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Unexpectedly low genetic divergences among populations of the threatened bog turtle (*Glyptemys muhlenbergii*)

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Abstract We used mitochondrial DNA sequence comparisons to assess range-wide population structure and historical patterns of differentiation among populations of the bog turtle (*Glyptemys muhlenbergii*). This species is one of North America's smallest and most endangered pond turtles, and is currently found in three largely disjunct groups of populations: in the southern U.S., in the northeast, and in the Finger Lakes and Lake Ontario Plains region of western and central New York State. All the New York sites and most of the northeastern sites were glaciated during the Pleistocene. We surveyed 2793 bases pairs of mitochondrial DNA spanning three genes (cytb, nd4, and d-loop) in 41 individuals from 21 populations throughout most of the bog turtle's distribution. We found surprisingly low levels of divergence among populations, even in southern populations that have been hypothesized as refugia during times of climate change. Our data suggest populations of bog turtle's suffered a bottleneck, followed by a rapid post-Pleistocene expansion into northern segments of the species' range. We discuss historical changes in habitat availability and climate that may have influenced the historical deployment of lineages in this species, and possible life history traits and habitat dynamics that might also contribute to the overall low genetic diversity across its range.

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Introduction

The spatial genetic structure of an organism is an outcome of the combined evolutionary forces acting within populations (e.g. drift and selection) and historical biogeographical events such as vicariance and changes in habitat that affect the distribution of evolving lineages (e.g. Hewitt 2000; Austin et al. 2002). In addition, species-typical life history characteristics and ecologies such as clutch size, longevity, dispersal capacity, and habitat specificity can increase or decrease the rate and direction of among-population genetic differentiation (Scribner et al. 1986, 1993; Nevo and Beiles 1991; Peterson and Denno 1998a, b; Hoelzel 1999). For example, all else being equal, we would expect that specialists occupying fragments or patches of habitat are more likely to exhibit higher genetic divergence among subdivided populations, particularly if populations have small effective population sizes (Nunney 1991). Intraspecific molecular phylogenies and population genetic studies are widely used to evaluate the distribution of genetic diversity among populations and regions and can offer insight into historical effects of isolation, biogeographic changes, and demographic and life history characteristics that contribute to rangewide genetic structure (Avise et al. 1987; Moritz 1994).

Much of eastern North America was glaciated during the climatic cycles of the late Pleistocene and the effect of these climatic and habitat changes have been demonstrated in many ectotherms with northern

distributions (Bernatchez and Dodson 1991; Holman and Andrews 1994; Hewitt 2000; Austin et al. 2002; Zamudio and Savage 2003). The common genetic signature for most of these species is a pattern of rapid population expansion from southern refugia, with large recolonized regions that are genetically homogeneous (Hewitt 1996, 2000; Bernatchez and Wilson 1998). Despite this common genetic pattern, the variance in degree and scale of population differentiation is high; with the accumulation of phylogeographies for more taxa, it is becoming clear that biogeographical processes alone are not sufficient to explain patterns among all species examined. Therefore, to fully understand genetic diversity in post-glacial populations we will ultimately have to examine the contribution of life history, ecology, and demography in the origin and maintenance of population differentiation (Nevo and Beiles 1991; Ross 1999; Austin et al. 2004).

In this study, we use mitochondrial DNA (mtDNA) sequence data to reconstruct the phylogenetic history of bog turtles (*Glyptemys muhlenbergii*, formerly *Clemmys muhlenbergii*; Holman and Fritz 2001), a rare and endangered semi-aquatic turtle distributed in the eastern U.S. Bog turtles range as far north as northern New York, well into regions covered by ice sheets during the last glacial maximum (Shackleton and Opdyke 1977; Shackleton et al. 1984); therefore, northernmost populations must have resulted from post-glacial colonization from southern refugia. A previous preliminary rangewide survey of genetic variation suggested genetic uniformity within bog turtles (Amato et al. 1997). Nonetheless, because of habitat requirements, life history and demographic characteristics, we would predict that this species should exhibit high levels of population differentiation when compared to other ectotherms with similar distributions. Of all North American turtles with northerly distributions, the bog turtle has the most disjunct and fragmented distribution at local and rangewide scales (Fig. 1; Conant and Collins 1998). This species is a habitat specialist, living in open canopy wetlands (bogs and fens) and wet meadows (Tryon and Herman 1990; Carter et al. 1999; USFWS 2001). These habitats are patchily distributed especially in the northern regions of the species' range (Klemens 1990, 1993; Tryon and Herman 1990). The range of the bog turtle is also highly discontinuous at a regional scale and remaining populations can be grouped into: (1) southern populations in Virginia, North Carolina, South Carolina, Georgia, and Tennessee; (2) northeastern populations in Delaware, Maryland, Connecticut, Massachusetts, New Jersey, eastern Pennsylvania, and eastern New York and (3) Lake Plains/Prairie Peninsula populations in western and central New York. A fourth historical population group in western Pennsylvania is believed to be extirpated (Fig. 1; Breisch 1988; Conant and Collins 1998; USFWS 2001).

