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High Connectivity Observed in Populations of Ringed Sawbacks, *Graptemys oculifera*, in the Pearl and Bogue Chitto Rivers Using Six Microsatellite Loci

Daniel L. Gaillard[1,2], Will Selman[3], Robert L. Jones[4], Brian R. Kreiser[1], Carl P. Qualls[1], and Keri Landry[5]

***Graptemys oculifera* is endemic to the Pearl River drainage system in Louisiana and Mississippi, and due to this limited range it is vulnerable to environmental changes. Many impacts to this drainage system are due to anthropogenic activities, and alterations may negatively impact the connectivity among populations of *G. oculifera*. Previous studies show populations below the Ross Barnett Reservoir might be undergoing population declines, suggesting limited movement among populations. In addition to anthropogenic effects, the drainage geomorphology might also play a role in shaping population connectivity, as the sister species of *G. oculifera*, *G. flavimaculata*, contains two distinct populations (mainstem Pascagoula and Escatawpa River) and possible subpopulations within the Pascagoula (upper Leaf River, upper Chickasawhay River, and lower sections of the Pascagoula River). We used six polymorphic microsatellite loci to analyze the population genetics of *G. oculifera* at eight sites in the Pearl River drainage. The STRUCTURE program found support for one genetic group; however, our AMOVA analysis detected small but significant genetic differentiation in our three-group analysis. Both the MIGRATE and Isolation-by-Distance analyses supported a stepping stone model of gene flow, not panmixia. We suggest management agencies should consider these genetic data when developing management plans. Although we did not detect any influence of the reservoir on population connectivity, likely due to the long generation times of turtles, we suggest mark–recapture and/or radio-telemetry studies be performed on populations near the reservoir to determine if cross reservoir movements occur.**

THERE are an estimated 335 globally recognized chelonian species, and 60% of those assessed are considered threatened, endangered, or critically endangered (Turtle Taxonomy Working Group [TTWG], 2014), making chelonians one of the most endangered taxonomic groups (International Union for the Conservation of Nature [IUCN]: IUCN Red List of Threatened Species. Version 2011.2. www.iucnredlist.org). The main causes of turtle declines across the globe are habitat loss and degradation, pollution, introduced species, and unsustainable use by humans (Gibbons et al., 2000). The southeastern United States is considered one of three global chelonian biodiversity hotspots (Buhlmann et al., 2009), with similar threats to the regional turtle fauna that are found globally.

The genus *Graptemys* is the most diverse North American turtle genus, with nine of the 14 recognized species exclusive to single river drainages across the southeastern United States (Lindeman, 2013). All species are highly aquatic, primarily inhabiting rivers, streams, and their associated habitats (i.e., oxbow ponds, bayous). Turtles within the genus are particularly susceptible to anthropogenic impacts including channelization, impoundments, desnagging (Lindeman, 1999; Jones and Selman, 2009), and also collection for the pet trade (U.S. Fish and Wildlife Service [USFWS], 2002). Since many species of *Graptemys* are endemic to single river drainages in the southeastern United States, many of these species are threatened by impacts to their respective watersheds (Ernst and Lovich, 2009).

Graptemys oculifera (Ringed Sawback) is endemic to the Pearl River watershed of central and southern Mississippi as well as southeastern Louisiana (Jones and Selman, 2009). Although it is one of the most abundant turtles in this watershed (Lindeman, 1998; Shively, 1999), it is federally listed as threatened (USFWS, 1986), state endangered in Mississippi (Mississippi Department of Wildlife, Fisheries, and Parks, 2012), state threatened in Louisiana (www.wlf.louisiana.gov/wildlife/rare-animal-fact-sheets; accessed 9 September 2014), and Vulnerable on the IUCN Red List (www.iucnredlist.org; accessed on 12 July 2014). These listings are primarily attributable to its limited inhabited area (<87.5 km^2; Jones and Selman, 2009) and population declines observed in the 1980s (Stewart, 1988). One of the primary threats to the species is that the Pearl River has been altered by channelization and damming, and there are other flood control and/or navigational projects proposed for the river (Stewart, 1988). Also, the Pearl River has historically been excessively polluted by municipal and industrial waste downstream of Jackson, Mississippi (Hinds County; McCoy and Vogt, 1980). Along with these threats, the Ross Barnett Reservoir, situated immediately north of Jackson (Fig. 1), is presumed to be a barrier to turtle movements (Jones and Selman, 2009). McCoy and Vogt (1980) designated two areas needed for preservation of the species, a 64 km stretch of the Pearl River above and below Carthage (Leake County, MS; upstream of the reservoir) and a 158 km stretch of the Pearl River from Georgetown (Copiah County,

