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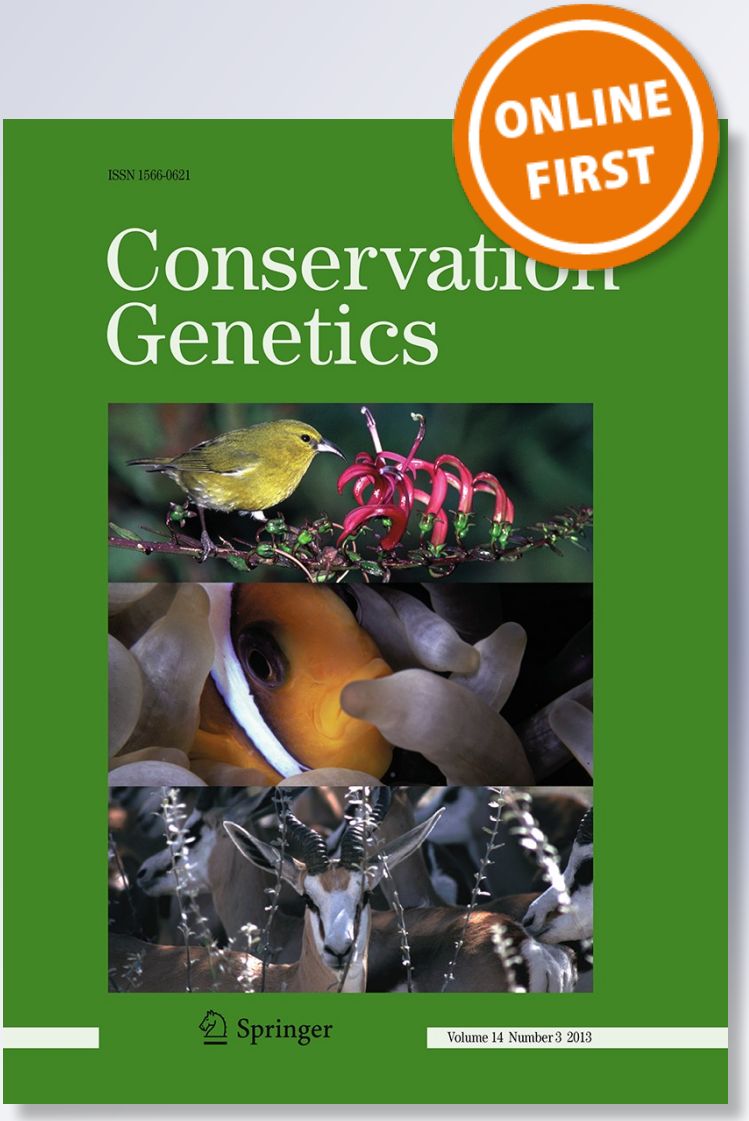
Conservation Genetics

ISSN 1566-0621

Conserv Genet
DOI 10.1007/s10592-013-0507-6



Springer

Conserv Genet
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RESEARCH ARTICLE

Conservation genetics of the yellow-blotched sawback *Graptemys flavimaculata* (Testudines: Emydidae)

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Received: 13 February 2013 / Accepted: 12 June 2013

Abstract Turtles within the genus *Graptemys* are increasingly becoming a group of conservation priority due to (1) the number of species endemic to single Gulf of Mexico rivers and (2) human alterations of riverine habitat. The yellow-blotched sawback (*Graptemys flavimaculata*) is a federally threatened species that is endemic to the Pascagoula River system of southeastern Mississippi, USA. Currently, there is nothing known about genetic variation and population structure in *G. flavimaculata*. We used microsatellite data to analyze population genetic structure, assess historical demography, and determine effective population size at six sites throughout the Pascagoula River system. Considerable genetic diversity was found within each site (mean allelic richness: 6.65–8.08) and two analyses found no evidence of genetic bottlenecks. All of the pairwise F_{ST} values, while low (average = 0.026), were significant, with most sites possessing one or more private alleles. Pairwise F_{ST} values with the Escatawpa River site were larger (0.030–0.047), which likely reflect its historical isolation. Genetic distance was correlated to geographic distance between sites, with the exception of the Escatawpa River site; a similar pattern was also found with estimates of recent rates of migration among sites. While an analysis of molecular variance indicated that most variation was partitioned within rather than among sites, STRUCTURE analysis strongly supported the recognition of two distinct groups (mainstem Pascagoula vs. Escatawpa), with the possibility of additional substructure within the mainstem Pascagoula.

Keywords *Graptemys flavimaculata* · River turtle · Conservation genetics · Microsatellites · Pascagoula River · Escatawpa River

Introduction

The intermingling of species conservation and genetics is becoming increasingly important as many habitats are altered by anthropogenic means and/or species are being directly exploited by humans (e.g., food or the pet trade). Conservation genetics provides a set of tools to assess the viability of individual populations, the connectivity among populations across various landscape scales, and the evolutionary uniqueness of certain lineages (Gibbs and Amato 2000). To date, relatively few studies have taken a conservation genetic approach with freshwater turtles, even though freshwater turtles (along with tortoises) are considered one of the most endangered animal taxa in the world (IUCN 2012). Of the 228 turtle species assessed by the IUCN, 134 species (59 %) are officially considered Threatened, with 76 species labeled as critically endangered or endangered (van Dijk et al. 2012).