In addition to a fragmented distribution, the bog turtle exhibits life history characteristics that should promote high levels of genetic differentiation among populations. Bog turtles are one of North America's smallest and rarest turtle, measuring 80–120 mm total carapace length (Klemens 1990, 1993; Ernst et al. 1994). They are long-lived and persist in small isolated populations, usually composed of less than 50 individuals (Klemens 1990, 1993; Tryon 1990). Bog turtles also have relatively low vagility (Ernst 1977; Chase et al. 1989), with infrequent instances of dispersal over longer distances or unsuitable habitat (Carter et al. 2000). Combined, we expected that restricted movement and high habitat specificity would limit gene flow and that small population sizes would further enhance genetic divergence among isolated populations (Walker and Avise 1998; Clark et al. 1999).

Over the past 20 years, this species has experienced a 50% population decline due to loss and alteration of wetland habitats, invasive wetland plant species, hydrological changes, toxic and organic pollution, and illegal collection (Groombridge 1982; Tryon and Herman 1990; USFWS 2001). In addition, habitats used by this species typically undergo ecological succession, from open canopy fens and bogs to closed canopy swamps, leading to the loss of suitable habitat that is not currently replaced (Klemens 1993; Herman and Tryon 1997). Locally extirpated populations are usually not repopulated, either because modified habitat patches can no longer support bog turtle populations, or because limited dispersal capacity reduces the likelihood that metapopulation dynamics can be sustained. In 1997, the US Fish and Wildlife Service listed northern allopatric populations as ''threatened'' under the Endangered Species Act due to habitat specialization and dwindling population sizes; at the same time southern populations were also listed due to similarity of appearance (USFWS 2001). Bog turtles are also listed as a CITES I species, which ranks them as one of the most imperiled turtles in the world (Turtle Conservation Fund 2002).

Given the conservation concern for this species, their current patchy distribution, and their unique life history characteristics, we surveyed rangewide genetic variability to determine intraspecific patterns of genetic diversity. Specifically, our goals were to examine the degree of genetic differentiation among regional population groups, the genetic signatures of past demographic processes such as post-Pleistocene colonization,

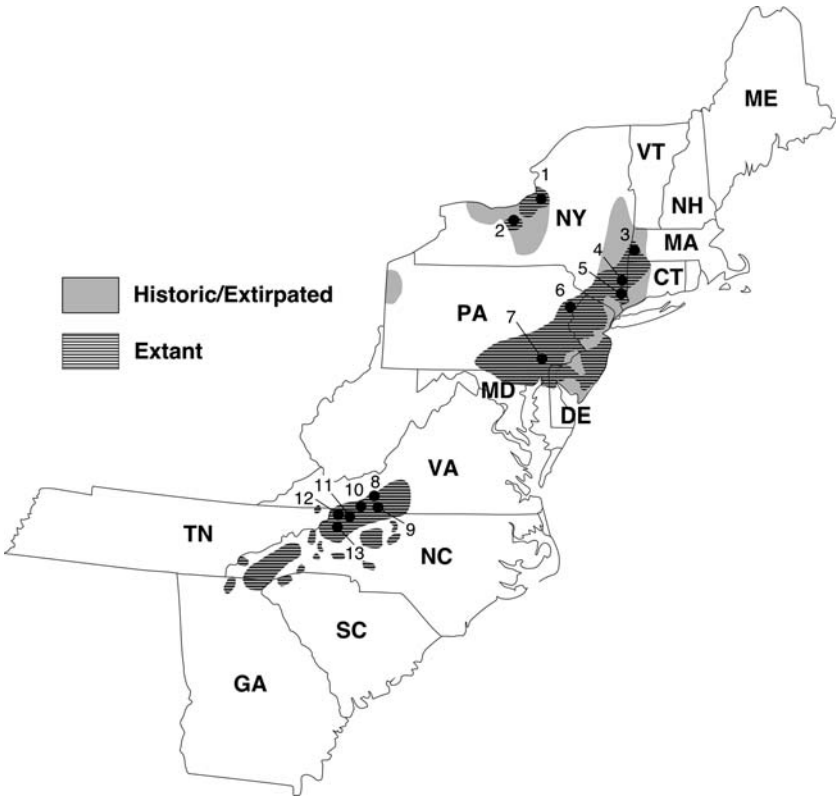

Fig. 1 The geographic range of *Glyptemys muhlenbergii* can be divided in to three geographically isolated segments occupying the southern, northeastern, and the Lake Plains/Prairie Peninsula regions of the species' range. Numbered dots identify counties where single or multiple populations were sampled for this study. Populations sampled correspond to those in Table 1.

and compare our results to patterns observed in other turtle species with similar distributions.