[1] Department of Biological Science, Box #5018, University of Southern Mississippi, Hattiesburg, Mississippi 39406; E-mail: (BRK) brian.kreiser@usm.edu; and (CPQ) carl.qualls@usm.edu.
[2] College of Life Sciences, Peking University, Summer Palace Rd. No. 5 Golden Life Sciences Building, Beijing, China 100871; E-mail: chinaturtle@126.com. Send reprint requests to this address.
[3] Rockefeller Wildlife Refuge, Louisiana Department of Wildlife and Fisheries, 5476 Grand Chenier Hwy, Grand Chenier, Louisiana 70643; E-mail: wselman@wlf.la.gov.
[4] Mississippi Department of Wildlife, Fisheries, and Parks, Mississippi Museum of Natural Science, 2148 Riverside Drive, Jackson, Mississippi 39202; E-mail: bob.jones@mmns.state.ms.us.
[5] Louisiana Department of Wildlife and Fisheries, Louisiana Natural Heritage Program, 2000 Quail Drive, Baton Rouge, Louisiana 70808; E-mail: klandry@wlf.la.gov.
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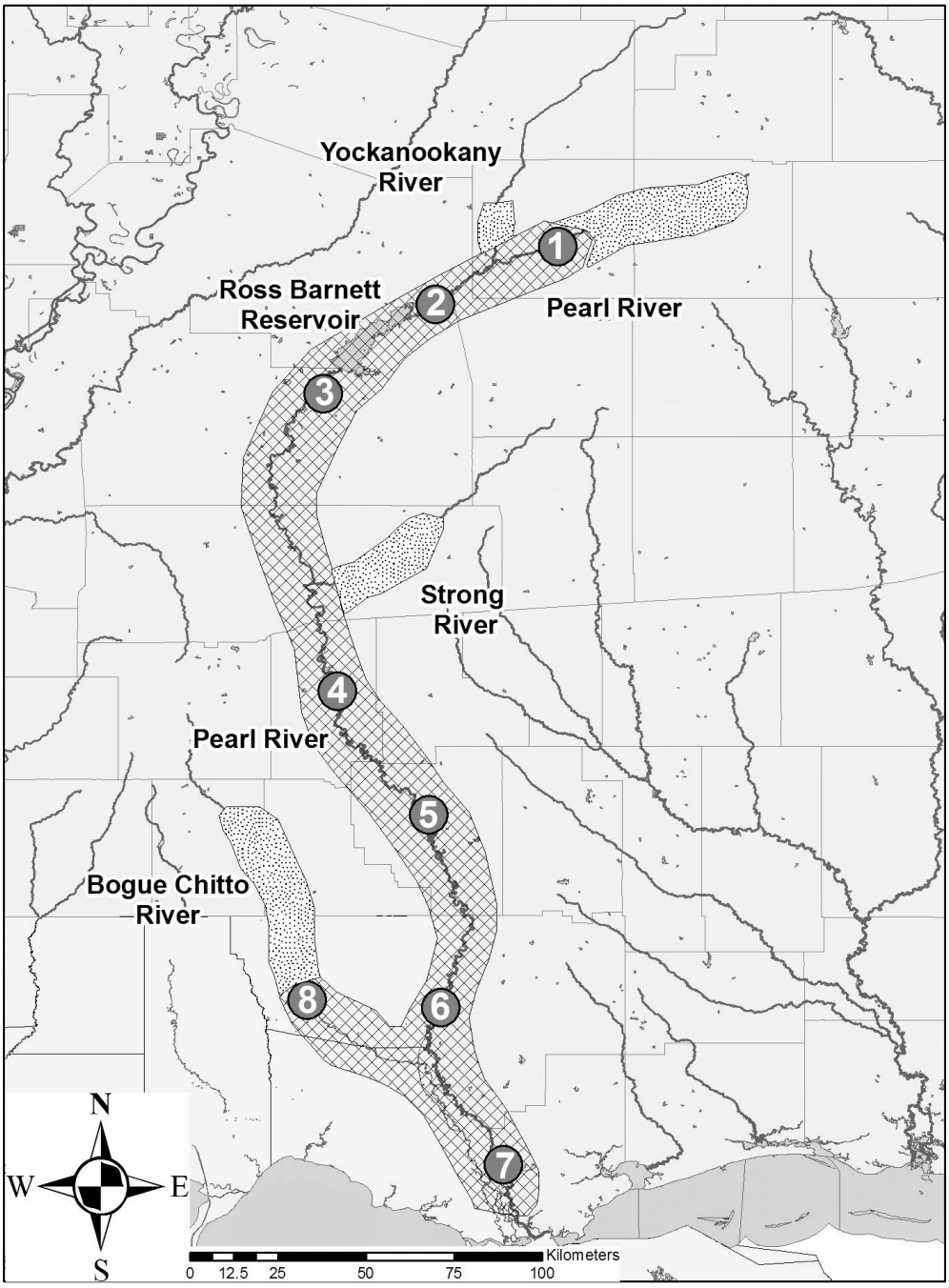

Fig. 1. The approximate range of *Graptemys oculifera* in Mississippi and Louisiana (crosshatching represents areas amenable to sampling and stippling represents logistically difficult areas to sample within the range). Sample collection localities: 1) Carthage, 2) Ratliff Ferry, 3) Lakeland, 4) Monticello, 5) Columbia, 6) Bogalusa, 7) Napoleon, and 8) Franklinton.

MS) to Bogalusa (Washington Parish, LA; downstream of the reservoir).

While previous studies have provided much needed information on the ecology and population status of *G. oculifera* (e.g., McCoy and Vogt, 1980; Stewart, 1988; Jones and Hartfield, 1995; Lindeman, 1998; Jones, 2006), there are no data that directly address the extent of genetic connectivity or lack thereof among populations. Genetic data are increasingly being applied to address conservation issues and provide additional insight into the biology of threatened turtle species. A recent study on *G. flavimaculata* found genetic differentiation within the Pascagoula River drainage, to which it is endemic (Selman et al., 2013a). Therefore, the objectives in this study were to 1) determine if population structure exists within *G. oculifera*, 2) assess the impact of the Ross Barnet Reservoir on gene flow, 3) compare levels of genetic diversity among sites, and 4) characterize the demographic history through testing for genetic bottlenecks and estimating effective population size. Our hope is that these data will prove useful in better understanding this species and ultimately help guide future management efforts.

