The Population Ecology, Molecular Ecology, and Phylogeography of the Diamondback Terrapin (Malaclemys terrapin)

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The Population Ecology, Molecular Ecology, and Phylogeography of the Diamondback Terrapin (Malaclemys terrapin)

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The Diamondback Terrapin (Malaclemys terrapin) is a turtle found in brackish water habitats along the Gulf and Atlantic coastlines of the North American continent. Historically, terrapins have had a complex relationship with humans, including over-harvesting, habitat loss and degradation, and translocations. Furthermore, ecological and molecular studies of terrapins yield conflicting results with respect to terrapin population biology. Resolving these conflicts is integral to understanding how past human activities have influenced the contemporary distribution and abundance of terrapin genetic diversity. In Chapter 1, I surveyed the terrapin literature and identified incongruences between ecological and molecular studies. I finish Chapter 1 with recommendations for future molecular and ecological terrapin studies. In Chapter 2, I demarcated metapopulation structure and quantified gene flow between populations in Chesapeake Bay. I detected four populations with weak structure, high admixture, high genetic diversity, and genetic signatures of anthropogenic translocation. In Chapter 3, I quantified the effective population sizes of the Chesapeake Bay populations, with both ecological and molecular approaches. Using mark-recapture, Bayesian model testing, and approximate Bayesian computation, I recovered incongruent results among methods. I then used mark-recapture data to rule out spurious molecular estimates, finding that
coalescent models more accurately estimate effective population size in Chesapeake Bay. 

In Chapter 4, I used a range-wide dataset to locate major terrapin populations, quantified historical and contemporary gene flow, and tested for bottlenecks. I used Bayesian model testing and discriminant analysis of principal components (DAPC) as well as historical terrapin literature to show the terrapin’s phylogeographic structure was best explained by anthropogenic translocation events in the early 1900’s. In Chapter 5, I revisited a classic terrapin phylogeographic hypothesis with approximate Bayesian computation and report evidence of a younger divergence date between Gulf and Atlantic populations. This younger divergence date implies the terrapin’s mitochondrial genome has a faster rate of molecular evolution than previously estimated.
DEDICATION

I dedicate this work to my wife, Michelle, who has always supported my aspirations and probably won’t read this. Tugboat.
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My advisers have shown an inexhaustible supply of patience and understanding, and I owe much of my growth as a scientist to them. Thank you, Willem and Shawn, for building my confidence, refining my skill sets, and polishing my writing. The guidance of a caring adviser has no substitute.

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CHAPTER 1: INTRODUCTION

The Diamondback Terrapin (Malaclemys terrapin) is one of the most charismatic and recognizable turtle species in North America, populating brackish water habitats along the Gulf and Atlantic coasts from Corpus Christi, TX to Cape Cod, MA, as well as a small disjunct population in Bermuda. Historically, the terrapin has been divided into seven subspecies: the Texas Diamondback Terrapin (Malaclemys terrapin littoralis), the Mississippi Diamondback Terrapin (Malaclemys t. pileata), the Ornate Diamondback Terrapin (M. t. macrospilota), the Mangrove Diamondback Terrapin (M. t. rhizophorarum), the Eastern Florida Diamondback Terrapin (M. t. tequesta), the Carolina Diamondback Terrapin (M. t. centrata), and the Northern Diamondback Terrapin (M. t. terrapin). All subspecies were delimited based on regional variation in size, color, and shape. The recognition of seven morphologically variable subspecies suggests substantial amounts of phylogeographic and population genetic structure should be present within the terrapin.

The natural history and ecology of the terrapin added weight to this thesis. Ecological studies show that terrapins generally exhibit low vagility, reside in small home ranges, and demonstrate high nest site philopatry (Auger 1989; Lovich and Gibbons 1990; Roosenburg 1996; Spivey 1998; Roosenburg et al. 1999). Indeed, Diamondback Terrapins have been recaptured in the same creek or river system for decades (Gibbons et al. 2001). On the other hand, not all females demonstrate perfect nest site fidelity, and some individuals are known to disperse long distances (8 km; Sheridan et al. 2010). Moreover, the dynamics of juvenile dispersal are largely unknown.
Thus, the ecological data are mixed in their predictions on levels of expected population genetic structure.

Unfortunately, during the 19th and 20th Centuries, before patterns of terrapin diversity were well studied, populations along the East Coast underwent severe demographic declines as a consequence of demand for terrapin meat (Coker 1906). The result was regional and local extirpations of terrapin populations. Moreover, as natural populations declined, government and private terrapin farms were created to meet the demand for their meat (Barney 1924; Hildebrand and Hatsel 1926; Hildebrand 1929; see Chapter 13). Farm-raised terrapins were collected from poorly documented locations, often locally but also from other parts of the terrapin’s range (Coker 1920). For example, as terrapin populations in Chesapeake Bay diminished, terrapins from North Carolina were translocated to replenish the dwindling stock (Coker 1920). Terrapins from South Carolina were moved to North Carolina and into Chesapeake Bay (Coker 1920). Additionally, Gulf terrapins were also translocated into south Atlantic populations (Coker 1920; Hildebrand 1933). Indeed, terrapins were moved around their range with such frequency that Coker (1920) quipped “Had these terrapin carried hand-bags, they might have displayed an array of hotel stickers to shame the traveler returned from Europe.” When demand for terrapin meat subsided, farms purportedly simply released their terrapins. The ability of released terrapins to interbreed with local populations and the numbers and success of the translocated terrapins remains unknown.

Thus, the terrapin has a complicated history with humans, which creates significant challenges to understanding the patterns of genetic variation. Most genetic work has
focused primarily on conservation needs and management strategies. I survey studies of genetic variation in the Diamondback Terrapin. Specifically, I address the terrapin’s phylogeographic and population genetic structure, and the abundance and distribution of terrapin genetic diversity, examining the relationship between terrapin genetic studies and ecological findings. I end with recommendations for future genetic work.

Phylogeographic Studies

The Diamondback Terrapin has extensive phenotypic variation among its seven putative subspecies. Accordingly, there was the expectation of underlying high levels of genetic structure across its range. Lamb and Avise (1992) conducted the first analysis of genetic structure throughout the terrapin’s range using mitochondrial DNA (mtDNA) and 18 restriction enzymes. They recovered 74 restriction fragments from 53 terrapins, with six haplotypes. Compared to other vertebrates, this level of divergence was unusually low (uncorrected $p = 0.001\%$), and geographic structure was weak, with only one phylogeographic break near Cape Canaveral, FL (referred to as the “Gulf/Atlantic divide” in the literature; Figure 1.1).

In contrast with taxonomy, Lamb and Avise (1992) did not recover any of the subspecies as diagnosable entities with molecular data. However, the genetic divide near Cape Canaveral did correspond with a noticeable change in terrapin morphology. Terrapins to the north lack knobs on their medial keels while terrapins in the south and in the Gulf possess knobby keels.

One possible cause of the discordance between subspecific designations based on morphology and terrapin phylogeography may be that terrapins exhibit low levels of
mtDNA variation. Previous research suggested that Chelonians exhibit low rates of nuclear and mitochondrial evolution relative to other vertebrates (Avise et al. 1992; Broham 2002; Shaffer et al. 2013). The slow-down in terrapin mtDNA evolutionary rate was inferred from concordant phylogeographic breaks among taxa (Lamb and Avise 1992; Avise et al. 1992). For instance, the Eastern Oyster (*Crassostrea virginica*) and Seaside Sparrow (*Ammodramus maritimus*) exhibit a phylogeographic break in the Gulf, while the Horseshoe Crab (*Limulus polyphemus*) exhibits a phylogeographic break near Cape Canaveral, FL, suggesting these taxa share a common vicariant history with the terrapin (Avise et al. 1992). However, relative to these other taxa, the terrapin demonstrates a paucity of mtDNA variation and thus would require a much slower rate of mtDNA evolution relative to other vertebrates (approximately 14-fold) if they shared this vicariant event (Avise et al. 1992). However, Avise et al. (1992) also noted that terrapins might simply have been isolated near Cape Canaveral more recently.

Perhaps as a consequence of the low genetic variation found by Lamb and Avise (1992), no further phylogeographic work was conducted on the terrapin for over a decade, until revisited by Hauswaldt (2004) and Hauswaldt and Glenn (2005). Hauswaldt (2004) surveyed the mtDNA control region, a rapidly evolving marker in most vertebrates, across the terrapin’s range. She recovered 28 mtDNA haplotypes, with unique haplotypes in Maryland, Texas, and Florida. The phylogeographic break found by Lamb and Avise (1992) was recovered, and haplotypes for the Atlantic and Gulf Coast formed separate clades. However, limited phylogenetic structure was recovered in both
clades, and even the Atlantic/Gulf divide lacked strong support (bootstrap < 70%; Hillis and Bull 1993).

In a parallel study, Hauswaldt and Glenn (2005) quantified genetic diversity across the terrapin’s range using six nuclear microsatellites (TerpSH; Hauswaldt and Glenn 2003). Population structure, population assignment, and bottleneck detection were the primary foci of the work. Most sampling took place along the Atlantic coast (NY, NJ, MD, NC, and three locations in SC), with additional sampling in the Gulf (FL and TX). Across the terrapin’s range, they found high levels of heterozygosity ($H_o$; 0.70-0.87) and large numbers of alleles at most loci ($A$; 7.5-13), except in Florida ($H_o = 0.56; A = 6.5$) Using an analysis of molecular variance (AMOVA; Excoffier et al. 1992), Hauswaldt and Glenn (2005) found that little variance could be explained by grouping all sampling localities (16.8%), while partitioning populations into Gulf and Atlantic groups explained the most, but still little, variance (25.1%). In addition, Hauswaldt and Glenn (2005) demonstrated that individuals in some populations could be correctly assigned to their population of origin using Bayesian assignment tests. Terrapins in the Gulf had higher instances of correct assignment (71-100%) than populations in the Atlantic (10-75%). Interestingly, terrapins along the Atlantic were more similar to Texas populations than Florida populations, consistent with the observation that terrapin translocations occurred during the early 20th Century. Surprisingly, the program BOTTLENECK (Piry et al. 1999) found no evidence of demographic bottlenecks in any population, including populations known to have been historically over-harvested.
More recently, Hart et al. (2014) used 12 nuclear microsatellites (Gmu; King and Julian 2004) to revisit phylogeographic structure throughout the terrapin’s range. Populations in the North Atlantic, Gulf, and Florida showed low levels of heterozygosity (0.32-0.47), while mid-Atlantic populations showed moderate levels (0.66-0.71). Genetic clusters (populations) were inferred using the Bayesian clustering program STRUCTURE (Pritchard et al. 2000). STRUCTURE operates by dividing individuals into clusters such that conformity to Hardy-Weinberg equilibrium is maximized while linkage disequilibrium is minimized. Hart et al. (2014) initially detected the presence of two genetic clusters. The first consisted of terrapins from Atlantic populations while the second consisted of terrapins from Florida. STRUCTURE plots indicated that populations sampled from the Gulf (LA and TX) were admixed with samples from the Atlantic and Florida. Within the Atlantic cluster, two subclusters were evident: Massachusetts vs. the remaining Atlantic populations. Further analysis showed that Louisiana and Texas formed a cluster, while Florida formed the final cluster, for a total of four clusters. Hart et al. (2014) also found support for these clusters using neighbor-joining phenograms, AMOVAs, and $F_{ST}$ calculations. In contrast with previous studies, evidence of population contraction was detected using BOTTLENECK, with each locality exhibiting some signature of a bottleneck. Hart et al. (2014) concluded that precipitous drops in population sizes had occurred.
Regional and Population Level Studies

Atlantic Coast

While phylogeographic studies did not recover large genetic discontinuities or incipient species, the investigation of patterns of genetic variation within and among populations has been an ongoing research interest. Such studies aim to reveal smaller scale patterns of differentiation, quantify directions and levels of gene flow, probe demographic history, and direct conservation and management.

Terrapins were historically harvested at unsustainable levels (Garber 1990), and the genetic impacts of this exploitation are a common thread running through terrapin research. To date, a range-wide, coordinated conservation plan for terrapins is not in place; rather, conservation efforts are left to individual states, with inconsistent enforcement (Glenos 2013; Hart et al. 2014). To investigate the problem of illegal harvests, Lester (2007) conducted a covert study to identify the source populations of terrapins sold at the Fulton Fish Market in New York City. She obtained blood samples from 63 terrapins in 2004, and, using assignment tests and 12 Gmu microsatellites, determined that terrapins had been collected along much of the East Coast, from South Carolina to New York. The majority originated from Chesapeake Bay (34/63), especially from Sandy Island Cove, MD. Maryland closed their legal terrapin fishery in 2007 due to its negative impacts on local populations (Roosenburg et al. 2008). How illegal markets currently affect terrapin populations remains unclear.

Many studies have examined genetic variation within and among sets of populations, with an eye towards documenting population structure and habitat use (e.g.,
Elucidating how habitat features impact patterns of gene flow has important implications for metapopulation dynamics and conservation planning. In a creative study, Sheridan (2010) used six Gmu microsatellite markers and computational modeling to compare and contrast barriers to dispersal in Barnegat Bay, NJ. First, she measured genetic differentiation among sampling localities using $F_{ST}$ and $G'_{ST}$ ($F_{st}$ for multiple alleles) statistics. She then estimated gene flow among populations under a diversity of models. Simple models quantified genetic differentiation as a function of straight-line and shoreline distances. Complex models included landscape features, and assigned costs to traverse them. Open, deep and shallow waters, emergent wetlands, development, roads, and riprap (rocks used to armor shorelines) were investigated for their propensity to facilitate or impede gene flow. Sheridan (2010) found that open water and developed landscapes were the biggest impediments to gene flow, while shallow water and emergent wetlands facilitated genetic exchange. These results are consistent with ecological studies of terrapin movement (Roosenburg et al. 1999).

Populations located at the edge of a species range commonly have smaller population sizes and lower levels of genetic diversity than more centrally located populations (Kirkpatrick and Barton 1997). McCafferty et al. (2013) investigated this phenomenon in terrapins by examining populations near the northern edge of the range in Massachusetts. Populations in this region are at the terminus of the terrapin’s range, but also may have undergone bottlenecks as a consequence harvesting. To investigate this, McCafferty et al. (2013) combined nuclear sequence data (class 1 major histocompatibility complex, MHC1) with six Gmu microsatellite loci. Despite their study
populations being at the northern edge of the terrapin’s range, McCafferty et al. (2013) found moderate levels of heterozygosity (0.62-0.71), contrary to the lower levels typically associated with range margins and population bottlenecks. Using STRUCTURE, microsatellites demarcated two populations, while $F_{st}$ statistics documented low to moderate levels of population differentiation (0.03-0.11). The first STRUCTURE cluster spanned from Wellfleet Harbor and Sandy Neck, MA, while samples in Sippican Harbor, MA constituted a second cluster. In contrast with microsatellites, no variation was recovered at the nuclear locus, MCH1. McCafferty et al. (2013) proposed this result was the consequence of the locus operating under purifying selection, although recent range expansion or low effective population size could lead to the same lack of variation. Translocation may also affect population structure in this region. In 1972, approximately 100 terrapins from New Jersey were released in Buzzards Bay (Lazell 1976).

Not all population level studies have recovered variation among populations. In the Hudson River of New York, Piermont Marsh harbors a terrapin population that is small and broadly distributed, with few high quality nesting sites. This population may be isolated from neighboring populations, and Wiktor et al. (2001) hypothesized that terrapins in Piermont Marsh may be genetically differentiated from other populations in the region. To test this hypothesis, they compared terrapins from Piermont Marsh with terrapins from Rhode Island, Connecticut, and New Jersey using two nuclear inter-simple sequence repeat (ISSR) loci. In contrast with their predictions, they found little genetic differentiation between Piermont Marsh and other populations in the North Atlantic. Wiktor et al. (2001) concluded the Piermont Marsh population must be connected to
others via high levels of gene flow, most likely from populations in the Hudson River. An alternative explanation is that the Piermont Marsh population has been founded relatively recently. That is, it could in fact be currently isolated demographically, yet this isolation may not yet have left a genetic signature.

Most recently, Converse et al. (2015) quantified historical and contemporary gene flow among terrapin populations in Chesapeake Bay. Using 12 Gmu microsatellite loci and STRUCTURE, they found four terrapin populations that were weakly structured, yet harbored moderate to high levels of heterozygosity (0.69-0.79). Using MIGRATE (Beerli 2008) and BAYESASS (Wilson and Rannala 2003) to estimate historical and contemporary gene flow, respectively, Converse et al. (2015) showed large increases in gene flow into Chesapeake Bay, as well as large increases between the Patuxent River and Kent Island, potentially due to terrapin translocation events. By contrast, levels of gene flow decreased over time among most populations within the Bay. They attributed this latter pattern to habitat loss and the impacts of a large crabbing industry, which negatively impacts terrapin dispersal, especially of juveniles and males (Roosenburg et al. 1997; Roosenburg and Green 2000).

**Gulf Coast**

Until recently, populations in the Gulf were understudied by molecular ecologists. The work of Hart (2005, 2014) and Hauswaldt and Glenn (2005) suggested that Gulf Coast populations contained lower levels of genetic diversity than populations in the mid-Atlantic, but both studies included limited sampling in the Gulf region. Coleman (2011) was the first to include a wide sampling of Gulf populations. Using 12
Gmu microsatellites and STRUCTURE, he identified three clusters of populations: Florida, South Carolina, and the Gulf (TX, LA, AL). STRUCTURE suggested little admixture among clusters. While earlier studies found mixed support for population bottlenecks, Coleman (2011) found strong support for bottlenecks in populations in the Gulf, Florida, and Atlantic using M-ratios (all M < 0.40). Moderate levels of heterozygosity (0.50-0.65) and significant deviations from Hardy-Weinberg equilibrium in the Alabama and South Carolina populations provided support for genetic bottlenecks. His findings differed from Hauswaldt and Glenn (2005), who found much higher levels of heterozygosity (0.56 - 0.80) and no evidence for bottlenecks in Gulf populations.

Work on Gulf Coast terrapins continued with Glenos (2013) whose sampling was most dense in Texas, but included Louisiana and Alabama. She did not find significant differentiation between the two sampling localities within Texas, suggesting terrapins along the Texas coast constitute a single population. On the other hand, she found terrapins from Alabama and Louisiana to be genetically separated from the Texas population. Consistent with Coleman (2011), her 12 Gmu microsatellites exhibited low levels of heterozygosity (0.39-0.52), few private alleles, and low numbers of alleles (3.0-5.5) in all populations.

The value of diversity studies is always highlighted by environmental disasters, such as the Deepwater Horizon oil spill in 2010, which deeply impacted coastal ecology in Louisiana. Drabeck et al. (2014) studied the effects of the oil spill on terrapin populations. Using four TerpSH and 12 Gmu microsatellites, they found no meaningful population structure among Louisiana terrapins, nor did they detect population
contractions using the program BOTTLENECK, in contrast with Coleman (2011) and Hart et al. (2014). However, consistent with Lamb and Avise (1992) and Hart et al. (2014), Drabeck et al. (2014) found Florida, the Gulf, and Atlantic terrapin populations to be diagnosable entities. AMOVAs showed that most genetic variance (7.47%) was explained by combining Gulf and Atlantic terrapin populations while excluding Florida populations. Interestingly, similar to Hauswaldt and Glenn (2005), they also found evidence for terrapin translocations during the 20th Century, as Gulf Coast terrapins were more similar to coastal Atlantic populations than adjacent populations in the Florida Keys. This finding may be due to the undesirable traits of Florida terrapins. Florida terrapins were considered insipid in taste and too small to sell or hybridize; rather, terrapins from Texas were preferred for shipment to the Atlantic seaboard (Hay 1917; Hildebrand 1933).

Further work on Louisiana terrapins was conducted by Petre et al. (2015). Twenty-six sampling localities (analyzed as eight sites) along the Louisiana coastline were examined for population structure, genetic diversity, population connectivity, and bottlenecks, using 13 Gmu microsatellites. While STRUCTURE did not detect any meaningful population structure, isolation-by-distance was detected along Louisiana’s coastline and MIGRATE-n found a stepping stone model best explained patterns of gene flow. Petre et al. (2015) found fairly high levels of heterozygosity (0.74-0.77) and allele number (5.8-10.4) among populations. Despite the high levels of genetic diversity, BOTTLENECK found that two of the eight sites exhibited the genetic signature of population contraction.
In summary, while many ecological studies document high levels of population structure, phylogeographic and regional genetic studies have found more limited genetic variation among populations. This contrast could be due to genetic and ecological studies quantifying structure on different time scales, with ecological studies documenting real time population structure that is yet to manifest itself in the genome. It remains possible that the terrapin recently underwent a rapid range expansion across much of its range, resulting in limited phylogeographic structure. Terrapin populations along the coasts may also have experienced sporadic episodes of increased gene flow, including human-mediated gene flow.

