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Spatiotemporal analysis of gene flow in Chesapeake Bay Diamondback Terrapins (*Malaclemys terrapin*)

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Abstract

There is widespread concern regarding the impacts of anthropogenic activities on connectivity among populations of plants and animals, and understanding how contemporary and historical processes shape metapopulation dynamics is crucial for setting appropriate conservation targets. We used genetic data to identify population clusters and quantify gene flow over historical and contemporary time frames in the Diamondback Terrapin (*Malaclemys terrapin*). This species has a long and complicated history with humans, including commercial overharvesting and subsequent translocation events during the early twentieth century. Today, terrapins face threats from habitat loss and mortality in fisheries bycatch. To evaluate population structure and gene flow among Diamondback Terrapin populations in the Chesapeake Bay region, we sampled 617 individuals from 15 localities and screened individuals at 12 polymorphic microsatellite loci. Our goals were to demarcate metapopulation structure, quantify genetic diversity, estimate effective population sizes, and document temporal changes in gene flow. We found that terrapins in the Chesapeake Bay region harbour high levels of genetic diversity and form four populations. Effective population sizes were variable. Among most population comparisons, estimates of historical and contemporary terrapin gene flow were generally low ($m \approx 0.01$). However, we detected a substantial increase in contemporary gene flow into Chesapeake Bay from populations outside the bay, as well as between two populations within Chesapeake Bay, possibly as a consequence of translocations during the early twentieth century. Our study shows that inferences across multiple time scales are needed to evaluate population connectivity, especially as recent changes may identify threats to population persistence.

Keywords: conservation genetics, contemporary gene flow, historical gene flow, metapopulation, population admixture, population structure, translocation

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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. 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 & Gilpin 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.*

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2001; Bowler & Benton 2005; Epps *et al.* 2005; Banks *et al.* 2013; Barr *et al.* 2015). The extent to which habitat fragmentation decreases population connectivity, however, is dependent upon the interaction between landscape features and organismal dispersal behaviour (Gu *et al.* 2002; Caizergues *et al.* 2003; Braunisch *et al.* 2010; Callens *et al.* 2011; Crispo *et al.* 2011; Castillo *et al.* 2014). In many cases, populations that are currently isolated by habitat fragmentation may not have been isolated in the past (Newmark 2008; Chiucchi & Gibbs 2010; Epps *et al.* 2013; Husemann *et al.* 2015). In contrast to 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 & Gibbs 2010; Epps *et al.* 2013).

In this article, we 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 & Barbour 1989). During the nineteenth and early twentieth centuries, terrapins were unsustainably harvested, resulting in severe population contractions and local extirpations (Garber 1988; Garber 1990). To help preserve dwindling populations and supplement terrapin harvests, governmental and private entities constructed terrapin breeding farms (Coker 1906; Barney 1924; Hildebrand & 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 favour, 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 & Roosenburg 2005; Dorcas *et al.* 2007; Grosse *et al.* 2009), habitat loss and fragmentation (Roosenburg 1991; Wood & Herlands 1997) and predator introductions (Feinberg & 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 favour of females (Roosenburg 1991; W. Roosenburg unpublished 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 & Kuchta *in press*). Ecological data show terrapins reside in small home ranges of 0.54–3.05 km^2 (Spivey 1998; Butler 2002) and can remain in the same study site for over a decade (Lovich & 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 to 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 & 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 article, we report on a study of metapopulation dynamics of the Diamondback Terrapin in Chesapeake Bay. Specifically, we 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, we identify possible instances of terrapin translocation. By comparing historical and contemporary levels of genetic connectivity, we 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 & Kuchta *in press*).