Methods

Population sampling

We sampled *G. muhlenbergii* from 21 localities distributed across the three disjunct segments of the species' range, including 11 sites in the South, 7 sites in the Northeast, and 3 sites in the Lake Plains region of New York (Fig. 1, Table 1). We chose *Glyptemys insculpta* and *Chrysemys picta* as successively distant outgroup taxa (Holman and Fritz 2001; Feldman and Parham 2002). Our sample of *G. insculpta* was obtained from the Royal Ontario Museum (accession number 1523); all *C. picta* sequences were obtained from Genbank (AF069423).

Molecular methods

We extracted DNA from blood samples collected in New York using a standard digestion with Proteinase K and lysis buffer followed by phenol–chloroform organic clean up (Sambrook and Russell 2001). Samples from North Carolina and Massachusetts were extracted with the Puregene DNA Isolation Kit (T. King pers comm). A previous study of genetic variability in this species (Amato et al. 1997) compared 291 bp of the 16S mtDNA gene, a gene known to evolve relatively slowly in vertebrates (Lopez et al. 1997; Pesole et al. 1999). To maximize our chances of capturing even low levels of diversity within this species, we sampled more populations throughout the range and targeted gene regions with higher rates of evolution. We assayed individuals for variation at three mitochondrial gene fragments: (1) the 5′ segment of the NADH dehydrogenase subunit 4 and the adjacent Histidine, Serine, and Leucine transfer RNAs (hereafter referred to as nd4), (2) the complete cytochrome *b* and partial sequence of the adjacent Threonine transfer RNA (hereafter referred to as cytb), and (3) a partial sequence at the 5′ end of the control region within the displacement loop (hereafter referred to as d-loop). We used the polymerase chain reaction (PCR) to amplify the nd4 fragment using primers ND4 and Leu (Arévalo et al. 1994), the cytb gene using primers M (Shaffer et al. 1997) and GLUDG (Palumbi et al. 1991), and the partial d-loop fragment using primers Des1 and Des2 (Starkey et al. 2003). Reactions were performed in a total volume of

Table 1 Sampling localities, sample sizes (*N*), and geographic distribution of unique haplotypes in *Glyptemys muhlenbergii* populations included in our study. Localities correspond to those in Fig. 1.

Region	State	Locality	County	Haplotypes	*N*
Lake Plains	New York	1a	Oswego	A	5
	New York	1b	Oswego	A/C	3/1
	New York	2	Seneca	A/B	1/1
Northeast	Massachusetts	3	Berkshire	A	2
	New York	4	Columbia	A	2
	New York	5a	Dutchess	A	1
	New York	5b	Dutchess	A	2
	New York	5c	Dutchess	A	2
	Pennsylvania	6	Monroe	A	1
	Pennsylvania	7	Chester	A	1
South	Virginia	8	Floyd	E	2
	Virginia	9	Patrick	E	2
	Virginia	10	Carroll	E	2
	North Carolina	11	Surrey	E	1
	North Carolina	12a	Alleghany	E*	1
	North Carolina	12b	Alleghany	D	2
	North Carolina	12c	Alleghany	D/E	1/1
	North Carolina	13a	Wilkes	D	2
	North Carolina	13b	Wilkes	D	2
	North Carolina	13c	Wilkes	D	2
	North Carolina	13d	Wilkes	D	1
Total					41

*Haplotype designation based on 5 of 6 variable sites for cytb and d-loop.

25 µl, each containing 100 ng template DNA, 1× PCR Buffer, 0.75 mM dNTPs, 1.5 mM $MgCl_2$, 1 uM primer, and 0.625 units of Taq polymerase. PCR conditions were the same for all three fragments: 95°C initial denaturation for 5 min, 35 amplification cycles of denaturation at 94°C for 1 min, annealing at 50°C for 1 min, extension at 72°C for 1 min, and a final 5 min extension at 72°C. Exonuclease I (10 units) and SAP (1 unit) were used to remove unincorporated oligonucleotides and dNTPs. For each successful amplification we performed cycle sequencing reactions with Big Dye terminator sequencing kits according to manufacturer's protocol (Applied Biosystems, Perkin Elmer, Foster City, CA). Cycle sequencing reaction conditions were 25 cycles of 96°C (30 s), 50°C (15 s), and 60°C (4 min). We used the same sequencing primers used in the original amplification, with the exception of an internal sequencing primer designed for cytb (Cmprimus; 5′-TGAGGCCAAATATCCTTCTGAGGTGCCACCG-3′). We sequenced each gene in both directions to avoid base-calling ambiguities. Products were column purified to remove non-incorporated terminator dye using Sephadex™ G-50 and products were electrophoresed on a 6% denaturing polyacrylamide gel on an ABI 377 automated sequencer. For 41 bog turtle samples we sequenced 889 bp of the nd4-tRNA fragment, 1179 bp of the cytb–tRNA fragment and 725 bp of d-loop (corresponding to positions 10920–11809, 14399–15583, and 15889–16563 of the complete mtDNA genome for *C. picta*; Mindell et al. 1999). We detected very low levels of nucleotide diversity in our initial rangewide sampling of bog turtles, therefore, in addition to the 41 individuals sequenced at all gene fragments, we also sequenced one or two target genes for an additional 33 individuals to determine whether the low levels of observed variability could be explained by low sample sizes. Electropherograms were checked by eye using the editing program Sequencher v.4.1 (GeneCodes, Michigan).