Turtles within the genus *Graptemys* (family Emydidae; map turtles and sawbacks) are highly aquatic, freshwater turtles. Of the 14 species in the genus, 8 are endemic to single river drainages along the Gulf of Mexico in the southeastern United States (Ernst and Lovich 2009;

Electronic supplementary material The online version of this article (doi:10.1007/s10592-013-0507-6) contains supplementary material, which is available to authorized users.

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Published online: 20 June 2013

Lindeman 2013). *Graptemys*, like many other freshwater species, are extremely susceptible to human alterations of rivers, including river impoundments, channelization, water quality degradation via pollution or sedimentation, and operations that remove basking logs or snags (reviewed by Buhlmann and Gibbons 1997; Mitchell and Klemens 2000; Moll and Moll 2004). Consequently, due to their restricted distributions and observed population declines, many of these turtles are also recognized as imperiled or in need of conservation (Ernst and Lovich 2009).

The yellow-blotched sawback (*Graptemys flavimaculata*) is endemic to the Pascagoula River system of southeastern Mississippi, USA (Selman and Jones 2011; Fig. 1). Population declines of *G. flavimaculata* in the 1980s led to federal listing as a threatened species in 1991 (U.S. Fish and Wildlife Service 1991). Along with inhabiting the Pascagoula River, *G. flavimaculata* also occurs in its tributaries, including the Leaf, Chickasawhay, and Escatawpa Rivers (Cliburn 1971; U.S. Fish and Wildlife Service 1993; Selman and Qualls 2009). During recent surveys, individuals were also detected in new drainage localities, with most of these being smaller creeks within the Pascagoula River system (Selman and Qualls 2009).

The Pascagoula River system is the only major river system in the lower United States that remains relatively unimpacted by impoundments and diversions (Dynesius and Nilsson 1994). Even though it is considered a "free flowing" river system, recent surveys of *G. flavimaculata* determined that their distribution is noticeably patchy; turtles were not equally distributed along river stretches within the Pascagoula River system, with fewer individuals present in the headwaters of rivers and smaller tributary creeks (Selman and Qualls 2009). Population estimates range from 80 to 120 turtles per river km (rkm) in moderate-sized rivers to 280–600 turtles per rkm in larger rivers (Selman and Qualls 2009); little is known about population sizes in small rivers and large creeks. Additionally, following Hurricane Katrina in 2005, lower Pascagoula River populations declined 47 % (Selman and Qualls 2008), presumably due to a loss or eradication of prey items due to decreased water quality (i.e., increased salinity, decreased dissolved oxygen).

Several studies with freshwater turtles have investigated genetic differentiation and population structure throughout the species' range and used this information to make conservation recommendations. North American study species include the western pond turtle (*Emys marmorata*—Gray 1995; Spinks and Shaffer 2005), the painted turtle (*Chrysemys picta*—Starkey et al. 2003), and the alligator snapping turtle (*Macrochelys temminckii*—Echelle et al. 2010). All of these studies detected considerable genetic variation over the entire species' ranges, as well as identifying critical populations at risk or those

Fig. 1 Pascagoula River system of southeastern Mississippi, USA and sampling sites for *G. flavimaculata* (*circles*). The *black cross-hatching* represents the approximate range of *G. flavimaculata* within the Pascagoula River system as described by Selman and Qualls (2009)

populations that represented significant evolutionary lineages. This would be expected as all of these species have relatively large distributions, with *E. marmorata*'s range extending along much of the Pacific Coast, *C. picta*'s range extends across most of North America, and *M. temminckii*'s range is throughout much of the southeastern United States (Ernst and Lovich 2009). *E. marmorata and C. picta* are also known to move extensively overland to other aquatic habitats (Bury 1972; House et al. 2010).

To our knowledge, no research has focused on the population genetic structure of a riverine turtle species that occurs exclusively within a single drainage, including any of the endemic *Graptemys* species of Gulf river drainages. In fact, many aspects of the basic life history and ecology of these endemic *Graptemys* species remain poorly understood (Ernst and Lovich 2009). Our goals were to determine: (1) Is there population structure within the range of *G. flavimaculata*?; (2) If so, what does this tell us about the ecology and life history of the species?; and (3) How does this apply to current and future species

management plans? These genetic data, in conjunction with recent work on the distribution and abundance of *G. flavimaculata* (Selman and Qualls 2009), should provide a better understanding of the biology of this threatened species and help guide management, monitoring, and recovery efforts.

Materials and methods

Sample collection

From 2005 to 2009, we collected tissue or blood samples from 241 individuals at six sites within the Pascagoula River basin of southeastern Mississippi (Fig. 1). Study sites included: (1) upper Leaf River (Forrest County; $n = 52$), (2) lower Leaf River (Perry County; $n = 22$), (3) upper Chickasawhay River (Clarke County; $n = 21$), (4) lower Chickasawhay River (Greene County; $n = 60$), (5) lower Pascagoula River (Jackson County; $n = 54$), and (6) Escatawpa River (Jackson County; $n = 32$). Turtles were trapped by submerging basking traps (made of ¾″ PVC coated crawfish wire) from observed turtle basking structures, and we also captured turtles by dip net while turtles were basking on emergent deadwood or swimming near the water surface. For specific information on capture techniques see Selman and Qualls (2009) and Selman et al. (2012).

Once captured, samples were collected by one of two methods. Blood was collected from individuals from the coccygeal vein (tail) using a heparinized 1 mL syringe and a 26 ½ gauge needle. Blood samples (appox. 0.75–1 mL) were stored on ice for 4–6 h and then centrifuged. Plasma and blood cells were then separated and frozen. Alternatively, tails tips were collected and stored in 100 % ethanol. For each individual, capture locality data was collected via GPS (Garmin GPS 72), as well as other information including sex and morphometrics for related studies.