Insights into Reproductive Ecology Provided by Genetic Studies

Large documented declines in terrapin populations during the early 20th Century were a major impetus to study the patterns of terrapin genetic diversity. By contrast, studies of terrapin reproductive biology received less attention. Two themes within reproductive biology have been studied by molecular ecologists: natal philopatry and multiple paternity. Natal philopatry is the propensity for offspring to return to their birth location to nest. Whether or not emerging terrapin hatchlings exhibit natal philopatry was unknown until recently, though it had been documented in the Mississippi Map Turtle (Graptemys pseudogeographica kohni), a closely related species (Freedberg et al. 2005). High levels of natal philopatry are important for studies of genetic differentiation because they represent a mechanism by which genetic structure among populations can evolve. If mothers and their daughters return to the same nesting locality each season, genetic evidence should complement ecological findings of nest site fidelity by mothers. In
Barnegat Bay NJ, Sheridan et al. (2010) documented natal philopatry in terrapins using six Gmu microsatellites and spatial autocorrelation analysis. They calculated genetic correlations among terrapins, and then surveyed for relationships with geographic distance. The only demographic group with a positive genetic autocorrelation was nesting females (at 0-50 m), indicating they exhibit spatial structure that could result from nest-site philopatry. Sheridan et al. (2010) also found that many juvenile males were transient at their study site. High levels of juvenile movement may be partly responsible for the lack of population genetic structure found in terrapins.

A clutch of Diamondback Terrapin hatchlings share a mother, but not necessarily a father. Multiple paternity in terrapins was not well understood until advances in genetic techniques allowed researchers to estimate paternity accurately but highly suspect as terrapins can store sperm for up to four years (Hildebrand and Hatsel 1926). The first study of multiple paternity in terrapins was conducted by Hauswaldt (2004), who surveyed six TerpSH microsatellite in 439 hatchlings from 26 mothers in Oyster Bay, NY. She found a majority of nests (82%) were sired by one male. This result was somewhat surprising, and suggested multiple paternity in the terrapin was uncommon relative to other Chelonians (e.g., Galbraith et al. 1993; Valenzuela 2000; Ireland et al. 2003).

Like any trait, multiple paternity can vary by region or population, or even over time in the same location. Following Hauswaldt (2004), Sheridan (2010) surveyed six Gmu microsatellites in 1,608 hatchlings and 1,558 adults from four sites in Barnegat Bay, NJ and Poplar Island in Chesapeake Bay. She recorded higher levels of multiple paternity
than did Hauswaldt (2004), with a mean of 29%. However, the occurrence of multiple paternity varied widely among nesting locations, from 12.5 to 45.7%. Moreover, this variation was correlated with population sex ratio: populations with female-biased sex ratios (4.7:1) demonstrated the highest levels of multiple paternity, while populations with extreme female-biased sex ratios (9:1) had lower levels.

Discussion

Studies of genetic variation have taught us much about terrapin biology. At the broadest scale, range-wide phylogeographic analyses by Lamb and Avise (1992), Hauswaldt (2004), Hauswaldt and Glenn (2005), and Hart et al. (2014) have shown that genetic structure across the range of the terrapin is limited, and do not support the existence of seven subspecies, at least if one views subspecies as incipient evolutionary lineages. Thus, the subspecific designations in terrapins are in need of re-evaluation.

Genetic studies of the terrapin are made more difficult by limited sequence variation and the specter of frequent human translocations. In general, mtDNA exhibits higher levels of structure than nuclear markers (microsatellites excluded), making mtDNA useful for phylogeography. However, in Chelonians, mtDNA is thought to mutate more slowly than in other vertebrates (Avise et al. 1992; Bromham 2002; Shaffer et al. 2013), resulting in less structure and weaker inference. Ultimately, many nuclear loci from next generation sequencing resources are needed to elucidate the evolutionary history of the terrapin.

At the range-wide and regional scales, a lack of genetic structure may be a consequence of the terrapin rapidly expanding its range from a refugium. Because the
terrapin’s range closely follows the Gulf and Atlantic coasts, it is essentially structured as a linear stepping stone, which should produce isolation-by-distance. STRUCTURE assumes no isolation-by-distance (Pritchard 2010), and yet isolation-by-distance is present at regional and range-wide scales in terrapins (Hauswaldt and Glenn 2005; Hart et al. 2014). Violation of the assumptions of $F_{ST}$ statistics (equal population sizes, island migration model, etc.) may also obfuscate population structure estimates. For this reason, it is important that future work take full advantage of the latest advances in population genetics, especially coalescent theory, which represents a powerful stochastic model for evaluating demographic history (Wakeley 2008). For example, Bayesian skyline plots could be used to examine variation in effective population sizes through time. Alternatively, historical events could also be investigated using Approximate Bayesian Computations, which allow complex models to be constructed and tested against genetic data (Csilléry et al. 2010). For instance, models including admixture, population divergence dates, population sizes, and translocation could be assembled and tested against one another.

Among population genetic studies, a shared finding is that terrapin genetic diversity is the lowest in southern populations, especially in Florida. This finding is in contrast with many taxa in the Eastern U.S., especially terrestrial taxa, which display the highest levels of genetic diversity in the south and the lowest levels in the north (Avise 2000; Soltis et al. 2006). In terrapins, the legacy of terrapin harvests may contribute to lowered genetic diversity. However, historical factors likely contributed as well. For example, if the terrapin had a Pleistocene refugium along the Atlantic coast and only
recently (within the last several thousand years) expanded into the Gulf, Gulf populations would be expected to exhibit low levels of genetic diversity.

By contrast, genetic diversity is highest in the mid-Atlantic region, possibly due to translocations into the region. Translocation is supported by Bayesian population assignment tests, which often assign Atlantic terrapins to Gulf populations (Hauswaldt and Glenn 2005). In addition, Drabeck et al. (2014) found that Atlantic populations were genetically more similar to Gulf populations than neighboring populations. Alternatively, the mid-Atlantic could be the origin of the terrapin’s historical range. If the terrapin originated in this portion of its range, genetic diversity is expected to be higher there while peripheral populations (the North Atlantic, Florida, and the Gulf) are expected to exhibit lower genetic diversity.

A common finding among genetic studies is that Florida populations stand out as distinctive. Hart et al. (2014), Drabeck et al. (2014), and Hauswaldt and Glenn (2005) all found Florida terrapins to be genetically differentiated from neighboring populations in the Atlantic and Gulf. The cause of this differentiation is not entirely clear, but terrapin populations in Florida exhibit much lower levels of heterozygosity (0.363; Hart et al. 2013) and fewer private alleles than populations in other regions, consistent with a severe genetic bottleneck, inbreeding, or small population sizes. One possible cause is the Suwanne Seaway, which separated peninsular Florida from the North American continent by sea level rise during the Miocene and Pliocene (Felder and Staton 1994). This Seaway would have isolated terrapin populations in Florida, while facilitating gene flow between
Gulf and Atlantic populations. The Suwanne Seaway could also account for the Gulf/Atlantic genetic divide first documented by Lamb and Avise (1992).

It is interesting to note that the specific microsatellite markers that terrapin studies have used influence the recovered patterns of diversity. Two sets of microsatellite primers are commonly used: TerpSH (Hauswaldt and Glenn 2003) and Gmu (King and Julian 2004). These two sets of markers differ in mutation rate. Hauswaldt (2004) estimated her loci to have an average mutation rate of $2.72 \times 10^{-3}$ mutations$^{-1}$ site$^{-1}$ generation$^{-1}$, while loci developed by King and Julian (2004) have a mean mutation rate of $4.34 \times 10^{-4}$ mutations site$^{-1}$ generation$^{-1}$ (Converse et al. 2015), approximately 10-fold slower. Accordingly, studies that use the TerpSH microsatellites consistently recover higher levels of diversity (Hauswaldt and Glenn 2005; Drabeck et al. 2014) than do studies that use the Gmu microsatellites (Hart et al 2014; Drabeck et al. 2014). Inference of terrapin phylogeographic structure is also affected by marker choice, with mtDNA recovering two phylogeographic groups (the Gulf/Atlantic divide; Lamb and Avise 1992; Hauswaldt 2004), and microsatellites recovering four population clusters (Gulf, Atlantic, MA, FL; Hart et al. 2014). While incongruence between mitochondrial and nuclear loci is not uncommon, terrapin studies have routinely recycled the same markers. The development of new high-resolution genetic markers is sorely needed.

Evidence for population bottlenecks is highly variable among studies, even though there is good historical evidence for demographic bottlenecks across the terrapin’s range (Garber 1990). Louisiana terrapins are an excellent example of the discordance among studies. Hart et al. (2014) and Coleman (2011) report bottlenecks in this region,
Petre et al. (2015) found evidence that varied by site, and Drabeck et al. (2014) found no evidence of a bottleneck in this region. In some cases, outbreeding due to terrapin translocations among populations may have obfuscated genetic evidence of a bottleneck. More work on population bottlenecks and their detection is warranted.

Finally, while many ecological studies have shown terrapins to exhibit high site fidelity and low dispersal, which should promote the evolution of population genetic structure, population genetic studies have consistently recovered low levels of genetic structure. Several hypotheses have been put forth to explain this discrepancy between ecology and genetics. Hauswaldt and Glenn (2005) hypothesized that terrapins form mating aggregations in the early spring and disperse far from their home range to participate. This behavior would homogenize local population structure such as generated by nest site fidelity. Ecological work by Seigel (1980) and Butler (2002) support this interpretation. However, work by Sheridan (2010) suggests that terrapins do not travel long distances to form mating aggregations. Converse et al. (2015) hypothesize that mating aggregations homogenize populations at the local level, while male-biased dispersal homogenizes populations at larger spatial scales. Since most populations are strongly female-biased, low levels of male migration can result in high levels of admixture, and thereby reduce population structure. I recommend that future ecological work focus on population sex ratios, juvenile dispersal, and adult dispersal in relation to mating aggregations, as these elements of terrapin biology have the potential to harmonize ecological and molecular findings.
It is important that future genetic work also take full advantage of advances in genomics to more accurately test models of population structure and population history. Thankfully, a large volume of high quality loci is on the horizon, as scientists at the University of Maryland have recently sequenced the entire mitochondrial and nuclear genomes of the terrapin (Mihai Pop, Center for Bioinformatics and Computational Biology, University of Maryland, pers. comm.). With a reference genome, a study could now easily include tens of thousands of single nucleotide polymorphisms or microsatellite loci. Hyper-variable sequence regions unique to terrapins also could be identified and used. Indeed, it is now possible to sequence whole genomes from throughout the range of the terrapin.

The Diamondback Terrapin has a complicated history, and it is important that future studies incorporate the latest advances in genomics, computational resources, and population genetic theory. Our understanding of the history of the terrapin is incomplete, and a range-wide phylogeographic study that takes advantage of next generation sequencing resources is needed to define management units, identify possible Pleistocene refugia, and better document population history. Populations in Florida are of particular interest as they are genetically distinct from both Gulf and Atlantic populations, perhaps as a consequence of the Suwanne Seaway. On a finer scale, landscape genetic studies that document contemporary movement patterns would inform conservation efforts. For instance, models of gene flow that include electrical circuit theory (McRae and Beier 2007) could be used to investigate the effects of crab pots on population connectivity. Additionally, studies of contemporary gene flow could include historical estimates, as
these provide important context for interpreting patterns of connectivity (Epps et al. 2013; Converse et al. 2015). Finally, more studies on natal philopatry and juvenile dispersal are needed, because these elements of terrapin biology will have an overarching impact on population genetic structure. By taking advantage of the latest developments in DNA sequencing and population genetic theory, and by conducting work on natural history and ecology in parallel, our understanding of the evolutionary history of the Diamondback Terrapin stands to become re detailed than ever.

Figure 1.1: Approximate sampling localities for range-wide, regional, and local Diamondback Terrapin studies. Each noted locality may represent multiple individuals sampled and/or multiple sampling sites within the same general area.
CHAPTER 2: SPATIOTEMPORAL ANALYSIS OF GENE FLOW IN CHESAPEAKE BAY DIAMONDBACK TERRAPINS

Introduction

The current genetic structure among a set of populations is the product of contemporary and historical processes, and distinguishing between the two is paramount for effective population management (Epps et al. 2013). Around the world, the fragmentation of habitats is a ubiquitous threat to biodiversity because it decreases population connectivity (dispersal and gene flow) relative to historical levels, thereby impacting metapopulation dynamics (Hanski and Gilpen 1997; Frankham et al. 2002). Reductions in gene flow and small effective population size ($N_e$) caused by habitat fragmentation diminish metapopulation viability by decreasing genetic diversity and increasing inbreeding (Lande 1995; Templeton et al. 2001; Bowler and Benton 2005; Banks et al. 2013; Barr et al. 2015). However, the extent to which habitat fragmentation decreases population connectivity, however, is dependent upon the interaction between landscape features and organismal dispersal behavior (Gu et al. 2002; Caizergues et al. 2003; Braunisch et al. 2010; Callens et al. 2011; Crispo et al. 2011). In many cases, populations that are currently isolated by habitat fragmentation may not have been isolated in the past (Newmark 2008; Chiucchi and Gibbs 2010; Epps et al. 2013; Husemann et al. 2015). In contrast with habitat fragmentation, the anthropogenic translocation of individuals between populations reduces genetic differentiation, increases diversity within populations, and may obscure estimates of genetic connectivity (Templeton et al. 1986; Moritz 1999; Weeks et al. 2011). Disentangling how historical
and contemporary processes affect current patterns of genetic diversity is a formidable challenge, but can be achieved by temporal sampling (Husemann et al. 2015), or by separately estimating contemporary and historical processes (Chiucchi and Gibbs 2010; Epps et al. 2013).

In this chapter, I examine population structure and connectivity in the Diamondback Terrapin (*Malaclemys terrapin*). Terrapins inhabit North American coastal and brackish waters, with a range that extends from Texas to Massachusetts (Ernst and Barbour 1989). During the nineteenth and early twentieth centuries terrapins were unsustainably harvested, resulting in severe population contractions and local extirpations (Garber 1998; 1990). To help preserve dwindling populations and supplement terrapin harvests, governmental and private entities constructed terrapin breeding farms (Coker 1906; Barney 1924; Hildebrand and Hatsel 1926; Hildebrand 1929). Terrapins from Chesapeake Bay were the preferred variety for consumption (Hay 1917; Hildebrand 1929) and the demand for “Chesapeakes” resulted in terrapins from North Carolina and possibly other populations to be imported into Chesapeake Bay terrapin farms (Coker 1920). Terrapin meat eventually fell out of favor, and as breeding farms closed terrapins were reportedly released into local waters. The amount of admixture from these translocated terrapins is unknown.

While terrapin harvesting in Maryland has been discontinued (Roosenburg et al. 2008), terrapins still face a myriad of threats, including mortality from boat strikes (Roosenburg 1991, Cecala et al. 2008), drowning in crab and eel pots (Roosenburg et al. 1997; Radzio and Roosenburg 2005; Dorcas et al. 2007; Grosse et al. 2009), habitat loss
and fragmentation (Roosenburg 1991; Wood and Herlands 1997), and predator introductions (Feinberg and Burke 2003). As male terrapins are smaller and disperse longer distances than do females (Sheridan 2010), they are particularly vulnerable to dispersal related mortality. Terrapin populations in Chesapeake Bay exhibit highly skewed sex ratios in favor of females (Roosenburg 1991; Roosenburg unpubl. data), making successful male dispersal important for maintaining genetic connectivity.

The consequences of habitat fragmentation and increased mortality on connectivity and population genetic structure are not entirely clear, however, and ecological and molecular findings are discordant with respect to levels of connectivity (Converse and Kuchta, 2017). Ecological data show terrapins reside in small home ranges of 0.54 – 3.05 km² (Spivey 1998; Butler 2002) and can remain in the same study site for over a decade (Lovich and Gibbons 1990; Gibbons et al. 2001). Furthermore, ecological data suggest terrapins form structured breeding assemblages, with females returning to the same nesting beach each season (Auger 1989; Roosenburg 1994; Mitro 2003) and hatchlings demonstrating natal philopatry (Sheridan et al. 2010). In contrast with these studies, genetic studies indicate that terrapin populations are weakly differentiated (Hart et al. 2014), with limited structure at both regional and local scales (Hauswaldt and Glenn 2005; Sheridan et al. 2010; Glenos 2013; Drabeck et al. 2014; Petre 2014).

The complex history terrapins share with humans in Chesapeake Bay makes it important to quantify levels of population genetic structure, including a comparison of contemporary and historical levels of connectivity. In this chapter, I report on a study of metapopulation dynamics of the Diamondback Terrapin in Chesapeake Bay. Specifically,
I estimate the following: i) the number of genetic populations in Chesapeake Bay; ii) levels of genetic diversity within and among populations; iii) effective population sizes; and iv) levels of contemporary and historical gene flow among populations. In addition, I identify possible instances of terrapin translocation. By comparing historical and contemporary levels of genetic connectivity, I examine the impact of habitat fragmentation and population translocations on patterns of genetic variation, and help resolve the discordance between ecological and molecular studies (Converse and Kuchta, 2017).

Materials and Methods

Sampling Localities and Microsatellite Genotyping

A total of 617 terrapins from 15 localities throughout Chesapeake Bay and nearby coastal bays were captured between 2003-2005 (Figure 2.1; Table 2.1), a dataset provided by Timothy L. King at the U.S. Geological Survey. Terrapins were captured using fyke nets or collected in winter refugia during hibernation (Haramis et al. 2011) and were marked with Passive Integrated Transponder tags to prevent resampling.

Twelve microsatellite loci were assayed that were developed for the bog turtle (*Glyptemys muhlenbergii*) but also amplify in other Emydid turtles (King and Julian 2004). PCR chemistry and protocols for the loci are detailed in King and Julian (2004). I used MICRO-CHECKER version 2.2.3 (Van Oosterhout et al. 2004), including 10,000 Monte Carlo simulations to test for the presence of null alleles and estimate 95% confidence intervals. No evidence of null alleles was detected at any locus.
Population Structure and Genetic Diversity

I used STRUCTURE version 2.3 (Pritchard et al. 2000) to infer the number of genotypic clusters in the Chesapeake Bay region. STRUCTURE identifies populations by maximizing conformity to Hardy-Weinberg equilibrium (HWE) while simultaneously minimizing linkage disequilibrium within K user-defined clusters. I ran STRUCTURE from K = 1 to K = 15 populations, with each value of K repeated ten times with randomly generated starting seeds. Each Markov Chain Monte Carlo (MCMC) run consisted of 550,000 iterations, with the first 250,000 discarded as burn-in. I used the admixture model, the correlated allele frequencies prior, the LOCprior, the LOCISPOP prior, fixed λ, and inferred α. I used sampling localities (Figure 2.1) as priors for the LOCprior. STRUCTURE results were collated and ΔK computed via the Evanno method (Evanno et al. 2005) using STRUCTURE HARVESTER web version 0.6.94 (Earl and vonHoldt 2011). Label switching and multimodality on preferred values of ΔK were addressed using CLUMPP version 1.1.2 (Jakobsson and Rosenberg 2007), and the final results were visualized using DISTRUCT version 1.1 (Rosenberg 2003). I repeated this procedure within STRUCTURE clusters to detect substructure. I also partitioned genetic variance using an analysis of molecular variance (AMOVA) in the software ARLEQUIN version 3.5.1.3 (Excoffier and Lischer 2010). Populations were partitioned by landscape features (river, bay, and coast), sampling locality, and STRUCTURE clusters. Significance was assessed using 1,000 permutations. I further estimated population differentiation by quantifying D_{est} (Jost 2008) in the R package DEMEtics version 0.8-7 (Gerlach et al. 2010) between all STRUCTURE clusters. I determined significance and estimated 95%
confidence intervals using 1,000 bootstrap replicates and Bonferroni correction (Dunn 1961). I used FSTAT version 2.9.3 (Goudet 1995) to estimate allelic richness, allele count, and linkage disequilibrium for all sampling localities, and ARLEQUIN to estimate heterozygosity and deviations from HWE.

**Mutation Rate**

Since coalescent estimates of historical gene flow and effective population sizes are scaled by mutation rate, I estimated a mutation rate ($\mu$) using approximate Bayesian computation (ABC) in popABC version 1.0 (Lopes et al. 2009). Mutations were modeled using the stepwise-mutation model (SMM; Kimura and Ohta 1978) and were measured in mutations site$^{-1}$ generation$^{-1}$. Demographic parameters were estimated under the isolation-migration model (Nielsen and Wakeley 2001; Hey and Nielsen 2004). Priors for this analysis are summarized in Table 2.2. I modeled my mutation rate prior using a log-normal distribution centered at $1 \times 10^{-3}$ (SD = 0.5; Hedrick 1996; Whittaker et al. 2003). Genetic tree topology was modeled under a uniform prior. I simulated 2,500,000 genetic trees and ran the ABC-rejection algorithm with a tolerance of 0.0004, retaining the 1,000 closest simulated points. I did not run an ABC-regression analysis as some of the summary statistics exhibited multicollinearity, violating the assumptions of local linear regression (Beaumont et al. 2002). Following the rejection step, I estimated the mode, 2.5% quantile, and 97.5% quantile for $\mu$ in R.

**Effective Population Size**

I used MIGRATE version 3.6.5 (Beerli 2008) to jointly estimate $\theta$ ($= 4N_e\mu$) while estimating $M$ (see below) and used the mutation rate estimated by popABC to convert $\theta$
into $N_e$. I also estimated effective population sizes using ONeSAMP v. 1.2 (Tallmon et al. 2008), which employs ABC and eight common summary statistics (e.g. observed heterozygosity, Wright’s $F_{IS}$) to estimate $N_e$ for a single population (Tallmon et al. 2008). For these analyses, I ran each STRUCTURE population individually and set lower and upper boundaries for $N_e$ to 2 and 1,000, respectively.

