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Population genetics of the diamondback terrapin (*Malaclemys terrapin*)

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Abstract

We examined the population genetic structure of the diamondback terrapins (*Malaclemys terrapin*), within and among estuaries. Based on mark-recapture studies, these estuarine turtles have high site fidelity that is likely to make them vulnerable to local extinctions. We tested if observed site fidelity of adults would be reflected in intraestuarine population genetic structure of six highly polymorphic microsatellite loci (five tetranucleotide and one dinucleotide). No evidence was found for population structuring within the Charleston estuary nor among three different estuaries in South Carolina. We then examined four other terrapin populations from North Carolina to New York, as well as from the Florida Keys and from Texas. With increasing geographical distance, genetic differentiation increased from South Carolina through New York, but overall values were low. The dinucleotide locus contributed significantly more to the genetic differentiation of some population comparisons than any of the other loci. Interestingly, terrapins from South Carolina to New York were much more genetically similar to those from Texas ($\rho = 0.154$) than to those from Florida ($\rho = 0.357$). We attribute this pattern to extensive translocations of terrapins during the early 20th century to replenish diminished populations and to provide turtle farms with stocks. Terrapins collected in Texas were especially sought for shipment to the northeastern US because of their larger size. Our study indicates no population structure within or among adjacent estuaries. Thus, the mark-recapture information from adult and subadult feeding locations is a poor predictor of population genetic structure. Additionally, it appears that past human activities may have drastically altered the genetics of current populations. Finally, our data suggest that translocation of eggs or head starting of terrapins within estuaries or among adjacent estuaries is acceptable from a genetic standpoint.

Keywords: estuary, microsatellites, population differentiation, population genetics, South Carolina, turtle

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Introduction

The diamondback terrapin (*Malaclemys terrapin*) is the only turtle species in North America that is endemic to brackish coastal marshes (Ernst *et al*. 1994). Terrapins are thought to play an important role in the salt marsh ecosystem (Hurd *et al*. 1979; Tucker *et al*. 1995) where they feed on mollusks and crustaceans and have been shown to have a significant impact on densities of the salt marsh periwinkle, *Littorina irrorata* (Levesque 2000). Ernst *et al*. (1994) recognized seven subspecies of *M. terrapin* which range from Cape Cod, Massachusetts, to southern Texas. Hartsell 2001, however, was not able to distinguish between the two northern subspecies *Malaclemys terrapin terrapin* and *Malaclemys terrapin centrata*, nor the two most western subspecies, *Malaclemys terrapin littoralis* and *Malaclemys terrapin pileata* using morphological characters. Lamb & Avise (1992) used restriction digest analysis of mitochondrial DNA to genotype 53 terrapins from 14 sites between Louisiana and Massachusetts using 18 restriction enzymes. Only one restriction site was geographically informative: one haplotype ranged from near Cape Canaveral, Florida, northwards, and the other extended south and westwards to Louisiana.

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This finding supports the function of Cape Canaveral as an ecological transition zone which has been found in a number of other coastal marine species (reviewed in Lamb & Avise 1992).

Intense harvest of terrapins as a gourmet food item in the late 19th and early 20th century caused populations to decline drastically and even become commercially extinct in many regions along the East Coast (Coker 1906). The United States Bureau of Fisheries established terrapin farms in Maryland and North Carolina to ensure the supply of animals (Coker 1906, 1920; Barney 1924; Hildebrand & Hatsel 1926; Hildebrand 1929). In addition to these governmental farms, private entrepreneurs were also breeding terrapins (Hildebrand & Hatsel 1926). Demand for terrapin meat decreased in the 1930s, and the species was able to become abundant again in many parts of its range (Hoff 1972). In some parts of the range, harvest of these turtles still persists (Garber 1990). Roosenburg (1990) estimated that between 8000 and 12 000 terrapins are caught annually in Maryland. In addition to harvest, terrapins also face threats which are difficult to quantify, such as pollution, habitat destruction and fragmentation. Other threats have been well documented, such as incidental drowning in commercial and recreational crab pots (Bishop 1983; Roosenburg *et al*. 1997, 1999; Hoyle & Gibbons 2000; Gibbons *et al*. 2001; Tucker *et al*. 2001) and mortality on roads (Wood & Herlands 1997). Terrapin population crashes linked to crabbing activity can take place over only a few years (Roosenburg *et al*. 1997; Gibbons *et al*. 2001), but only a few studies have investigated recent long-term terrapin population trends. Mitro (2003) did not find much change in the number of nesting terrapins at a site in Rhode Island during a 10-year period. However, local extirpations have been documented from Florida (Seigel 1993) and South Carolina (Gibbons *et al*. 2001; Tucker *et al*. 2001).