Laboratory methods

Selman et al. (2009) screened microsatellite loci originally developed for other related emydid turtles and identified six loci that reliably amplified and were variable in *G. flavimaculata* (*Malaclemys terrapin—TerpSH2*, *TerpSH5*, *TerpSH7* [Hauswaldt and Glenn 2003]; *Glyptemys muhlenbergii—GmuB08*, *GmuD70*, *GmuD88* [King and Julian 2004]). Total genomic DNA was extracted from individuals from each site with the DNeasy Tissue Kit (QIAGEN Inc.). Amplifications were conducted in a total volume of 12.5 μL using 50 mM KCl, 10 mM Tris–HCl (pH 8.3), 0.01 % gelatin, 200 μM dNTPs, 2 mM $MgCl_2$ (4 mM for *TerpSH7*), 0.5 units of *Taq* polymerase (Promega Co.), 0.3 μM of the M13 tailed forward primer (Boutin-Ganache et al. 2001), 0.3 μM of the reverse primer, 0.1 μM of the labeled M13 primer (LICOR Co.), 20–150 ng of template DNA, and water to the final volume. PCR cycling conditions consisted of an initial denaturing step of 94 °C for 2 min followed by 35 cycles of 30 s at 94 °C, 1 min at 52–60 °C, and 1 min at 72 °C. See Selman et al. (2009) for the annealing temperature used for each locus. A final elongation step of 10 min at 72 °C ended the cycle. Alleles were visualized on acrylamide gels using a LICOR 4300 DNA analyzer and scored using Gene Image IR v. 3.55 (LICOR Co.).

Data analyses

Genetic variation at each site was described using standard measures, including number of alleles (A), observed heterozygosity (H_o), and expected heterozygosity (H_e) as calculated by GenAlex 6.3 (Peakall and Smouse 2006). Loci were tested for Hardy-Weinburg equilibrium and linkage disequilibrium using GENEPOP for the web (Raymond and Rousett 1995; http://genepop.curtin.edu.au/). Program FSTAT 2.9.3 (Goudet 1995) was used to calculate genetic differentiation among populations (θ—Weir and Cockerham's (1984) unbiased estimator of F_{ST}), perform significance testing of F_{ST} values, and determine allelic richness (A_R). Critical values for the significance testing of the F_{ST} values were determined using the false discovery rate method of Benjamini and Hochberg (1995) as implemented by PAIRWISE v. 1.5 (available at http://www.public.asu.edu/~mwwatkin/Watkins3.html). This approach may be more realistic in detecting significant population structure in a conservation context (Narum 2006). We used separate ANOVAs to statistically compare measures of genetic diversity (A_R, H_o and H_e) among the six sites. We also used t tests to compare these same diversity measures among groups of sites representing the core (Pascagoula, lower Leaf, and lower Chickasawhay) and peripheral portions of the range (upper Leaf, upper Chickasawhay, and Escatawpa). We used JMP 9.0.2 (SAS Institute, Cary, NC) for ANOVAs and t tests.

We used several methods to determine if population structure existed between collection localities for *G. flavimaculata*. STRUCTURE 2.3.3 (Pritchard et al. 2000) uses a Bayesian approach to partition individuals into some number of genetically discrete populations that are in Hardy–Weinberg and linkage equilibrium. We tested values of K (number of populations) from 1 to 8 using a model of admixed ancestry and assuming allele frequencies were correlated between groups. We selected the Hubisz et al. (2009) option to use sampling location as part of the analysis. For each value of K we performed 20 separate replicates with a burn-in of 50,000 generations followed by

a subsequent 200,000 generations. The most appropriate value of K was determined by examining the probability scores for each value of K and by the ΔK method (Evanno et al. 2005) as calculated by Structure Harvester v 6.92 (Earl and von Holdt 2012). We averaged all 20 runs at the best values of K with CLUMPP v. 1.1.2 (Jakobsson and Rosenberg 2007) and visualized the results with DISTRUCT v. 1.1 (Rosenberg 2004). Furthermore, an analysis of molecular variance (AMOVA; Excoffier et al. 1992) as implemented by ARLEQUIN v. 3.11 (Excoffier et al. 2005) was used to examine the distribution of molecular variation within and among populations. Geographic distance between sites in river kilometer was traced from Google-Earth imagery. Our limited number of sites made significance testing of a Mantel test problematic (Jackson and Somers 1989) and therefore, we did not specifically test for isolation by distance but correlations between genetic and geographic distances will be discussed.

We estimated the migration rate (m) among sites over the past several generations using BAYESASS v. 3.0.1 (Wilson and Rannala 2003). This Markov chain Monte Carlo method does not require that populations be in Hardy–Weinberg or migration-drift equilibrium. Initial runs were used to determine the delta values for allele frequency, migration rate, and inbreeding that resulted in acceptance rates for proposed changes falling between 0.2 and 0.4 as per the authors' recommendation, which we found to be 0.4, 0.4, and 0.7, respectively. The program was run four times using different initial seed values for 1×10^7 iterations with a burn in of 1×10^6. Samples were collected every 100 generations and this was used to infer the posterior probability distributions of migration rates. To check for convergence, the parameter estimates were compared across the four independent runs and the trace files of the log probability were visualized using Tracer v. 1.5 (Rambaut and Drummond 2007).