MATERIALS AND METHODS

Sample collection.—A total of 229 individuals was collected from eight sites on the Pearl and Bogue Chitto rivers in Mississippi and Louisiana (see Fig. 1 for sampling site locations). Pearl River study sites included: (1) Carthage (CH; Leake

County, MS; $n = 27$), (2) Ratliff Ferry (RF; Madison and Rankin counties, MS; $n = 30$), (3) Lakeland (LD; Hinds and Rankin counties, MS; $n = 30$), (4) Monticello (MT; Lawrence County; $n = 29$), (5) Columbia (COL; Marion County; $n = 58$), (6) Bogalusa (BOG; Pearl River County, MS and Washington Parish, LA; $n = 32$), and (7) Napoleon (NAP; Pearl River County, MS and St. Tammany Parish, LA; $n = 11$). We also sampled from a single site on the Bogue Chitto River at (8) Franklinton (FK; Washington Parish, LA; $n = 12$). Sampling sites at CH, RF, LD, MT, and COL were selected for this study because they are long-term study sites of *G. oculifera* of Robert L. Jones. The BOG, NAP, and FK sites were chosen to obtain a more complete representation of the potential genetic variation within the range of *G. oculifera*. Unfortunately, there are gaps in our sampling including the extreme upper reaches of the Pearl and Bogue Chitto rivers and also in smaller tributaries like the Strong and Yockanookany rivers, which were not easy to access and navigate. Two sites, NAP and FK, proved difficult to sample due to low population densities, and thus resulted in smaller sample sizes. Individuals were collected by the use of dip nets, basking traps, and by hand (as described by Selman et al., 2012); fyke nets were also used at Franklinton (Vogt, 1980). We obtained tissues samples by either clipping the terminal end of the tail or by using a new 23G1 gauge (Becton Dickinson PrecisionGlide) hypodermic needle and drawing 0.2 ml of blood from the coccygeal vein. The tail clips were preserved by placing them into 100% ethanol and blood was preserved in a 1.5 ml centrifuge tube with approximately 0.5 ml of SED tissue preservation buffer (Seutin et al., 1991) and frozen at –20°C.

Laboratory methods.—Genomic DNA was extracted from each sample using the DNeasy Tissue Kit (QIAGEN). We genotyped all individuals for seven loci that cross-amplified reliably in *G. oculifera* as described by Selman et al. (2009; *Terp*SH1, *Terp*SH2, *Terp*SH3, *Terp*SH5, *Terp*SH7, and *Gmu*8) and one other locus, Cc7 (FitzSimmons et al., 1995). Polymerase chain reactions (PCR) were performed on a Veriti Thermal Cycler (Applied Biosystems) in 12.5 μl reactions consisting of 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 0.01% gelatin, $MgCl_2$ as noted (1 mM–Cc7, 1.25 mM–*Gmu*8, 1.5 mM–*Terp*SH1 and *Terp*SH2, 2 mM–*Terp*SH3 and *Terp*SH7, and 2.5 mM–*Terp*SH5), 200 μM dNTPs, 0.1875 units of *Taq* polymerase (New England Biolabs), 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 M13 labeled primer (LI-COR), 20–100 ng of template DNA and water to the final 12.5 volume. PCR cycling conditions consisted of an initial denaturing step of 94°C for 2 min followed by 35 cycles of 30 sec at 94°C, 1 min at 56°C (58°C for *Terp*SH3) and 1 min at 72°C with a final elongation step of 10 min at 72°C. PCR products were run on a LI-COR 4300 DNA sequencer along with a 50–350 bp size standard (LI-COR) and scored using Gene Image IR v. 3.55 (LI-COR).

Data analysis.—Basic summary statistics (number of alleles—Na, observed heterozygosity—H_o, expected heterozygosity—H_e) were calculated using GenAlEx 6.41 (Peakall and Smouse, 2006), and allelic richness (A_R) was calculated by using FSTAT 2.9.3 (Goudet, 2001). Genetic differentiation between sites (θ, the unbiased estimator of F_{ST}; Weir and Cockerham, 1984) along with significance testing was also calculated with FSTAT 2.9.3. Loci were tested for Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) using GENEPOP on the web (Raymond and Rousset, 1995; http://genepop.curtin.edu.au/) with a sequential Bonferroni correction (Rice, 1989) applied to both tests. Microchecker (Oosterhout et al., 2004) was also used to test for null alleles. We statistically compared measures of genetic diversity (A_R, H_o, H_e, and Na) among sites using ANOVAs using the VEGAN package (Oksanen, 2010) in R version 3.1.1 (R Core Team, 2013). We tested two different *a priori* models of population structure with an analysis of molecular variance (AMOVA; Excoffier et al., 1992) as implemented in ARLEQUIN v. 3.11 (Excoffier et al., 2005). For this analysis, the significance was assessed by 1000 random permutations of the data. The first model was comprised of two groups representing sites above (Carthage and Ratliff Ferry) and below the reservoir (Lakeland, Monticello, and Columbia), while the second model added a third group representing the southern portion of the Pearl River system (Napoleon, Bogalusa, and Franklinton). We also tested for significant differences in H_o, H_e, A_R, and Na among sites and between groupings using ANOVA. All significance values were set to $\alpha = 0.05$.