The vulnerability of terrapin populations to local extirpations has been associated with their high site fidelity. Based on a 17-year mark-recapture study on more than 1000 terrapins at Kiawah Island, South Carolina, Gibbons *et al*. (2001) concluded that subadult and adult terrapins show high site fidelity to specific tidal creeks and rarely move between adjacent creeks. Throughout that study, only terrapins that are 3 years old and above were sampled. Currently, little is known about juvenile dispersal (J.W. Gibbons, personal communication). Terrapins are known to form mating aggregations (Seigel 1976), but it is not known how far animals travel to these. At Kiawah Island, mating aggregations were not sampled (J.W. Gibbons, personal communication). Tucker *et al*. (2001) had found that females were more likely than males to move among the five tidal creeks studied at Kiawah Island, among which the largest distance between two sites was about 3 km, but overall transition probabilities were low, ranging from zero to 0.398 ($\bar{x}$ = 0.05). The authors concluded that migration rates were too low to allow recolonization of a creek that had suffered extirpation.

The main purpose of this study was to test if the high site fidelity of subadult and adult terrapins would manifest in population genetic structure. We hypothesized that genetic differentiation among terrapins from different sites within an estuary would be significant and began our study focusing on terrapins from the Charleston estuary in South Carolina. As we were unable to find significant genetic differentiation at this geographical scale, we continued our study by including terrapins from other estuaries in South Carolina, as well as from other states, to establish how geographical distance influences genetic differentiation in this species. Such population genetic survey would likely reflect some impact humans have had on terrapins during the past 200 years of harvesting, artificial propagation and release, and translocation of animals. The results from this study allow us to make recommendations for conservation and management of terrapins, in particular to evaluate potential genetic effects of translocating animals within and among sites.

Methods

Populations studied

A total of 130 terrapins were sampled from four sites in the Charleston estuary, South Carolina, during 2001: Ashley River (16 F, 18 M); Wando River (11 F, 15 M), Cooper River (9 F, 19 M), and Charleston Harbor (20 F, 22 M) (Fig. 1). The largest distance between any two collection sites in the Charleston estuary (Ch) was approximately 30 km by water (South Carolina Department of Natural Resources Inshore Fisheries collection site 19 in the Ashley River and site 30 in the Wando River). Altogether, 190 additional terrapins were sampled from the following eight locations (sample size and population abbreviation in parenthesis): ACE Basin, South Carolina (N = 20; ACE), Cape Romain, South Carolina (N = 21; Cr), Beaufort, North Carolina (N = 20; NC), the Patuxtent River, Maryland (N = 56; MD), Stone Harbor, New Jersey (N = 26; NJ), Oyster Bay, New York (N = 21; NY) the Florida Keys (N = 12; FL), and from Nueces Bay, Texas (N = 14; TX) (Fig. 1). In this study, we will refer to terrapins from South Carolina to New York as the East Coast terrapins.

Microsatellite analysis

DNA was extracted from blood (Ch, NC, MD, FL, and TX), tail tip (ACE and Cr), and leg muscle (NJ) using standard phenol–chloroform method (Sambrook *et al*. 1989). DNA was genotyped using five tetranucleotide loci and one dinucleotide locus. The isolation protocol, primers, and polymerase chain reaction (PCR) conditions have been described previously (Hauswaldt & Glenn 2003). The advantage of this method is that one does not need to fluorescently label one primer per locus, but instead, designs one of the locus-specific primers with a M13 or CAG

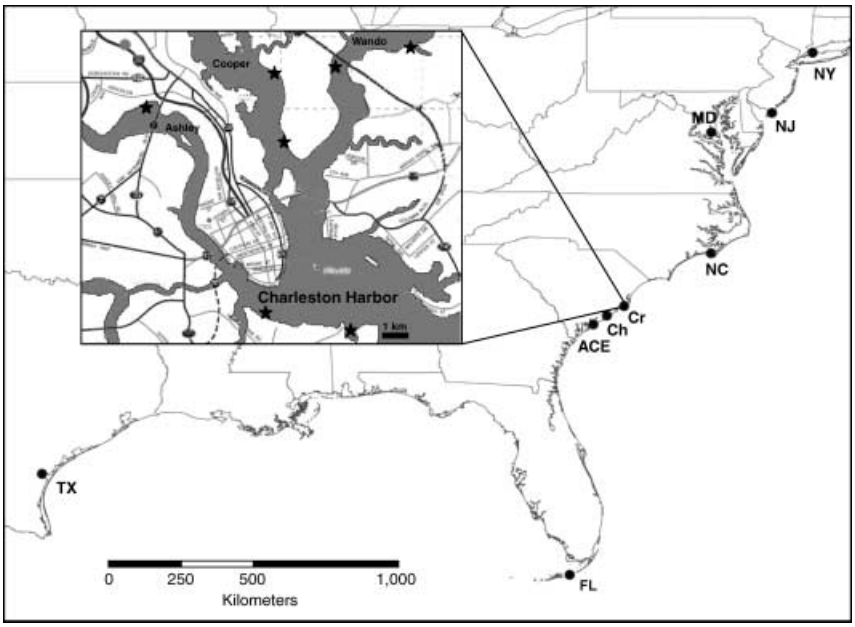

Fig. 1 Map of the southeastern USA indicating sampling locations. Insert shows Charleston Harbor estuary (stars indicate several of the sampling sites).