Another important way that genetic data can be analyzed in a conservation context is through characterizing the demographic history of a population including effective population size (N_e) and population bottlenecks. We estimated N_e for each site using LDNE (Waples and Do 2008), which uses a bias correction (Waples 2006) of the linkage disequilibrium method (Hill 1981). Parameters for this analysis included a monogamous model of mating, excluded alleles with a frequency <0.02, and 95 % confidence intervals were estimated by jackknifing. By way of comparison, we summarized the density of *G. flavimaculata* at three of six sites from previously published population estimates and basking density surveys from all six sites (Selman and Qualls 2009).

During a genetic bottleneck, allelic diversity is lost faster than population heterozygosity, which produces an excess of heterozygosity relative to the observed number of alleles (Cornuet and Luikart 1996). We tested for evidence of genetic bottlenecks at all six sites with BOTTLENECK ver. 1.2 (Piry et al. 1999) using a two-phase mutation model with a 95 % proportion of stepwise mutation model, 10 % variance, and run with 1,000 iterations (e.g. Di Rienzo et al. 1994; Piry et al. 1999; Jones et al. 2004). We thereafter used a one-tailed, Wilcoxon Sign Rank Test to determine if significant differences existed between detected heterozygosity in comparison to expected heterozygosity at equilibrium. We also performed the M ratio test (Garza and Williamson 2001), which compares the number of alleles with their size distribution to look for evidence of a bottleneck. Parameter values used in this test included a proportion of one-step mutations of 90 % and an average size of non one-step mutations (Δg) of 3.5. We performed each test with two values of θ (1 and 10) corresponding to the suggested mutation rate of 5.0×10^{-4} and N_e values of 500 and 5,000, respectively. The significance of each value of M was determined by comparison to the critical value obtained from 95 % threshold of 10,000 simulations of an equilibrium population.

Results

None of the six sample sites deviated from Hardy–Weinberg equilibrium expectations nor demonstrated linkage disequilibrium after sequential Bonferroni correction (Rice 1989). Generally, the Pascagoula, upper Leaf, lower Leaf, and lower Chickasawhay sites had higher allelic richness (averaged > 7.5 alleles per locus) compared to the upper Chickasawhay and Escatawpa sites (averaged < 6.9 per locus; Table 1). Observed and expected heterozygosity values were high, ranging from 0.737 to 0.879 and 0.746 to 0.814, respectively. Both the upper Chickasawhay and Escatawpa sites had lower observed and expected heterozygosity levels relative to the other four sites (Table 1). However, we found no significant differences among our six sample sites for A_R ($F_{5,30} = 0.51$, $p = 0.76$), H_o ($F_{5,30} = 1.26$, $p = 0.31$), and H_e ($F_{5,30} = 0.76$, $p = 0.58$). We also found no significant differences between core versus periphery sites in A_R ($t = -1.33$, $p = 0.19$) or H_e ($t = -1.34$, $p = 0.19$), but H_o was significantly higher at core sites relative to periphery sites ($t = -2.10$, $p = 0.04$). Genetic diversity measures within populations (number of alleles, allelic richness, private alleles, observed heterozygosity, and expected heterozygosity) are presented in Table 1 and allelic frequencies among sites are presented in Supplemental Fig. 1.

Most pairwise F_{ST} values were relatively small (Table 2), with the smallest value observed between the lower Chickasawhay and Pascagoula sites ($F_{ST} = 0.006$, 144.9 river km). Almost all of the higher F_{ST} values were

Table 1 Genetic measures among six sample sites for the yellow-blotched sawback (*Graptemys flavimaculata*) with six tested microsatellite loci

Locus	Genetic measure	Pascagoula	Upper Leaf	Lower Leaf	Upper Chickasawhay	Lower Chickasawhay	Escatawpa
TerpSH2	N	54	51	22	21	60	32
	A	8	8	8	4	8	4
	A_R	6.793	6.419	7.239	3.840	5.828	3.968
	H_O	0.704	0.686	0.727	0.429	0.600	0.625
	H_E	0.748	0.768	0.673	0.532	0.682	0.631
TerpSH5	N	54	52	19	21	59	31
	A	9	8	9	7	8	8
	A_R	7.498	7.003	8.841	6.712	7.063	7.348
	H_O	0.833	0.750	0.895	0.857	0.831	0.806
	H_E	0.786	0.733	0.742	0.723	0.796	0.780
TerpSH7	N	54	52	21	20	59	32
	A	11	10	8	8	10	9
	A_R	9.549	7.970	7.838	7.884	8.161	7.604
	H_O	0.889	0.750	0.857	0.950	0.915	0.750
	H_E	0.865	0.830	0.832	0.810	0.846	0.811
GmuB08	N	54	51	22	21	60	31
	A	6	6	5	5	6	6
	A_R	5.264	4.894	4.970	4.857	4.736	5.398
	H_O	0.704	0.745	0.909	0.714	0.867	0.613
	H_E	0.757	0.717	0.728	0.681	0.715	0.625
GmuD70	N	54	48	18	20	58	32
	A	11	13	9	10	12	8
	A_R	9.738	10.654	9.000	9.784	9.370	7.412
	H_O	0.870	0.979	0.944	0.750	0.810	0.750
	H_E	0.868	0.884	0.856	0.859	0.852	0.763
GmuD88	*N*	53	49	18	21	57	32
	A	11	9	10	7	12	10
	A_R	9.615	8.083	10.000	6.840	9.634	9.693
	H_O	0.906	0.857	0.944	0.762	0.860	0.875
	H_E	0.860	0.813	0.847	0.800	0.825	0.868
Total Private Alleles		1	2	0	1	3	2
Average *A*		9.333	9.000	8.166	6.833	9.333	7.500
Average A_R		8.076	7.504	7.981	6.653	7.465	6.904
Average H_O		0.817	0.795	0.879	0.744	0.813	0.737
Average H_E		0.814	0.791	0.780	0.734	0.786	0.746