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 1–10 using a model of admixed ancestry and assuming correlated allele frequencies between groups and with site location used as a prior (Hubisz et al., 2009). For each value of K, we performed 20 replicates with a burn-in of 100,000 generations followed by a subsequent 500,000 generations. We determined the best value of K by comparing the probability scores for each value of K and by examining the ΔK values (Evanno et al., 2005) calculated by the program Structure Harvester v 6.92 (Earl and von Holdt, 2012).

We used a Mantel's test to determine if there was a relationship between geographic and genetic distance (i.e., isolation by distance). Geographic distances were derived from river distance (rkm) between sites as traced in Google-Earth using the path measuring tool (Carthage, MS; Canton, MS; Flowood, MS; Monticello, MS; Columbia, MS; Napolean, MS; Bogalusa, LA; Franklinton, LA) along the mid river channel while genetic distances were represented by θ, the unbiased estimator of F_{ST}. Negative values were set to zero. These two distance matrices were analyzed by the Isolation-By-Distance Web Services program (Jensen et al., 2005; http://ibdws.sdsu.edu/~ibdws/) with 1000 randomizations and remaining settings left as default conditions.

We investigated several different patterns of gene flow by using Migrate-n (version 3.6.4; Beerli and Felsenstein, 2001; Beerli, 2006) to estimate the marginal likelihood of the model and then using the Bayes factor to rank the probabilities of each one (Beerli and Palczewski, 2010). Three models of gene flow were tested: the first model represented panmixia, the second model (full) had gene flow possible among any of the sites, while the third allowed for gene flow only between adjacent sites. Since site 8 (FK) was in a tributary, we modified the stepping stone model so that migration was possible between this site and each of its closest neighbors (sites 6 and 7). For each Migrate analysis, we used the Brownian mutation model, which is appropriate for microsatellite loci. The starting genealogy was derived from a UPGMA derived tree and initial theta and M values were derived from the F_{ST} calculation. Priors for theta were

kept as uniform with minimum, maximum, and delta values set to 0.01, 100.0, and 9.99, respectively. Static heating was applied to four independent chains using temperature settings of 1.0, 1.5, 3.0, and 1,000,000.0. Each analysis was replicated four times with a total of 100,000 steps per run, recorded every 100 generations, of which 10,000 were discarded as the burn-in. After completion of the run, stationarity was assessed by examining the effective sample size (ESS) and distribution of each parameter, where values >1000 and a unimodal posterior distribution were taken to indicate that convergence was reached.

Two models were used to test for potential population bottlenecks. Model one followed the STRUCTURE output that found support for a single population comprising all individuals studied. Model two followed the groupings in the AMOVA Three Group analysis. Population bottlenecks were tested by using the program BOTTLENECK (Piry et al., 1999). As population numbers decline, the number of alleles declines at a faster rate than does heterozygosity, thus an excess of heterozygosity can be a sign of a genetic bottleneck (Cornuet and Luikart, 1996). The two-phase model of mutation (TPM) was used (Piry et al., 1999) with variance for TPM set to 30% and proportion stepwise mutation set to 70% and was run for 1000 iterations. The significance of heterozygosity excess was calculated by using the Wilcoxon sign rank test. Another method to look for evidence of a bottleneck is the *M* ratio test (Garza and Williamson, 2001), which compares the number of alleles with their size distribution. We used the following parameters as per the recommendations of the authors (Garza and Williamson, 2001): a proportion of one-step mutations of 90%, an average size of non one-step mutations (Δg) of 3.5, and a value of 10 for θ. The critical value for M for each population was obtained from the 95% threshold of 10,000 simulations of an equilibrium population.

Lastly, we calculated effective population size (N_e) for each site using NeEstimator v. 2.01 (Do et al., 2013). Estimates of N_e where derived from the bias corrected (Waples, 2006) linkage disequilibrium method (Hill, 1981) with 95% confidence intervals estimated by jackknifing. Mating was assumed to be random, and alleles with a frequency <0.02 were excluded from the analysis. We then compared these values with the results of published (Jones and Hartfield, 1995; Lindeman, 1998) or unpublished studies of population densities of *G. oculifera* at the same locations.

RESULTS

One locus, *Terp*SH1, showed consistent deviations from HWE and the presence of null alleles and was excluded from further analysis. No other loci deviated from HWE or demonstrated linkage disequilibrium after a Bonferroni correction. The summary of genetic diversity measures for each site is found in Table 1. Two of the lower sites (NAP and FK) tended to have fewer alleles per locus, although this trend was not as pronounced in the values for allelic richness. Most loci had expected and observed heterozygosity values ranging between 0.6–0.8, but these values for *Gmu*08 and Cc7 were typically lower at many of the sites. There were no significant differences found among sites for the genetic diversity indices tested (H_o, $F = 0.432$, $P = 0.876$; H_e, $F = 0.457$, $P = 0.859$; Na, $F = 0.411$, $P = 0.89$; A_R, $F = 0.096$, $P = 0.998$), nor were there any significant differences found between groups from our AMOVA models (Table 2). Private alleles were relatively uncommon, with only three sites (RF, COL, BOG) possessing a total of four private alleles.