oligonucleotide tag. Each locus was amplified with a three-primer system in which only the M13 and CAG primers were fluorescently labelled with FAM, HEX, or NED. The choice of fluorescent dye did not affect fragment size (J.S. Hauswaldt, unpublished). After amplification, 0.5 μL of up to three different PCR products were combined with 0.2 μL CXR ladder (Promega), and 2.5 μL Blue Dextran/Formamide solution (Promega). After denaturing, 1.5 μL of this cocktail was loaded on a 0.2 mm thick 4.5% polyacrylamide gel (12 cm well-to-read length) and fragments were separated on an ABI 377 automated sequencer over 1.5 h. Microsatellite fragments were scored with GENESCAN version 3.0 and GENOTYPER version 2.5 (Perkin Elmer, Applied Biosystems). Fragment sizes were entered into GENALEX version 5.04 (Peakall & Smouse 2001) to calculate summary statistics (Table 1) and to facilitate conversion of data into formats required for other software. The allelic data (as PCR product size) is available at http://www.uga.edu/srel/DNA_Laboratory/index.htm. To test for size homoplasy, we cloned and sequenced one allele for three loci from three homozygous individuals from three different locations (TerpSH1, allele 298, Ch, Cr, TX; TerpSH3, allele 165, ACE, MD, TX; TerpSH7, allele 104, Cr, NJ, FL).

Statistical analyses

Allelic richness was calculated with FSTAT version 2.9.3.2 (Goudet 2001). Tests of Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) were performed using GENEPOP version 3.4 (updated from Raymond & Rousset 1995). Means are reported as ± SE. ANIMALFARM (Landry *et al*. 2002) was used to test for equal contribution of all loci to estimators of genetic distance based on the stepwise mutation model (SMM). We used BOTTLENECK version 1.2.02 (Cornuet *et al*. 1999) to test for evidence consistent with recent population bottlenecks or expansions by recognizing significant heterozygosity excess or deficiency for each population using a Wilcoxon sign–rank test. We conducted the tests using 1000 iterations using the SMM of microsatellite evolution, as well as a two-phased mutation model (TPM). We chose a mix of 90% : 10% stepwise : infinite allele model with 10% variance as in Jones *et al*. (2004). We also calculated the *M* ratio (Garza & Williamson 2001) as implemented in AGARST (Harley 2001). This ratio of the total number of alleles to the overall range in allele size can also be used to indicate a population bottleneck. Compared to methods that measure the deficit of rare alleles, *M* reflects a reduction in effective population size for a longer time (Garza & Williamson 2001).

We calculated two measures of population subdivision, ρ and θ, and two measures of genetic distance $(\delta\mu)^2$ and D_{SA}. RSTCALC (Goodman 1997) was used to calculate ρ values and $(\delta\mu)^2$. ρ is an unbiased estimator of R_{ST} (Slatkin 1995), a measure of the proportion of total variance in alleles that is distributed among populations. Permutation tests and bootstrapping as implemented in RSTCALC were used to test the significance of the ρ values. Values for θ, estimators of F_{ST} according to Weir & Cockerham (1984), were calculated using FSTAT. Although θ is based on the infinite allele model of allele evolution and is therefore often not as appropriate for the microsatellite loci as ρ and $(\delta\mu)^2$, it is less biased when using small sample sizes (Ruzzante 1998). $(\delta\mu)^2$ is a measure of genetic distance that is based on the squared differences of mean allele size between two populations averaged over all loci (Goldstein *et al*. 1995). This parameter increases linearly with the time

of separation when populations are in mutation drift equilibrium and it assumes an SMM of microsatellite evolution. Takezaki & Nei (1996) concluded that a large number of loci are required to make accurate estimates of population differentiation based on $(\delta\mu)^2$ because of the large variance associated with this parameter. We therefore also calculated another measure of genetic distance, the proportion of shared alleles (D_{SA}) using MSA (microsatellite analyser version 3.15) (Dieringer & Schlötterer 2003). We tested for correlations between all pairs of measures as well as between genetic parameters and physical distance using a Mantel test with 100 000 iterations in MANTEL version 1.15 (Cavalcanti).