**Historical Gene Flow**

I used MIGRATE to estimate gene flow levels in Chesapeake Bay prior to European colonization (historical gene flow, $M$: proportion of migrants per generation, scaled by mutation rate). Since MIGRATE operates in a coalescent framework, it estimates gene flow over long periods of time, up to $\sim 4N_e$ generations (thousands of years) for larger populations (Beerli 2009). I used populations demarcated by STRUCTURE as *a priori* population assignments in MIGRATE. To improve speed, I used a Brownian motion model to approximate a stepwise- mutation model. Using slice sampling, I ran four statically heated parallel chains (heated at 1.0, 1.5, 3.0 and 1,000,000) for 30,000,000 iterations, sampling every 3,000 iterations, and excluded 7,500,000 iterations as burn-in. MCMC estimates of $M$ were modeled with a uniform prior containing lower and upper boundaries of 0 and 2,000. $F_{ST}$ values were used for initial estimates of $M$. A full migration model was used, which facilitates comparisons with gene flow estimates made in BAYESASS. I considered parameter estimates to be accurate if an effective sample size (ESS) of 1,000 or greater was observed (Beerli, *pers. comm.)*
Contemporary Gene Flow

Contemporary rates of gene flow (m: proportion of migrants per generation) in Chesapeake Bay were estimated using BAYESASS version 3.0 (Wilson and Rannala 2003). BAYESASS estimates all pairwise migration rates among populations. According to Wilson and Rannala (2003), BAYESASS estimates gene flow “…over the last several generations.” Following Chiucchi and Gibbs (2010), I assumed this to mean approximately five generations. Using a generation time of 12 years (Roosenburg, *unpubl. data*) BAYESASS is quantifying gene flow within the last 60 years or so, a time period characterized by extensive anthropogenic influences, including habitat loss and fragmentation. I used populations demarcated by STRUCTURE as *a priori* population assignments. I ran 10 MCMC simulations (Faubet et al. 2007) with different starting seeds for 20,000,000 iterations, sampling every 2,000 iterations; 10,000,000 iterations were excluded as burn-in. Chain mixing delta parameters were adjusted in pilot runs to maintain a MCMC state-change acceptance ratio of 20-40%, the empirically recommended window (Rannala 2011). I diagnosed MCMC stationarity for each run in TRACER version 1.5. (Rambaut and Drummond 2007) and used a Bayesian deviancy measure (Spiegelhalter et al. 2002) to determine which run best fit the data with R (Meirmans 2014). I took the starting seed from this best-fit run and ran a MCMC for 50,000,000 iterations, sampling every 2,000, with the first 20,000,000 iterations excluded as burn-in. I visualized MCMC stationarity for this final run in TRACER. The ESS for all parameters was >200.
Comparison of Historical and Contemporary Gene Flow

I tested for a relationship between historical and contemporary gene flow by conducting a Mantel test in the R package VEGAN version 2.2-1 (Oksanen et al. 2013) using 100,000 permutations. To compare historical estimates of gene flow generated by MIGRATE ($M = m_h/\mu$) to contemporary estimates of gene flow from BAYESASS, I multiplied the $M$-values generated by MIGRATE by the mutation rate estimated in popABC. I then subtracted these values from the contemporary estimates of gene flow from BAYESASS ($\Delta m = m - m_h$). The resulting value, $\Delta m$, denotes temporal changes in gene flow. Negative values of $\Delta m$ indicate reduced gene flow in the present, positive values increased gene flow, and values near zero no change.

Population Bottlenecks

Since estimates of $\theta$ and $M$ are sensitive to fluctuations in effective population size (Beerli 2009), I conducted tests to detect bottlenecks. I tested for bottlenecks at two generational time scales. First, I tested for bottlenecks using a mode-shift test, which is capable of detecting bottlenecks “…within the past few dozen generations,” (Luikart and Cornuet 1998). Older bottlenecks were tested for using a Wilcoxon’s sign-rank test, which detects bottlenecks 25-250 generations in the past (Cornuet and Luikart 1996). Bottleneck tests were conducted in the program BOTTLENECK version 1.2.02 (Piry et al. 1999). I ran BOTTLENECK under the SMM and the two-phase model (TPM) and tested for heterozygosity excess. Under the TPM, I set 95% of all mutations to be single-step with 12% variance within multi-step mutations, following the recommendation of Piry et al. (1999). All tests were conducted using 50,000 permutations and analyzed by
STRUCTURE cluster. Because small sample sizes can lead to low statistical power in detecting bottlenecks (Peery et al. 2012), I also pooled all samples together and reran all tests for the entire Chesapeake Bay (n = 617).

Results

Population Structure and Genetic Diversity

No loci were found to be in linkage disequilibrium, and across all populations and loci, 7 of 165 loci were out of HWE at \( \alpha = 0.05 \), but only one locus in one population (MD1) was out of HWE after Bonferroni correction. Population genetic metrics are provided in Table 2.3.

Preliminary runs of STRUCTURE did not detect genetic structure within Chesapeake Bay. This was because locus D21 was nearly monomorphic. Removing this locus ameliorated the problem, and all results presented have locus D21 removed. In total, I identified four terrapin populations (Figure 2.1): the Patuxent River, Kent Island, the coastal bays, and inner Chesapeake Bay. Initial runs of STRUCTURE found the Patuxent River (MD3, MD4) to form the first cluster, with the remaining localities forming a second cluster (Figure 2.1, \( \Delta K = 2 \)). Analysis of the second cluster (Figure 2.1, \( \Delta K = 3 \)) revealed it was composed of three subclusters: Kent Island (MD2, MD8), the coastal bays (VA2, VA3, VA4), and inner Chesapeake Bay (MD1,5,6,7,9,10,11, VA1).

AMOVAs indicated that most of the genetic diversity in Chesapeake Bay is found within populations. STRUCTURE clusters explained the most genetic variation (0.96% \( P = 0.0031 \)), while landscape features (0.88% \( P = 0.0154 \)) and sampling locality (0.88% \( P < 0.001 \)) explained slightly less. Estimates of \( D_{\text{est}} \) among STRUCTURE clusters identified
significant levels of population differentiation among all clusters (Table 2.4). Kent Island and inner Chesapeake Bay were estimated to be the most similar ($D_{est} = 0.0155$) while the Patuxent River and the coastal bays were estimated to be the most dissimilar ($D_{est} = 0.0654$).

**Mutation Rate**

ABC posterior estimates of $\mu$ solved at a mode of $4.3 \times 10^{-4}$ mutations site$^{-1}$ generation$^{-1}$ (Figure 2.2). This estimate of $\mu$ is similar to a commonly assumed microsatellite mutation rate of $5.0 \times 10^{-4}$ (e.g. Estoup et al. 2002; Faubet et al. 2007; Chiuchhi and Gibbs 2010).

**Effective Population Size**

Estimates of $N_e$ from MIGRATE produced a range of effective population sizes in Chesapeake Bay (Figure 2.3A). Inner Chesapeake Bay was estimated to have the largest effective population size, at $N_e = 758$ (95% CI: 476 - 1398). The Patuxent River was the next largest, at $N_e = 144$ (8 - 261), followed by Kent Island, at $N_e = 109$ (19 - 261), and the coastal bays, at $N_e = 98$ (0 - 238). ONeSAMP generated the same order of population sizes, but differed in its estimates. Inner Chesapeake Bay was estimated to have an effective size of 302 (265 - 361), the Patuxent River = 254 (195 - 467), Kent Island =154 (139 - 188), and the coastal bays = 100 (78 - 158).

**Historical Gene Flow**

Estimates of historical gene flow rates revealed similar but low levels of gene flow among all populations (Figure 2.3A). Historical gene flow levels from the coastal
bays to inner Chesapeake Bay were the lowest of all routes ($m = 0.0082$), while levels of gene flow from the Patuxent River to Kent Island were the highest ($m = 0.0181$).

**Contemporary Gene Flow**

Contemporary levels of gene flow in Chesapeake Bay showed much more variation than did historical levels (Figure 2.3B). Gene flow leaving inner Chesapeake Bay was the lowest of all contemporary levels ($m = 0.0010$), while gene flow emigrating from the coastal bays into inner Chesapeake Bay was the highest ($m = 0.3201$). Gene flow between Kent Island and the Patuxent River was also markedly higher than gene flow between other populations ($m = 0.2958$ and $m = 0.1584$, respectively). Of the six paired gene flow routes, only two were found to be asymmetrical (non-overlapping 95% CI): gene flow between Kent Island and the Patuxent River, and gene flow between the coastal bays to inner Chesapeake Bay.

**Comparison of Historical and Contemporary Gene Flow**

A Mantel test did not detect a relationship between historical and contemporary gene flow ($r = 0.86, P = 0.16667$). Three rates were found to increase substantially through time (Figure 2.3C, solid lines). Contemporary gene flow levels from Kent Island to the Patuxent River and gene flow from the Patuxent River to Kent Island were much higher than historical levels ($\Delta m = +0.2831$ and $\Delta m = +0.1403$, respectively), as were gene flow levels from the coastal bays to inner Chesapeake Bay ($\Delta m = +0.3119$). I removed these routes and performed another Mantel test, but found no significant relationship ($r = 0.86, P = 0.125$), as all other routes showed varying degrees of gene flow reduction, approximately $\Delta m \approx -0.01$. The Patuxent River to inner Chesapeake Bay
(Δm = -0.0059) and the coastal bays to the Patuxent River (Δm = -0.0084) showed the least change in gene flow levels over time.

Population Bottlenecks

BOTTLENECK tests failed to detect any signatures of heterozygosity excess in Chesapeake Bay. Similarly, a mode-shift test failed to detect any bottlenecks, with all populations assuming a normal “L-shaped” distribution. When the data were pooled to correct for low power associated with small sample sizes (Peery et al. 2012), the same results were recovered.

Discussion

Given the extent of habitat fragmentation and its contribution to the ongoing biodiversity crisis, conservation efforts are often aimed at evaluating and ameliorating levels of connectivity between populations (Wilcox and Murphy 1985; Hanski and Gilpen 1997; Beier et al. 2008; Newmark 2008). Many such studies assume that population connectivity was higher prior to anthropogenic changes, but this is not always the case, and there is commonly a disconnect between ecological estimates of dispersal and levels of genetic fragmentation (Kuchta and Tan, 2006; Epps et al. 2013).

I delineated four terrapin populations in the Chesapeake Bay region that exhibited high levels of heterozygosity and allelic diversity and weak structure (Figure 2.1). Historical estimates of migration indicate that gene flow was limited among all populations (m_h ≈ 0.01; Figure 2.3A). By contrast, contemporary estimates of migration were more variable (Figure 2.3B). While most populations remained connected by low levels of gene flow, substantial increases in gene flow were detected between Kent Island
and the Patuxent River (Δm = +0.2831 and Δm = +0.1403) and from the coastal bays into inner Chesapeake Bay (Δm = + 0.3119; Figure 2.3C).

The documented increases in contemporary gene flow may have been human-mediated, as terrapins are known to have been translocated into and around Chesapeake Bay to supplement terrapin farms. Terrapins were first brought into Chesapeake Bay from North Carolina around 1909, and were reportedly released when the terrapin farms closed (Hildebrand and Hatsel 1926; Hildebrand 1933). This transport of terrapins could explain my substantial increase in gene flow from the coastal bays to inner Chesapeake Bay (Δm = +0.3119; Figure 2.3C). Alternatively, the increase in gene flow could be due to natural processes. Hauswaldt and Glenn (2005) demonstrated that 75% of Chesapeake Bay terrapins could be correctly assigned to their population of origin using only six microsatellite loci, and that Chesapeake Bay populations have higher numbers of private alleles than neighboring populations. If translocations from North Carolina represented a large influx of genetic variation, one might expect assignment tests to confound Chesapeake Bay terrapins with terrapins from North Carolina, which was not the case. Increased spatial sampling is needed to determine whether my documented increase in contemporary gene flow into Chesapeake Bay is due to the natural movement of individuals or is due to translocation from North Carolina or another source.

The Patuxent River and Kent Island also exhibited large temporal increases in gene flow between them (Figure 2.3C). This too may be caused by translocation, as the largest terrapin farm in Chesapeake Bay was located on the Patuxent River at one time, and reportedly “…consist[ed] of a large salt water lake, which could accommodate
thousands of terrapins…” (Carpenter 1891). So far as I know, this farm was stocked prior to imports of terrapins from North Carolina, and thus the terrapins farmed on the Patuxent River were most likely from Chesapeake Bay. Populations located near Kent Island and the Patuxent River represent nearby sources for the farm. Thus, anthropogenic translocation could be the cause of the detected contemporary increases in gene flow. Alternatively, it is possible that the increases in contemporary gene flow are the product of natural increases in genetic connectivity, despite the high levels of habitat fragmentation in the region. Relative to the eastern shoreline, the western shore of Chesapeake Bay lacks jutting peninsulas (Figure 2.1). This lack of peninsulas may act as a conduit of gene flow between Kent Island and the Patuxent River, as movement between these populations is not as circuitous as dispersal along the eastern shore. However, this requires dispersal distances that are not commonly documented in ecological studies.

In contrast with the increases in contemporary gene flow discussed above, the majority of populations exhibited decreased contemporary gene flow. Within Chesapeake Bay, substantial habitat modification has occurred within the last century. In particular, shorelines have become reinforced with riprap to prevent erosion. Female terrapins prefer to nest on sandy beaches (Roosenburg 1994) and usually return to the same location each nesting season (Szerlag and McRobert 2006). Moreover, studies show that offspring exhibit natal philopatry (Sheridan et al. 2010). As sandy beaches are lost to shoreline development, females are restricted to fewer nesting locations, increasing population fragmentation. In addition, even where terrapins can nest, the mortality risk for eggs,
hatchlings, and adult females has increased, as raccoons (*Procyon lotor*) and other mesopredators thrive in human-modified landscapes (Crooks and Soulé 1999). Some nesting locations suffer mortality rates as high as 92% for nests (Feinberg and Burke 2003) and 10% for adult females (Seigel 1980).

While gravid females face higher mortality during terrestrial nesting excursions, juvenile females and males of all age classes experience increased mortality in aquatic habitats as fisheries bycatch. Crab pots are used to harvest Blue Crabs (*Callinectes sapidus*), and males and juvenile females (both of which are smaller than adult females) commonly become entrapped in crab pots and drown (Roosenburg et al. 1997; Roosenburg and Green 2000). While several states now require a bycatch reduction device (BRD) to exclude terrapins, Maryland only requires them in recreational crab pots. However, BRD compliance for recreational crab pots in Maryland is under 35% (Radzio et al. 2013). Terrapins are characterized by male-biased dispersal (Sheridan et al. 2010), and thus males in particular experience an increased risk of mortality due to fishery activities. I suggest that the increased mortality risk of dispersing males has lowered contemporary gene flow rates among populations.

While it is well documented that terrapins underwent population contractions due to overharvesting and other factors (Garber 1989; 1990), I failed to find evidence of a population bottleneck in Chesapeake Bay. This surprising result may be a consequence of translocation, which would have subsidized populations by reintroducing genetic diversity, and may have confounded efforts to detect a genetic bottleneck (Hauswaldt and Glenn 2005; this study). Indeed, natural populations can be greatly effected by
translocation, with potential benefits (Westemeier et al. 1998) or unforeseen consequences (Frankham et al. 2002). More work on bottleneck detection using genetic data is badly needed as bottleneck tests using heterozygosity excess may fail to detect bottlenecks in populations known to have experienced substantial declines (Peery et al. 2012; Funk et al. 2010).

The Diamondback Terrapin has been the focus of much conservation attention, and a number of ecological studies indicate that terrapins generally exhibit limited dispersal and high levels of philopatry, which over time would lead to the buildup of genetic structure (Roosenburg 1994; Spivey 1998; Gibbons et al. 2001; Butler 2002; Sheridan et al. 2010; Converse and Kuchta, 2017). By contrast, genetic studies find that terrapin populations are weakly differentiated, even at regional scales (Hauswaldt and Glenn 2005; Sheridan et al. 2010; Drabeck et al. 2014; Hart et al. 2014; Petre et al. 2015). One hypothesis to reconcile these data is that terrapins migrate large distances (several kilometers) to mating aggregations, which would prevent the buildup of genetic structure among populations (Hauswaldt and Glenn 2005). However, work by Sheridan (2010) suggests that terrapins do not travel long distances to mating aggregations. I propose a modification to Hauswaldt and Glenn’s (2005) hypothesis: that mating behavior and population sex ratios jointly function to limit genetic structure. Under this hypothesis, terrapins form mating aggregations near their home ranges, which homogenizes populations at the local level. Similarly, dispersal by male terrapins promotes admixture among mating aggregations. Male-biased dispersal has large genetic consequences because populations in Chesapeake Bay contain highly unequal sex ratios. For example,
at Poplar Island and the Patuxent River, female terrapins outnumber males five to one (5:1) and three to one (3:1), respectively (Roosenburg, *unpubl. data*). Biased sex ratios allow dispersing males to disproportionately contribute their genetic material to host populations. Furthermore, female terrapins mate multiple times, store sperm, and lay clutches of mixed paternity (Hauswaldt 2004; Sheridan 2010), increasing the odds of mating with immigrant males. It also remains possible that ecological studies document structure that is genetically nascent (Landguth et al. 2010).

My results have important implications for the management of species in heavily modified landscapes. Anthropogenic habitat fragmentation is an ongoing contributor to the biodiversity crisis, and the study of metapopulation connectivity is crucial for setting appropriate conservation targets (Wilcox and Murphy 1985). However, current population genetic structure is the product of the joint influence of contemporary and historical processes, and thus to assess contemporary changes in connectivity it is necessary to consider the historical context. Contrary to my initial hypothesis of substantial decreases in contemporary gene flow among terrapin populations as a consequence of habitat loss and fragmentation, I documented enormous increases in gene flow into Chesapeake Bay and between two populations within Chesapeake Bay. I hypothesize that this is due to translocation events associated with terrapin farming. Without an estimate of historical levels of connectivity, however, it would not have been clear that the high contemporary gene flow estimates were a recent phenomenon; indeed, I may have interpreted the relatively low estimates of contemporary gene flow among most other populations as indicative of reduced dispersal! Incorporating historical

My results confirm the importance of taking historical factors into account when quantifying genetic connectivity in highly impacted landscapes.
Table 2.1: Summary of sampling locations and sample sizes.

<table>
<thead>
<tr>
<th>Locality</th>
<th>Sample Size</th>
<th>Latitude</th>
<th>Longitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herring Bay, MD (MD1)</td>
<td>25</td>
<td>38.754362</td>
<td>-76.550857</td>
</tr>
<tr>
<td>Kent Island, MD (MD2)</td>
<td>38</td>
<td>38.936302</td>
<td>-76.363836</td>
</tr>
<tr>
<td>Patuxent River, MD (MD3)</td>
<td>63</td>
<td>38.444332</td>
<td>-76.607811</td>
</tr>
<tr>
<td>Buzzard’s Island, Patuxent River, MD (MD4)</td>
<td>60</td>
<td>38.489969</td>
<td>-76.652694</td>
</tr>
<tr>
<td>Sandy Island Cove, Nanticoke River, MD (MD5)</td>
<td>55</td>
<td>38.260110</td>
<td>-75.947966</td>
</tr>
<tr>
<td>Back Cove, Smith Island, MD (MD6)</td>
<td>17</td>
<td>38.021666</td>
<td>-75.998875</td>
</tr>
<tr>
<td>Janes Island, MD (MD7)</td>
<td>56</td>
<td>38.007513</td>
<td>-75.849861</td>
</tr>
<tr>
<td>Marshy Creek, Kent Island, MD (MD8)</td>
<td>64</td>
<td>38.954972</td>
<td>-76.227814</td>
</tr>
<tr>
<td>Northeast Cove, Bloodsworth Island, MD (MD9)</td>
<td>43</td>
<td>38.167177</td>
<td>-76.062002</td>
</tr>
<tr>
<td>Tylerton, Smith Island, MD (MD10)</td>
<td>64</td>
<td>37.964927</td>
<td>-76.020185</td>
</tr>
<tr>
<td>St. Jerome’s Creek, MD (MD11)</td>
<td>16</td>
<td>38.134924</td>
<td>-76.347049</td>
</tr>
<tr>
<td>Mobjack Bay, VA (VA1)</td>
<td>45</td>
<td>37.325137</td>
<td>-76.350877</td>
</tr>
<tr>
<td>Wachapreague, VA (VA2)</td>
<td>38</td>
<td>37.602862</td>
<td>-75.686380</td>
</tr>
<tr>
<td>Location</td>
<td>ID</td>
<td>Latitude</td>
<td>Longitude</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>----</td>
<td>---------------</td>
<td>---------------</td>
</tr>
<tr>
<td>Metompkin Island, VA (VA3)</td>
<td>20</td>
<td>37.752026</td>
<td>-75.546442</td>
</tr>
<tr>
<td>Cedar Island, VA (VA4)</td>
<td>13</td>
<td>37.633496</td>
<td>-75.612748</td>
</tr>
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</table>
Table 2.2: Summary of the parameters and priors used in popABC. 2,500,000 genetic trees were simulated and a tolerance of 0.0004 was applied, resulting in 1,000 simulated data points. ICB = inner Chesapeake Bay, Patuxent = Patuxent River, Kent = Kent Island, and CoB = coastal bays.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Prior</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \mu )</td>
<td>Mutation Rate ( (\text{site}^{-1} \text{ generation}^{-1}) )</td>
<td>Lognormal ((-3.0,0.5,0.5,0.5))</td>
</tr>
<tr>
<td>( N_{e1} )</td>
<td>Effective Population Size, Kent Island (individuals)</td>
<td>Uniform ((0, 5000))</td>
</tr>
<tr>
<td>( N_{e2} )</td>
<td>Effective Population Size, Patuxent (individuals)</td>
<td>Uniform ((0, 5000))</td>
</tr>
<tr>
<td>( N_{e3} )</td>
<td>Effective Population Size, ICB (individuals)</td>
<td>Uniform ((0, 5000))</td>
</tr>
<tr>
<td>( N_{e4} )</td>
<td>Effective Population Size, CoB (individuals)</td>
<td>Uniform ((0, 5000))</td>
</tr>
<tr>
<td>( N_{eA1} )</td>
<td>Ancestral Population Size (individuals)</td>
<td>Uniform ((0, 10000))</td>
</tr>
<tr>
<td>( N_{eA2} )</td>
<td>Ancestral Population Size (individuals)</td>
<td>Uniform ((0, 10000))</td>
</tr>
<tr>
<td>( N_{eA3} )</td>
<td>Ancestral Population Size (individuals)</td>
<td>Uniform ((0, 10000))</td>
</tr>
<tr>
<td>( m_{1} )</td>
<td>Kent -&gt; Patuxent Migration Rate (fraction of immigrants)</td>
<td>Uniform ((0, 0.1))</td>
</tr>
<tr>
<td>( m_{2} )</td>
<td>Patuxent -&gt; Kent Migration Rate (fraction of immigrants)</td>
<td>Uniform ((0, 0.1))</td>
</tr>
<tr>
<td>( m_{3} )</td>
<td>ICB -&gt; CoB Migration Rate (fraction of immigrants)</td>
<td>Uniform ((0, 0.1))</td>
</tr>
<tr>
<td>( m_{4} )</td>
<td>CoB -&gt; ICB Migration Rate (fraction of immigrants)</td>
<td>Uniform ((0, 0.1))</td>
</tr>
<tr>
<td>( m_{A1} )</td>
<td>Ancestral Migration Rate (fraction of immigrants)</td>
<td>Uniform ((0, 0.1))</td>
</tr>
</tbody>
</table>
Table 2.2: continued

<table>
<thead>
<tr>
<th></th>
<th>Ancestral Migration Rate (fraction of immigrants)</th>
<th>Uniform (0, 0.1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$m_{A2}$</td>
<td>$T_{ev,1}$</td>
<td>Splitting Event 1 (years)</td>
</tr>
<tr>
<td>$T_{ev,2}$</td>
<td>Splitting Event 2 (years)</td>
<td>$T_{ev,1} + \text{Uniform (0, 5000)}$</td>
</tr>
<tr>
<td>$T_{ev,3}$</td>
<td>Splitting Event 3 (years)</td>
<td>$T_{ev,2} + \text{Uniform (0, 5000)}$</td>
</tr>
<tr>
<td>$T$</td>
<td>Generation Time</td>
<td>12 years (constant)</td>
</tr>
<tr>
<td>$Top$</td>
<td>Tree Topology (18 possible arrangements)</td>
<td>Uniform (0, 18)</td>
</tr>
</tbody>
</table>
Table 2.3: Mean observed ($H_o$) and expected ($H_e$) heterozygosities and number of alleles ($N_A$) for each locus.