Pairwise F_{ST} values (Table 3) ranged from zero (negative values) to 0.099. In general, as the distance between sites increased so too did F_{ST} values, with these values also being significantly different from zero after adjusting for multiple comparisons. Comparisons of BOG and FK to the upper two sites (CH and RF) were an exception as the pairwise F_{ST} values were both small and not significantly different from zero. In both of the two *a priori* models of genetic structure for the AMOVA, the amount of variation partitioned among groups was less than 1%. The Reservoir model was not significant ($P = 0.393$), but the Three Group model was significant ($P = 0.038$), suggesting there is genetic structure across the range, albeit extremely limited. The results of the STRUCTURE analysis failed to detect any evidence of strong genetic differentiation, with a K of 1 having the highest likelihood score (average lnL = –2969.76; SD = 0.14).

The Mantel test detected a significant positive correlation between geographic and genetic distance ($P = 0.014$, $Z = 199.133$, $r = 0.4125$, Fig. 2). The results of the model testing with Migrate-n agreed with the Mantel test. The stepping stone pattern, where gene flow was restricted to adjacent regions, had the highest probability ($P = 1.0$) of any of the three models with a marginal likelihood of –3995.62. Of the other two models, the full migration model clearly had the lowest marginal likelihood (lmL = –17442.31) as compared to the panmixia model (lmL = –4932.57). All but three of the parameters for mutation scaled migration rates (M) produced unimodal posterior distributions, which we report along with 95% highest posterior density (Fig. 3). In general, M values among sites in both upstream and downstream directions were fairly consistent with overlapping 95% posterior densities.

For all of our tests (individual sites or pooled sites), the one-tailed Wilcoxon test for heterozygosity under the two-phase mutation model in the BOTTLENECK program did not detect any evidence of significant heterozygosity excess after a Bonferroni correction was applied. Likewise, the M ratio test did not suggest a history of bottlenecks in any of the populations, as none of the values were lower than the critical values determined by simulation. The M ratio value averaged across the six loci ranged from a low of 0.67 (NAP) to a high of 0.86 (FK). Estimates of N_e (Table 4) ranged from a low of 97.6 (CH) to 1456.2 (CO). Two sites (MT and FK) had negative estimates, which the software reports as infinity. This may be the result of either a truly large N_e or the consequence of limited sampling, but in either case the lower bound of the 95% confidence interval can be used for the purposes of inferring plausible values of N_e (Waples and Do, 2010). In general, sites from the upper and lower portions of the range tended to have smaller lower confidence interval limits, with the two southern sites (NAP and FK) having the lowest values. Sites tended to demonstrate similar trends in population estimates or density per river kilometer (Table 4) as seen in the estimates of N_e, although the ranking of sites by these metrics was not exactly the same.

DISCUSSION

Population structure.—Our eight sample sites from the mainstem Pearl River and Bogue Chitto River are all within a single river system and cover a relatively small spatial scale

Table 1. Summary genetic measures among eight sample sites for the Ringed Sawback (*Graptemys oculifera*) for six microsatellite loci. Table includes locus, number of alleles (Na), allelic richness (A_R), observed heterozygosity (H_o), and expected heterozygosity (H_e).

Locus	Site	CH	RF	LD	MT	COL	BOG	NAP	FK
Sample size		27	30	30	29	58	32	11	12
*Terp*SH2	N	27	29	30	29	57	30	11	12
	Na	7	4	7	6	7	6	3	3
	A_R	4.529	3.480	4.542	3.996	4.139	4.090	3.000	2.917
	H_o	0.481	0.621	0.600	0.586	0.684	0.567	0.727	0.333
	H_e	0.613	0.563	0.553	0.586	0.606	0.602	0.541	0.531
*Terp*SH3	N	26	28	30	28	56	30	11	12
	Na	4	4	3	3	3	3	3	4
	A_R	3.414	3.771	2.999	3.000	2.990	2.995	3.000	3.993
	H_o	0.577	0.571	0.667	0.571	0.679	0.621	0.727	0.417
	H_e	0.553	0.596	0.629	0.666	0.626	0.606	0.632	0.552
*Terp*SH5	N	27	30	29	25	57	31	11	12
	Na	6	6	4	6	5	5	3	5
	A_R	4.774	5.043	3.956	4.964	4.670	4.822	3.000	4.913
	H_o	0.815	0.767	0.655	0.680	0.737	0.742	0.364	0.583
	H_e	0.711	0.658	0.666	0.718	0.711	0.724	0.434	0.663
*Terp*SH7	N	26	30	30	29	54	32	11	12
	Na	9	8	8	8	8	8	5	7
	A_R	7.020	6.388	6.234	6.081	6.283	5.838	5.000	6.746
	H_o	0.731	0.733	0.833	0.621	0.759	0.813	0.727	0.750
	H_e	0.782	0.751	0.751	0.737	0.783	0.711	0.760	0.743
*Gmu*08	N	24	28	28	29	54	32	11	12
	Na	2	4	3	3	4	3	4	2
	A_R	2.000	2.786	2.848	2.972	3.055	2.567	4.000	2.000
	H_o	0.042	0.393	0.400	0.310	0.130	0.344	0.636	0.333
	H_e	0.353	0.494	0.451	0.599	0.548	0.334	0.479	0.375
Cc7	N	27	26	26	28	58	32	11	12
	Na	2	2	2	2	2	2	2	1
	A_R	1.999	1.999	1.992	2.000	1.999	2.000	2.000	1.000
	H_o	0.370	0.423	0.231	0.393	0.431	0.438	0	0
	H_e	0.346	0.334	0.260	0.448	0.357	0.451	0.165	0
Total private alleles		0	1	0	0	1	2	0	0
Average Na		5.0	4.7	4.5	4.7	4.8	4.5	3.3	3.7
Average A_R		4	3.9	3.7	3.8	3.9	3.7	3.3	3.6
Average H_o		0.503	0.585	0.564	0.527	0.570	0.587	0.530	0.403
Average H_e		0.560	0.566	0.522	0.626	0.605	0.572	0.502	0.477