Analyses of molecular variance (AMOVAS; Excoffier *et al*. 1992) were conducted with ARLEQUIN 2001 (Schneider *et al*. 2000). One AMOVA was performed under the null hypothesis of no genetic structure. Subsequent AMOVAS examined population structure after combining populations in various ways to test for geographical structure.

Assignment tests were performed with GENECLASS version 2.0 (Piry *et al*. 2004). Two Bayesian-based tests (Rannala & Mountain 1997; Baudouin & Lebrun 2000) and one frequency-based test (Paetkau 1995) were used to calculate the probability of each individual's assignment to one of the nine populations. With each test, we only considered the population assignment that was ranked first.

Results

Allelic patterns

All loci were polymorphic and observed heterozygosities were at least 0.5 in all populations, except for Florida (Table 1

Table 1 Summary statistics for all loci across populations of diamondback terrapins

	ACE Basin, SC					Charleston, SC					Cape Romain, SC				
	N	*A*	H_O	H_E	A_R	*N*	*A*	H_O	H_E	A_R	*N*	*A*	H_O	H_E	A_R
Terp1	20	11	0.65*	0.87	8.96	128	17	0.84	0.89	9.42	21	13	0.81	0.89	10.18
Terp2	20	11	0.85	0.89	9.40	130	11	0.93	0.87	8.36	21	11	0.95	0.87	9.08
Terp3	20	8	0.70	0.67	5.86	130	9	0.76	0.76	6.49	21	8	0.86	0.78	6.50
Terp5	20	7	0.85	0.81	6.33	129	10	0.85	0.84	7.01	21	8	0.90	0.80	6.53
Terp7	20	11	0.90	0.86	8.93	130	14	0.82	0.87	8.06	21	10	0.81	0.86	8.03
Terp8	20	13	0.80	0.87	9.60	129	21	0.83	0.90**	10.23	21	13	0.90	0.87	9.81
Avg.	20	10.25	0.79	0.83	8.18	129.3	13	0.84	0.85	8.26	21	10.50	0.87	0.85	8.35

	NC					MD					NJ				
	N	*A*	H_O	H_E	A_R	*N*	*A*	H_O	H_E	A_R	*N*	*A*	H_O	H_E	A_R
Terp1	20	10	0.8	0.86	8.58	56	13	0.77	0.8	7.58	26	12	0.81	0.88	9.34
Terp2	20	9	0.75	0.85	7.71	56	15	0.87	0.84	8.05	26	10	0.73	0.82	7.03
Terp3	20	7	0.75	0.78	5.91	56	13	0.73	0.85	8.57	26	7	0.69	0.7	4.92
Terp5	20	6	0.75	0.72	5.28	56	7	0.71	0.73	5.29	26	8	0.85	0.8	6.47
Terp7	17	8	0.71	0.82	7.43	56	13	0.91	0.86	8.18	26	11	0.85	0.88	8.79
Terp8	20	11	0.75	0.84	8.68	55	15	0.82	0.81	8.41	26	13	0.73	0.83	8.76
Avg.	19.50	8.50	0.75	0.81	7.27	55.83	12.67	0.80	0.82	7.68	26.0	10.17	0.78	0.82	7.55

	NY					FL					TX					
	N	*A*	H_O	H_E	A_R	*N*	*A*	H_O	H_E	A_R	*N*	*A*	H_O	H_E	A_R	$\bar{x}$
Terp1	20	10	0.65	0.87	7.97	12	11	0.92	0.84	10.08	14	11	0.86	0.81	9.12	12
Terp2	21	6	0.62	0.74	5.40	12	9	0.92	0.83	8.44	14	8	0.93	0.86	7.82	10
Terp3	21	6	0.86	0.75	5.14	12	10	0.67	0.75	8.67	14	6	0.79	0.8	5.89	8.2
Terp5	21	5	0.52	0.59	4.20	12	3	0.58	0.64	3.00	14	6	0.86	0.77	5.43	6.7
Terp7	20	11	0.90	0.83	8.09	12	2	0.18	0.15	1.98	14	7	0.79	0.74	6.48	9.6
Terp8	21	11	0.65	0.82	8.23	10	4	0.10*	0.53	4.00	11	7	0.54	0.66	6.72	12
Avg.	20.67	8.17	0.70	0.77	6.50	11.67	6.50	0.56	0.62	6.03	13.50	7.50	0.80	0.77	6.91	9.75

N = number of individuals genotyped, *A* = number of alleles, H_O = observed heterozygosity, H_E = expected heterozygosity, A_R = allelic richness.

denote populations that showed deviations from Hardy–Weinberg expectations after Bonferroni adjustment ($P < 0.05$, **$P < 0.01$).

$\bar{x}$ = average number of alleles/locus across all populations.