Appendix includes locus, number of individuals genotyped at locus (*N*), number of alleles (*A*), allelic richnes (A_R), observed heterozygosity (H_o), and expected heterozygosity (H_e)

associated with comparisons that included the Escatawpa site, with the highest between the Escatawpa and upper Leaf River sites (F_{ST} = 0.047, 256.7 river km). Although many of the pairwise F_{ST} values were small, they were all significantly different from zero after adjusting for multiple comparisons. Additionally, every site except the lower Leaf had at least one private allele with the most observed at the lower Chickasawhay site ($n = 3$). The results of the range-wide AMOVA indicated that most of the genetic variation was partitioned within populations (97.84 %) rather than among populations (2.16 %), although the global F_{ST} value (0.013) was significant ($p < 0.0001$).

The results of the STRUCTURE analysis were generally consistent with the patterns observed in the F_{ST} values. Two groups were identified by the ΔK analysis as best representing the structure in the data, although there was also weaker evidence for additional population structure (Supplemental Fig. 2). At K = 2 (average lnL = −5,322.4; SD = 2.98), the Escatawpa site formed its own group and most individuals from all other sites had strong membership coefficients (q scores) in the other group (Fig. 2; Table 3). To further explore potential population subdivision, we performed another STRUCTURE analysis using all the sites except the Escatawpa River. The ΔK analysis supported the

Table 2 Pairwise F_{ST} values (below diagonal) and geographic distances (above diagonal; in river kilometer) among 6 sample sites for *Graptemys flavimaculata* within the Pascagoula River system

	Pascagoula	Upper Leaf	Lower Leaf	Upper Chickasawhay	Lower Chickasawhay	Escatawpa
1. Pascagoula	–	222.4	145.2	346.4	144.9	34.3
2. Upper Leaf	0.014	–	77.2	366.6	165.1	256.7
3. Lower Leaf	0.016	0.015	–	289.4	87.9	179.5
4. Upper Chickasawhay	0.021	0.041	0.034	–	201.5	380.7
5. Lower Chickasawhay	0.006	0.012	0.013	0.021	–	179.2
6. Escatawpa	0.036	0.047	0.030	0.039	0.043	–

All F_{ST} values deviated significantly from zero

recognition of three groups (average lnL = −4,641.3; SD = 20.2), although only two sites (upper Chickasawhay and Pascagoula) possessed a high level of membership in only one of these groups (Fig. 2; Table 3); the third group detected in the data corresponded to individuals from the upper Leaf site. Individuals from the lower Leaf and lower Chickasawhay sites both showed admixed ancestry split among two or more groups.

Estimates of contemporary rates of migration between sites tended to be low, with most individuals being derived from the source site (Table 4). The highest migration rates tended to be among sites located in the upper portion of the Pascagoula River drainage. Lower levels of migration were detected between these sites and the lower Pascagoula, as well as between any of the sites and the Escatawpa site. However, these values should be treated with caution as the 95 % confidence levels were overlapping in many cases. Likewise, the accuracy of these estimates may be limited when there are low levels of differentiation among populations (Wilson and Rannala 2003).

Testing for a population bottleneck, a one-tailed Wilcoxon's test for heterozygosity excess did not reveal a significant bottleneck in all populations ($p > 0.42$) except the lower Pascagoula River site ($p = 0.039$). This value was not significant, however, after applying a sequential Bonferroni correction (Rice 1989). Similarly, the M ratio test did not detect evidence of bottlenecks at any of the sites. The M ratio value averaged across the six loci ranged from 0.785 to 0.926, and none of these were lower than the critical values from the simulations using a θ of 1 or 10. The Escatawpa site had a N_e estimated of 18.9 (CI

Table 3 Average group membership scores (q) for each site from the STRUCTURE analysis for K = 2 and K = 3, with the latter being a separate analysis without the Escatawpa River site

	K = 2		K = 3		
	1	2	1	2	3
Pascagoula	0.908	0.092	0.086	0.135	0.779
Upper Leaf	0.981	0.019	0.640	0.233	0.127
Lower Leaf	0.766	0.234	0.114	0.344	0.542
Upper Chickasawhay	0.591	0.409	0.026	0.842	0.132
Lower Chickasawhay	0.951	0.049	0.054	0.336	0.610
Escatawpa	0.055	0.945	–	–	–