<table>
<thead>
<tr>
<th>Locus</th>
<th>$H_o$</th>
<th>$H_e$</th>
<th>$N_A$</th>
</tr>
</thead>
<tbody>
<tr>
<td>B91</td>
<td>0.49</td>
<td>0.53</td>
<td>2.29</td>
</tr>
<tr>
<td>B08</td>
<td>0.84</td>
<td>0.84</td>
<td>7.71</td>
</tr>
<tr>
<td>D93</td>
<td>0.52</td>
<td>0.53</td>
<td>4.57</td>
</tr>
<tr>
<td>A18</td>
<td>0.76</td>
<td>0.73</td>
<td>6.07</td>
</tr>
<tr>
<td>D87</td>
<td>0.84</td>
<td>0.84</td>
<td>10.57</td>
</tr>
<tr>
<td>B67</td>
<td>0.40</td>
<td>0.42</td>
<td>2.07</td>
</tr>
<tr>
<td>D90</td>
<td>0.87</td>
<td>0.83</td>
<td>9.00</td>
</tr>
<tr>
<td>D55</td>
<td>0.86</td>
<td>0.84</td>
<td>10.50</td>
</tr>
<tr>
<td>D114</td>
<td>0.76</td>
<td>0.73</td>
<td>8.14</td>
</tr>
<tr>
<td>D121</td>
<td>0.90</td>
<td>0.89</td>
<td>11.86</td>
</tr>
<tr>
<td>D62</td>
<td>0.81</td>
<td>0.82</td>
<td>8.71</td>
</tr>
</tbody>
</table>
Table 2.4: Estimates of population differentiation. Values below the diagonal are $D_{est}$ values, with 95% CIs in brackets. Values above the diagonal represent P-values, with Bonferroni corrections shown in parentheses.

<table>
<thead>
<tr>
<th></th>
<th>Kent Island</th>
<th>Patuxent River</th>
<th>Coastal Bays</th>
<th>Inner CB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kent Island</td>
<td>-</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.006)</td>
<td>(0.006)</td>
<td>(0.006)</td>
</tr>
<tr>
<td>Patuxent River</td>
<td>0.0443</td>
<td>-</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[0.0380 – 0.0523]</td>
<td>(0.006)</td>
<td>(0.006)</td>
</tr>
<tr>
<td>Coastal Bays</td>
<td>0.0374</td>
<td>0.0654</td>
<td>-</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[0.0320 – 0.0486]</td>
<td>[0.0576 – 0.0747]</td>
<td>(0.006)</td>
</tr>
<tr>
<td>Inner CB</td>
<td>0.0155</td>
<td>0.0291</td>
<td>0.0333</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[0.0116 – 0.0219]</td>
<td>[0.0254 – 0.0343]</td>
<td>[0.0274 – 0.0414]</td>
</tr>
</tbody>
</table>
Figure 2.1: Sampling localities and STRUCTURE results. Top left: the distribution of Terrapins (shaded red), and the location of the study (black box). Main figure: four terrapin populations were demarcated. Triangles indicate the Patuxent River, stars represent Kent Island, squares represent the coastal bays, and circles represent inner Chesapeake Bay. At $\Delta K = 2$, the Patuxent River forms a cluster while the remaining sampling localities form a second cluster. This second cluster is composed of three subclusters ($\Delta K = 3$), which contains Kent Island, inner Chesapeake Bay, and the coastal bays.
Figure 2.2: Approximate Bayesian computation posterior (solid line) and hyperprior (dotted line; log-normal) distributions for \( l \), the mutation rate used to convert \( \theta \) into \( N_e \) and \( M \) into \( m_h \) (MIGRATE), for comparisons with \( m \) from BAYESASS. The mode is \( 1.9 \times 10^{-3.36} \), or \( 4.39 \times 10^{-4} \) mutations site\(^{-1}\) generation\(^{-1}\).
Figure 2.3: Gene flow in Chesapeake Bay. KI = Kent Island, PR = the Patuxent River, ICB = inner Chesapeake Bay, and CoB = the coastal bays. Thin lines represent estimates of m or Δm of <0.01, intermediate lines represent estimates of 0.01–0.05, and thick lines represent estimates of >0.05. (A) Results from MIGRATE. Numbers within boxes denote Ne, while values above arrows indicate proportion of immigrants (m_h). (B) Contemporary gene flow rates determined by BAYESASS. Numbers within boxes indicate the proportion of individuals to remain within the population, and values above arrows indicate the proportion of immigrants (m) to their respective populations. (C) Historical gene flow rates subtracted from contemporary rates (m – m_h = Δm). Dashed arrows indicate gene flow routes that have reduced contemporary gene flow (-Δm) and solid arrows indicate routes that have increased contemporary gene flow (+Δm).
CHAPTER 3: EFFECTIVE POPULATION SIZE IN DIAMONDBACK TERRAPINS: INCONGRUENCE BETWEEN MOLECULAR AND ECOLOGICAL ESTIMATES

Introduction

Effective Population Size

Effective population size \( (N_e) \) is a fundamental concept in evolutionary biology, and is used in many fields from population genetics and phylogenetics to conservation genetics and molecular ecology. Proposed by Wright (1931), the effective population size was defined as "the number of breeding individuals in an idealized population that would show the same amount of dispersion of allele frequencies under random genetic drift or the same amount of inbreeding as the population under consideration." Frankham et al. (2010) recently defined the effective population size as “The size of an ideal population that loses heterozygosity (or becomes inbred or drifts) at the same rate as the observed population.” The concept of an “ideal population” is central to any definition of effective population size.

A commonly used model of the “ideal population” is the Wright-Fisher model (Fisher 1922; Wright 1931; Crow 1987, 2010). In this model, generations do not overlap, each individual reproduces a mean of one offspring (to maintain constant population size), the population is panmictic, two alleles with frequencies \( p \) and \( q \) are present, and selection and mutation are absent. These assumptions were made to evaluate how allele frequencies behave solely under the process of genetic drift (Tran et al. 2013). The original Wright-Fisher model has since been expanded to incorporate overlapping generations and multiple alleles across loci (Moran 1958; Tran et al. 2013). More recently, effective population has been scaled by gene genealogies (the coalescent),
allowing population genetic processes to be incorporated into higher-level (phylogenetic) analyses (Nordberg and Krone 2002; Sjödin et al. 2005; Wakeley 2009). Indeed, the concept of the effective population has permeated into many fields of biology, including species demarcation (de Queiroz 2007; Kuchta and Wake 2016).

Although the effective population size is a fundamental evolutionary concept and assumes a simple population model, there is no universal statistic for its estimation (Frankham et al. 2010). Rather, several statistics exist to estimate \( N_e \), which can be derived from sex-ratios, organismal ploidy, allele frequencies, levels of inbreeding, and other approaches, as well as combinations of these statistics (Fisher 1922; Wright 1931, 1938; Crow 2010). A sample of these statistics is found in Table 3.1.

The estimation of \( N_e \) from multiple methods can create confusion with respect to which approach is best suited to a specific study system. This confusion is compounded by reference to “the” effective population size, which implies a population can only exhibit a single effective population size (Sjödin et al. 2005). Moreover, \( N_e \) is sometimes incorrectly associated with the census population size (\( N_c \)), which has a singular value at one point in time (Nordberg and Krone 2002). Thus, the complexities in estimating \( N_e \) can lead to misconceptions. In addition, the assumptions made by “idealized” populations are violated by all populations to varying degrees (Frankham et al. 2010). For example, Elephant Seals (Mirounga angustirostris) are extremely polygynous, and consequently deviate from assumptions of Fisherian sex-ratios and panmixia (Fabiani et al. 2004). Thus, unless there is a clear biological basis for choosing a particular model, which is
often not the case, it is useful for studies investigating effective population size to utilize a variety of approaches.

**Study System**

Diamondback Terrapins (*Malaclemys terrapin*) inhabit brackish water habitats along the Gulf and eastern coastlines of the United States (Ernst and Barbour 1989). Terrapin populations typically exhibit weak structure at the local and regional scales, and genetic diversity tends to be higher in Atlantic populations than in Gulf populations (Hauswaldt and Glenn 2005; Hart et al. 2014; Converse and Kuchta 2017). Near Chesapeake Bay, four genotypic clusters have been delimited (Converse et al. 2015): Kent Island (KI), the Patuxent River (PR), inner Chesapeake Bay (ICB) and the coastal bays (CoB) constitute the known genotypic clusters. However, the degree of population structure is weak and exhibit high levels of admixture. All populations are characterized by high levels of heterozygosity, high mean number of alleles, and high allelic richness (Figure 3.1; Converse et al. 2015). Gene flow is highest between KI and the PR, and from the CoB to ICB (Converse et al. 2015).

Poplar Island (PI) is situated in Chesapeake Bay approximately 26 km south of the Bay Bridge and is located within the ICB genotypic cluster (Figure 3.1; Converse et al. 2015). Poplar Island is actively under construction as an ecological restoration site, with wetlands and upland forests in different stages of completion. Because female terrapins nest on sandy beaches (Roosenburg 1994), PI has vast areas with suitable nesting habitat. Female terrapins lay hundreds of nests on PI each year, and their nests enjoy relatively low levels of mortality due to the absence of mammalian nest predators.
(Feinberg and Burke 2003; Roosenburg et al. 2014). Furthermore, the construction of artificial wetlands has created habitat capable of sustaining terrapin populations on PI for the foreseeable future. The combination of optimal nesting habitat, low nest mortality, suitable habitat for hatchling and adult terrapins, and a lack of predators creates unique circumstances that allow biologists to study terrapin population dynamics in fine detail. Nesting and demographic studies as well as survivorship experiments among cohorts have been ongoing on PI for over a decade (Roosenburg et al., unpublished data).

Thus, terrapin populations in Chesapeake Bay provide an excellent opportunity to estimate effective population sizes using molecular and ecological approaches, especially for populations that exhibit weak structure. In this article, I compare estimates of effective population size generated from molecular and ecological data, and assess congruence between the two approaches. I further estimate $N_e$ from molecular data using a variety of methods, including approximate Bayesian computation (ABC), model testing in a Bayesian framework, and frequentist-based methods, to test for levels of concordance and discordance when estimating $N_e$ with different methodologies.

Methods

Molecular Data

I used 11 polymorphic microsatellite loci developed in a species that is closely related to the Diamondback Terrapin, the Bog Turtle (*Glyptemys muhlenbergii*), for all molecular estimates of effective population size (King and Julian 2004), a dataset given to me by Timothy King. I used the genotypic clusters demarcated by Converse et al. (2015) (KI, PR, ICB, CoB) as a priori population assignments for all molecular estimates.
of effective population size. PCR chemistry and protocols for these loci are detailed in King and Julian (2004).

To estimate effective population sizes in a Bayesian model-testing framework, I used MIGRATE v. 3.6.11 (Beerli 2008) and compared 12 models of population structure (Figure 3.2). Each model used uniform distributions to model $\Theta (=4N_e\mu)$ and $M (=m/\mu)$, bounded between 0.001 and 100, and 0 and 2000, respectively. Individual models were explored using a Metropolis coupled Markov chain Monte Carlo (MC$^3$) algorithm composed of 20000 retained steps, with a 50% burn-in and 500 step sampling interval. To improve speed, I ran a Brownian motion model to approximate a stepwise-mutation model, and randomly subsampled 40 individuals at each locus. To facilitate model comparisons, I used an identical starting seed for each model, whereas each model was composed of the same random genotypic subsample at each locus. I tested for the best-fit model using approximate Bézier scores (Beerli and Palczewski 2010), which require the posterior be explored with at least four heated parallel chains (heated at 1.0, 1.5, 3.0, and 1,000,000, respectively). For comparative purposes, I also used harmonic means to select the best-fit model. I replicated each model five times, for a total of 100000 recorded steps per model. To probe model selection consistency, I reran these analyses independently for a second time. I then took the best-fit models from the first and second analyses as determined by Bézier scores and harmonic means and reran the analyses using 25 replicates (Figure 3.3). To convert $\Theta$ into $N_e$, I multiplied $\Theta$ values from the best-fit model by a mutation rate of $4.34 \times 10^{-4}$ mutations site$^{-1}$ generation$^{-1}$ (Converse et al. 2015).

I further estimated effective population sizes using approximate Bayesian
computation (ABC; Beaumont et al. 2002) with the softwares ONeSAMP v. 1.2 (Tallmon et al. 2008) and DIYABC v. 2.04 (Cornuet et al. 2014). In contrast with MIGRATE, ONeSAMP estimates \( N_e \) one population at a time, rather than jointly estimating \( \Theta \) values. ONeSAMP uses a suite of eight common summary statistics (e.g. Wright’s \( F_{IS} \), observed heterozygosity) to infer \( N_e \). For these analyses, I set the lower boundary of \( N_e \) to 2 and the upper boundary to 1000, the minimum and maximum values allowed.

For DIYABC, I investigated a single scenario where terrapins colonized Chesapeake Bay from south to north (northward range expansion). Since ABC models are computed relatively quickly, I analyzed the entire genotypic dataset as opposed to a subsample. I modeled individual locus mutation rates with a gamma distribution under shape 2, with lower and upper boundaries of \( 1 \times 10^{-5} \) and \( 1 \times 10^{-2} \), respectively. The overall mean mutation rate was modeled with a uniform distribution bounded between \( 1 \times 10^{-4} \) and \( 1 \times 10^{-3} \). A generalized stepwise model was used for microsatellite mutation, which allows repeats to increase or decrease by one or more motifs under a geometric distribution (Cornuet et al. 2008). Model parameters for \( N_e \) and divergence dates are provided in Table 3.2. I used 34 summary statistics to generate 1000000 simulated data points, retained the closest 0.1% of simulated data, and then applied polychotomous logistic regression to the retained data (Beaumont et al. 2002). Effective population sizes and divergence date estimates were logit transformed and observed under original model parameters. To test goodness-of-fit, I used the model checking function in DIYABC (Cornuet et al. 2014). This function checks the distribution of simulated data points under the selected model and displays its estimates, which must fall within the observed
summary statistics to be deemed congruent. Following the recommendations of Cornuet et al. (2010), I used a new set of summary statistics to evaluate goodness-of-fit and did not use summary statistics applied to parameter estimates or model selection (Table 3.2).

Finally, I estimated effective population sizes in NeEstimator v. 2.0 (Do et al. 2014) using heterozygosity excess (Zhdanova and Pudovkin 2008), linkage disequilibrium (Waples and Do 2010), and molecular coancestry (Nomura 2008). I used a $P_{\text{crit}}$ value of 0.05 for all analyses. The $P_{\text{crit}}$ value is the lowest frequency at which an allele occurs. If the $P_{\text{crit}}$ value is too low, estimates of effective population size may become biased, as the analysis may incorporate alleles found in a single heterozygote in the population (Do et al. 2014).

**Ecological Approaches**

I captured terrapins and collected mark-recapture data on Poplar Island (Figure 3.1; 38.760886, -76384044) from 2005-2015. Terrapins were captured with fyke nets, modified crab pots, dip nets, and by hand (Figure 3.1), from May-August each summer. I deployed 2-4 fyke nets regularly and used other trapping methods opportunistically. Fyke nets were deployed at 07:30 on Mondays, checked at 07:30 Tuesday through Friday, and removed Friday. Trapping locations were rotated every two weeks to ensure thorough sampling of wetland cells, Poplar Harbor, as well other areas adjacent to Poplar Island.

Captured terrapins were marked with two systems. The first was a passive integrated transponder (PIT) tag (Biomark, Boise, ID, USA), which emits a unique 10 digit alphanumeric code. This code is read by a PIT tag reader (Biomark, Boise, ID, USA) allowing for reliable individual identification. Betadine (Purdue Products L.P.,
Stamford, CT, USA) was used to sterilize the integument in the right femoral pocket, and PIT tags were injected subcutaneously. NewSkin (Prestige Brands, Tarrytown, NY, USA) was used to seal the PIT tag injection site.

Wing bands (“PI-tags,” National Band & Tag Company, Newport, KY, USA) were employed as the second system to identify individuals. PI-tags were engraved with a four digit numeric code proceeded by “PI” (e.g., PI 1234). A small hole was drilled in the ninth right marginal scute of captured individuals and the PI-tag secured in place with needle-nose pliers. Tissue displaced during shell boring was collected and stored in a marked tube containing 95% ethanol. Although PI-tags have fewer combinations relative to PIT tags, they do not require an additional reader to be interpreted, which facilitates quick identification of captured individuals in the field.

In addition to individual markings, I measured (in mm) several morphological traits for all captured terrapins: head width (HW), plastron length (PL), plastron width (PW), carapace height (CH), carapace length (CL), and right pectoral plastron scute (RP), as well as mass (in grams), and age (in years). Individuals were sexed, with individuals that were sexually ambiguous or too young to sex scored as juveniles. If an individual had 10 or more visible annuli reflecting clearly visible growth in recent years, I documented its age as >10 because growth lines in older individuals become unreliable. I did not age younger individuals if the growth rings were too obscured to provide and accurate estimate of age. I assessed the accuracy of aging by confirming the age of recaptures of marked individuals in addition to the recaptures of more than 400 marked hatchlings originally marked at time of hatching.
To estimate the census population size at Poplar Island, I evaluated several models in MARK v. 8.0 (White and Burnham 1999). I created two versions of my mark-recapture dataset: the first dataset included males and females as separate groups, while the second dataset scored males, females, and juveniles as separate groups. For my MARK analyses, I estimated \( N_c \) with models assuming an open population.

I estimated \( N_c \) with POPAN (Schwarz and Arnason 1996) and Burnham Jolly-Seber models (Jolly 1965; Seber 1965; Burnham 1991). Open population models use survival rates and capture probabilities (\( p \)) to estimate \( N_c \) (Cooch and White 2014). They also allow individuals to enter or leave the population in question. However, because permanent emigration and individual death result in the same observation (no further recaptures), they are assumed under a single parameter, \( \Phi \) (apparent survivorship). Open population models further assume that capture and survival probabilities are equal between marked and unmarked individuals, and that the area of the study does not change over time.