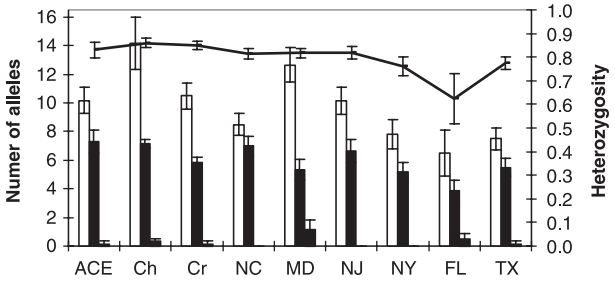

Fig. 2 Allelic patterns across nine populations of *Malaclemys terrapin*. Shown are mean (± SE) of the total number of alleles per locus (open bars), alleles with frequency < 5% (black bars), number of private alleles (hatched bars), and heterozygosity.

and Fig. 2). In the FL population, TerpSH7 and TerpSH8 yielded only two and four alleles respectively, and observed heterozygosities were 0.14 and 0.10, respectively. There was no evidence for linkage among any of the loci. Two loci did not conform with Hardy–Weinberg expectations in three populations after Bonferroni corrections (Table 1). Allelic richness was highest for TerpSH1 (9.03 ± 0.29) and lowest for TerpSH5 (5.50 ± 0.43). In regard to the different populations, allelic richness was largest in Cr (8.35 ± 0.65) and lowest in FL (6.03 ± 1.40). Overall, the number of private alleles was low (Fig. 2). The number of private alleles per individual was highest in FL (0.25), followed by MD (0.13) and TX (0.07). No private alleles were found in NC, NJ, and NY. Considering the frequency of occurrence per locus, private alleles were most predominant for TerpSH3; this locus had four private alleles in MD, three of which occurred at frequencies above 5%. All fragments from each of the three populations tested for size homoplasy were 100% identical in sequence. Under neither the SMM nor the TPM was there any evidence for a genetic bottleneck or population expansion for any of the nine populations after *P* values were adjusted for multiple tests. Neither did the *M* ratio reveal a genetic bottleneck (all *M* values above 0.7, data not shown).

Based on the ANIMALFARM test, loci contributed equally to genetic distance coefficients, except for TerpSH8. The disproportionate variance of this locus was particularly noticeable when contrasting NY to the other East Coast populations. When considering terrapins from SC to NY, the contribution of TerpSH8 was significantly different from equal contribution (Sidak adjusted $P = 0.001$). Although the contribution of this locus was still high when excluding NY, it was not statistically significant. Although the range of allele sizes at this locus was similar among the East Coast populations, allelic distribution was not. In terrapins from SC to MD, the allelic distribution was unimodal and the most frequently occurring alleles were between 198 and 204 base pairs (online supplement). In NJ and NY, however, alleles were distributed bimodally: the first peak (allele 202) was in the same size range as that in more southern populations, and the other peak was at allele 230. In the NJ population, 32% of all alleles were at allele 202 and 19% at allele 230. In the NY population, the distribution was even more skewed towards the larger allele: 50% of all alleles were evenly distributed between alleles 202 and 230. Only four alleles were found for this locus in FL, one of which (218) was in very high proportion (65%). In TX, allele 206 occurred in highest proportion (55%). It is important, however, to keep in mind the small number of individuals that were scored for the FL and TX populations, in particular for this locus. As TerpSH8 seemed to differ substantially among some of the populations, we performed all subsequent statistical analyses with and without this locus.

Genetic variation within and among populations

We were unable to find population genetic structure among terrapins from the Charleston estuary at any level we tested: among males and females collected from the same site, among animals collected during different months from the same site, among different sites within the same river, and among rivers. Pairwise ρ values between terrapins from the Ashley, Cooper, and Wando Rivers, and Charleston Harbor ranged from −0.003 to −0.011 (complete results not shown). Therefore we combined all samples from the Charleston area into one group, Ch ($N = 130$).

Among the three SC estuaries (ACE, Ch, and Cr), there was no significant population genetic structure (Table 2). East Coast terrapins were much more similar genetically to terrapins from TX than to those from FL. The average θ between the East Coast and FL (0.164 ± 0.005) was almost twice as large as between the East Coast and TX (0.084 ± 0.009). When considering all populations and loci, ρ values for 31 of 36 pairwise comparisons were significantly different from zero ($P < 0.05$) (30 without TerpSH8) (Table 2). Overall, ρ and θ where highly correlated ($r = 0.94$, $P < 0.0001$). Although numeric values for ρ were much larger than those for θ, most pairwise comparisons were declared significantly different from zero for both measures. The pattern of differentiation was consistent even with the variation in sample size.