Materials and methods

Sampling localities and microsatellite genotyping

We sampled 617 terrapins from 15 localities throughout Chesapeake Bay and nearby coastal bays between 2003 and 2005 (Fig. 1; Appendix S1, Supporting Information). Terrapins were captured using fyke nets or collected in winter refugia during hibernation (Haramis *et al.* 2011). Terrapins were marked with passive integrated

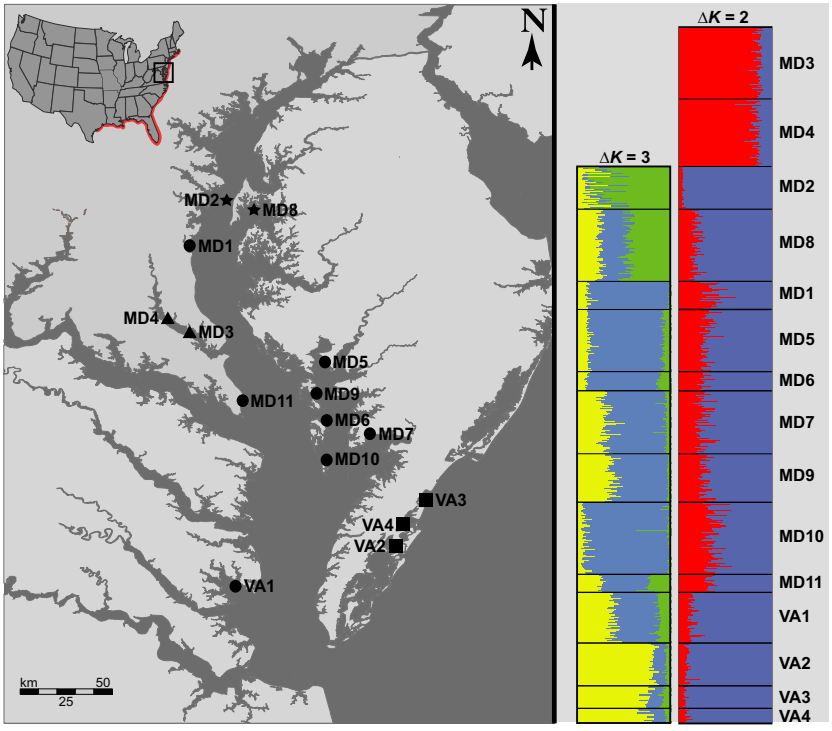

Fig. 1 Sampling localities and STRUCTURE results. Top left: the distribution of Diamondback Terrapins (shaded red), and the location of the study (black box). Main figure: four terrapin populations were demarcated. Triangles indicate the Patuxent River, stars represent Kent Island, squares represent 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.

transponder tags to prevent resampling. Blood samples were preserved on FTA cards (Whatman, Inc., Clifton, NJ, USA). DNA was isolated using Puregene DNA extraction kits (Qiagen, Venlo, Netherlands) and resuspended in TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA).

We assayed individuals at 12 microsatellite loci (Appendix S2, Supporting Information) developed for the bog turtle (*Glyptemys muhlenbergii*), which amplify in other Emydid turtles (King & Julian 2004). Each PCR consisted of 100–200 ng of genomic DNA, 0.88 μL PCR buffer (59 mM Tris-HCl, pH 8.3; 15 mM $(NH_4)_2SO_4$; 9 mM ß-mercaptoethanol; 6 mM EDTA), 3.75 mM $MgCl_2$, 0.31 mM dNTPs, 0.15–0.25 mM of forward and reverse primers, and 0.4 U AmpliTaq. All samples were brought up to a total volume of 20 μL with deionized water. Each forward primer was 5′ modified with FAM, NED or HEX fluorescent labels (Applied Biosystems, Waltham, MA, USA). The following amplification conditions were used: 94 °C for 2 min; 35 cycles of 94 °C denaturation for 45 s, 56 °C annealing for 45 s, 72 °C extension for 2 min; final extension of 72 °C for 10 min. Thermal cycling was performed in an MJ DNA Engine PTC 200 (MJ Research, Watertown, MA, USA).