POPAN models estimate \( N_c \) from \( \Phi \), \( p \), and the probability of entering the population (PENT). I used the logit link function for parameters \( \Phi \) and \( p \), the log link function for \( N \) (the number of groups for which to estimate \( N_c \)), and the multinomial logit (mlogit) link function for all PENT parameters, as recommend by Cooch and White (2014). I tested several POPAN models assuming time-dependent or constant survivorship (e.g., \( \Phi(t) \), \( \Phi(.) \)), constant and time-dependent capture probabilities (e.g., \( p(.) \), \( p(t) \)), and constant and time-dependent entrance probabilities (e.g., \( \text{PENT}(.) \), \( \text{PENT}(t) \)). I also tested models by sex and with combinations of these assumptions. To facilitate
model selection, I used AIC$_c$ scores (Akaike 1973, 1974) and selected the best-fit model for each dataset.

Burnham Jolly-Seber models use the parameters $\Phi$, $p$, and $\lambda$ (growth rate) to estimate $N_c$, and add an initial population size parameter ($N_0$) at the beginning of the analysis. The PENT parameter from the POPAN model is incorporated in the $\lambda$ parameter in the Burnham Jolly-Seber model (Cooch and White 2014). I used the logit link function for $\Phi$, $p$, and $\lambda$, and the log link function for $N$, as recommend by Cooch and White (2014). To assess model fit, I used AIC$_c$ scores for all analyses.

Results

*Molecular Estimates of $N_e$*

Model testing in MIGRATE indicates that the Against Flow (Conduit) model is the best-fit model for Chesapeake Bay population structure (Bézier = -167283.87, Figure 3.2, Table 3.3). All population models greatly outperformed the No Flow model (Figure 3.2, Table 3.3). From MIGRATE, Kent Island was found to have the smallest effective population size (Table 3.4; mean = 516, median = 678, mode = 330; Figure 3.4), followed by the Patuxent River (5670, 4399, 1105), the coastal bays (17210, 17616, 17733), and inner Chesapeake Bay (36095, 36609, 33430).

Effective population size estimates in DIYABC were less variable within populations (Table 3.4; Figure 3.4). DIYABC estimated the Patuxent River to have the smallest effective population size (mean = 4650, median = 4390, mode = 3660), followed by Kent Island (7100, 7510, 9220), the coastal bays (13500, 13300, 13200), and inner Chesapeake Bay (32700, 33100, 35300). The other ABC software, ONeSAMP, generated
effective population size estimates that were approximately 5-10% of those from DIYABC and MIGRATE (Table 3.4; Figure 3.4). The coastal bays were found to have the smallest effective size (mean = 98, mode = 96), followed by Kent Island (156, 154), the Patuxent River (263, 254), and inner Chesapeake Bay (305, 302).

Effective population size estimates from N\textsubscript{E}Estimator varied by analysis. Effective population size estimates using linkage disequilibrium estimated Kent Island to be the smallest (mean = 266), followed by the coastal bays (551), the Patuxent River (1697) and inner Chesapeake Bay (+\infty). Estimates of effective population size using molecular coancestry estimated Kent Island to be the smallest (36), followed by inner Chesapeake Bay (67). Molecular coancestry found the effective sizes of the Patuxent River and the coastal bays to both be infinity (+\infty). Finally, effective population sizes estimates derived from heterozygosity excess were uninformative, as all populations were estimated to have N\textsubscript{e} = +\infty (Table 3.4; Figure 3.4).

Ecological Estimates of N\textsubscript{e}

From 2006 to 2015, there were 3576 female captures, 532 males, and 1224 juveniles, for a total of 5332 unique captures (Table 3.5). Mark-recapture data suggest that the terrapin population on PI is growing, as the number and proportion of juveniles captured increased steadily (Table 3.5). Using non-Fisherian sex-ratios and raw mark-recapture data, the effective population size near PI is approximately 1822.

My estimates of N\textsubscript{e} using Burnham Jolly-Seber open population models consistently failed to reach numerical convergence (Cooch and White 2014). During likelihood estimation, Burnham Jolly-Seber models apply penalty constraints to keep
parameters consistent relative to one another (Cooch and White 2014). A majority of my models failed to converge, and thus did not generate results. However, several simpler models did reach numerical convergence, but their estimates were not reliable (e.g. $N_c$ of $9.0 \times 10^{13}$).

AICc scores from the POPAN analyses show that the $\Phi_{(t,s)} P_{(t,s)} P_{\text{E}}(t) N_s(s)$ model is the best-fit model for both the male-female and male-female-juvenile datasets (Table 3.6). POPAN estimates of $N_c$ with the male-female dataset indicate there are approximately 943 males in the population [812 – 1080 95% CI; 59.95 SE] and 4533 females [4375 – 4697; 81.98], for a total ($N_c$) of 5476 adults [5208 – 5765] (Table 3.7). When juveniles are included, the male population is estimated to be 936 [833 – 1068; 68.36], the female population is 4680 [4511 – 4856; 88.00], and juveniles 1673 [1621 – 1727; 26.98], for a total ($N_c$) of 7289 individuals [6944 – 7663] (Table 3.7). These estimates do not reflect the number of hatchlings in the population.

Using the $N_c$ estimates from POPAN and non-Fisherian sex-ratios, the PI population has an effective size of 3120 [2753 – 3534] when juveniles are accounted for, and 3122 [2799 – 3481] when they are not (Table 3.7).

**Discussion**

*Molecular Approaches*

I found considerable variation in effective population size among my molecular estimates using the same dataset (Table 3.4; Figure 3.4). ONcSAMP and NeEstimator generated small estimates of $N_c$ relative to MIGRATE and DIYABC for all genotypic clusters (Table 3.4; Figure 3.4). More congruence was observed between MIGRATE and
DIYABC for the CoB and ICB clusters, but these methods diverged in their estimates for the PR and KI clusters (Table 3.4; Figure 3.4). For NeEstimator, I could not determine Ne estimates from molecular coancestry and linkage disequilibrium, or for any Ne estimates from heterozygosity excess.

There are several explanations for the observed incongruence among molecular approaches. The time-scale over which Ne was estimated varied greatly by analysis. For example, MIGRATE operates in a coalescent framework, while NeEstimator does not (Beerli 2008; Do et al. 2010). Consequently, MIGRATE estimates effective population sizes over evolutionary time, which can approach 4Ne generations (thousands of years) if the population is sufficiently large (Beerli 2009). In contrast, NeEstimator estimates the effective population size over “contemporary time,” which Do et al. (2014) define as “…the time periods encompassed by the samples.” Thus, MIGRATE’s estimates of Ne predate European colonization of Chesapeake Bay, while NeEstimator’s estimates of Ne encompass 2003 - 2005, the time window my genetic samples were collected (Converse et al. 2015).

Differences in prior distributions also affect estimates of Ne. MIGRATE and ONeSAMP both estimate Ne from \( \theta \) (=4Neµ), but differ greatly in how the \( \theta \) prior can be set. MIGRATE allows \( \theta \) to assume several prior distributions, while ONeSAMP only estimates \( \theta \) under a uniform distribution. Furthermore, the uniform distributions used by these two programs are not the same. MIGRATE allows the lower and upper boundaries of the uniform prior to be customized, while ONeSAMP does not. For MIGRATE, I set the \( \theta \) prior to be uniform between 0.001 and 100 (P. Beerli, personal communication), while
ONeSAMP bounded the distribution between 2 and 12 (Tallmon et al. 2008). Combined with summary statistics, ONeSAMP’s estimates of \( N_e \) cannot exceed 1000 (Tallmon et al. 2008), well below MIGRATE’s upper estimate of \( \sim 57500 \) given the mutation rate (Converse et al. 2015). Likewise, differences in summary statistics may influence estimates of \( N_e \) for ABC approaches. DIYABC allows up to 12 summary statistics for microsatellites, while ONeSAMP uses the same eight summary statistics for all analyses (Tallmon et al. 2008; Cornuet et al. 2014).

The population model assumed for each analysis also influences estimates of \( N_e \). Gene flow, inbreeding, mutation rates, and recombination rates all affect \( N_e \) (Frankham et al. 2010). MIGRATE incorporates gene flow into its \( N_e \) estimates, while DIYABC, ONeSAMP, and \( N_e \)Estimator do not (Table 3.8; Beerli 2008; Tallmon et al. 2008; Do et al. 2010; Cornuet et al. 2014). MIGRATE, DIYABC, and ONeSAMP incorporate mutation rates, but differ in how they are modeled. MIGRATE uses either a stepwise-mutation model (Kimora and Ohta 1978) or a Brownian motion model to approximate a stepwise-mutation model, while DIYABC assumes a geometric distribution of motif mutation (Cornuet et al. 2008). ONeSAMP does not allow the mutation rate to be modeled (Tallmon et al. 2008). Only DIYABC and \( N_e \)Estimator (linkage disequilibrium) incorporate recombination rates into \( N_e \) estimates, and only ONeSAMP incorporates inbreeding into its \( N_e \) estimates (Table 3.8).

Harmonic means did not agree with Bézier scores for the best-fit model (Table 3.8). Harmonic means are known to poorly approximate likelihood calculations using MCMC (Beerli and Palczewski 2010), and MCMC methods are often used in population
genetics. As computation power increases, other likelihood approximations (e.g. Bézier scores) are more feasible.

Weak population structure and high admixture levels may have impacted my estimates of $N_e$ and was a cause of incongruence among the methods. High admixture is usually a result of high gene flow (Wakeley 2009). In my study, MIGRATE was the only software that quantified gene flow when estimating $N_e$. Thus, in weakly structured populations, molecular estimates of $N_e$ that ignore gene flow may be greatly impacted. Although DIYABC does not estimate gene flow, it was most similar to MIGRATE (Table 3.4; Figure 3.4). This congruence is most likely attributable to the population models of both analyses. $N_e$ estimates from MIGRATE assumed the Against Flow (Conduit) model (Figure 3.2), while DIYABC assumed a one way, south to north colonization model.

Ecological Approaches

Burnham Jolly-Seber open population models often failed to generate estimates of $N_c$, most likely not due to a disconnect between model assumptions and biological reality. For the PI population, the assumptions of the Burnham Jolly-Seber model are reasonable (and similar to POPAN models). The complexity of the likelihood function, coupled with penalty constraints, prevented my models from generating estimates of $N_c$.

POPAN estimates of $N_c$ from both terrapin datasets recovered the same model $(\Phi_{(t,s)} P_{(t,s)} P_E N_{(s)})$ using AICc scores (Table 3.6). In these models, all parameters vary by time, and all parameters vary by sex, with the exception of $P_E$. Previous work (Sheridan et al. 2010) suggest that males disperse further than females, but my data suggests males and females (and juveniles) have the same probability of entering the PI
population. While it is often stated that effective population sizes are ~10% of the census size (Frankham et al. 2010), my mark-recapture data with ecological \( N_e \) estimates suggests the ratio of \( N_e \) to \( N_c \) on PI is 56% [52 – 59%] when juveniles are not factored in, and 43% [40 – 45%] when they are (Table 3.7). As the PI population continues to grow, this percentage may decrease, especially if sex-ratios deviate further from Fisherian ratios.

**Incongruence Between Molecular and Ecological Estimates of \( N_e \)**

For the ICB genotypic cluster, I observed incongruence between ecological and molecular estimates of \( N_e \) (Table 3.4, Table 3.7; Figure 3.4). Differences in temporal and geographic scale are most likely responsible for this incongruence. MIGRATE, DIYABC, and ONeSAMP incorporate mutation rates to estimate \( N_e \), and thus their estimates are over coalescent time (Wakeley 2009). Recent changes in population dynamics are difficult to detect with these methods. Indeed, precipitous and recent changes in population dynamics can introduce ambiguity in coalescent histories, and can result in specious parameter estimates (Beerli 2009). By contrast, ecological mark-recapture methods operate over contemporary time, and are sensitive to recent population changes (Besbeas et al. 2002; Baker et al. 2004). Thus, my genetic approaches may be estimating historical effective population sizes, while my ecological approaches estimate contemporary effective population sizes. However, simultaneous differences in geographic scale complicate comparison between the molecular and ecological estimates of \( N_e \).

Terrapin populations are known to exhibit weak genetic structure but demonstrate nest-site and home range fidelity (Auger 1989; Lovich and Gibbons 1990; Spivey 1998;
Gibbons et al. 2001; Hauswaldt and Glenn 2005; Sheridan et al. 2010; Hart et al. 2014; Petre et al. 2015; Converse et al. 2015; Converse and Kuchta 2017). Over regional scales, this may be of large consequence for estimates of $N_e$. All of the molecular approaches assumed the ICB cluster exhibited no substructure, and thus estimates of $N_e$ reflect the entire ICB genotypic cluster. On the other hand, the open population models and mark-recapture data do not make this assumption. The POPAN and Burnham Jolly-Seber models explicitly state they assume the population in question experiences emigration and immigration (Jolly 1965; Seber 1965; Burnham 1991; Schwarz and Arnason 1996). Consequently, the mark-recapture data may be estimating $N_e$ for a sub-population within the ICB cluster that cannot be detected genetically due to a complete lack of population structure. Thus, the two approaches (molecular and ecological) may be estimating $N_e$ over two geographic scales as well as two time scales.

**Congruence between Ecological and Molecular Estimates**

Weak population structure may be a cause of incongruence between the molecular estimates of $N_e$, adding uncertainty as to which inferences are to be trusted. High admixture and weak population structure likely impact estimates of $N_e$ that do not incorporate population connectivity, and Chesapeake Bay terrapin populations exhibit these characteristics (Converse et al. 2015). Since these populations are connected by high levels of gene flow, the $N_e$ estimates from MIGRATE ($\sim 35000$) are likely the most accurate. DIYABC inferences of $N_e$ for this genotypic cluster are similar to MIGRATE ($\sim 33000$). Indeed, the mark-recapture data suggest the terrapin population near PI harbors at least $\sim 6900$ individuals, and an $N_e$ of at least $\sim 2700$. This result renders estimates of $N_e$
from ONeSAMP (~300) and NeEstimator (~67) for the entire ICB cluster suspect. My data suggest the ICB cluster has an historic size of ~35000, and the local population near PI has a contemporary Ne of ~3100.

**Effective Population Size in Cheloniens**

Studies of effective population size in Cheloniens have revealed considerable variation within and among species. Within Chesapeake Bay, Hart et al. (2014) recovered 95% confidence intervals for KI terrapins at Ne = 7533 – +∞. This interval falls near or within my estimates of 7100 (mean), 7500 (median), and 9220 (mode) from DIYABC (Table 3.4; Figure 3.4). Likewise, Hart et al. (2014) estimated a 95% CI for PR to be 247 – +∞, consistent with my estimates from ONeSAMP (mean = 263, median = 254; Table 3.4; Figure 3.4). Among species of turtles, estimates of Ne vary widely. Pacific Green Turtles (*Chelonia mydas*) near Michoacan, Mexico exhibit a female effective size of 1900 – 2300 (Chassin-Noria et al. 2004); in Australasia, Ne estimates range from 300 – 125000 (Dethmers et al. 2006). Flatback Turtles (*Nanator depressus*) in Australia exhibit an effective size of 1200 – 4200 (Theissinger et al. 2009). In Michigan, USA, Eastern Box Turtles (*Terrapene carolina*) populations range from Ne = 6675 – 9516 (Marsack and Swanson 2009). Northwest Atlantic Leatherback Turtles (*Dermochelys coriacea*) are estimated to have undergone precipitous drops in effective population size, from Ne ~ 3500000 to Ne = 70 (Molfetti et al. 2013). My estimates of Ne in terrapins generally fall within estimates of effective size for other Cheloniens.

My study documented incongruence among molecular approaches for estimates of Ne, and suggests that studies should use more than one metric to evaluate Ne. The
gene
tic diversity within a population is the result of many complex biological phenomena
over varying time scales, and currently no single population genetic model can
encompass this complexity. Consequently, populations are studied with several simpler
models, which may make different underlying assumptions. My study demonstrates that
ecological data can help rule out spurious estimates of $N_e$ when molecular data conflict.

Table 3.1: A sample of equations used to estimate effective population sizes from
different types of data. Variables shown are allele frequency ($p$), the number of males
($N_m$), the number of females ($N_f$), and time ($t$).

<table>
<thead>
<tr>
<th>Type</th>
<th>Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allele frequencies</td>
<td>$N_e = \frac{p(1 - p)}{2\text{var}(p)}$</td>
</tr>
<tr>
<td>Non-Fisherian sex-ratios</td>
<td>$N_e = \frac{4N_m \times N_f}{N_m + N_f}$</td>
</tr>
<tr>
<td>Inbreeding</td>
<td>$N_e = \frac{1}{2 \times \left(1 - \frac{P_{t+1}}{P_t}\right)}$</td>
</tr>
<tr>
<td>Temporal variation (harmonic mean)</td>
<td>$\frac{1}{N_e} = \frac{1}{t} \sum_{i=1}^{t} \frac{1}{N_i}$</td>
</tr>
</tbody>
</table>
Table 3.2: Parameters used to determine $N_e$ in DIYABC. $N_e$ is measured in individuals and $T_n$ in years.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Distribution</th>
<th>Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>$N_{e1}$ (Kent)</td>
<td>$U(10, 10000)$</td>
<td>None</td>
</tr>
<tr>
<td>$N_{e2}$ (Pat)</td>
<td>$U(10, 10000)$</td>
<td>None</td>
</tr>
<tr>
<td>$N_{e3}$ (ICB)</td>
<td>$U(10, 50000)$</td>
<td>None</td>
</tr>
<tr>
<td>$N_{e4}$ (CoB)</td>
<td>$U(10, 25000)$</td>
<td>None</td>
</tr>
<tr>
<td>$T_1$ (Pat, Kent)</td>
<td>$U(0, 5400)$</td>
<td>$T_2 \geq T_1$</td>
</tr>
<tr>
<td>$T_2$ (ICB, Pat, Kent)</td>
<td>$T_1 + U(0, 5400)$</td>
<td>$T_3 \geq T_2$</td>
</tr>
<tr>
<td>$T_3$ (CoB, ICB, Pat, Kent)</td>
<td>$T_2 + U(0, 5400)$</td>
<td>$T_3 &gt; T_1$</td>
</tr>
</tbody>
</table>
Table 3.3: Population models and their respective Bézier scores and harmonic means (HM). Models are found in Figure 3. Scores marked with an asterisk (*) denote the best-fit model from each run. These best-fit models were compared among each other a third time, with 25 replicates. The Against Flow (Conduit) model was found to fit the data best.

<table>
<thead>
<tr>
<th>Model</th>
<th>Bézier 1</th>
<th>HM 1</th>
<th>Bézier 2</th>
<th>HM 2</th>
<th>Bézier 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Against Flow (Conduit)</td>
<td>-696,050.01*</td>
<td>-2,900.31</td>
<td>-741,885.21</td>
<td>-12,516.79</td>
<td>-167,283.87*</td>
</tr>
<tr>
<td>Against Flow</td>
<td>-1,126,892.35</td>
<td>-13,185.13</td>
<td>-1,131,493.31</td>
<td>-3,672.51</td>
<td>NA</td>
</tr>
<tr>
<td>Coastal Escape (Conduit)</td>
<td>-1,403,164.66</td>
<td>2,426.40</td>
<td>-1,378,049.31</td>
<td>-2,626.78</td>
<td>NA</td>
</tr>
<tr>
<td>Central Conduit</td>
<td>-1,406,497.91</td>
<td>-2,570.59</td>
<td>-1,430,924.77</td>
<td>-2,415.99</td>
<td>NA</td>
</tr>
<tr>
<td>With Flow</td>
<td>-1,532,529.78</td>
<td>-22,073.41</td>
<td>-454,854.51*</td>
<td>-3,122.33</td>
<td>-940,968.69</td>
</tr>
<tr>
<td>Tributary Trap (Conduit)</td>
<td>-1,863,853.60</td>
<td>-2,225.91</td>
<td>-1,188,523.09</td>
<td>-2,344.78</td>
<td>NA</td>
</tr>
<tr>
<td>Full Matrix</td>
<td>-1,918,529.84</td>
<td>-2,857.01</td>
<td>-1,757,393.44</td>
<td>-2556.35</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td>Coastal Escape</td>
<td>-2,057,439.59</td>
<td>-2,382.84</td>
<td>-621,170.64</td>
<td>-2,889.32</td>
<td>NA</td>
</tr>
<tr>
<td>Central Sink</td>
<td>-2,295,251.84</td>
<td>-2,337.15</td>
<td>-1,839,373.89</td>
<td>-2,180.14*</td>
<td>-1,468,248.55</td>
</tr>
<tr>
<td>Tributary Trap</td>
<td>-2,386,171.33</td>
<td>-2,138.72*</td>
<td>2,062,235.94</td>
<td>-2,739.67</td>
<td>-1,576,769.90</td>
</tr>
<tr>
<td>With Flow (Conduit)</td>
<td>-6,136,159.11</td>
<td>-969,642.15</td>
<td>-4,999,347.08</td>
<td>-608,126.09</td>
<td>NA</td>
</tr>
<tr>
<td>No Flow</td>
<td>-27,133,017.74</td>
<td>-18,303,896.36</td>
<td>-49,470,297.80</td>
<td>-39,417,145.05</td>
<td>NA</td>
</tr>
</tbody>
</table>
Table 3.4: Effective population size estimates from molecular data. Effective population sizes are listed under mean/median/mode. Values in brackets denote 95% confidence intervals. ONeSAMP does not report modes while NeEstimator only reports means. LD = linkage disequilibrium, HE = heterozygosity excess, and MoCo = molecular coancestry.