Isolation by distance

The parameters ρ, θ, or D_{SA} were significantly correlated with physical distance only if FL was excluded ($r = 0.86$, 0.89, 0.88, respectively, $P < 0.001$ for all). In contrast, $(\delta\mu)^2$ was significantly correlated with distance when all populations were considered ($r = 0.73$, $P = 0.008$). D_{SA} was highly correlated with ρ and θ ($r = 0.94$ and 0.98, respectively, $P < 0.001$), but not as highly correlated with $(\delta\mu)^2$ ($r = 0.55$, $P = 0.039$).

	ACE	Ch	Cr	NC	MD	NJ	NY	FL	TX
AC	—	**0.012**	0.031	**0.022**	0.046	0.114	0.146	0.409	0.183
CH	**0.001**	—	**−0.002**	0.023	0.028	0.099	0.126	0.356	0.145
Cr	**−0.001**	**0.003**	—	0.058	0.051	0.134	0.147	0.302	0.091
NC	**0.016**	0.019*	**0.015**	—	0.045	0.052	0.080*	0.427	0.234
MD	0.037	0.032	0.027	0.011	—	0.069	0.136	0.409	0.216
NJ	0.029	0.025	0.029	0.025*	0.040	—	**0.010**	0.396	0.295
NY	0.062	0.049	0.060	0.022*	0.048	0.039	—	0.358	0.280
FL	0.173	0.141	0.149	0.170	0.167	0.179	0.168	—	0.181
TX	0.079	0.063	0.049	0.087	0.098	0.098	0.116	0.152	—

Table 2 θ (lower diagonal) and ρ values averaging over variance components (upper diagonal) values for the diamondback terrapin populations based on six microsatellite loci

Pairwise comparisons with ρ values are averaged over loci. All values except those in bold are significant after sequential Bonferroni correction ($P < 0.05$). Stars indicate comparisons that were only significantly different from zero when TerpSH8 was included.

Analyses of molecular variance (AMOVAS)

To test higher level structuring among terrapin populations, several AMOVAS were conducted with and without TerpSH8 (the value without TerpSH8 is in parenthesis). Among all nine populations, FL contributed most to genetic differentiation. When all populations were grouped together and all loci were considered, 16.8% (7.7%) of the variation was among populations. Grouping TX and FL terrapins separate from all other populations resulted in the largest amount of variation partitioned out among groups, 25.1% (19.5%) and 8.5% (2.7%) of the variation among populations within groups.

For the East Coast populations, among population variance was 9.8% (3.0%). Among the four Carolina populations, genetic differentiation was not significant. Including populations north of NC rendered AMOVAS statistically significant. When grouping NJ and NY together and comparing this group to all other East Coast terrapins, among group variation was 18.3% (1.15%) and variation among populations within the groups was 2.0% (2.6%). The variation among these two groups was significant when the dinucleotide locus was included, but was not significant when it was excluded.

Assignment tests

The two Bayesian-based assignment tests were slightly better in assigning individuals to their source population than the frequency based test (54.7% and 53.4% correctly assigned vs. 49.1%). All individuals of the FL, 75% of the MD, and 71% of the TX populations were assigned to their source population (Fig. 3). Only 10% of the ACE terrapins were correctly assigned. Exclusion of TerpSH8 had the most significant effect on the population from Charleston, resulting in a reduction of correct assignment by 10% (data not shown). The NC population is the most diverse among the Carolinas; only 11 of the 20 individuals were assigned to the Carolina genotype. The break between the Carolinas and any other population north of it is clearly recognizable (Fig. 3).

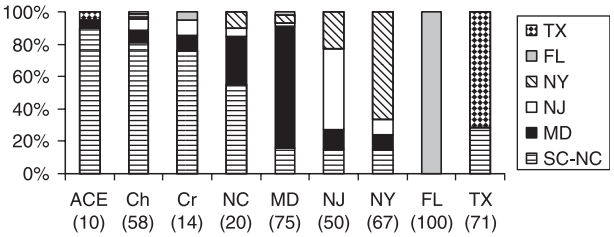

Fig. 3 Assignment scores with all six loci according to Baudoin & Lebrun (2000). Percent of correct assignment to the source population is in parentheses.

Discussion

Genetic diversity within the Charleston estuary

The population genetic analysis of diamondback terrapins within the Charleston estuary revealed no genetic structure despite distances of up to 30 km between sampling sites. This result contrasts long-term mark-recapture data that indicate high site fidelity of adult and subadult terrapins to individual tidal creeks (Gibbons *et al*. 2001) or stretches of rivers (Roosenburg *et al*. 1999). Terrapins mate in late March/early April and mating takes place in large aggregations (Seigel 1976). For example, at Grice Cove, Charleston Harbor, hundreds of terrapin heads can be observed in a relatively small area during the breeding season (D.W. Owens, personal communication). During the summer months, however, we were never able to see more than five terrapin heads at the same site. For the long-term mark-recapture study at Kiawah Island, however, mating aggregations were not sampled (J.W. Gibbons, personal communication) and almost three times as many sampling events took place during the summer as in the spring. We