Analyses

We aligned cytb, nd4, and d-loop sequences separately using ClustalW (Thompson et al. 1994) in the MegAlign v. 6.1.2 program of the Lasergene sequence analysis software (DNASTAR, Inc., Madison, Wisconsin). For each gene, we used gap penalty to gap length ratios ranging from 2/3 to 20 (2/3, 1, 2, 5, 10, 16.6, 20) to identify any regions of ambiguous homology (Gatesy et al. 1993). All other alignment parameters were held constant at the default values.

Low levels of nucleotide diversity precluded meaningful phylogenetic reconstruction. Therefore, we used statistical parsimony (Templeton et al. 1992), implemented in the program TCS version 1.13 (Clement et al. 2000), to examine the evolutionary relationships among haplotypes. We calculated haplotype diversity ($h = 1–\Sigma\, fi^2$, where f is the frequency of the ith haplotype; Nei 1987; Avise 2000) and nucleotide diversity, the average number of nucleotide differences per site between sequences, $\Pi = \Sigma\, fi\, fj\, pij$ where p_{ij} is the sequence divergence between the ith and jth haplotype;

Nei 1987; Avise 2000) to evaluate the genetic signature of possible historical demographic changes in bog turtle populations. We quantified h and Π at two scales: range wide (all populations) and regionally (with northern and southern populations analyzed separately).

Mismatch distributions of individual pairwise differences between mtDNA haplotypes provide information about historical demography of populations (Rogers and Harpending 1992; Rogers et al. 1996). Theoretical studies have shown that population growth has a strong affect on patterns of genetic polymorphism (Rogers and Harpending 1992). Populations in long and stable demographic equilibrium have multimodal distributions, whereas the distribution is unimodal (approximately Poisson) in populations that have passed through demographic expansions (possibly following a bottleneck). To test for population expansion, we conducted a mismatch distribution test using Arlequin v. 2.0 (Schneider et al. 2000) to calculate the expansion parameters τ, θ_1, and θ_0. These parameters are fitted by a generalized least square procedure and represent age of expansion ($\tau = 2\mu t$) expressed in units of mutational time, and population size before ($\theta_0 = 2\mu N_0$) and after ($\theta_1 = 2\mu N_1$) expansion, where N_0 and N_1 are the effective female population sizes. The fit of mismatch distributions to the expansion model was assessed with 1000 Monte Carlo simulations and we calculated the upper and lower boundaries of the mismatch at $\alpha = 0.01$. The significance probability was calculated by comparing the sum of square deviations (SSD) between the observed data and those from the simulated expansion model; a significant P value rejects the fit of the data to the expansion model. We also calculated Tajima's D (Tajima 1989) and Fu's F_S (Fu 1997). Historical population growth predicts significantly negative D and F_S values (Tajima 1989; Fu 1997). The significance of any deviation from values expected under demographic stationarity was tested with 1000 bootstrap replicates in Arlequin v. 2.0. Because the northern (including both Northeastern and Lake Plains populations) and southern regions of the species range may have very different histories, we also considered them separately in analyses.

Results

We detected only five haplotypes among the 41 *G. muhlenbergii* individuals sampled for all three genes combined (total 2793 bp), a surprisingly low level of genetic variation. We did not find any polymorphism among all nd4 sequences (100% similarity); we detected four polymorphic nucleotide sites in the cytb fragment (three transitions and one transversion; average pairwise similarity = 99.84%) and two nucleotide transitions in the d-loop (average pairwise similarity = 99.87%; Table 2). We combined cytb (1179 bp) and d-loop (725 bp) fragments and aligned the total 1904 nucleotides for analyses. Because our initial results were surprising, we increased our sampling by sequencing 33 additional samples (primarily from the northern part of the range) at one or two of our target gene fragments to confirm that the pattern was not a result of small sample sizes. Additional sampling revealed no novel or undetected haplotypes and the data were entirely consistent with the findings of the original sample. Northern bog turtles had genetic profiles consistent with haplotype A (15 additional individuals were sequenced at nd4, 12 individuals at cytb, and 5 at a combination of two genes (nd4/cytb, cytb/d-loop or nd4/d-loop). We sequenced cytb for one additional turtle from the south and that individual had a sequence profile consistent with haplotype D. We continued our analyses with the 41 samples for which we had complete data at all three gene fragments, since they apparently encompass all the diversity found throughout the range of this species.

Sequence alignment using ClustalW resulted in zero alignment-invariant sites and zero alignment-ambiguous sites for cytb, and 2 alignment-invariant sites and 14 alignment-ambiguous sites for d-loop. All alignment

Table 2 Variable nucleotide sites for *Glyptemys muhlenbergii* across three mitochondrial genes.