<table>
<thead>
<tr>
<th>Method</th>
<th>Ne ICB</th>
<th>Ne KI</th>
<th>Ne PR</th>
<th>Ne CoB</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIGRATE</td>
<td>36095/36609/33430</td>
<td>516/678/330</td>
<td>5670/4399/1105</td>
<td>17210/17616/17733</td>
</tr>
<tr>
<td></td>
<td>[15000 – 58140]</td>
<td>[0 – 1473]</td>
<td>[0 – 44380]</td>
<td>[16318 – 19147]</td>
</tr>
<tr>
<td>DIYABC</td>
<td>32700/33100/35300</td>
<td>7100/7500/9220</td>
<td>4650/4390/3660</td>
<td>13500/13300/13200</td>
</tr>
<tr>
<td></td>
<td>[15600 – 46700]</td>
<td>[3010 – 9720]</td>
<td>[1650 – 8700]</td>
<td>[7350 – 19800]</td>
</tr>
<tr>
<td>ONeSAMP</td>
<td>305/302/NA</td>
<td>156/154/NA</td>
<td>263/254/NA</td>
<td>98/96/NA</td>
</tr>
<tr>
<td></td>
<td>[265 – 361]</td>
<td>[139 – 188]</td>
<td>[195 – 467]</td>
<td>[78 – 157]</td>
</tr>
<tr>
<td>NeEstimator</td>
<td>LD</td>
<td>+∞/NA/NA</td>
<td>266/NA/NA</td>
<td>1697/NA/NA</td>
</tr>
<tr>
<td></td>
<td>[+∞ – +∞]</td>
<td>[149 – 861]</td>
<td>[375 – +∞]</td>
<td>[182 – +∞]</td>
</tr>
</tbody>
</table>
Table 3.4: continued

<table>
<thead>
<tr>
<th></th>
<th>+∞/NA/NA</th>
<th>+∞/NA/NA</th>
<th>+∞/NA/NA</th>
<th>+∞/NA/NA</th>
</tr>
</thead>
<tbody>
<tr>
<td>HE</td>
<td>[73 - +∞]</td>
<td>[23 - +∞]</td>
<td>[23 - +∞]</td>
<td>[27 - +∞]</td>
</tr>
<tr>
<td>MoCo</td>
<td>67/NA/NA</td>
<td>36/NA/NA</td>
<td>+∞/NA/NA</td>
<td>+∞/NA/NA</td>
</tr>
<tr>
<td></td>
<td>[5 – 205]</td>
<td>[27 – 668]</td>
<td>[+∞ – +∞]</td>
<td>[+∞ – +∞]</td>
</tr>
</tbody>
</table>
Table 3.5: Unique individual captures by sex and year.

<table>
<thead>
<tr>
<th>Year</th>
<th>Females</th>
<th>Males</th>
<th>Juveniles</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>2006</td>
<td>62</td>
<td>27</td>
<td>30</td>
<td>119</td>
</tr>
<tr>
<td>2007</td>
<td>117</td>
<td>36</td>
<td>14</td>
<td>167</td>
</tr>
<tr>
<td>2008</td>
<td>180</td>
<td>18</td>
<td>26</td>
<td>224</td>
</tr>
<tr>
<td>2009</td>
<td>402</td>
<td>35</td>
<td>32</td>
<td>469</td>
</tr>
<tr>
<td>2010</td>
<td>290</td>
<td>29</td>
<td>195</td>
<td>514</td>
</tr>
<tr>
<td>2011</td>
<td>439</td>
<td>40</td>
<td>144</td>
<td>623</td>
</tr>
<tr>
<td>2012</td>
<td>552</td>
<td>109</td>
<td>105</td>
<td>766</td>
</tr>
<tr>
<td>2013</td>
<td>696</td>
<td>102</td>
<td>164</td>
<td>962</td>
</tr>
<tr>
<td>2014</td>
<td>475</td>
<td>56</td>
<td>211</td>
<td>742</td>
</tr>
<tr>
<td>2015</td>
<td>363</td>
<td>80</td>
<td>303</td>
<td>746</td>
</tr>
<tr>
<td>Total</td>
<td>3576</td>
<td>532</td>
<td>1224</td>
<td>5332</td>
</tr>
</tbody>
</table>
Table 3.6: Top: the five best POPAN models according to AICc scores for the male-female only dataset. Bottom: the five best POPAN models according to AICc scores for the male-female-juvenile dataset

<table>
<thead>
<tr>
<th>Model</th>
<th>AICc</th>
<th>ΔAICc</th>
<th>AICc Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>M+F dataset</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\Phi_{t,s} p_{t,s} \text{PENT}_t \text{N}_s$</td>
<td>8953.64</td>
<td>0.00</td>
<td>1.00</td>
</tr>
<tr>
<td>$\Phi_{t,s} p_t \text{PENT}_t \text{N}_s$</td>
<td>9024.03</td>
<td>70.39</td>
<td>0.00</td>
</tr>
<tr>
<td>$\Phi_{t,s} p_{t,s} \text{PENT}_t \text{N}_s$</td>
<td>9145.55</td>
<td>191.91</td>
<td>0.00</td>
</tr>
<tr>
<td>$\Phi_{t,s} p_{t,s} \text{PENT}_{t,s} \text{N}_s$</td>
<td>9309.62</td>
<td>355.98</td>
<td>0.00</td>
</tr>
<tr>
<td>$\Phi_{t,s} p_{t,s} \text{PENT}_t \text{N}_s$</td>
<td>9374.05</td>
<td>420.41</td>
<td>0.00</td>
</tr>
<tr>
<td><strong>M+F+J dataset</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\Phi_{t,s} p_{t,s} \text{PENT}_t \text{N}_s$</td>
<td>11691.04</td>
<td>0.00</td>
<td>1.00</td>
</tr>
<tr>
<td>$\Phi_{(t,s)} p_{t,s} \text{PENT}_t \text{N}_s$</td>
<td>11967.82</td>
<td>276.74</td>
<td>0.00</td>
</tr>
<tr>
<td>$\Phi_{(t,s)} p_{t,s} \text{PENT}_t \text{N}_s$</td>
<td>12135.00</td>
<td>443.97</td>
<td>0.00</td>
</tr>
<tr>
<td>$\Phi_{t,s} p_t \text{PENT}_t \text{N}_s$</td>
<td>12295.70</td>
<td>604.67</td>
<td>0.00</td>
</tr>
<tr>
<td>$\Phi_{t,s} p_{t,s} \text{PENT}_{t,s} \text{N}_s$</td>
<td>12333.59</td>
<td>642.56</td>
<td>0.00</td>
</tr>
</tbody>
</table>
Table 3.7: Top: Census population estimates \((N_c)\) from POPAN using both datasets. M = male, F = female, J = juvenile. Values in brackets denote 95% confidence intervals. Bottom: Effective population size estimates \((N_e)\) for the male-female and male-female-juvenile datasets.

<table>
<thead>
<tr>
<th>Dataset</th>
<th>(N_c) Males</th>
<th>(N_c) Females</th>
<th>(N_c) Juveniles</th>
<th>(N_c) Total</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>(N_e)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>M+F</td>
<td>3120</td>
</tr>
<tr>
<td>M+F+J</td>
<td>3122</td>
</tr>
</tbody>
</table>
Table 3.8: Summary of evolutionary processes and population dynamics incorporated by population genetic software. “Custom” indicates the software allows the user to model the parameter in question.

* For estimates of $N_e$ derived from linkage-disequilibrium.

<table>
<thead>
<tr>
<th>Software</th>
<th>Gene Flow</th>
<th>Recombination</th>
<th>Inbreeding</th>
<th>Coalescent</th>
<th>Mutation</th>
<th>Population Models</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIGRATE</td>
<td>Y/custom</td>
<td>N</td>
<td>N</td>
<td>Y</td>
<td>Y/custom</td>
<td>Y/custom</td>
</tr>
<tr>
<td>DIYABC</td>
<td>N</td>
<td>Y/custom</td>
<td>N</td>
<td>Y</td>
<td>Y/custom</td>
<td>Y/custom</td>
</tr>
<tr>
<td>ONeSAMP</td>
<td>N</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>NeEstimator</td>
<td>N</td>
<td>Y*</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
</tbody>
</table>
Figure 3.1: Left: Sampling localities (diamonds) by genotypic clusters from Converse et al (2015). Sampling localities located the PR genotypic cluster are listed in yellow font, KI in blue, ICB in purple, and CoB in red. Poplar Island (PI) is found within the and is denoted with a star. Right: Arial view of Poplar Island (PI) in 2013. Wetland cells are located to the east and planned upland forests to the west. Poplar Harbor is outlined in yellow, with Jefferson Island located to the north and Coaches Island to the south. Fyke net trapping locations are shown in red.
Figure 3.2: Twelve (12) MIGRATE models of Chesapeake Bay population structure.
Figure 3.3: Analysis flow for testing MIGRATE models. Each model was replicated five times, and evaluated with Bézier scores, harmonic means, and log Bayes factor (LBF). This initial analysis step was replicated a second time. The best models from these two runs were then replicated 25 times and tested against one another using Bézier scores and LBF.
Figure 3.4: Effective population sizes in Chesapeake Bay. MIGRATE = blue; DIYABC = red; ONeSAMP = purple; NeEstimator—linkage disequilibrium = black, sold; NeEstimator—heterozygosity excess = black, long-dash; NeEstimator—molecular coancestry = black, short-dash.
CHAPTER 4: TURTLE SOUP, PROHIBITION, AND THE POPULATION GENETIC STRUCTURE OF DIAMONDBACK TERRAPINS

Introduction

Turtle soup was a popular food item in the United States during late nineteenth and early twentieth centuries. Although many turtle species were consumed, diamondback terrapins (*Malaclemys terrapin*) were considered a delicacy and were highly sought. The historical market price for terrapins demonstrates their popularity: a dozen larger terrapin sold for $70.00 during 1915-1920 (Coker 1920), or ~$830 today.

Recipes for turtle soup varied, but many contained sherry. However, in 1920 the United States ratified the Eighteenth Amendment, banning the production, sale, and transport of alcoholic beverages (Prohibition). The sherry used to make turtle soup became difficult to procure, and demand for turtle soup plummeted. After the market crashed, several terrapin farms purportedly dumped their stocks into local waters.

Prior to Prohibition, intense demand for turtle soup resulted in the decline of terrapins across large portions of their range, particularly along the Atlantic seaboard (Kennedy 2017). Due to their larger size, female terrapins were preferred (Coker 1920).

To combat extirpation and supplement the turtle soup market, terrapin farms were established by private businesses and governmental agencies to explore domestication and cultivation (Hay 1917; Hildebrand & Hatsel 1926; Hildebrand 1929, 1933). Terrapins from the mid- and north Atlantic were preferred for their superior taste and size. In particular, terrapins from Chesapeake Bay were the favorite and were sold with the moniker “Chesapeakes” (Hay 1917; Coker 1920). In 1891, approximately 40,000 kg
of terrapin were harvested from Chesapeake Bay alone, but by 1920 terrapin harvests plummeted to ~370 kg (Cook 1989). As wild stocks dwindled, terrapins were translocated from other regions in an effort to maintain stock solvency (Coker 1920). Terrapins from North and South Carolina (“Carolinas”) were frequently shipped to other states, while terrapins from Florida were derided as too small and insipid in flavor (Hay 1917). Terrapins from Texas achieved a desirable size but lacked the flavor of “Chesapeakes” and “Carolinas,” and some terrapin farms hybridized terrapins with the goal of creating a quick-growing, yet flavorful, terrapin (Hildebrand 1933). Hybridization experiments between terrapins from Texas and the Carolinas began in 1914 at the U.S. Fisheries Biological Station in Beaufort, North Carolina, and the first hybrid individuals were confirmed in 1919 (Hildebrand 1933). However, Prohibition followed these hybridization experiments. As terrapin farms along the Atlantic coast closed, they purportedly released their mixed stocks into local waters. The population admixture that resulted is poorly known.

Previous work has detected hints of the influence of translocation on patterns of terrapin genetic diversity (Converse and Kuchta 2017). Terrapins from South Carolina were shown to be more similar to Texas populations, while Florida populations were identified as genetically distinct (Hauswaldt & Glenn 2005; Hart et al. 2014; Drabeck et al. 2014). In addition, dramatic increases in contemporary gene flow into Chesapeake Bay were interpreted to be the result of translocation (Converse et al. 2015). Alternatively, documented patterns of genetic diversity could be due to natural movements. For example, the Suwannee Seaway is a hypothetical embayment that
existed during the early Miocene and late Pliocene (Bert 1986; Webb 1990; Randazzo & Jones 1997; Lee & Ó Foighil 2003). This seaway ran through the northern part of present-day Florida, potentially facilitating gene flow between the Gulf and the Atlantic while isolating populations in peninsular Florida (Converse and Kuchta 2017).

Here I use microsatellite data to examine patterns of genetic variation across the terrapins’ range and report evidence of human-mediated gene flow that is consistent with historical accounts of terrapin translocation. I accomplish this by: i) inferring population structure in a Bayesian framework, as well as with discriminate analysis of principal components (DAPC); ii) estimating both historical and contemporary levels of gene flow and comparing them to infer changes in gene flow over time; iii) testing alternative models of population structure, including stepping-stone models and models that include translocation events; and iv) surveying for population bottlenecks. Based on previous studies and historical documentation, I hypothesized: i) admixture between Atlantic and Gulf populations, but not with Florida populations; ii) some non-adjacent populations (e.g. Texas and New Jersey) would show increases in contemporary gene flow, consistent with translocation; iii) population genetic models incorporating translocation would outperform other models; and iv) genetic tests for bottlenecks would fail to detect population contractions in populations known to have experienced serious decline, due to human-mediated influxes of genetic diversity.
Methods

Sampling and Population Structure

I analyzed six polymorphic microsatellite loci (Hauswaldt and Glenn 2003) from 12 sampling localities throughout the Gulf and Atlantic seabords (Figure 4.1A), a dataset donated by Susanne Hauswaldt. This dataset includes sampling from Texas (TX), Florida (FL), South Carolina (SC), North Carolina (NC), Maryland (MD), New Jersey (NJ), and New York (NY) during 2001 (Figure 4.1A). Tests of allelic richness, heterozygosity, and number of alleles, as well as lab procedures and PCR protocols are available in Hauswaldt and Glenn (2005).

I demarcated coastal population structure with two methods. The first was a Bayesian analysis in the program STRUCTURE v. 2.3 (Pritchard et al. 2000). The second was a discriminate analysis of principal components (DAPC) in the R package adegenet v. 1.4-2 (Jombart 2008). STRUCTURE delineates genetic clusters by maximizing conformity to Hardy-Weinberg equilibrium while simultaneously minimizing linkage disequilibrium among loci for K user-defined clusters, while DAPC utilizes k-means to maximize variation between groups after PCA transformation. For STRUCTURE, I ran K from 1 to 12 (sampling locations), replicating each value of K ten times, each with a random starting seed. Individual runs were composed of a Markov chain Monte Carlo (MCMC) algorithm of 700,000 steps, with the first 50% removed as burn-in. I used the admixture model, the LOCprior, and LOCISPOP prior for all runs. Preferred values of ΔK were computed with the Evanno method (Evanno et al. 2005) in STRUCTURE HARVESTER v. 0.6.94 (Earl & vonHoldt 2011). Output from STRUCTURE HARVESTER
was processed in CLUMPP v. 1.1.2 (Jakobsson & Rosenberg 2007) to control for labeling switching and multimodality. DISTRUCT v. 1.0 (Rosenberg 2003) was used to visualize the data. I ran STRUCTURE in a hierarchical fashion: first I ran an initial analysis to detect basal levels of structure (Evanno et al. 2005), and then I searched for additional structure within each identified population individually.

For DAPC, I first optimized the number of PC axes to avoid under- or over-fitting my population genetic models. I accomplished this with cross-validation, which uses stratified random sampling and divides the dataset into a training set and a validation set. I partitioned the training set to be 90% of the data and the validation set to 10%, and employed 100 replicates. The cross-validation method estimated 60 axes should be retained. The Bayesian information criterion (BIC) was used to choose the optimum number of clusters for DAPC analysis. Four discriminate functions (DF) were retained for each analysis.

**Historical and Contemporary Gene Flow**

I used MIGRATE v. 3.6.5 (Beerli 2009) to estimate historical gene flow levels ($M = m_h/\mu$: proportion of migrants per generation, scaled by mutation rate). For these analyses I treated sampling localities as populations with the exception of South Carolina. Because South Carolina lacked structure among its sampling localities (Figure 4.1G; see results), I treated all South Carolina sampling localities as a single population. I randomly subsampled 40 individuals from each population and slice sampled the posterior distribution with a MCMC of 5,000,000 steps with the first 50% removed as burn-in. Each MCMC consisted of four statically heated parallel chains sampled every 500
iterations. Five independent replicates were run, totaling 25,000,000 MCMC steps. Estimates of $\theta (=4N_e\mu$: effective population size, scaled by mutation rate) were modeled under a uniform distribution bounded between 0.0001 and 100, and historical gene flow estimates were bounded between 0 and 2000.

For estimates of contemporary gene flow ($m$: proportion of migrants per generation), I used BAYESASS v. 3.0 (Wilson & Rannala 2003). I ran 10 independent MCMC simulations with random starting seeds (Faubet et al. 2007) for 30,000,000 steps, and sampled every 3,000 steps. I discarded the first 50% as burn-in. I used TRACER v. 1.5 (Rambaut & Drummond 2007) to visualize MCMC simulations, and used R scripts to calculate a Bayesian deviancy measure to determine the run that best fit the data (Spiegelhalter et al. 2002; Meirmans 2014).

**Temporal Changes in Gene Flow**

Since MIGRATE uses the coalescent, it estimates gene flow over long periods of time, approximately $4N_e$ generations (several thousand years) in the past (Beerli 2009). Thus, its gene flow estimates pre-date translocation events in the early twentieth century. By contrast, BAYESASS estimates gene flow “…over the last several generations” (Wilson & Rannala 2003), where “several” is commonly interpreted as roughly five generations (e.g. Chiucchi & Gibbs 2010; Converse et al. 2015). With a generation time of 12 years (Roosenburg, unpublished data), the contemporary gene flow estimates of BAYESASS are within the last 60 years or so. These estimates post-date known terrapin translocation events.
To estimate temporal changes in gene flow, I compared historical and contemporary estimates. First, I multiplied the historical gene flow estimates from MIGRATE \((M = m_h/\mu)\) by a mutation rate \((\mu)\) of \(2.72 \times 10^{-3}\). This mutation rate was estimated explicitly for the microsatellites used by Hauswaldt (2004). The resulting values are subtracted from the contemporary gene flow estimates \((m)\) generated by BAYESASS. Thus, \(\Delta m = m - m_h\). Positive values of \(\Delta m\) indicate increased levels of contemporary gene flow, negative values indicate reduced contemporary gene flow, and values near zero indicate no temporal change in gene flow.

*Testing Coastal Population Structure*

I compared eight models of gene flow in MIGRATE, generated with the methods described above (Figure 4.2). My initial model was a linear stepping-stone (Model A), which restricted gene flow to adjacent populations. I then added or removed gene flow routes from Model A based on results from STRUCTURE, DAPC, and my \(\Delta m\) estimates. Models B-F incorporated gene flow from translocation events with varying connectivity to Florida. Models G and H modeled the Suwannee Seaway. I used approximate Bézier scores and log Bayes factors (LBF) to determine which model best explained coastal population structure.

*Bottlenecks*

BOTTLENECK v. 1.2.02 (Piry et al. 1999) was used to test for bottlenecks. Locus mutation was modeled with the stepwise-mutation model (SMM) and the two-phase model (TPM). The TPM modeled 95% of mutations as single-step while multi-step mutations were modeled with 12% variance (Piry et al. 1999). Each test consisted of
20,000 permutations. I tested for bottlenecks by sampling locality and by STRUCTURE cluster. I used the Wilcoxon signed-rank test, which detects bottlenecks 25 - 250 generations in the past (Cornuet & Luikart 1996), and a mode-shift test, which detects bottlenecks “…within the past few dozen generations” (Luikart & Cornuet 1998).

Results

*Population Structure — STRUCTURE*

Initial runs of STRUCTURE found \(\Delta K = 2\) best describes coastal population structure (Figure 4.1B). However, \(\Delta K\) scores of 4 and 7 are comparable, and I also report them for comparative purposes (Figure 4.1C,D). As \(\Delta K\) identifies basal levels of hierarchical structure (Evanno et al. 2005), I also tested for substructure within groups for \(\Delta K = 2\), which included northern and southern groups. Inference of population substructure within the northern group (NC, MD, NJ, NY) revealed \(K = 2\) or \(K = 3\) subclusters (Figure 4.1E,F). This is due to two solutions of \(K\) having overlapping likelihood scores. If \(K = 2\) is adopted, NJ and NY constitute a cluster (Figure 4.1E), while if \(K = 3\) is adopted, they form separate subclusters (Figure 4.1F). The southern group (TX, FL, SC) included \(\Delta K = 3\) subclusters (Figure 4.1G), one for each state. I did not detect substructure among SC sampling localities. Thus, in total there are either five or six genotypic clusters, dependent upon \(K = 2\) or \(K = 3\) for north Atlantic terrapins.

*Population Structure — DAPC*

BIC indicated the optimum number of clusters is six (Figure 4.3). DAPC for the six-cluster analysis is shown in Figure 4.4, and membership probabilities are provided in Figure 4.5. Mid- and north Atlantic terrapins form clusters 1, 3, and 6; cluster 4 diagnoses
Chesapeake Bay (MD) terrapins, and cluster 2 includes populations located in the Gulf (TX, FL). Cluster 5 is composed of SC terrapins and links cluster 4 with clusters 1, 3, and 6. Cluster 2 exhibits no overlap with any cluster.