therefore propose that terrapins move substantial distances during the spring to sites of mating aggregations, but afterwards return to their home sites. This behaviour would explain the lack of genetic structure within an estuary, but would also agree with high site fidelity observed from mark-recapture studies during the summer months. Some movement also takes place during the nesting season; female terrapins have been observed to travel up to 8 km to a suitable nesting site (Hurd *et al*. 1979) but there has been no evidence for any long-distance migration. At this point, hardly anything is known about the movement of hatchlings and juveniles (J.W. Gibbons, personal communication). Research on the number of mating aggregations per estuary and fidelity of terrapins to such aggregations is also needed. Thus, two very different aspects of the terrapin life history seem to promote gene flow, at least within estuaries – (i) juvenile dispersal, and (ii) mating aggregations.

The diamondback terrapin is the only nonmigrating species of aquatic turtle where results from a long-term mark-recapture study have now been compared with a population genetic study on a similar geographical scale. In the giant Amazon River turtle (*Podocnemis expansa*), Sites *et al*. (1999) found a significant amount of within-river system gene flow. Their finding was in agreement with a mark recapture study that indicated migration distances of up to 100 km (Sites *et al*. 1999). In a recent study on stream dwelling salmonids, a similar finding was made: although a 5-year mark-recapture study had indicated low migration rates (zero to 4.1%) between two spawning areas, no genetic differentiation was found (Wilson *et al*. 2004). The authors point out the different merits of direct and indirect measures of dispersal, and stress the importance of incorporating both approaches to better assess dispersal. Whereas mark-recapture data provides an instantaneous migration rate, important to an ecological and demographic context, population genetic structure provides an insight into a longer-term evolutionary context.

Population structure among different estuaries

Population genetic structure along the East Coast was comparatively low. Whereas Lamb & Avise (1992) found no difference among terrapins between northern Florida and Massachusetts using restriction fragment analysis of mitochondrial DNA, microsatellite markers expectedly showed much more resolution. When interpreting F_{ST} values (as estimated by θ), according to criteria in Hartl and Clark (1997), there was weak genetic differentiation among terrapins between SC and NJ, moderate differentiation between NY or TX and most other populations, and great differentiation between FL and any other population. However, as explained by Balloux and Lugon-Moulin (2002 and references within) the effect of polymorphism can greatly decrease the expectations for F_{ST}, rendering a low F_{ST} (θ) value (e.g. 0.019 between Charleston and NC) as statistically significant genetic structure. In highly structured populations, F_{ST} will greatly underestimate differentiation.

Among terrapins from the Carolinas we were unable to detect an isolation by distance effect nor significant genetic structure based on AMOVA. The results of our study are not in conflict with a subspecies delineation between the terrapin (*Malaclemys terrapin centrata*) and the northern terrapin (*Malaclemys terrapin terrapin*). However, we cannot confirm whether the subspecies break is at Cape Hatteras (Ernst *et al*. 1994), because our sampling regime was not intended to address this level of phylogeographical structure. It remains unclear what has led to the low level of genetic differentiation among estuaries. It seems unlikely to us, although it is possible, that juvenile dispersal or mating aggregations would be homogenizing genetic variance among populations of terrapins in different estuaries. Unfortunately, direct observation of such long-range dispersal would be very difficult. We hypothesize that longer-term evolutionary processes, such as recolonization following the last glacial maxima (cf. Walker & Avise 1998), are more important in establishing the observed patterns.

The importance of the dinucleotide locus (TerpSH8) to measures of genetic distance emphasizes the need for careful examination of individual loci in any population genetic structure using microsatellites. We found that the program, ANIMALFARM (Landry *et al*. 2002) provides a convenient means to test for unequal contribution of loci. Although the range of allele sizes for TerpSH8 did not differ among populations from SC and NJ or NY, the pattern of allele frequencies was very different, changing from unimodal (SC — MD) to bimodal (NJ and NY). It would be interesting to find out if the bimodal pattern is unique to the NJ/NY area, and what the allele distribution for terrapins from populations north of NY is for this locus. This pattern may be the result of drift, a founder effect of terrapins recolonizing this region following the last glacial maxima, a higher mutation rate of this locus, selection of linked genes, or a combination of these. Additional studies surveying more loci, especially with a larger proportion of dinucleotide loci, are warranted.

Population differentiation among East Coast, Florida, and Texas terrapins

East Coast terrapins were genetically more similar to those from TX than to those from FL, despite the much larger geographical distance. Although we have to be careful not to overinterpret our data in light of the limited number of loci we used and the small sample sizes for FL and TX, independent statistical tests do indicate the same relationship between East Coast and TX vs. East Coast and FL terrapins. We explain this phenomenon with the extensive translocations of terrapins that took place approximately 70–100 years

ago rather than with a complex natural phylogeographical process.