Fragment analysis and allelic designations followed King *et al.* (2006). Capillary electrophoresis was conducted on an ABI Prism 3100 Genetic Analyzer using GeneScan-500 ROX size standard (Thermo Fisher Scientific – Applied Biosystems, Waltham, MA, USA). Fragment size data were generated using GENESCAN software version 3.7 (Applied Biosystems). GENOTYPER software version 3.6 (Applied Biosystems) was used to score, bin and assign genotypes for each individual. We used MICRO-CHECKER version 2.2.3 (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

We 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. We ran STRUCTURE from $K = 1$ to $K = 15$ populations, with each value of K run 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. We used the admixture model, the correlated allele frequencies prior, the LOCprior, the LOCISPOP prior, fixed λ and inferred α. We used sampling localities (Fig. 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 &

vonHoldt 2011). Label switching and multimodality on preferred values of ΔK were addressed using CLUMPP version 1.1.2 (Jakobsson & Rosenberg 2007), and the final results were visualized using DISTRUCT version 1.1 (Rosenberg 2003). We repeated this procedure within STRUCTURE clusters to detect substructure. We also partitioned genetic variance using an analysis of molecular variance (AMOVAS) in the software ARLEQUIN version 3.5.1.3 (Excoffier & Lischer 2010). Populations were partitioned by landscape features (river, bay and coast), sampling locality and STRUCTURE clusters. Significance was assessed using 1000 permutations. We 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. We determined significance and estimated 95% confidence intervals using 1000 bootstrap replicates and Bonferroni correction (Dunn 1961). We 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

Because coalescent estimates of historical gene flow and effective population sizes are scaled by mutation rate, we estimated a mutation rate (μ) using approximate Bayesian computation (ABC) in POPABC version 1.0 (Lopes *et al.* 2009). Mutations were modelled using the stepwise-mutation model (SMM; Kimura & Ohta 1978) and were measured in mutations^{-1} site^{-1} generation^{-1}. Demographic parameters were estimated under the isolation–migration model (Nielsen & Wakeley 2001; Hey & Nielsen 2004). Priors for this analysis are summarized in Table 1. We modelled our mutation rate hyperprior using a log-normal distribution centred at 1×10^{-3} (SD = 0.5; Hedrick 1996; Whittaker *et al.* 2003). Genetic tree topology was modelled under a uniform prior. We simulated 2 500 000 genetic trees and ran the ABC-rejection algorithm with a tolerance of 0.0004, retaining the 1000 closest simulated points. We 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, we estimated the mode, 2.5% quantile, and 97.5% quantile for μ in R.

Effective population size

We used MIGRATE version 3.6.5 (Beerli 2008) to jointly estimate θ ($=4N_e\mu$) while estimating M (see below) and used the mutation rate estimated by POPABC to convert θ into N_e. We also estimated effective population sizes using ONESAMP v. 1.2 (Tallmon *et al.* 2008), which uses 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, we ran each STRUCTURE population individually and set lower and upper boundaries for N_e to 2 and 1000, respectively.

Historical gene flow

We 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). Because MIGRATE operates in a coalescent framework, it estimates gene flow over long periods of time, up to ~$4N_e$ generations (thousands of years) for larger populations (Beerli 2009). We used populations demarcated by STRUCTURE as a priori population assignments in MIGRATE. To improve speed, we used a Brownian motion model to approximate a stepwise-mutation model. Using slice sampling, we ran four statically heated parallel chains (heated at 1.0, 1.5, 3.0 and 1 000 000) for 30 000 000 iterations, sampled every 3000 iterations, and excluded 7 500 000 iterations as burn-in. MCMC estimates of M were modelled with a uniform prior containing lower and upper boundaries of 0 and 2000. F_{ST} values were used for initial estimates of M. A full migration model was used, which facilitates comparisons with geneflow estimates made in BAYESASS. We considered parameter estimates accurate if an effective sample size (ESS) of 1000 or greater was observed (P. Beerli, personal communication)