Haplotype	cytb				d-loop	
	14404	14624	14625	15392	16317	16360
A	C	G	T	T	T	C
B	.	.	G	.	.	.
C	.	.	.	.	C	.
D	T	.	.	.	.	.
E	T	A	.	C	.	T

All individuals sequenced were monomorphic for the nd4 fragment, thus the six variable sites were found in cytb (4 sites) and d-loop (2 sites) only. Numbers correspond to nucleotide positions in the *C. picta* mitochondrion (Mindell et al. 1999); dots indicate bases equal to those in haplotype A. Sequences for each gene fragment have been accessioned in Genbank (DQ499646–DQ499656).

ambiguous sites were indel mutations between the outgroups and bog turtle sequences, and none corresponded to polymorphic sites within bog turtle samples.

Despite this low rangewide and regional nucleotide diversity, we did find fixed genetic differences between populations in the northern and southern portions of the range. Bog turtle populations in the Lake Plains/Prairie Peninsula region included one of three haplotypes found at unequal frequencies: haplotypes A (82%), B (9%) and C (9%). Populations in the northeast were fixed for haplotype A. In the south haplotypes D and E were observed in approximately equal frequency (56% D and 44% E). Haplotype A, the most common haplotype in the northern segments of the species' range, is a single nucleotide transitional mutation from the widespread southern haplotype D (Fig. 2, Table 2). Haplotypes B and C are a single mutational step from the widespread northern haplotype A and these two haplotypes are each restricted to single populations in the Lake Plains region (Fig. 2, Table 1). One North Carolina individual had a genetic profile consistent with Haplotype E across 2554 of 2793 bp (including 5/6 variable sites) but this haplotype assignment is tentative because of missing data at the cytb 'T/C' positional transversion (14404; Table 2). Due to exceedingly low levels of variation, even a single error at polymorphic sites can affect patterns of geographic genetic diversity, therefore the sequence for this individual was not included in the diversity or mismatch analyses. The outgroup taxa were too divergent for inclusion within the haplotype network, and exceeded the 95% confidence interval for statistical parsimony. *Glyptemis insculpta*, the immediate outgroup to bog turtles (Feldman and Parham 2002) is 95 mutational steps from the nearest bog turtle haplotype.

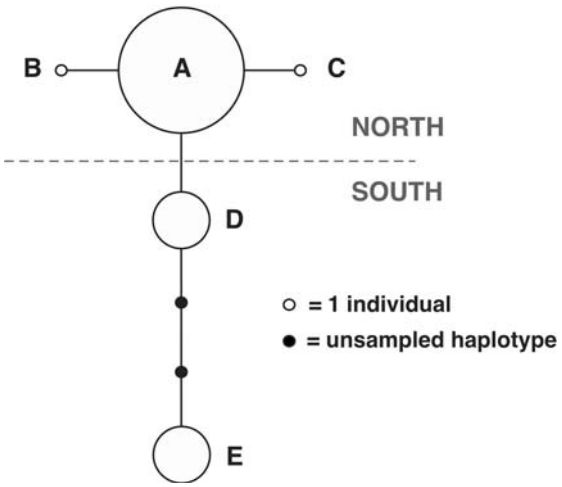

Fig. 2 TCS haplotype network for 5 haplotypes (cytb and d-loop fragments combined) detected in 41 *Glyptemys muhlenbergii* individuals. Each line represents a single mutational change. Each haplotype is represented by a circle and the area of the circle is proportional to the number of individuals with that haplotype. Black circles indicate intermediate haplotypes not sampled in this study.

Rangewide haplotype diversity was moderately high ($h = 0.664$) while nucleotide diversity was exceptionally low ($\Pi = 0.000279$). We found regional differences in these measures of genetic diversity; the two northern population groups are characterized by lower haplotype and nucleotide diversity ($h = 0.169$, $\Pi = 0.00003$) compared to southern populations ($h = 0.5$, $\Pi = 0.000452$). Mismatch analyses significantly supported the hypothesis of range-wide population expansion, but the patterns differed among regions; we detected a signature of rapid population expansion for northern populations but not for southern populations using goodness-of-fit test, Tajima's D, and Fu's F_S (Fig. 3, Table 3).

Discussion

Possible causes of low genetic diversity

We predicted deep intraspecific divergences and population structure among bog turtle populations due to a number of intrinsic and extrinsic factors, including fragmented range, historical barriers to gene exchange, small population sizes, high longevity, low dispersal capacity, and low reproductive rate. Indeed, we found significant differentiation (no shared haplotypes between northern and southern regions; Table 3); however, contrary to our predictions we observed extremely shallow divergence suggesting alternative evolutionary forces have shaped the genetic composition of this species. We discuss four possible scenarios that could explain the extremely low levels of divergences observed in this species: (1) slow rate of mtDNA evolution, (2) selective sweep of the mtDNA genome, (3) population bottleneck due to Pleistocene recolonization, and (4) an interaction between life-history and habitat specificity that reduces the fixation of geographic variation in this system.