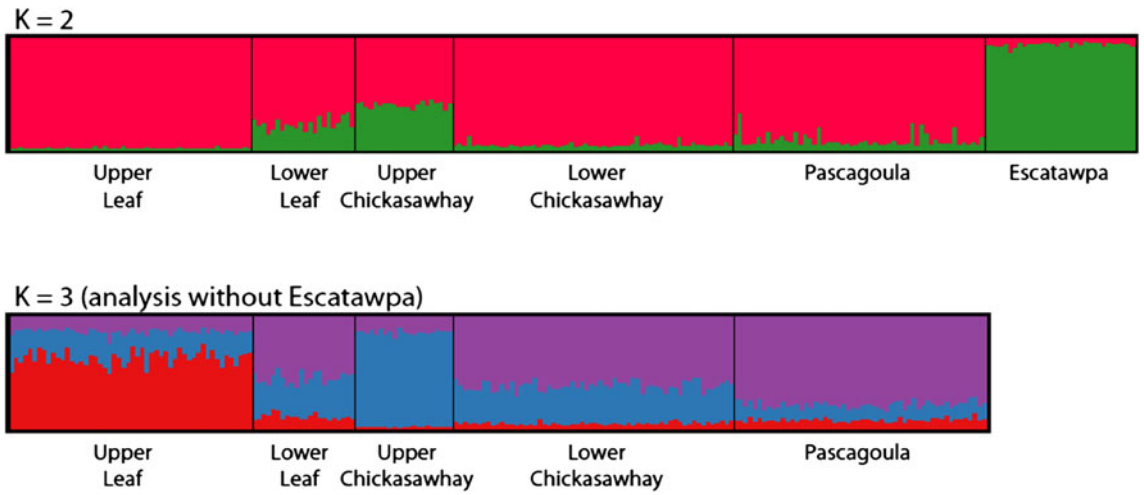

Fig. 2 Bar plots of membership coefficients from the STRUCTURE analysis of *Graptemys flavimaculata* for values of K = 2 and K = 3. For the latter, the Escatawpa site was not included to focus on determining structure within the mainstem Pascagoula River

Table 4 Rates of recent migration between sites of *Graptemys flavimaculata* as estimated with the program BAYESASS

Migration from	Migration into					
	Pascagoula	Upper Leaf	Lower Leaf	Upper Chick.	Lower Chick.	Escatawpa
Pascagoula	**0.695 (0.654–0.736)**	0.026 (0.000–0.081)	0.015 (0.000–0.046)	0.026 (0.000–0.073)	0.194 (0.096–0.292)	0.044 (0.000–0.101)
Upper Leaf	0.017 (0.000–0.050)	**0.724 (0.602–0.846)**	0.01 (0.000–0.030)	0.016 (0.000–0.047)	0.22 (0.081–0.359)	0.012 (0.000–0.036)
Lower Leaf	0.031 (0.000–0.090)	0.039 (0.000–0.112)	**0.685 (0.650–0.720)**	0.042 (0.000–0.120)	0.124 (0.008–0.240)	0.079 (0.000–0.171)
Upper Chick.	0.023 (0.000–0.066)	0.024 (0.000–0.075)	0.021 (0.000–0.060)	**0.768 (0.643–0.893)**	0.126 (0.000–0.269)	0.039 (0.000–0.110)
Lower Chick.	0.045 (0.000–0.106)	0.021 (0.000–0.074)	0.011 (0.000–0.035)	0.031 (0.000–0.084)	**0.876 (0.796–0.956)**	0.016 (0.000–0.043)
Escatawpa	0.03 (0.000–0.079)	0.016 (0.000–0.049)	0.034 (0.000–0.089)	0.042 (0.000–0.116)	0.025 (0.000–0.070)	**0.853 (0.773–0.933)**

The values represent the migration rate into a given site along with the 95 % confidence intervals in parentheses. Values along the diagonal (in bold) are the proportion of individuals derived from the source site

Chick. Chickasawhay River

Table 5 N_e estimates as calculated by LDNe (lowest allele freq used = 0.02; jackknife to estimate 95 % CI)

Site	Ne	95 % CI	Population estimate	Basking density
Upper Leaf	–	248.8–∞	80–120	23.7
Lower Leaf	–	144.0–∞	*	60.0
Upper Chickasawhay	–	76.0–∞	*	3.0
Lower Chickasawhay	397.6	118.4–∞	93	24.9
Pascagoula	–	196.6–∞	281-602	67
Escatawpa	18.9	12.8–29.5	*	4.2

The data for population estimates and basking density is from Selman and Qualls (2009) and expressed in number of turtles per river km. A dash (–) indicates that the software returned a negative value for N_e, which is likely due to a large N_e at the site. An asterisk (*) indicates a site that was not estimated via mark-resight methods

12.8–29.5), while the lower Chickasawhay was 397.6 (95 % CI 118.4–∞). Values of N_e at four of the six sites were negative values suggesting that the estimate of N_e is infinity (LDNe software documentation). In general, lower estimates of N_e correlated with lower population levels observed through visual surveys (Table 5).

Discussion

Population structure

Our sites from the mainstem Pascagoula and Escatawpa are all part of a continuous river system across a relatively small spatial scale. The presence of genetic structure in *G. flavimaculata* is therefore somewhat surprising. Both the significant pairwise F_{ST} values and presence of private alleles at all sites but one indicated that the population within the main river system is not entirely panmictic. Furthermore, in the STRUCTURE analysis at K = 2, the Escatawpa site was clearly different from the sites on the mainstem Pascagoula (the significance of this is discussed below). Sites from the upper Chickasawhay and lower Leaf demonstrated some degree of admixture with the Escatawpa group, but this likely reflects underlying structure across the mainstem Pascagoula sites as demonstrated by the K = 3 STRUCTURE model. The K = 3 model excluding the Escatawpa site further identified the upper sites of both the Leaf and Chickasawhay as distinct, as well as the lower portions of the Pascagoula River system as being somewhat distinct (including lower Chickasawhay, lower Leaf, and Pascagoula sites). Furthermore, estimates of contemporary levels of migration were generally low with high proportions of the individuals being derived from their own site.

