrapins" (Selman et al. 2014). The wildlife refuge contains approximately 30,000 hectares maintained as saline, brackish or freshwater marshes. This expansive refuge may have been important to maintaining genetic diversity and a presumably healthy population. It is worth noting that the highest estimate of effective population size in western Louisiana was for the RWR group.

Genetic diversity observed (e.g., average observed heterozygosity = 0.76) in this study was comparable to what has been reported in east coast populations (New Jersey and Charleston Harbor), which were intensely studied using similar loci (Table 5). However, the genetic diversity in Louisiana is much higher than reported for Mobile and Galveston Bays. These studies did have smaller sample sizes, which could have influenced the estimate of genetic diversity. Conversely, there could be a biological

Table 5 Comparison of this study and previous genetic studies on terrapins of similar geographic scale

Study	Sample size	Number of loci	H_O	H_E	Location
This study	566	12	0.74–0.77	0.74–0.78	All of Louisiana
Sheridan et al. (2010)	1558	6	0.82	0.81	NJ
Hauswaldt and Glenn (2005)	130	6	0.84	0.85	Charleston Harbor, SC
Hart et al. (2014)	120	12	0.66	0.68	NC
Coleman (2011)	53	12	0.51	0.51	Mobile, AL
Glenos (2013)	61	12	0.43	Not reported	Galveston, TX

reason for the discrepancy such that population reductions in Louisiana may have been smaller or the populations recovered more rapidly. Further, the expansive coastal wetlands of Louisiana are greater than those of other Gulf Coast states and this could have also moderated the genetic impacts of historical harvesting through overall larger population sizes in the region. Because the marsh is so expansive, consequently there was likely a greater possibility for some more remote populations to be relatively unaffected.

Conclusions

The results of this study suggest further research should focus on investigating movements between sites. Detecting individuals utilizing active river deltas with large riverine influxes (e.g., Atchafalaya Delta) would explain our findings of a highly connected population even with potential freshwater barriers. If individuals are not found in these areas, it would suggest that populations on either side of the freshwater barriers are periodically connected via delta switching or due to stochastic events like the movement of individuals by storms and hurricanes. Long term mark and recapture studies (Selman et al. 2014, Pearson et al., unpublished data) should be continued before and after these large events to determine if and when individuals move to adjacent sample sites or across longer distances.

Although there was no discrete genetic structuring across Louisiana, it is clear there are some differences east and west of the Atchafalaya Delta (e.g., F_{ST} values, effective population sizes and genetic bottlenecks). This may in part be a product of historical and contemporary human activities and may dictate the use of different management strategies for terrapins in Louisiana. We suggest that coastal habitat management plans should focus on preserving or protecting suitable saltmarsh and nesting habitats used by terrapin along the Louisiana coastline, including both Chenier (western) and Deltaic (eastern) regions. This would preserve the current stepping stone and IBD gene flow pattern we observed, as well as connect habitats that may provide source individuals to prevent localized extirpations. In the Chenier Plain there are two large wildlife refuges, which already protect large areas of habitat suitable for terrapins by limiting the amount of development and human use (Selman et al. 2014). However, in eastern Louisiana, the MRD is subject to higher rates of wetland loss, habitat fragmentation and is mostly privately owned (Couvillion et al. 2011). The instability of this habitat creates uncertainty in the persistence of terrapin populations east of the Atchafalaya River. Coastal restoration projects and coastline protection measures outlined in the Coastal Master Plan for Louisiana (CPRA 2012) will likely support terrapin habitats in both regions. Therefore, existing population data (Selman et al. 2014, Pearson et al., unpublished data) and genetic data provided herein could be incorporated into this framework to develop a more sound coastal management plan for terrapins in Louisiana.

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