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 & Rannala 2003). BAYESASS estimates all pairwise migration rates among populations. According to Wilson & Rannala (2003), BAYESASS estimates gene flow '…over the last several generations.' Following Chiucchi & Gibbs (2010), we assumed this to mean roughly five generations. Using a generation time of 12 years (W. Roosenburg, unpublished 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. We used populations demarcated by STRUCTURE as a priori population assignments. We ran 10 MCMC simulations (Faubet *et al.* 2007) with different starting seeds for 20 000 000 iterations, sampling every 2000 iterations; 10 000 000 iterations were excluded as burn-in. Chain mixing delta parameters were adjusted in pilot runs to

Table 1 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 1000 simulated data points. ICB = inner Chesapeake Bay, Patuxent = Patuxent River, Kent = Kent Island and CoB = coastal bays

Parameter	Description	Prior
μ	Mutation Rate (site^{-1} generation^{-1})	Lognormal (−3.0,0.5,0.5,0.5)
N_e1	Effective Population Size, Kent Island (individuals)	Uniform (0, 5000)
N_e2	Effective Population Size, Patuxent (individuals)	Uniform (0, 5000)
N_e3	Effective Population Size, ICB (individuals)	Uniform (0, 5000)
N_e4	Effective Population Size, CoB (individuals)	Uniform (0, 5000)
N_eA1	Ancestral Population Size (individuals)	Uniform (0, 10 000)
N_eA2	Ancestral Population Size (individuals)	Uniform (0, 10 000)
N_eA3	Ancestral Population Size (individuals)	Uniform (0, 10 000)
m1	Kent -> Patuxent Migration Rate (fraction of immigrants)	Uniform (0, 0.1)
m2	Patuxent -> Kent Migration Rate (fraction of immigrants)	Uniform (0, 0.1)
m3	ICB -> CoB Migration Rate (fraction of immigrants)	Uniform (0, 0.1)
m4	CoB -> ICB Migration Rate (fraction of immigrants)	Uniform (0, 0.1)
m_A1	Ancestral Migration Rate (fraction of immigrants)	Uniform (0, 0.1)
m_A2	Ancestral Migration Rate (fraction of immigrants)	Uniform (0, 0.1)
$T_{ev}1$	Splitting Event 1 (years)	Uniform (0, 5000)
$T_{ev}2$	Splitting Event 2 (years)	$T_{ev}1$ + Uniform (0, 5000)
$T_{ev}3$	Splitting Event 3 (years)	$T_{ev}2$ + Uniform (0, 5000)
τ	Generation Time	12 years (constant)
Top	Tree Topology (18 possible arrangements)	Uniform (0, 18)

maintain a MCMC state-change acceptance ratio of 20–40%, the empirically recommended window (Rannala 2011). We diagnosed MCMC stationarity for each run in TRACER version 1.5. (Rambaut & Drummond 2007) and used a Bayesian deviancy measure (Spiegelhalter *et al.* 2002) to determine which run best fit the data with R (Meirmans 2014). We took the starting seed from this best-fit run and ran a MCMC for 50 000 000 iterations, sampled every 2000 iterations, with the first 20 000 000 iterations excluded as burn-in. We visualized MCMC stationarity for this final run in TRACER. The ESS for all parameters was >200.

Comparison of historical and contemporary gene flow

We 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, we multiplied the M-values generated by MIGRATE by the mutation rate estimated in POPABC. We then subtracted these values from the contemporary estimates of gene flow from BAYESASS ($\Delta m = m - m_h$). The resulting value, Δm, denotes temporal changes in gene flow. Negative values of Δm indicate reduced gene flow in the present, positive values indicate increased gene flow, and values near zero indicate no change.

Population bottlenecks

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

Results

Population structure and genetic diversity

Measures of genetic diversity showed high levels of heterozygosity, allelic richness and allele counts for

sampling localities relative to other regions (Hauswaldt & Glenn 2005; Hart *et al*. 2014; Appendix S3, Supporting Information). Sampling localities had mean expected and observed heterozygosities between 0.69 and 0.78, a mean of 6–8 alleles per locus and mean allelic richness between 5.8 and 6.5. No loci were found to be in linkage disequilibrium. 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 (Appendices S3 and S4, Supporting Information).