compared to the ranges of many species. Thus, it is not surprising that we detected minimal population structure across the sites sampled. Strong differentiation was not evident as the STRUCTURE analysis found one genetic group was present. However, other analyses indicated that there is not panmixia across the sites and that subtle genetic differences are present, including the significant pairwise F_{ST} values, the presence of private alleles, a small but significant portion of the genetic variation explained by the AMOVA three group model, a pattern of isolation by distance, and a "modified stepping stone" model of migration.

Table 2. Mean values for H_o, H_e, A_R, and Na for the reservoir and three group models and the ANOVA results for each genetic diversity measure followed by the *P* value in parentheses.

	Mean H_o	Mean H_e	Mean A_R	Mean Na
Reservoir				
Above	0.544	0.563	3.93	4.83
Below	0.53	0.556	3.67	4.25
F value	0.034(0.855)	0.016(0.899)	0.271(0.605)	0.67(0.417)
Three group				
North	0.544	0.563	3.93	4.83
Middle	0.554	0.594	3.8	4.67
South	0.507	0.517	3.55	3.83
F value	0.22(0.803)	0.938(0.399)	0.253(0.777)	1.026(0.367)

Table 3. Distances in river kilometers between sites are above diagonal and pairwise F_{ST} values below the diagonal for the eight sites for *Graptemys oculifera*. See the text for site abbreviations.

	CH	RF	LD	MT	CO	BOG	NAP	FK
CH	—	25.6	64.3	177.3	227.3	286.7	366.7	350.3
RF	–0.006	—	38.7	151.7	201.7	261.1	341.1	331.5
LD	0.003	0.008	—	113	163	222.4	302.4	292.8
MT	0.026	0.022*	0.025	—	50.0	109.4	189	179.4
CO	0.018	0.026*	0.022*	0.005	—	59.4	139.4	139.8
BOG	–0.025	0.012	0.026*	0.032	0.026*	—	80.0	70.5
NAP	0.076*	0.099*	0.076*	0.066*	0.039*	0.060*	—	80.1
FK	0.009	0.036	0.050*	0.071*	0.039*	0.031	0.041	—

* indicates a *P* value significantly different from zero

Genetic differentiation among sites that were not immediately adjacent to one another, as measured by $F_{ST,}$ tended to be significantly different from zero. The two sites from the southern edge of the range (NAP and FK) had the highest pairwise F_{ST} values—seven were higher than the greatest pairwise F_{ST} value for *G. flavimaculata* (Selman et al., 2013a). Admittedly, the high F_{ST} for these sites may be influenced by the small sample sizes (n = 11 and 12, respectively), which can lead to errors in estimates of fixation indices (Nei and Chesser, 1983). Another outlier is the very low pairwise F_{ST} values for comparisons between the upper two sites and BOG and FK. The similarity between sites at such a great distance likely reflects the influence of sampling error (e.g., the FK site) or genetic drift, particularly at the BOG site. The allele frequencies for several loci at BOG (data not shown) are more similar to CH and RF than they are to adjacent sites.

Other than a few outliers, the pattern of increasing genetic differentiation with increasing river distance among sites clearly matched the expectation of isolation by distance. Isolation by distance has been found in other species of *Graptemys*, such as *G. caglei* in the Guadalupe River (Ward et al., 2013), *G. geographica* in the Trent-Severn Waterway (Bennet et al., 2010), and qualitatively in *G. flavimaculata* (Selman et al., 2013a). The modified stepping stone model, which is based on the isolation by distance pattern, was also selected as the best model in the Migrate-n analysis. The results of the Migrate analysis should be interpreted with some caution given the number of loci we had available for this study. However, the general conclusions we draw from the Migrate analysis are congruent with the isolation by distance results. We can add the observation that migration between sites in general seemed to be symmetrical without an apparent downstream bias (Fig. 3). While there are currently no movement data on *G. oculifera*, a study on the movement of the sister species, *G. flavimaculata,* shows a similar pattern of unbiased movement between upstream and downstream sites (Jones, 1996).

Our work may not have fully characterized the extent and patterns of genetic differentiation among sites. Gaps in our sampling include upper reaches of the Pearl and Bogue Chitto rivers and also in smaller tributaries like the Strong and Yockanookany rivers. Populations of *G. flavimaculata* in the upper portions of the Chickasawhay and Leaf rivers were genetically distinct compared to populations in the lower stretches of the river (Selman et al., 2013a). Selman et al. (2013a) postulated that near the upstream limits, habitat suitability is "patchy" (Shively and Jackson, 1985), and, therefore, populations are less continuous than in lower portions of the river system. However, we faced the same logistical constraints Selman et al. (2013a) pointed out in their study in that many areas of these upstream reaches of the Pearl River are difficult to sample for a variety of reasons including a lack of available boat launches, poor navigability (i.e., high deadwood density and shallow water), and lower densities of *G. oculifera*. Furthermore, many of these upstream populations that are not exposed to regular boating traffic are more wary of canoes or boats in the river (Selman et al., 2013b).