Slow rate of turtle mitochondrial DNA evolution is one potential explanation for the genetic homogeneity among bog turtle populations (Amato et al. 1997; King and Julian 2004). Although turtle species vary significantly in their degree of intraspecific differentiation (Walker and Avise 1998), an increasing number of rangewide studies have revealed relatively low levels of variation in many freshwater species, despite broad geographic distributions and potential barriers to gene flow (Weisrock and Janzen 2000; Starkey et al. 2003; Spinks and Shaffer 2005). In a now classic survey of

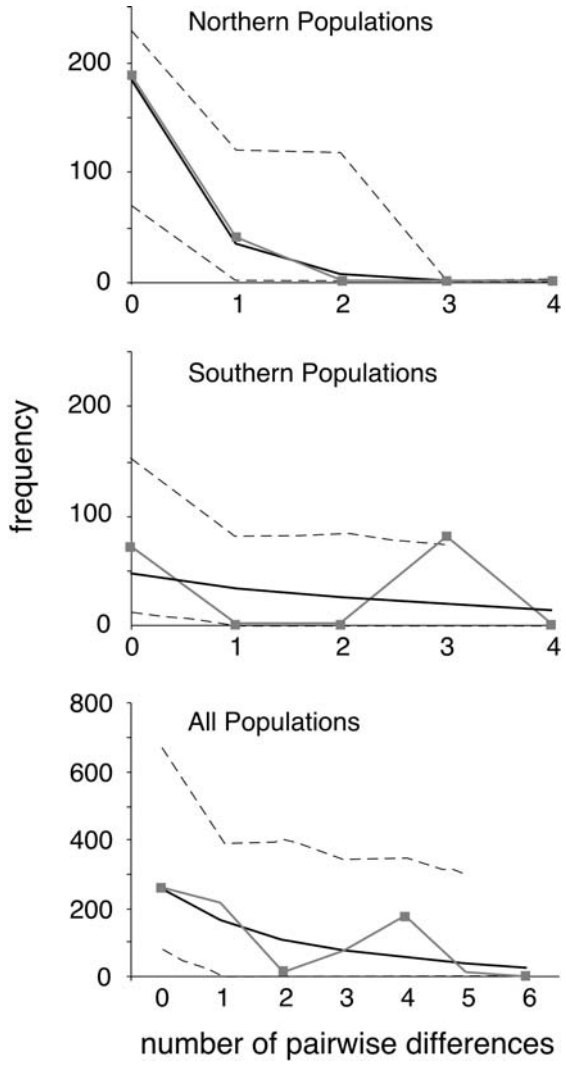

Fig. 3 Pairwise mismatch distributions for northern populations (Northeast and Lake Prairie populations combined), southern populations, and all populations of *Glyptemys muhlenbergii* included in this study. Gray lines/squares represent the observed frequencies of pairwise differences among haplotypes, bold lines show expected values for populations that have undergone historical demographic expansion, and dashed lines represent upper and lower 99% confidence intervals derived from the simulated number of polymorphic sites. The distribution of polymorphism in northern populations suggests population expansion; corresponding results of the goodness of fit and neutrality tests are reported in Table 3.

levels of genetic polymorphism in 22 species of turtles, divergences among clades varied from 0.010 to 0.070% (Walker and Avise 1998). In contrast, the bog turtles sampled in our study exhibit a net sequence divergence ranging from 0.001 to 0.003%, an order of magnitude smaller than those previously reported. Thus, a ''turtles-pace'' rate of mitochondrial evolution (Avise et al. 1992) probably contributes, but it is likely not the sole explanation for the extremely low genetic variability in this species.

Distinction between selective sweeps and population bottlenecks (demographic factors) is possible through assay of genetic diversity across multiple, unlinked loci (Galtier et al. 2000; Hahn et al. 2002). Recent analysis of genetic variability at nuclear microsatellite loci (King and Julian 2004) detected polymorphism within a single population from Maryland, suggesting that not all bog turtle loci lack genetic variability. However, it is not yet known whether genetic variation at microsatellite markers is partitioned geographically. Given differences in mutation rates between microsatellites and mtDNA genes, it is difficult to assess whether the variability at microsatellite loci represents historical variation, or whether those levels of differentiation might be expected to accumulate at hypervariable loci in the time since a population bottleneck reduced variability at all loci. We did not sample additional unlinked nuclear loci for this study. Instead, we evaluate the likelihood of a selective sweep and/or bottleneck in bog turtles using coalescent theory (mismatch analyses) and diversity indices, and consider each within the framework of the geologic and landscape history.