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, we identified four terrapin populations (Fig. 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 (Appendix S5, Supporting Information), with the remaining localities forming a second cluster (Fig. 1, $\Delta K = 2$). Analysis of the second cluster (Fig. 1, $\Delta K = 3$) revealed it was composed of three subclusters (Appendix S5, Supporting Information): 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 variance in Chesapeake Bay is found within populations (Appendix S6, Supporting Information). 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_{est} among STRUCTURE clusters identified significant levels of population differentiation among all clusters (Table 2). 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 μ solved at a mode of 4.3×10^{-4} mutations^{-1} site^{-1} generation^{-1} (Fig. 2). This estimate of μ is similar to a commonly assumed microsatellite mutation rate of 5.0×10^{-4} (e.g. Estoup *et al*. 2002; Faubet *et al*. 2007; Chiucchi & Gibbs 2010).

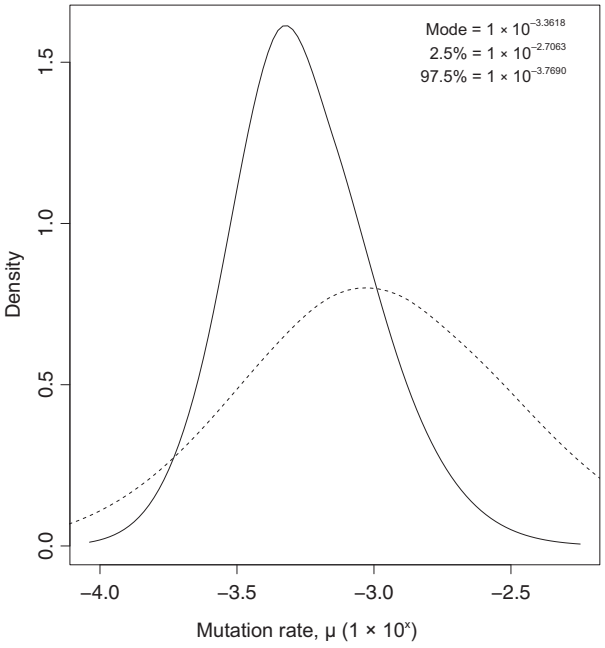

Fig. 2 Approximate Bayesian computation posterior (solid line) and hyperprior (dotted line; log-normal) distributions for μ, the mutation rate used to convert θ into N_e and M into m_h (MIGRATE), for comparisons with m from BAYESASS. The mode is $1 \times 10^{-3.36}$, or 4.3×10^{-4} mutations^{-1} site^{-1} generation^{-1}.

Effective population size

Estimates of N_e from MIGRATE produced a range of effective population sizes in Chesapeake Bay (Fig. 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).

Table 2 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

	Kent Island	Patuxent River	Coastal Bays	Inner CB
Kent Island	—	0.001 (0.006)	0.001 (0.006)	0.001 (0.006)
Patuxent River	0.0443 [0.0380–0.0523]	—	0.001 (0.006)	0.001 (0.006)
Coastal Bays	0.0374 [0.0320–0.0486]	0.0654 [0.0576–0.0747]	—	0.001 (0.006)
Inner CB	0.0155 [0.0116–0.0219]	0.0291 [0.0254–0.0343]	0.0333 [0.0274–0.0414]	—