Different patterns of genetic variation in G. oculifera and G. flavimaculata.—Even though *G. flavimaculata* and *G. oculifera* are sister species (Lamb et al., 1994; Stephens and Wiens, 2003) and occupy neighboring drainages, there are dramatic differences in the pattern of genetic variation exhibited by the two species. Most of their life history and ecology is relatively similar (Lindeman, 2013), so this is not likely to explain the differences observed. Instead, the two drainages in which they occur are likely the source of these differences as they are dramatically different in geomorphology. The Pascagoula River drainage is highly dendritic (i.e., has a "Y" shape) with two major tributaries—the Leaf and Chickasawhay rivers—whereas, the Pearl River has a linear formation with only minor tributaries. Several authors have suggested that the species radiation of *Graptemys* has been tightly linked to fluctuating sea levels and drainage isolation during the Pliocene and Pleistocene (reviewed by Lindeman, 2013). These events likely impacted coastal rivers differently depending on their geomorphology, which ultimately influenced the pattern of intraspecific variation in the species of *Graptemys* inhabiting these drainages. Thus, it is possible that during sea level maxima (likely later in the Pleistocene), the "Y" shape of the Pascagoula might have resulted in two refugia being formed by the major tributaries (Leaf and Chickasawhay rivers) for *G. flavimaculata*, but the Pearl is relatively straight and may have left only one refugium for *G. oculifera*. Isolation event(s) imposed by sea level fluctuations may have led to significant spatial separation of populations of *G. flavimaculata* within each refugium and subsequently to detectable levels of genetic differentiation in populations of *G. flavimaculata*, while not producing differentiation in *G. oculifera*. If correct, this hypothesis would predict more genetic differentiation in more highly dendritic river systems. For example, *G. nigrinoda* (Black-knobbed Sawback) would be expected to have greater genetic differentiation than both *G. flavimaculata* and *G. oculifera* due to