The population structure we observed may be related in part to the behavior of the species. Limited dispersal might be the product of individuals having relatively small, established home ranges within riverine habitats (Jones 1996). Within the more homogenous environment of the lower Pascagoula River, turtles occupy distinct home ranges (i.e., with little influence from river currents) and average linear home ranges for *G. flavimaculata* vary from less than 200 m to 6 river km. Jones (1996) also found that the only "overland' movements occurred between the river and other riverine associated habitats (i.e., oxbow lakes, bayous); most of these movements were short travels by females, and males only occurred in wooded riparian habitats during flood events (i.e., never observed walking on land). Selman and Qualls (2008) found additional support of strong home range fidelity following a hurricane storm surge and associated large flooding event; following the event, paint-marked turtles were found in similar areas as they were prior to the event and were not pushed downstream or pushed upstream.

Abiotic influences may also limit the dispersal capacity of *G. flavimaculata*. Even though the Pascagoula River is a continuous system, the preferred habitat of *G. flavimaculata* within the drainage is not continuously distributed (Selman and Qualls 2009). Within the northern reaches of

the Leaf and Chickasawhay rivers and within smaller tributaries, *G. flavimaculata* exhibits a very 'patchy' distribution, with relatively few individuals occupying isolated river stretches of suitable quality habitat (i.e., open canopy for better basking conditions, higher density of basking snags, moderate flow, and presence of nesting sandbars). Patches of suitable habitat are often separated by several kilometer of marginal or unsuitable habitat that includes a closed canopy, few snags, high flow with rapids, and no sandbars (Selman and Qualls 2009). It is unknown if individuals will move long distances through such unfavorable habitat. Therefore, these turtles have little propensity to disperse overland and even though they are capable of moving long, linear river distances, stretches of unsuitable habitat may prevent individuals from venturing to the next suitable habitat patch (Shively and Jackson 1985). We suspect that the high pairwise F_{ST} values, lower levels of genetic diversity, and small population size (Selman and Qualls 2009) found in the upper Chickasawhay site are likely a by-product of small pockets of favorable habitat separated by unfavorable habitat and limited dispersal by turtles among habitat patches.

Unfortunately, some important gaps exist in our sampling including the headwaters of the Leaf River, the 201.5 river km between the upper and lower Chickasawhay River sampling sites, and smaller tributaries. Sampling for individuals at additional sites in these reaches proved extremely difficult due to decreased river accessibility because of (1) lack of public boat ramps, (2) poor channel navigability (e.g., low river levels, high snag density), and (3) difficulty in obtaining adequate sample sizes from low density populations of *G. flavimaculata*. Although collecting samples in these sites would be difficult and extremely time consuming, it would help address some important questions that are left unanswered by our analysis. For example, additional sampling between the upper and lower Chickasawhay River sites would allow us to determine if the differentiation in the upper Chickasawhay river site is simply a product of isolation by distance or if there is a distinct barrier(s) to gene flow. Likewise, sampling in the headwaters of the Leaf and smaller tributaries (e.g., Tallahala, Bogue Homa, Bouie creeks) would provide a better indication of the role that habitat patchiness plays in limiting gene flow between sites.

While there are not many riverine turtle examples in the literature for comparison with our study, significant F_{ST} values and evidence of isolation by distance was found in a South American river turtle, *Podocnemis unifilis* (Yellow-Spotted Amazon River turtle; Escalona et al. 2009). Escalona et al. (2009) found that almost all pairwise F_{ST} values were significant among different sample populations, as well as significant evidence within the Orinoco and Amazon River basins for intradrainage isolation by distance among sites 86–690 rkm apart. Conversely, isolation by distance was not found in the *P. expansa* (Giant Amazon River turtle; Pearse et al. 2006). When comparing populations within river basins, Pearse et al. (2006) found that only 2 of the 11 pairwise F_{ST} values were significantly different from zero, while all values were significant for interdrainage comparisons. They concluded that extensive gene flow was occurring within drainages and little gene flow was occurring among drainages. Even though both of these *Podocnemis* species occur sympatrically within the Orinoco and Amazon River systems, they exhibit different patterns of genetic structure. The differences between the two species is likely due to several ecological differences, including the high vagility and the large seasonal movements of *P. expansa* that are associated with moving to feeding or nesting grounds (von Hildebrand et al. 1997). Vargas-Ramírez et al. (2012) also found considerable differentiation among sample sites for *P. lewyana* (Columbian River turtle), including significant differentiation within a single drainage (Magdalena River), but little evidence for isolation by distance. On a smaller scale, Bennett et al. (2010) found little genetic differentiation and a single, panmictic population of *Graptemys geographica* (common map turtle) across riverine sites separated by manmade locks and dams.

Significance of the Escatawpa river population

The Escatawpa River site was the most genetically distinct of the six sites we sampled, with this genetic differentiation not appearing to be a function of geographic distance. For example, while the Pascagoula River and Escatawpa River sites had one of the highest pairwise F_{ST} values ($F_{ST} = 0.036$) it also had the smallest separating geographic distance of all sites compared (34.3 river km). In comparison, the geographic distance between the upper Leaf River/upper Chickasawhay River is tenfold longer (366.6 river km), but these sites had a similar degree of genetic differentiation ($F_{ST} = 0.041$). Furthermore, the apparent geographic isolation of the Escatawpa River site is supported by the observation that this site had a lower average allelic richness relative to all other sites, except the upper Chickasawhay site.