In the late 1800 s and early 1900s, terrapins were intensely harvested for terrapin soup and in some areas, populations had already drastically declined (Hildebrand & Hatsel 1926). For example, in 1891 more than 89 000 pounds of terrapins were sold in Maryland fish markets, but by 1920, fishermen from Maryland were only able to harvest 823 pounds (Garber 1990). After the Delaware Bay terrapin populations were depleted, terrapins from Chesapeake Bay became the favourite for terrapin dishes (Coker 1920). When the Chesapeake Bay populations began to dwindle, some terrapin shippers began to replenish their stocks with terrapins from North and South Carolina, and even terrapins from the Gulf reached the markets in Maryland and New York (Coker 1920). Coker (1920; pg. 184) likens terrapins transported along the coast to overseas travellers: 'Had these terrapins carried handbags, they might have displayed an array of hotel stickers to shame the traveller who returned from Europe'. It is clear that terrapins were translocated along the east coast to replenish diminished populations. Unfortunately, it is not possible to quantify the numbers of animals that were shipped straight to the market, let along how many were released on purpose or were kept in holding pens, where they could have had a chance to escape and interbreed with local animals. Thus, it is possible that today's genotype pattern is reflecting historical mixing of terrapins from different sources. For example, according to the assignment tests, the NC population was the most mixed in respect to its genotypes; six of 20 animals were assigned to MD. The animals were collected from Beaufort, NC, near the site of the former terrapin farm where terrapins from the Chesapeake Bay were hybridized with those from the Carolinas and Texas (Hildebrand 1929, 1933). After the terrapin farms closed down in the 1930s, thousands of animals were released with little or no documentation (e.g. Anonymous 1947).

Only terrapins from FL could unmistakably be assigned back to their source population. Although measures of genetic differentiation for MD were in line with an isolation-by-distance effect, the assignment test revealed something unusual about the Chesapeake Bay population that was not shown with other statistical parameters: 75% of the MD terrapins were correctly assigned to their source population. This rate is the second highest of all populations. The Chesapeake Bay population was heavily exploited at the beginning of the last century (Coker 1920). MD-specific multilocus genotypes as well as the relatively large number of private alleles may be an indication for the Chesapeake Bay population having had a different population genetic history compared to other East Coast estuaries. It is possible that the decimation of the MD population did cause a genetic bottleneck, but that it cannot be traced with only six microsatellite loci. A mitochondrial marker is expected to have more power and thus may reveal additional insights to this matter.

Implications for diamondback terrapin conservation

Feeding site fidelity of adults and subadults within an estuary was not reflected in population genetic structure. We reconcile our results with previous findings of high site-fidelity by noting the temporal and spatial restrictions of the mark-recapture studies. We suggest that gene flow among different sites could take place during the spring, when terrapins assemble in mating aggregations. However, until we understand more about the demography of this species, in particular, the behaviour of hatchling and juveniles, we cannot determine which life history stage is most responsible for causing genetic homogeneity. Previous studies indicated that migration rates were too low to allow recolonization of a creek that had suffered extirpation (Tucker *et al*. 2001). Our study by no means suggests a lower level of threat of local extirpations or increase likelihood of recolonization. Just because it is likely that animals move around during the spring in search of mates does not mean that they would disperse and successfully colonize a new or formerly inhabited site in an estuary.

Although population genetic structure is relatively low along the East Coast, it is still important to avoid further dilution of possible local genetic adaptation by translocating terrapins too far (e.g. beyond adjacent estuaries). As with any management plan that involves translocation of animals, caution is necessary: just because microsatellite data indicate genetic homogeneity among different populations, it does not mean that they are also homogeneous at loci that affect fitness (Wilson *et al*. 2004) nor should other factors that suggest against translocations of adults be ignored. Headstarting programs for diamondback terrapins have been established in Massachusetts, New Jersey, Delaware, and Maryland, but concerns about stock mixing have delayed some headstarting programs (M. Whilden, personal communication). Our data suggest that it is acceptable to release juveniles with parents from within the same estuary or from nearby estuaries.

To better define the management units, a denser sampling regime with an increased number of loci is necessary. It would be interesting to conduct an extensive phylogeographical study of *Malaclemys terrapin* along its entire range. In particular, studies to determine genetic differentation among terrapins from the other parts of the Gulf Coast and throughout Florida would be valuable. If terrapins from Florida have not been part of any translocation, we would expect to find an isolation-by-distance effect along the coastline of FL. It may be necessary to include genetic markers that are less variable than microsatellites. Ideally, both mitochondrial and nuclear sequences should be used for such a study, because it was mainly the larger female

terrapins that were harvested and translocated, and therefore it may be possible to detect gender biased gene flow among the populations.

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