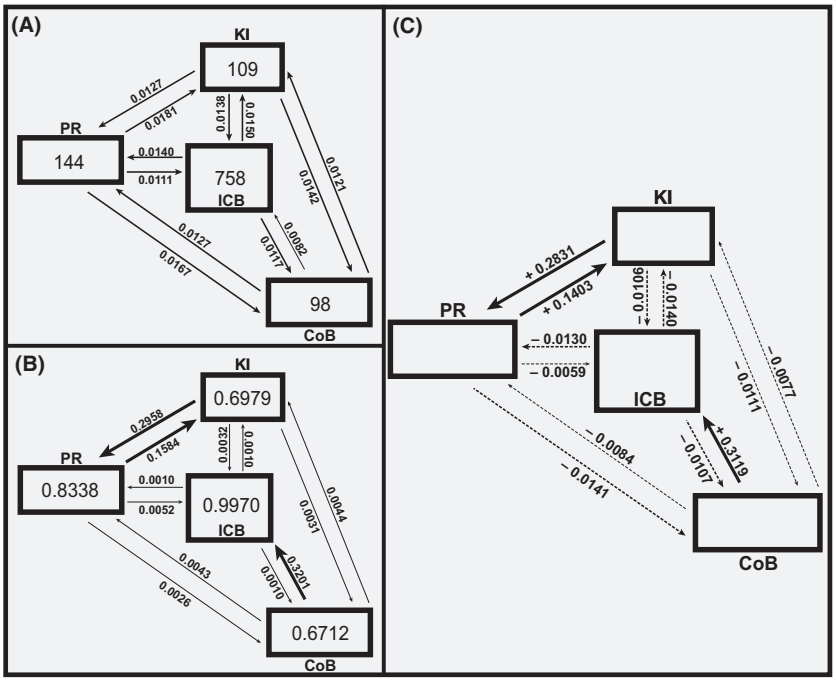

Fig. 3 Gene flow in Chesapeake Bay. KI = Kent Island, PR = the Patuxent River, ICB = inner Chesapeake Bay, and CoB = the coastal bays. Thin lines represent estimates of m or Δm of <0.01, intermediate lines represent estimates of 0.01–0.05, and thick lines represent estimates of >0.05. (A) Results from MIGRATE. Numbers within boxes denote N_e, 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 ($m - m_h = \Delta m$). Dashed arrows indicate gene flow routes that have reduced contemporary gene flow ($-\Delta m$) and solid arrows indicate routes that have increased contemporary gene flow ($+\Delta m$).

Historical gene flow

Estimates of historical geneflow rates revealed similar but low levels of gene flow among all populations (Fig. 3A). Historical geneflow 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 (Fig. 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 geneflow routes, only two were found to be asymmetrical (nonoverlapping 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 (Fig. 3C, solid lines). Contemporary geneflow 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 geneflow levels from the coastal bays to inner Chesapeake Bay ($\Delta m = +0.3119$). We 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 geneflow reduction, approximately $\Delta m \approx -0.01$. The Patuxent River to inner Chesapeake Bay ($\Delta m = -0.0059$) and the coastal bays to the Patuxent River ($\Delta m = -0.0084$) showed the least change in geneflow levels over time.

Population bottlenecks

Bottleneck tests failed to detect any signatures of heterozygosity excess in Chesapeake Bay terrapins (Appendix S7, Supporting Information). 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 (Appendix S7, Supporting Information).

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 & Murphy 1985; Hanski & Gilpin 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 & Tan 2006; Epps *et al.* 2013).

We delineated four terrapin populations in the Chesapeake Bay region that exhibited high levels of heterozygosity and allelic diversity (Fig. 1; Appendix 3, Supplementary Information) and weak structure (Fig. 1; Appendix S6, Supporting Information). Historical estimates of migration indicate that gene flow was limited among all populations ($m_h \approx 0.01$; Fig. 3A). By contrast, contemporary estimates of migration were more variable (Fig. 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 ($\Delta m = +0.2831$ and $\Delta m = +0.1403$) and from the coastal bays into inner Chesapeake Bay ($\Delta m = +0.3119$; Fig. 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 & Hatsel 1926; Hildebrand 1933). This transport of terrapins could explain our substantial increase in gene flow from the coastal bays to inner Chesapeake Bay ($\Delta m = +0.3119$; Fig. 3C). Alternatively, the increase in gene flow could be due to natural processes. Hauswaldt & 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 neighbouring 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 our 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 (Fig. 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 we 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 (Fig. 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 to 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 & 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 & Soulé 1999). Some nesting locations suffer mortality rates as high as 92% for nests (Feinberg & Burke 2003) and 10% for adult females (Siegel 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 & 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. We suggest that the increased mortality risk of dispersing males has lowered contemporary geneflow rates among populations.