Given the extent of glaciation and habitat change in North America, we assume that bog turtles found refuge in the southern portions of the range and subsequently expanded northward along with receding glaciers in the last 13,000–20,000 years (Shackleton and Opdyke 1977); therefore shifts in habitat could be important factors in regulating patterns of genetic variation in this species (Hewitt 1996, 2000). Low genetic diversity in populations of historically glaciated regions has been reported for many plant and animal taxa (Barrett and Kohn 1991; Lewis and Crawford 1995; Lenk et al. 1999; Vellend and Waterway 1999; Weisrock and Janzen 2000; Riberon et al. 2001; Alexandrino et al. 2002; Zamudio and Savage 2003). However, a broad survey of post-glacially distributed European plant and animal taxa showed incongruent post-pleistocene phylogeographic patterns, suggesting that recolonization cycles and rates differ among species (Taberlet et al. 1998). Thus, while geological history influences the diversity within and among populations, species-typical traits are important in ultimately shaping contemporary population structure.

Our analyses are consistent with the Pleistocene extinction and population expansion models (Hewitt 1999) that predict northern populations will have reduced genetic diversity and appear ''younger'' in evolutionary time due to their recent expansion (Hewitt 2000). The most common northern bog turtle haplotype (Haplotype A) is only a single mutational step from a widespread southern haplotype, suggesting a shared common ancestor (Fig. 2). Haplotype and nucleotide diversity values (Grant and Bowen 1998;

Table 3 Mismatch distribution models, Tajima's D, and Fu's F_S, detect different historical demographic processes in northern and southern segments of the bog turtle's range.

Population			
	North	South	Combined
Model parameters			
N	22	18	40
S	2	3	6
τ	3.00 (0.401–4.254)	4.02 (0.619–12.904)	4.87 (0.471–12.535)
θ_1	0.078 (0–0.802)	0.003 (0–8.667)	0.002 (0–6.861)
θ_0	0.227 (0–2300.227)	2.427 (0–4266.802)	2.088 (0.15–2396.462)
Goodness-of-fit test			
SSD	0.0011	0.2701	0.045
P	0.429*	< 0.001	0.326*
Tajima's D	–1.514	2.276	0.511
P	0.010*	0.992	0.633
Fu's F_S	–1.974	4.253	1.169
P	0.007*	0.976	0.811

We tested the significance of the model of population expansion using a goodness-of-fit test (Rogers and Harpending 1992; Schneider and Excoffier 1999), Tajima's D (Tajima 1989), and Fu's F_S (Fu 1997). The parameters of the model of population expansion, τ, θ_0, and θ_1, are the age of expansion, and population size before and after expansion. S is the number of segregating sites. We report goodness-of-fit tests for the mismatch distributions (based on SSD, sum of squared deviations) and significance of D and F_S. Non-significant values for SSD ($P > 0.05$) signify that the data do not deviate from that expected under the model of expansion. Significant negative D and F_S values are expected in cases of population expansion.

*Model significant for population expansion.

Avise 2000) further corroborate this scenario of post-glacial population expansion. The low nucleotide and haplotype diversity observed in northern populations could indicate a population expansion from a single (or few) haplotypes following population bottleneck. In contrast, southern populations exhibit higher nucleotide diversity, suggesting rapid population growth following a period of low effective population size. The difference between these two scenarios is based on the genetic diversity of founding populations and population sizes prior to the presumed bottleneck as well as sufficient time since the bottleneck to accumulate mutations (Grant and Bowen 1998). We suggest that northern bog turtle populations were recently founded by colonizers bearing few haplotypes while the source populations in the south were larger and more diverse. Accordingly, our mismatch distribution analyses and significance of D and F_S detected a signature of rapid population expansion for northern populations, but not for southern ones, suggesting that demographic processes in the north are unique (Fig. 3, Table 3). Mismatch analyses also supported a rangewide population expansion, however this pattern is likely driven by the highly skewed distribution of haplotypes in the north.

Our inferences about historical demography of the bog turtle are based on a small number of haplotypes and few segregating sites, raising the question whether our data can provide sufficient statistical power for tests of population expansion. A recent study using analyses of reduced data sets and simulations of expanding and stable populations, demonstrated that mismatch analyses, D, and F_S were robust and continued to show the expected significant patterns even with vastly reduced datasets (Pereira et al. 2001). As expected, the mean and variance of mismatch distributions changed, but the genetic patterns were generally retained even with only 10 segregating sites (the least diverse subsampled dataset in the study). A comparison of the performance of the three tests for population expansion suggested that Tajima's D may be more robust than F_S in cases of small sample sizes, but F_S is more sensitive when few polymorphic sites are present (Pereira et al. 2001). Our data set contains both small sample sizes and low polymorphism; however, given the robustness of these tests, repeatable results across tests of the bog turtle data, and the different patterns detected in northern and southern populations, we conclude that these patterns are likely not merely statistical artifacts.

Combined, our data suggest a historical population bottleneck with subsequent rapid expansion for northern turtle populations. Although we cannot eliminate selective sweep or slow mtDNA evolution as possible explanations of low genetic diversity in bog turtles, we do find genetic evidence compatible with historical bottlenecks and post-glacial colonization and hypothesize that the patterns of genetic diversity we observed most likely resulted from these demographic processes.