If DAPC is run on sampling localities (Figure 4.1A), an alternative population genetic structure is delimited that resembles the spatial distribution of my sampling localities (Figures 4.6, 4.7). Clusters 1-6 (SC1-6) overlap heavily. Cluster 7 (NC) deviates slightly from clusters 1-6. Cluster 8 (MD) overlaps with cluster 7 and clusters 1-6. Cluster 9 (NJ) falls above cluster 8, and cluster 10 (NY) falls above cluster 9. Cluster 11 (FL) exhibits no overlap with any cluster, while cluster 12 (TX) overlaps with SC populations.

**Historical and Contemporary Gene Flow**

The highest levels of historical gene flow were from NJ to NC (Table 4.1; \( m_h = 0.0775 \)) and the lowest were from NY to FL (\( m_h = 0.0128 \)). Contemporary gene flow estimates show SC to NC exhibited the highest levels of gene flow (Table 4.2; \( m = 0.1497 \)), while SC to FL demonstrated the lowest levels (\( m = 0.0037 \)).

Of the 42 gene flow routes, six exhibited increased levels of contemporary gene flow (Table 4.3; \( +\Delta m > 0.010 \)), 22 demonstrated reduced levels of contemporary gene flow \( (-\Delta m < -0.010) \), and 14 were relatively stable over time \( (-0.010 < \Delta m < 0.010) \). Four of the six gene flow routes estimated to have increased contemporary gene flow are found between adjacent populations. The two routes between non-adjacent populations to show higher levels of contemporary gene flow are from TX to SC \( (\Delta m = +0.0567) \) and from NC to NY \( (\Delta m = +0.0320) \).
**Models of Population Structure**

As the diamondback terrapin has a linear distribution (Figure 4.1), I used a stepping stone process as my null model of population connectivity (Figure 4.2A). To model translocation events, I modified the stepping stone model to include unidirectional gene flow between TX and SC, and NC and NY; these models differed only in their relative isolation of FL (Figure 4.2B-F). I modeled the Suwannee Seaway by modeling bidirectional gene flow between TX and SC, with FL either completely isolated (Figure 4.2G) or a sink population (Figure 4.2H). I found that the Atlantic Exchange model (Figure 4.2E), which modeled translocation events from TX to SC and NC to NY (gene flow between non-adjacent populations), and which included bidirectional gene flow between SC and FL but unidirectional gene flow between TX and FL, vastly outperformed all other models, including the linear stepping-stone model (Bézier scores = -13,724.32 vs. -72,456.30). Converting approximate Bézier scores into posterior probabilities confirmed the Atlantic Exchange model as the best-fit model (PP = 100%). All other models had estimated posterior probabilities near 0%.

**Bottlenecks**

I recovered no evidence for genetic bottlenecks for any sampling locality with the TPM or SMM, although the TPM for SC6 approached significance (Table 4.4; \( P = 0.055 \)). Mode-shift tests also failed to detect bottlenecks for any sampling locality. In addition, neither the TPM, SMM, nor a mode-shift test detected bottlenecks for any STRUCTURE cluster (Table 4.4).
Discussion

Over the last two centuries, the relationship between terrapins and humans has been complex, and the terrapin’s population genetic structure reflects this relationship. The demand for turtle soup resulted in historical population contractions and extirpations, and culminated in the construction of terrapin farms (Hay 1917; Hildebrand and Hatsel 1926; Hildebrand 1929, 1933). To get flavorful terrapins to market quickly, Texas and Carolina terrapins were hybridized at the North Carolina terrapin farm (Hildebrand 1933). Then, in 1920, the enactment of Prohibition restricted access to sherry, which drastically cut demand for turtle soup. Consequently, many terrapins were released into local waters, which promoted population admixture and may have resulted in the reintroduction of genetic diversity.

I documented population genetic structure that is consistent with historical accounts of terrapin translocation during the twentieth century (Hay 1917; Coker 1920). I recovered two or three genotypic clusters in the mid- and north Atlantic (MD, NJ-NY) and three genotypic clusters in the Gulf and southern populations (TX, FL SC), for a total of six genotypic clusters (Figure 4.1B-G; Figure 4.3). I also recovered the North American Gulf/Atlantic phylogeographic divide previously described in the terrapin and other taxa (Figure 4.1C; Avise et al. 1992; Lamb & Avise 1992; Hauswaldt 2004; Soltis et al. 2006; Converse and Kuchta 2017).

In addition to delineating population structure and quantifying population connectivity, I also found that a modified stepping-stone model with genetic exchange along the Atlantic seaboard and unidirectional gene flow from TX to SC and from NC to
NY best describes terrapin population structure (Figure 4.2E). This model of population connectivity outperformed a linear stepping-stone model, as well as models of the Suwannee Seaway, a natural conduit of gene flow between Gulf and Atlantic populations (Figure 4.2). In addition, I found Florida populations to be divergent from neighboring populations (Figure 4.1B-D, G, Figures 4.4, 4.6, Table 4.3), which complements accounts that Florida terrapins were not translocated due to their inferior size and taste (Hay 1917), as well as other studies that reported FL terrapins to be genetically distinct (Hauswaldt and Glenn 2005; Hart et al. 2014; Drabeck et al. 2014).

My Bayesian model comparisons supported bidirectional gene flow between SC and FL, and unidirectional gene flow from TX to FL (the Atlantic Exchange model, Figure 4.2E), but did not support alternative models of connectivity, such as the Suwannee Seaway (Figure 4.2).

I found that NC is highly admixed with other mid-north Atlantic populations (Figure 4.1C-F), which could be the result of human-mediated gene flow. For example, relative to historical levels of gene flow, I documented increased contemporary connectivity from NC to NY (Table 4.3), which is consistent with accounts of translocation (Coker 1920). North Carolina was the location of a terrapin breeding operation (Hildebrand 1926, 1929, 1933), while NY was the location of a large terrapin market (Coker 1920). With the volume of terrapins brought to market, it is possible some terrapins escaped or were released into local waters. North Carolina also exhibited increased contemporary gene flow into SC and Chesapeake Bay, MD (Table 4.3), consistent with a previous study that detected large increases of contemporary gene flow
into Chesapeake Bay (Converse et al. 2015). Finally, I observed admixture between TX and SC populations (Figure 4.1D,G; Figure 4.6), as well as increased levels of contemporary gene flow from TX into SC (Table 4.3), consistent with chronicled translocation and hybridization experiments (Coker 1920; Hildebrand 1933; Converse and Kuchta 2017).

Although I documented population genetic evidence of translocation between some non-adjacent populations, my study failed to find genetic evidence for some known instances of translocation. In particular, I did not detect increases in contemporary gene flow or admixture between TX and NC (Table 4.3, Figure 4.1B-D, Figures 4.4, 4.6). There are several possible explanations: translocation can fail (Short et al. 1992; Dodd & Seigel 2002), and released terrapins from TX may not have successfully interbred with local populations in NC. Alternatively, my sampling in NC may have not included admixed localities (Figure 4.1A). I also did not find increased contemporary gene flow from MD to NY, although I detected admixture between these populations (Table 4.3, Figure 4.1B-F, Figures 4.4, 4.6). Terrapins from MD may not have been released into local waters in NY, or they may have failed to interbreed. Despite current weak demand for turtle soup, terrapins from the Chesapeake Bay region continue to be sold at markets in New York, often illegally (Lester 2007; Roosenburg et al. 2008).

It is well documented that terrapin populations historically underwent severe contractions (Kennedy 2017), but I failed to detect any population bottlenecks in any region (Table 4.4). For example, the decline of terrapins from Chesapeake Bay is documented by shrinking harvests after decades of overexploitation (Cook 1989), but I
did not detect bottlenecks in this region. Indeed, previous work in Chesapeake Bay indicated terrapins exhibit relatively high levels of genetic diversity (Hauswaldt and Glenn 2005; Converse et al. 2015). It is possible that I failed to detect population bottlenecks because tests of heterozygosity excess have weak statistical power (Peery et al. 2012). Alternatively, the failure to detect bottlenecks may be the consequence of terrapin translocation events reintroducing genetic diversity into populations. If true, the enactment of Prohibition may have inadvertently benefited the terrapin in two ways. The first was the collapse of the turtle soup market, which slowed the harvesting of natural populations. The second was the closure of terrapin farms and the release of translocated individuals into local populations, which may have reintroduced genetic diversity and increased population viability (Frankham et al. 2010).

Thus, my study shows that population genetic structure in the diamondback terrapin possesses the signature of historical translocation events. Translocation among natural populations is known to increase levels of population admixture and genetic diversity (Puckett et al. 2013; Wagner et al. 2015). At least in some cases, increased levels of genetic diversity save populations from the negative consequences of inbreeding depression and lowered mean population fitness (Westemeier et al. 1998; Moritz 1999; Storfer 1999; Souty-Grosset and Grandjean 2008; Doody et al. 2009). However, translocated populations may exhibit high levels of genetic diversity but have low effective population sizes, suggesting several population genetic metrics are required to judge the efficacy of translocation (Olson et al. 2013). An alternative outcome of translocation is that it may harm populations by causing outbreeding depression, harm
locally adapted populations by moving them away from an adaptive peak (Laikre et al. 2010; Calsbeek et al. 2012), or introduce diseases (Maddocks et al. 2015). Nonetheless, given the extent of the current biodiversity crisis (Worm et al. 2006; Pongsiri et al. 2009; Bellard et al. 2012; Assis et al. 2016), including increasing population fragmentation (Templeton et al. 2001; Epps et al. 2005; Banks et al. 2013; Barr et al. 2015), conservation-oriented translocation has become increasingly pivotal to maintaining population viability (Pérez et al. 2012).

My study shows that convoluted genetic histories can be disentangled with modern population genetic tools and that translocation can leave an indelible fingerprint in populations. Anthropogenic influences increasingly disrupt population dynamics (Templeton et al. 2001; Epps et al. 2005; Epps and Keyghobad 2015; Converse et al. 2015); however, the indirect consequences of social and political activities are not always predictable. My study suggests the population genetic structure in the Diamondback Terrapin may be the byproduct of an interaction between market demand for turtle soup during the late nineteenth and early twentieth centuries, followed by the enactment of Prohibition in 1920, which resulted in the large scale release of captive terrapins into local waters.
Table 4.1: Historical gene flow ($m_h$) estimates among coastal populations. Gene flow is measured by the proportion of migrants per generation, ranging from 0.0 – 1.0

<table>
<thead>
<tr>
<th></th>
<th>TX</th>
<th>FL</th>
<th>SC</th>
<th>NC</th>
<th>MD</th>
<th>NJ</th>
<th>NY</th>
</tr>
</thead>
<tbody>
<tr>
<td>TX</td>
<td>-</td>
<td>0.0295</td>
<td>0.0282</td>
<td>0.0235</td>
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</tr>
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<td>-</td>
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<td>0.0263</td>
<td>-</td>
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</tr>
<tr>
<td>NC</td>
<td>0.0354</td>
<td>0.0207</td>
<td>0.0215</td>
<td>-</td>
<td>0.0221</td>
<td>0.0438</td>
<td>0.0254</td>
</tr>
<tr>
<td>MD</td>
<td>0.0180</td>
<td>0.0277</td>
<td>0.0719</td>
<td>0.0249</td>
<td>-</td>
<td>0.0451</td>
<td>0.0583</td>
</tr>
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<tr>
<td>NY</td>
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<td>0.0374</td>
<td>0.0280</td>
<td>0.0214</td>
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</tr>
</tbody>
</table>

Table 4.2: Contemporary gene flow (m) estimates among coastal populations. Gene flow is measured by the proportion of migrants per generation, ranging from 0.0 – 1.0

<table>
<thead>
<tr>
<th></th>
<th>TX</th>
<th>FL</th>
<th>SC</th>
<th>NC</th>
<th>MD</th>
<th>NJ</th>
<th>NY</th>
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<tbody>
<tr>
<td>TX</td>
<td>-</td>
<td>0.0268</td>
<td>0.0849</td>
<td>0.0184</td>
<td>0.0191</td>
<td>0.0207</td>
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<td>FL</td>
<td>0.0207</td>
<td>-</td>
<td>0.0233</td>
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<td>0.0172</td>
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<td>SC</td>
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<td>-</td>
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<td>0.0054</td>
<td>0.0077</td>
<td>0.0051</td>
</tr>
<tr>
<td>NC</td>
<td>0.0139</td>
<td>0.0133</td>
<td>0.1497</td>
<td>-</td>
<td>0.0511</td>
<td>0.0245</td>
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</tr>
<tr>
<td>MD</td>
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<td>0.0080</td>
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<td>NJ</td>
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<td>0.0630</td>
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<tr>
<td>NY</td>
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<td>0.0128</td>
<td>0.0324</td>
<td>0.0157</td>
<td>0.0286</td>
<td>0.0303</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 4.3: Temporal changes in gene flow (Δm) among all populations; bolded values denote increased gene flow between disjunct populations.

<table>
<thead>
<tr>
<th></th>
<th>TX</th>
<th>FL</th>
<th>SC</th>
<th>NC</th>
<th>MD</th>
<th>NJ</th>
<th>NY</th>
</tr>
</thead>
<tbody>
<tr>
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<td>-0.0323</td>
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<td>-0.0051</td>
<td>-0.0060</td>
<td>-0.0067</td>
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<td>-0.0026</td>
<td>-</td>
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<td>-0.0678</td>
</tr>
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<td>NC</td>
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<td>-0.0074</td>
<td>+0.1282</td>
<td>-</td>
<td>+0.0290</td>
<td>-0.0193</td>
<td>+0.0320</td>
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<tr>
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<td>-0.0197</td>
<td>-0.0201</td>
<td>-0.0148</td>
<td>-</td>
<td>-0.0306</td>
<td>-0.0453</td>
</tr>
<tr>
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<td>-0.0093</td>
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<td>-0.0621</td>
<td>+0.0137</td>
<td>-</td>
<td>+0.0738</td>
</tr>
<tr>
<td>NY</td>
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<td>-0.0172</td>
<td>-0.0011</td>
<td>-0.0217</td>
<td>+0.0006</td>
<td>+0.0089</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 4.4: BOTTLENECK results by sampling locality and STRUCTURE cluster, and their respective $P$-values; an “L-Shaped” distribution under a Mode-Shift test indicates a bottleneck was not detected.

<table>
<thead>
<tr>
<th>Sampling Locality</th>
<th>SMM</th>
<th>TPM</th>
<th>Mode-Shift</th>
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</thead>
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<tr>
<td>SC1</td>
<td>0.578</td>
<td>0.344</td>
<td>L-Shaped</td>
</tr>
<tr>
<td>SC2</td>
<td>0.281</td>
<td>0.922</td>
<td>L-Shaped</td>
</tr>
<tr>
<td>SC3</td>
<td>0.422</td>
<td>0.945</td>
<td>L-Shaped</td>
</tr>
<tr>
<td>SC4</td>
<td>0.500</td>
<td>0.281</td>
<td>L-Shaped</td>
</tr>
<tr>
<td>SC5</td>
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<td>0.500</td>
<td>L-Shaped</td>
</tr>
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<td>0.055</td>
<td>L-Shaped</td>
</tr>
<tr>
<td>NC</td>
<td>0.422</td>
<td>0.219</td>
<td>L-Shaped</td>
</tr>
<tr>
<td>MD</td>
<td>1.000</td>
<td>1.000</td>
<td>L-Shaped</td>
</tr>
<tr>
<td>NJ</td>
<td>0.922</td>
<td>0.719</td>
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<td>NY</td>
<td>0.945</td>
<td>0.922</td>
<td>L-Shaped</td>
</tr>
<tr>
<td>TX</td>
<td>0.719</td>
<td>0.781</td>
<td>L-Shaped</td>
</tr>
<tr>
<td>FL</td>
<td>0.961</td>
<td>0.961</td>
<td>L-Shaped</td>
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<tr>
<td>STRUCTURE Cluster</td>
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<tr>
<td>SC</td>
<td>0.961</td>
<td>0.422</td>
<td>L-Shaped</td>
</tr>
<tr>
<td>MD</td>
<td>1.000</td>
<td>1.000</td>
<td>L-Shaped</td>
</tr>
<tr>
<td>NJ/NY/NC</td>
<td>0.961</td>
<td>0.719</td>
<td>L-Shaped</td>
</tr>
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</table>
Table 4.4: continued

<table>
<thead>
<tr>
<th></th>
<th>TX</th>
<th>FL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.719</td>
<td>0.961</td>
</tr>
<tr>
<td>L-Shaped</td>
<td></td>
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</table>

L-Shaped
Figure 4.1: A) Black box: the distribution of the terrapin (red) along the North American coastline. Main: sampling localities and number of samples for each location. B) All terrapin populations under $\Delta K = 2$. C) All terrapin populations under $\Delta K = 4$. D) All terrapin populations under $\Delta K = 7$. Subsections B,C, and D depict the same analysis, explored under different genotypic partitions. E) Mid- and north Atlantic terrapins under $K = 2$. In this scenario, NY and NJ terrapins constitute a single population. F) Mid- and north Atlantic terrapins under $K = 3$. In this scenario, NY and NJ form separate populations. Subsections F and G depict the same analysis under two values of $K$ with similar likelihood scores. G) SC terrapins investigated with Gulf populations. TX and FL form separate populations and SC1-6 constitute a single cluster. No substructure is present within SC1-6. TX, FL, SC, and MD are diagnosable clusters. If NY and NJ form a single cluster (subsection E), there are five total clusters. If NY and NJ form separate clusters (subsection F), there are six total clusters.
Figure 4.2: The eight MIGRATE models of population structure derived from STRUCTURE, DAPC, and Δm results. Solid lines denote naturally occurring gene flow while dashed lines indicate gene flow possibly arising from translocation. Bézier scores are found below model names. Model A is a linear stepping-stone; Model B added gene flow from TX to SC and from NC to NY; Model C removed all gene flow to and from FL while retaining translocation; Model D treated FL as a sink population; Model E allowed gene flow with FL on the Atlantic coast; Model F allowed gene flow with FL on the Gulf Coast; Model G depicts the Suwannee Seaway with completely isolated FL populations; Model H depicts the Suwannee Seaway with FL as a sink population.
Figure 4.3. BIC results indicating DAPC analyses are optimized at six clusters.
Figure 4.4: DAPC for 60 retained axes and four discriminate functions. Six clusters are recovered with this model. The top half contains populations along the Atlantic coast while Gulf populations are found on the right half of the diagram. SC terrapins are present within each cluster.
Figure 4.5: Assignment probabilities for individuals under $K=6$ (Figure 4.4). Warmer colors indicate higher assignment probabilities for clusters.
Figure 4.6: DAPC for 60 retained axes on sampling localities and four discriminate functions. Clusters in the model resemble the spatial distribution of sampling localities along the Gulf and Atlantic seaboards. (Figure 4.1). FL exhibits no admixture with any cluster while TX shows overlap with populations found in SC.
Figure 4.7: Assignment probabilities for individuals under K = 12 (Figure 4.6). Warmer colors indicate higher assignment probabilities for clusters. Clusters 9 and 10 exhibit uncertainty when assigning individuals to either NJ or NY, consistent with STRUCTURE results (Figure 4.1E,F).
CHAPTER 5: SPLITTING DIAMONDS: DATING PHYLOGEOGRAPHIC
DIVERGENCE IN THE DIAMONDBACK TERRAPIN USING APPROXIMATE
BAYESIAN COMPUTATION.

Introduction

Divergence dating is an essential component of biogeographic inference. Because most taxa lack a complete evolutionary chronicle in the fossil record, divergence dates are commonly estimated using a molecular clock. However, divergence estimates from molecular data are sensitive to the rate of nucleotide substitution (Hillis et al. 1996), and rates of nucleotide substitution vary by gene and taxon, and the rate of evolution can itself evolve (Thorne 1998; Kuchta et al. 2016). Additionally, different molecular clock models generate different divergence date estimates, even within the same taxon.

Diamondback Terrapins (Malaclemys terrapin) are turtles that are found in brackish water habitats along the eastern North American continent from Texas to Massachusetts (Ernst and Barbour 1989). Terrapins are characterized by male-biased dispersal and prefer shallow water, near shore environments (Roosenburg 1991; 1994; Spivey 1998; Sheridan et al. 2010). Although seven subspecies are currently recognized, terrapins lack strong genetic structure at the local and regional scales (Hauswaldt and Glenn 2005; Glenos 2013; McCafferty et al. 2013; Hart et al. 2014; Drabeck and Chatfield 2014; Converse et al. 2015; Petre et al. 2015; Converse and Kuchta 2017). However, terrapins exhibit a genetic break near Cape Canaveral, Florida that coincides with morphological variation in their carapace shape (Lamb and Avise 1992; Avise et al. 1992; Converse and Kuchta 2017). Moreover, this “Gulf/Atlantic” phylogeographic
divide found in the terrapin is present in over 25 other taxa, including the Horseshoe Crab (Limulus polyphemus), the Dusky Seaside Sparrow (Ammodramus maritimus), the Hermit Crab (Pagurus longicarpus), and the Blacktip Shark (Carcharhinus limbatus) (Saunders et al. 1986; Avise and Nelson 1989; Young et al. 2002; Keeney et al. 2005; Soltis et al. 2006).