A possible geological explanation for the deeper divergence of the Escatawpa site is that the Escatawpa River historically flowed independently into the Gulf of Mexico at Grand Bay (approx. 16.5 km east of the Pascagoula River mouth; Otvos 2007; Fig. 1). The Pascagoula River only recently captured the Escatawpa River (within the last 11,500 years), with primary evidence for this event being ceased fluvial deposition into Grand Bay (Otvos 2007). We presume that prior to the connection of the two rivers, a population of *G. flavimaculata* occurred within the isolated

Escatawpa River that flowed into the Gulf of Mexico at Grand Bay. Therefore, the deeper genetic divergence of the Escatawpa River population is likely attributable to the historical geographic separation of the Escatawpa River relative to the Pascagoula River. Currently, unsuitable habitat, in the form of brackish marsh, exists in most of the 34.3 river km between the lower Pascagoula River and Escatawpa River sites. We suspect that this brackish marsh has helped to maintain the persistence of the genetic differentiation among the two sites since *G. flavimaculata* do not inhabit these areas (Selman and Qualls 2009) and likely do not traverse this unsuitable habitat. This geological scenario seems to explain the genetic divergence of the Escatawpa River population. However, we also recognize that genetic drift in a small isolated population could also lead to genetic differentiation on a much shorter time scale.

Whether a population, such as the Escatawpa population, represents an evolutionary significant unit or a management unit is typically determined in part with mitochondrial DNA data (Moritz 1994). However, turtles typically exhibit low levels of divergence in mitochondrial DNA, particularly in the *Graptemys* genus (e.g., Avise et al. 1992; Weins et al. 2010). For example, Ennen et al. (2010) sequenced 1,560 base pairs of mitochondrial DNA from two closely related *Graptemys* species (*G. flavimaculata*, $n = 6$; *G. oculifera*, $n = 6$) and found only 3–7 base pairs that differed between the haplotypes in the two species. Therefore, if mitochondrial data are not useful in delineating between two *Graptemys* species, it is not likely to prove useful in defining the evolutionary/management units within a single *Graptemys* species, such as defining the Escatawpa River population.

Was there a historical population bottleneck?

Our analyses indicate that there is little evidence for a recent or a historical genetic bottleneck for any of the sampled *G. flavimaculata* sites. Likewise, effective population sizes were not suggestive of past bottlenecks as estimates for all sites but the Escatawpa were large. Most turtle species that exhibit genetic bottlenecks have been subjected to high levels of human harvest, including *P. expansa* (Pearse et al. 2006). There are no historical records that indicate that *G. flavimaculata* has been subject to high historical or recent human harvest or consumption, even though there were observed range-wide population declines in the 1980s (McCoy and Vogt 1980; Stewart 1989). Early studies on the species do not point to extremely low historical population levels (Cliburn 1971), with one even stating that *G. flavimaculata* was "clearly the dominant turtle species in the Pascagoula and Chickasawhay Rivers" (Cagle 1954). Unfortunately, shooting of turtles by humans for target practice (i.e., "plinking turtles") continues within the Pascagoula River system, while incidental take from fisherman and intentional collection for the pet trade still likely occurs (Selman and Jones 2011).

Conclusions and conservation implications for the yellow-blotched sawback

The finding of population structure within the *G. flavimaculata* populations suggests that there is further need for conservation of the species and its riverine habitat on a drainage-wide scale. The presence of at least two distinct groups as determined by STRUCTURE, significant F_{ST} values between all sites, and the presence of private alleles at almost all sites indicates that *G. flavimaculata* does not represent a panmictic population as might be expected in a continuous river system such as the Pascagoula River. Future management of this species should take into account the differences among populations, particularly if reintroductions are needed in the future for this endangered species.

The Escatawpa River site is clearly genetically distinct relative to mainstem Pascagoula River sites. From a conservation and management perspective, this population is also considered the most imperiled population of *G. flavimaculata* (Selman and Jones 2011). The Escatawpa River population has lower population densities compared to most other sites (Table 4, Selman and Qualls 2009) and only occurs in a small portion of the river ($\sim$15–20 river km; W. Selman, pers. obs), thus the total population likely consists of fewer than 500 individuals. Further, it has the smallest estimated N_e of any sample site and therefore, is at a higher potential for inbreeding depression and loss of genetic variability relative to the other populations. Due to the higher levels of genetic differentiation and the historic and current geographic isolation of the Escatawpa River population, we suggest that future research should focus on this population.

Acknowledgments Most of all, we would like to thank many agencies that provided funding throughout this project: the Mississippi Department of Wildlife, Fisheries, and Parks; the U.S. Fish and Wildlife Service; the Chelonian Research Foundation Linnaeus Fund; and the Batchelor Foundation/Turtle Survival Alliance. We would like to thank all those who assisted with various aspects of this study, especially USM field assistants Dustin Strong, Chris Griffith, and Thomas Bocek. Lynn McCoy assisted with many logistical aspects, particularly when we encountered any boat or trailer problems while working at the sites in Jackson County. Jimmy Dale Odom was extremely generous to allow us to use his private boat ramp to access the upper Leaf River site, while Lynn and Bill Selman provided home cooking and lodging while working at the upper Chickasawhay River field site. Two anonymous reviewers provided helpful comments that allowed us to greatly improve the manuscript. This project was approved by the U.S. Fish and Wildlife Service; Mississippi Department of Wildlife, Fisheries, and Parks; and the University of Southern Mississippi Institutional Animal Care and Use Committee (IACUC #07032201).

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