While it is well documented that terrapins underwent population contractions due to overharvesting and other factors (Garber 1988; Garber 1990), we 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 re-introducing genetic diversity, and may have confounded efforts to detect a genetic bottleneck (Hauswaldt & 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 (Funk *et al.* 2010; Peery *et al.* 2012).

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 build-up of genetic structure (Butler 2002; Converse & Kuchta *in press*; Gibbons *et al.* 2001; Roosenburg 1994; Sheridan *et al.* 2010; Spivey 1998). By contrast, genetic studies find that terrapin populations are weakly differentiated, even at regional scales (Drabeck *et al.* 2014; Sheridan *et al.* 2010; Hauswaldt & Glenn 2005; Hart *et al.* 2014; Petre 2014). One hypothesis to reconcile these data is that terrapins migrate large distances (several kilometres) to mating aggregations, which would prevent the build-up of genetic structure among populations (Hauswaldt & Glenn 2005). However, work by Sheridan (2010) suggests that terrapins do not travel long distances to mating aggregations. We propose a modification to Hauswaldt & Glenn's (2005) hypothesis: that mating behaviour 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 exhibit highly unequal sex ratios. For example, at Poplar Island and the Patuxent River, female terrapins outnumber males nine to one (9:1) and three to one (3:1), respectively (W. Roosenburg, unpublished 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).

Our 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 & 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 our initial hypothesis of substantial decreases in contemporary gene flow among terrapin populations as a consequence of habitat loss and fragmentation, we documented enormous increases in gene flow into Chesapeake Bay and between two populations within Chesapeake Bay. We 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 geneflow estimates were a recent phenomenon; indeed, we may have interpreted the relatively low estimates of contemporary gene flow among most other populations as indicative of reduced dispersal! Incorporating historical processes greatly improves interpretation of contemporary processes (Vandergast *et al.* 2007; Hansen *et al.* 2009; Pavlacky *et al.* 2009; Epps *et al.* 2013; Husemann *et al.* 2015). Our results confirm the importance of taking historical factors into account when quantifying genetic connectivity in highly impacted landscapes.

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P.E.C. and S.R.K. designed and conceived the study. W.M.R., P.F.P.H. and G.M.H. collected field samples. T.L.K. supervised and conducted laboratory work and data collection. P.E.C. and S.R.K. analysed the data. P.E.C. and S.R.K. wrote the manuscript.

Data accessibility

Data used for this article (microsatellite data and all program input files) have been deposited in Dryad, doi:10.5061/dryad.nf8gf.

Supporting information

Additional supporting information may be found in the online version of this article.

Appendix S1 Sampling localities and their abbreviations. Sampling took place from 2003–2005.

Appendix S2. Summary of loci amplified for subsequent analyses.

Appendix S3 Observed heterozygosity (H_o), expected heterozygosity (H_e), allelic richness (A_R), and number of alleles (N_A) by locus for population MD1.

Appendix S4 Pairwise tests of linkage disequilibrium for all loci, based on 1000 permutations and an adjusted *P*-value of 0.00090.

Appendix S5 ΔK plot under the Evanno method for the initial run of STRUCTURE finding the Patuxent River as a genotypic cluster.

Appendix S5 ΔK plot under the Evanno method for the second run of STRUCTURE finding KI, ICB, and CoB as populations.

Appendix S6 AMOVA results indicating terrapin populations in Chesapeake Bay are weakly structured.

Appendix S7 *P*-values for bottleneck detection under each model in BOTTLENECK.