Although the terrapin shares a common phylogeographic pattern with other taxa, it is divergent in its lack of diversity in its mitochondrial DNA (mtDNA; Lamb and Avise 1992; Avise et al. 1992; Converse and Kuchta 2017). In a classic phylogeographic study, Avise et al. (1992) produced two hypotheses to explain this paucity of mtDNA variation. The first hypothesis proposed that a slow rate of evolution in mtDNA resulted in less accumulation of genetic diversity relative to other taxa in the Gulf region (Lamb and Avise 1992; Avise et al. 1992). Under this hypothesis, terrapin mtDNA exhibits a 14-fold decrease in evolutionary rate relative to other vertebrate taxa, and terrapin populations diverged into Gulf and Atlantic clusters when most other taxa did, approximately 350,000 - 1,100,000 years ago. (Lamb and Avise 1992; Avise et al. 1992). A slow rate of molecular evolution has been documented in the Painted Turtle (Chrysemys picta) using complete genome sequencing (Shaffer et al. 2013) and initial phylogeographic work on the terrapin revealed extremely low nucleotide diversity (Hauswaldt 2004). Thus, the hypothesis of slow molecular evolution in the terrapin (Lamb and Avise 1992; Avise et al. 1992) has received recent empirical support. The second hypothesis by Avise et al. (1992) proposed that terrapin mtDNA evolved at the “conventional” vertebrate rate, and terrapin populations thus diverged approximately 50,000 years ago. The estimates from
these hypotheses are based on a shared vertebrate mtDNA clock of 2% myr\(^{-1}\), which has
been much debated (Avise et al. 1992; Hillis et al. 1996; Yang 2014). In this article, I
revisit the competing phylogeographic scenarios for the terrapin postulated by Lamb and
Avise (1992) and Avise et al. (1992). I use microsatellite data and approximate Bayesian
computation (ABC) to evaluate three models of terrapin phylogeographic divergence.
From these models, I estimate divergence dates and effective population sizes, and
address hypotheses of slower rates of mtDNA evolution in the Diamondback Terrapin.

Materials

Sample Collection and Microsatellite Preparation

Susanne Hauswaldt donated a dataset composed of 12 localities along the Atlantic
and Gulf seaboards of the United States, including a total of 320 terrapins (Figure 5.1).
Terrapins were collected from ACE Basin, South Carolina (SC1), the Ashley River
(SC2), Charleston Harbor (SC3), the Wando River (SC4), the Cooper River (SC5), Cape
Romain (SC6), Beaufort, North Carolina (NC), the Patuxent River, Maryland (MD),
Stone Harbor, New Jersey (NJ), Oyster Bay, New York (NY), the Florida Keys (FL), and
Nueces Bay, Texas (TX). DNA from blood, tail tips, or leg muscle was extracted as
described in Hauswaldt and Glenn (2005). I then screened samples for six polymorphic
microsatellite loci using protocols described in Hauswaldt and Glenn (2003) and
Hauswaldt and Glenn (2005). Population genetic metrics including allelic richness,
heterozygosity, and number of alleles are detailed in Hauswaldt and Glenn (2005).
Coastal Population Structure

STRUCTURE version 2.3 (Pritchard et al. 2000) was used to demarcate genotypic clusters (K) among my sampling localities. I ran STRUCTURE from \( K = 1 \) to \( K = 12 \), with each value of \( K \) replicated 10 times with different and random starting seeds. Individual runs were composed of a Markov Chain Monte Carlo (MCMC) of 700,000 iterations, with 350,000 MCMC iterations discarded as burn-in. I used the admixture model, correlated allele frequencies model, inferred \( \alpha \), LOCprior, and LOCISPOP prior for all runs. STRUCTURE HARVESTER web version 0.6.94 (Earl and vonHoldt 2011) was used to collate STRUCTURE results and compute \( \Delta K \) using the Evanno method (Evanno et al. 2005). Preferred values of \( \Delta K \) were analyzed in CLUMPP version 1.1.2 (Jakobsson and Rosenberg 2007), to account for label switching and multimodality, and its output was visualized in DISTRUCT version 1.1 (Rosenberg 2003).

Divergence Dating — Microsatellite Modeling

I used approximate Bayesian computation (ABC; Beaumont et al. 2002) to estimate divergence dates between Gulf and Atlantic populations using DIYABC version 2.04 (Cornuet et al. 2014). I modeled individual locus mutation rates with gamma distributions bounded between \( 1 \times 10^{-5} \) and \( 1 \times 10^{-2} \) mutations site\(^{-1}\) generation\(^{-1}\) with shape 2, and modeled the mean microsatellite mutation rate with a uniform distribution bounded between \( 1 \times 10^{-4} \) and \( 1 \times 10^{-3} \). A generalized stepwise model was used for microsatellite mutation, which allows repeats to increase or decrease by one or more motifs under a geometric distribution (Cornuet et al. 2008). For parameter estimates,
scenario selection, and confidence testing, I used 12 one-sample and nine two-sample microsatellite summary statistics.

_Divergence Dating — Range Scenarios and Priors_

To test the phylogeographic hypotheses from Lamb and Avise (1992) and Avise et al. (1992), I used the three most discrete genotypic clusters recovered by STRUCTURE (MD, FL, TX; Figure 5.1) to simplify phylogeographic scenarios and decrease computation time. These locations were also selected based on sample size and lower levels of population admixture (Haudwaldt and Glenn 2005; this study). I converted divergence date estimates from generations to years with a generation time of 12 years (Converse et al. 2015; Roosenburg _unpubl. data_). I estimated all parameters under the ABC-regression algorithm (Beaumont et al. 2002). In total, I ran two separate phylogeographic analyses using DIYABC. The first analysis was composed of a single scenario with the Gulf/Atlantic divergence date modeled with a uniform prior bounded between 0 and 1,100,000 years (Figure 5.2A; Avise et al. 1992). Effective population sizes were modeled under uniform distributions bounded between 10 and 40,000, as determined by preliminary analyses. From this initial model, I generated 1,000,000 simulated data points, retained the closest 0.01% of simulated points, and applied polychotomous logistic regression to the retained data. Estimates for divergence dates and effective population sizes were logit transformed and quantified under the original parameter choice option (Cornuet et al. 2014).

For my second analysis, I took the estimates of the mode (“intermediate divergence”) and 5% and 95% confidence intervals (“young” and “old” divergences,
respectively) from the first analysis and centered Gaussian distributions around each, for a total of three models (Figure 5.2B). I parameterized standard deviations to be equal to 20% of each scenario’s divergence estimate and bounded each distribution by two standard deviations. The divergence date between Texas and Florida was modeled with a uniform prior bounded between 0 and the divergence estimate from each scenario (Figure 5.2B). For these analyses, I simulated 3,000,000 data points and modeled all remaining parameters as indicated in the initial analysis. I estimated the posterior probabilities of the scenarios and their model parameters by retaining the closest 0.01% of simulated data.

**Divergence Dating — Scenario Confidence and Scenario Checking**

To evaluate the effectiveness of my ABC analyses to discern among competing scenarios, I generated pseudo-observed datasets (pods). Each analysis consisted of 1,000 pods with parameter values drawn from prior distributions in previous ABC analyses. These pods generate relative posterior probabilities amongst the three scenarios. Using these relative posterior probabilities, I estimated type 1 and type 2 errors amongst competing scenarios. Type 1 error arises when the “true” scenario is rejected in favor of a “false” scenario, while type 2 error arises when a “false” scenario is selected over the “true” scenario. I estimated type 1 and type 2 errors for all possible model comparisons. Mean type 2 error was calculated over each respective scenario.

Finally, I used the model checking function in DIYABC to evaluate the goodness-of-fit for the preferred scenario. This function evaluates the consistency of the posterior parameter distributions from the preferred scenario with the observed dataset. For the model to be deemed accurate, the observed statistics must fall within the distribution of
simulated points under the preferred scenario (Cornuet et al. 2010). Following the recommendations of Cornuet et al. (2010), I used a new suite of summary statistics to evaluate my model’s goodness-of-fit, and avoided summary statistics from previous analyses for parameter estimation or model selection.

Results

Coastal Population Structure

STRUCTURE recovered two major clusters (K=2) along the Gulf and Atlantic coasts (Figure 5.1). The “Atlantic” cluster consisted of populations in the mid- and north Atlantic, while the “Gulf” cluster was composed of populations from South Carolina and the Gulf of Mexico. Analysis of substructure within the Atlantic cluster recovered either two or three clusters, with Maryland as the most discrete (Figure 5.1). Evaluation of the Gulf cluster populations recovered Texas, Florida, and South Carolina are discrete entities. No substructure was present within South Carolina populations. Based on these results, I selected Maryland (MD), Texas (TX), and Florida (FL) as target populations for downstream analyses. Thus, Atlantic populations are represented by Maryland, and Gulf populations by Florida and Texas.

Divergence Dating and Effective Population Size

My initial ABC scenario (Figure 5.2A) estimated a Gulf/Atlantic divergence of 157,200 years ago (95% CI = 65,760 - 775,200; Figure 5.2A). Texas and Florida are estimated to have diverged approximately 39,840 years ago [26,040 - 442,800]. For my second analysis (Figure 5.2B), I constructed three models and respectively centered
Gaussian distributions around 157,200 years ago (mode), 65,760 (5% CI), and 775,200 (95% CI) as described in the methods.

Results from my second analysis are presented in Table 5.1. Of the three competing scenarios, ABC posteriors indicated the intermediate divergence scenario was the preferred model (PP = 66.30%). The young divergence scenario was the next best (PP = 30.85%) followed by the old divergence scenario (PP = 2.85%). Under the intermediate divergence scenario, the Gulf/Atlantic split occurred 138,000 years ago [106,800 - 195,600]. The young divergence scenario estimated the split at 63,960 years ago [36,600 - 86,520], and the old divergence scenario estimated the split at 669,600 years ago [512,400 - 938,400]. Under the preferred scenario, Maryland exhibits an effective population size of 30,300 individuals [17,900 - 38,800], Texas exhibits an effective size of 15,700 individuals [6,040 - 35,600], and Florida 4,770 individuals [2,710 - 11,700].

Type 1 and Type 2 error summaries are found in Table 5.2. Error was highest between the intermediate and young divergence scenarios, as these models were closer to one another in parameter space. Error was lowest when comparing the young and old divergence scenarios, as these models were distant in parameter space.

*Microsatellite Mutation*

ABC estimates for the preferred intermediate divergence scenario recovered an overall mutation rate of $2.70 \times 10^{-4}$ mutations site$^{-1}$ generation$^{-1}$ [1.66 - 7.61 x 10$^{-4}$]. The young divergence scenario estimated the mutation rate to be $5.24 \times 10^{-4}$ [2.75 - 9.44 x 10$^{-4}$], and the old divergence scenario estimated the rate to be $1.26 \times 10^{-4}$ [1.08 - 4.08 x 10$^{-4}$].
Discussion

Lamb and Avise (1992) and Avise et al. (1992) recovered unusually low levels of mtDNA diversity in turtles and used phylogeographic inference to estimate slower rates of molecular evolution in Testudines. These seminal contributions delineated a link between molecular level processes and range-wide phylogeographic structure among disparate taxa. Moreover, these studies helped demonstrate that observation at the phylogeographic level could contribute to understanding molecular level phenomena.

Using molecular data and phylogeographic models evaluated using ABC, my study suggests that Gulf and Atlantic terrapin populations did not diverge 350,000 - 1,100,000 years ago, as hypothesized by Lamb and Avise (1992) and Avise et al. (1992). Consequently, my study casts further doubt on the postulated 14-fold slowdown in terrapin mtDNA molecular rate (Avise et al. 1992). However, my results suggest the terrapin has some degree of decreased rate of molecular evolution, which is consistent with a sister taxon (Painted Turtle) that exhibits a slower rate of molecular evolution (Shaffer et al. 2013). In addition, the terrapin’s nuclear and mitochondrial genomes have been sequenced as of late 2015 (Mihai Pop, pers. comm.). Once available, these data will help shed light on the terrapin’s rate of molecular evolution relative to other vertebrate taxa and help elucidate its phylogeographic history.

There are several explanations for the Gulf and Atlantic phylogeographic divide. According to historical estimates, sea levels were approximately 80 meters lower than contemporary levels 150,000 years ago (Grant et al. 2012). This period of lower sea levels coincides with the “intermediate divergence” model. Similarly, sea levels were
approximately 80 meters below contemporary levels around 65,000 years ago (Grant et al. 2012). This change in sea level coincides with the “young divergence” model. Lower sea levels would have increased the perimeter of peninsular Florida, possibly to the Florida Shelf. As a consequence, the dispersal distance between Gulf and Atlantic populations would have increased, leading to deceased genetic exchange.

Alternatively, the Gulf/Atlantic genotypic divide may be an indirect consequence of the Gulf Stream (Avise 2000). North of Cape Canaveral, Florida, the Gulf Stream deviates away from the Atlantic coast and towards the Atlantic Ocean, which may impede dispersal between Gulf and Atlantic populations. It has been postulated that this is the cause of the shared phylogeographic divergence among the terrapin and other coastal taxa (Saunders et al. 1986; Soltis et al. 2006). A third explanation by Wise et al. (2004) proposed that a combination of climate, currents, mangrove ecosystems, and sediments contribute to adversely to prevent dispersal along the eastern Florida coastline. These explanations are not mutually exclusive. It is possible that fluctuations in sea level caused Gulf and Atlantic populations to diverge, while the Gulf Stream and features of Florida’s eastern shoreline maintain a barrier to gene flow.

The incongruences between my divergence date estimates and those of Lamb and Avise (1992) could be a byproduct of marker choice. Lamb and Avise (1992) assayed mtDNA using restriction enzymes, while my study used nuclear microsatellites. Incongruences between maternally-inherited, haploid mtDNA and biparentally-inherited, diploid nuclear DNA are well documented (Hudson and Coyne 2002; Kuchta and Tan 2005, 2006; Schelly et al. 2006; Linnen and Farrell 2007; Kuchta et al. 2009; Kuchta et
al. 2016). Likewise, variation in marker mutation rate may partially explain my divergence date estimates. I estimated my loci to have an overall mutation rate of $2.70 \times 10^{-4}$ mutations site$^{-1}$ generation$^{-1}$, much faster than estimates of Chelonian mtDNA mutation of $2.01 \times 10^{-9}$ (Eo and DeWoody 2010).

I obtained three divergence date estimates for terrapin phylogeography using ABC analyses. The “intermediate divergence” model had the strongest support (PP = 66.30%) and suggests that the Gulf and Atlantic divide occurred ~138,000 years ago. The “young divergence” model was the next best (PP = 30.85%), and estimated the Gulf and Atlantic divide at 63,960 years ago. The “old divergence” model had the least support (PP = 2.85%), and estimated the divergence to have occurred 669,600 years ago (Table 5.1).

The estimates generated by “intermediate divergence” model fell in between the original divergence hypotheses of 50,000 or 350,000 - 1,100,000 years ago postulated by Lamb and Avise (1992) and Avise et al. (1992). However, the “young divergence” model estimated the Gulf and Atlantic divergence occurred 63,960 years ago, while the “old divergence” model recovered a divergence date of 669,000 years ago. Both of these estimates fell near or within the divergence windows postulated by Lamb and Avise (1992) and Avise et al. (1992) (Figure 5.2). In addition, ABC estimates of effective population size were congruent with geographic patterns for number of alleles, allelic richness, and observed heterozygosity (Hauswaldt and Glenn 2005; Hart et al. 2014). Maryland exhibited the largest effective population size and highest metrics for genetic diversity, followed by Texas and Florida (Table 5.1).
Type 1 error estimates indicated that the intermediate divergence scenario was the most likely to be rejected when it was the preferred model (39.2%), while the old divergence scenario was the least likely to be rejected when preferred (13.1%; Table 5.2). This pattern was also true of type 2 error. In my analyses, higher type 1 and 2 error was associated when models were fit with slightly overlapping priors (e.g. “intermediate divergence” and “young divergence” models; Figure 5.2B), and lower when priors were disparate (e.g. “young divergence” and “old divergence” models). While there is some uncertainty when choosing between “young” and “intermediate” divergence models, the “old divergence” model had poor support.

The paucity of mtDNA diversity in the Diamondback Terrapin presented a novel problem for terrapin phylogeography, and resulted in two competing hypotheses with implications on rates of molecular evolution (Lamb and Avise 1992; Avise et al. 1992; Converse and Kuchta 2017). Using ABC, my study found that neither of the two original divergence hypotheses were well supported, and instead indicates a divergence date of \(~138,000\) years ago. This divergence date suggests a faster rate of molecular evolution in the terrapin than previously estimated and also reveals the complexity of the terrapin’s phylogeographic history.
Table 5.1: Summary of the three competing models for Gulf-Atlantic divergence dates. Values in brackets indicate 95% confidence intervals. The intermediate model was centered on the mode from the initial analysis, the young model on the 5% CI, and the old model on the 95% CI.

<table>
<thead>
<tr>
<th>Model</th>
<th>Posterior Probability</th>
<th>Gulf/Atlantic Split (years)</th>
<th>TX/FL Split (years)</th>
<th>N_e MD</th>
<th>N_e TX</th>
<th>N_e FL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intermediate</td>
<td>66.30%</td>
<td>138,000</td>
<td>96,120</td>
<td>30,300</td>
<td>15,700</td>
<td>4,770</td>
</tr>
<tr>
<td>Young</td>
<td>30.85%</td>
<td>63,960</td>
<td>41,880</td>
<td>27,000</td>
<td>6,210</td>
<td>2,420</td>
</tr>
<tr>
<td></td>
<td>[23.49 – 38.21%]</td>
<td>[48,600 – 86,520]</td>
<td>[18,840 – 62,280]</td>
<td>[13,500 – 37,700]</td>
<td>[3,460 – 34,500]</td>
<td>[1,560 – 6,760]</td>
</tr>
<tr>
<td>Old</td>
<td>2.85%</td>
<td>669,600</td>
<td>214,800</td>
<td>36,700</td>
<td>33,200</td>
<td>11,800</td>
</tr>
<tr>
<td></td>
<td>[0 - 9.63%]</td>
<td>[512,400 – 938,400]</td>
<td>[88,560 – 648,000]</td>
<td>[27,600 – 39,600]</td>
<td>[15,300 – 38,200]</td>
<td>[5,340 – 21,400]</td>
</tr>
</tbody>
</table>
Table 5.2: Type 1 and type 2 errors for all model comparisons. “among models” indicates Type 1 and 2 errors when all models are evaluated simultaneously. “between models” denotes Type 1 and 2 errors between the two models listed. The first model listed for each comparison is assumed as the “true” model for error calculation.

<table>
<thead>
<tr>
<th>Model</th>
<th>Type 1 Error (%)</th>
<th>Type 2 Error (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>among models</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intermediate (mode)</td>
<td>39.2</td>
<td>16.6</td>
</tr>
<tr>
<td>Young (5% CI)</td>
<td>20.6</td>
<td>15.4</td>
</tr>
<tr>
<td>Old (95% CI)</td>
<td>13.1</td>
<td>4.5</td>
</tr>
<tr>
<td><strong>between models</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intermediate/Young</td>
<td>27.0</td>
<td>24.5</td>
</tr>
<tr>
<td>Intermediate/Old</td>
<td>8.5</td>
<td>13.2</td>
</tr>
<tr>
<td>Young/Intermediate</td>
<td>24.5</td>
<td>27.0</td>
</tr>
<tr>
<td>Old/Intermediate</td>
<td>13.2</td>
<td>8.5</td>
</tr>
<tr>
<td>Young/Old</td>
<td>2.3</td>
<td>4.9</td>
</tr>
<tr>
<td>Old/Young</td>
<td>4.9</td>
<td>2.3</td>
</tr>
</tbody>
</table>
Figure 5.1: Sampling localities and STRUCTURE results. Sample sizes are found on right in parentheses and correspond to sampling locality. Populations used in downstream ABC analyses are indicated in red. Faded into the background is a figure adopted from Avise et al. (1992), with blue circles denoting their Gulf cluster and white circles their Atlantic cluster. The genotypic and morphological transition near Cape Canaveral, FL, is represented by a dashed line. Two genetic clusters ($\Delta K=2$) were initially identified, with South Carolina populations found within the Gulf cluster. Texas, Florida, and South Carolina were identified as subclusters. Within the Atlantic cluster, Maryland formed a discrete entity while New Jersey and New York exhibited weak differentiation.
Figure 5.2: ABC models for Gulf/Atlantic divergence scenarios. Time (thousands of years) is found along the middle x-axis and applies to parts (A) and (B). A) Priors (dotted lines) and posteriors (solid lines) for the initial model. Blue lines represent the FL/TX divergence date and red lines denote the Atlantic/Gulf divergence date. Posteriors include modes (squares) and 5% and 95% confidence intervals (vertical lines). B) The three divergence scenarios constructed from the results from the initial model. All Atlantic/Gulf divergence dates (red lines) were modeled using Gaussian distributions. The top scenario is a Gaussian distribution centered on the mode from the initial analysis (157,200; sd = 31,440), the middle scenario is a Gaussian distribution centered on the 5% confidence interval (65760; sd = 13,152), and the bottom scenario is a Gaussian distribution centered on the 95% confidence interval (775,200; sd = 155,040). For each scenario, the TX/FL divergence date (blue lines) was modeled with a uniform prior bounded between zero and the mode for each Gaussian distribution. Ranges for the Atlantic and Gulf divergence dates are indicated in brackets.
LITERATURE CITED