Post-Pleistocene range expansion may account for the reduced genetic variability of bog turtle populations; however, other factors may also enhance genetic uniformity in long-lived vertebrates (Kuo and Janzen 2003, 2004; Mockford et al. 2004). Bog turtles inhabit open-canopy wetlands that are subject to ecological succession to closed-canopy, wooded swamplands rendering them inappropriate for this species (Tryon and Herman 1990; Klemens 1993). It is assumed that historically turtles compensated for these natural habitat shifts via migration (Carter et al. 2000) and that natural processes such as beaver activity or fires created new habitat patches, thus maintaining populations connected through low levels of gene flow. This mechanism has been proposed for other long-lived organisms that rely on low levels of gene flow to balance the genetic cost of isolation (Scribner et al. 1984; Mockford et al. 2004). In addition to being long-lived, bog turtles also have small population sizes, delayed sexual maturity, and low reproductive output (USFWS 2001); life history characters that inhibit rapid genetic change (Bromham 2002). It is possible that the turnover rate of suitable habitat exceeds the expected rate of genetic fixation in a population. In other words, the life history characteristics of this species, coupled with specialization on a ''short-lived'' habitat could result in a pattern of repeated extinction–recolonization dynamics in neighboring populations. This combination of life history and habitat turnover (promoting low fixation and multiple founder events) could potentially result in the repeated loss of polymorphism and retention of few mitochondrial haplotypes. Future finer-scale population genetic studies and modeling approaches could be used to investigate the feasibility of this scenario as a general explanation for low levels of diversity in taxa with this combination of characteristics.

Phylogeographic comparison with other turtles in eastern North America

Population genetic studies have been carried out in three North American freshwater turtles with distributions similar to that of the bog turtle: the painted turtle, *C. picta* (Starkey et al. 2003), softshell turtles, *Apalone spinifera* (Weisrock and Janzen 2000), and the common snapping turtle, *Chelydra serpentina* (Phillips et al. 1996; Walker et al. 1998). In general, these studies reveal a shared history of northward post-glacial colonization from more genetically diverse southern populations, although absolute degrees of genetic differentiation vary substantially among studies.

Apalone spinifera (softshell turtle) and *Chrysemis picta* (painted turtle) both exhibit higher genetic diversity and structure in southern populations relative to northern populations. A mitochondrial DNA phylogeny supports the conclusion that *A. spinifera* populations in the north were derived from southern populations as a result of post-Pleistocene glacial dispersal (Weisrock and Janzen 2000). Similarly, phylogenetic analyses of *C. picta* (painted turtle) populations across North America revealed four distinct clades, one of which is less genetically diverse and widespread in the northeastern U.S., the pattern expected after regional glacial extirpation and recolonization events (Starkey et al. 2003). Phylogeographic sampling of *Chelydra serpentina* (common snapping turtle) revealed low overall genetic variation and shallow population divergences; however, sampling for that study focused on south-eastern populations (Walker et al. 1998); therefore direct genetic comparisons are not possible with our data set. However, all three species show higher genetic diversity rangewide than we found in bog turtle populations, suggesting that the demographic changes that shaped genetic distribution in *G. muhlenbergii* were extreme, even when compared to other co-distributed turtles.

Analyses of fossil faunas suggest that the painted and the snapping turtle were early invaders of formerly glaciated areas at the end of the Wisconsinan (Holman and Andrews 1994), and these species have several behavioral, physiological, and reproductive adaptations for cold tolerance (Obbard and Brooks 1979, 1981a; Storey et al. 1988; St. Clair and Gregory 1990). Although these species are generally habitat generalists and are capable of significant overland dispersal (Obbard and Brooks 1981b; Ernst et al. 1994; Holman and Andrews 1994), a common pattern in phylogeographic structure of these widespread freshwater turtles is the apparent reduced levels of variability even in southern portion the range (Walker et al. 1998; Starkey et al. 2003). The population-level phylogeny of painted turtle suggests that this species was present in the Great Plains/Rocky Mountain region, was extirpated, and subsequently recolonized the region, perhaps due to a brief period of aridification approximately 14,000-years ago (Starkey et al. 2003). These extinction/colonization dynamics reduce genetic differentiation and are similar to the more localized recolonization processes we propose as a mechanism for reduced population-level variability in bog turtles inhabiting temporary successional habitats.

Conservation implications

Due to anthropogenic landscape changes, the loss of fens and bogs in the last century far exceeds the creation of

novel, suitable habitat patches (Herman 1989; Tryon and Herman 1990; Klemens 1990, 2000). Fragmentation of bog turtle habitat will further limit gene flow among remaining sub-populations, preventing the exchange of genetic diversity in this species. However, low genetic diversity seems to be a historical characteristic of this species, therefore, the lack of genetic variability is not likely the largest threat to the persistence of bog turtles. Preservation of extant habitats supporting populations with sustainable levels of recruitment and the restoration or creation of habitats in areas occupied historically are likely the most important measure necessary for conservation of this species.

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