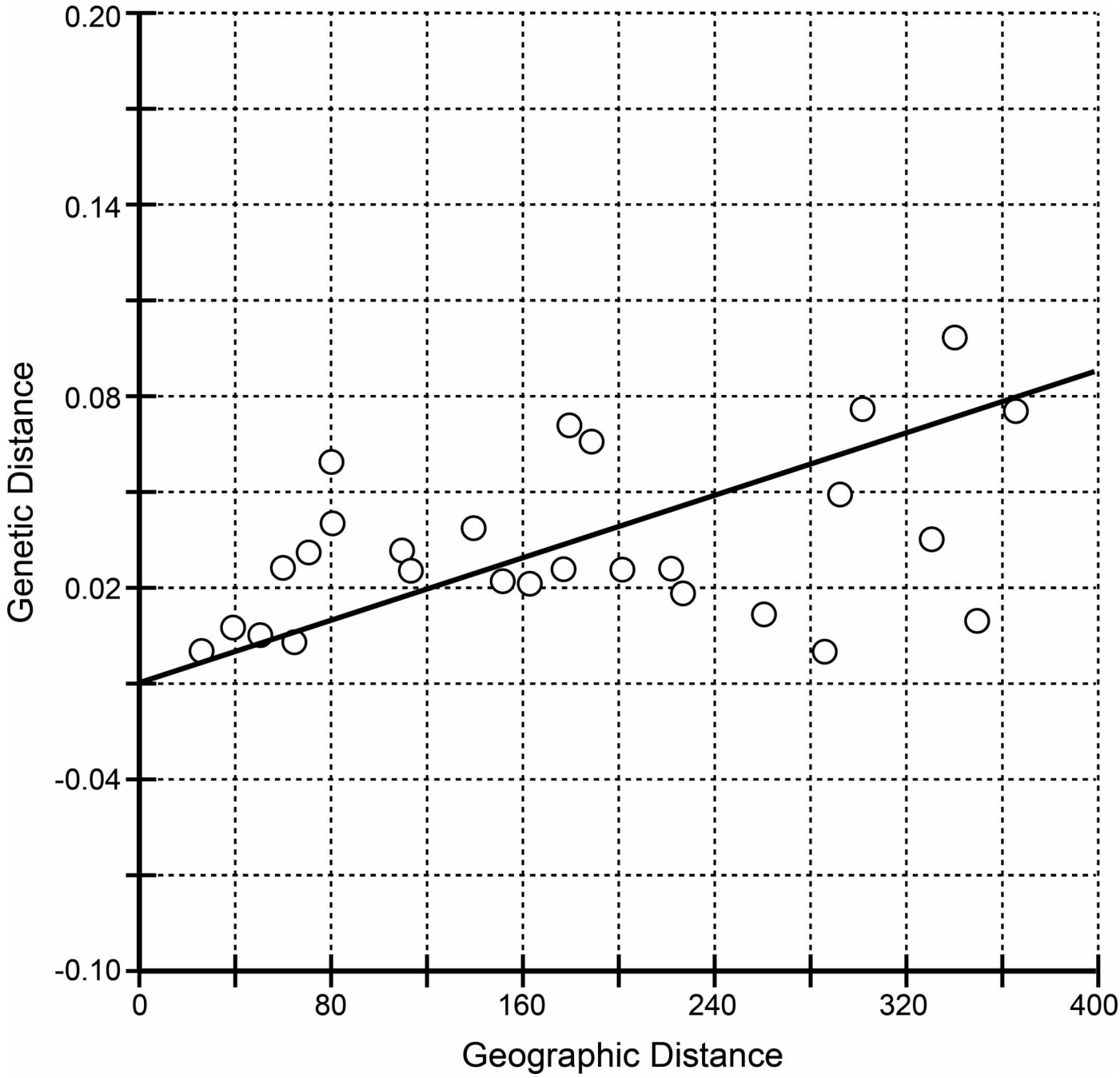

Fig. 2. Isolation by distance among sites of *Graptemys oculifera* as indicated by the significant positive correlation between pairwise genetic distances (F_{ST}) and geographic distance in river kilometers (Mantel test; $P = 0.014$, $Z = 199.133$, $r = 0.4125$). The two negative values of F_{ST} were set to zero.

the highly dissected Mobile River drainage (including large tributaries; Alabama, Black Warrior, Tombigbee, and Cahaba rivers). Although no microsatellite DNA data is available for *G. nigrinoda*, there is clinal variation in morphology found in this species within the Mobile Bay Basin (Cagle, 1954; Folkerts and Mount, 1969; Freeman, 1970; Ennen et al., 2014). This variation could represent limited gene flow between some populations; however, this hypothesis needs to be tested in future population genetic studies.

Lack of evidence for historical or contemporary bottlenecks.— Prior to being federally listed as threatened in 1986, surveys indicated that *G. oculifera* was in decline and not detected in some river reaches surveyed (McCoy and Vogt, 1980; Stewart, 1988). However, more recent studies have shown that *G. oculifera* can exceed 340 turtles per river km (Jones and Hartfield, 1995), with basking densities at most of our study sites ranging from 20.4 to 83.2 per river kilometer (Lindeman, 1998; Shively, 1999; Dickerson and Reine, unpubl.; Selman and Jones, unpubl.). Neither BOTTLENECK nor M ratio detected evidence of genetic bottlenecks in any of the groups analyzed. This suggests that the historical reduction in abundance was not severe enough or of long enough duration to produce a detectable genetic bottleneck. Furthermore, estimates of effective population size seem to generally correlate with current population estimates (Table 4). Similarly, this pattern was also found in *G. flavimaculata* that had observed population declines in the 1980s, but no evidence for population bottlenecks (Selman et al., 2013a).

Table 4. N_e estimates as calculated by NeEstimator (lowest allele frequency used = 0.02; jackknife to estimate 95% CI). When the software produced a negative value for N_e, this was reported as infinity. Population estimates for each site when available along with 95% CI are reported along with the density (per river kilometer) calculated from basking surveys. NA indicates that the information was not available for a particular site.

Site	N_e	95% CI	Pop. est.[a]	95% CI[a]	Basking density per km
Carthage	97.6	22.7–∞	408	254–671	12[a]
Ratliff Ferry	194.7	15.0–∞	1097	792–1535	98[a]
Lakeland	130.2	15.6–∞	689	553–885	13[a]
Monticello	∞	34.6–∞	837	710–1000	20[a]
Columbia	1456.2	64.2–∞	633	404–1014	10[a]
Bogalusa	104.5	21.0–∞	NA	NA	20.4[b]
Napoleon	130.5	6.3–∞	NA	NA	20–30[c]
Bogue Chitto	∞	9.1–∞	NA	NA	12.3[b]

[a] Jones and Hartfield, 1995
[b] Lindeman, 1998
[c] W. Selman, pers. obs.

Genetic structure relative to the Ross Barnett Reservoir.—We did not detect a genetic impact of Ross Barnett Reservoir in any of our analyses. No significant genetic bottlenecks or extremely low estimates of N_e were found at sites above the reservoir. Likewise, the reservoir currently does not seem to have an impact on genetic structure. The pairwise F_{ST} value between the sites immediately above and below the reservoir was small and not significantly different from zero. Likewise, the AMOVA model that grouped sites above and below the reservoir did not explain a significant amount of the variation. Estimates of migration rates between the two sites separated by the reservoir are not qualitatively different from those among other sites on the mainstem Pearl River. We also did not detect any significant differences in H_o, H_e, Na, and A_R between sites above and below the reservoir.

Although these results suggest there is not a currently recognizable impact of the Ross Barnett Reservoir on gene flow, this conclusion should be tempered by the fact that turtles have long generation times and enough generations may not have passed since the formation of this barrier for the consequences to be detected in our analyses (Landguth et al., 2010). In the roughly 50 years since the reservoir was constructed, there have likely only been 3–5 generations and any genetic differentiation between upstream and downstream sites would not have been achieved under best case scenarios following models by Landguth et al. (2010). A similar genetic study on *G. geographica* found that dams and locks built 100 years ago did not result in detectable population genetic structure or in significant differences in genetic diversity indices in the Trent-Severn Waterway in Ontario (Bennett et al., 2010). They too suggested that the long generation times of turtles could be the reason for not detecting any population differentiation.

Ward et al. (2013) found weak genetic structure in populations of *G. caglei* in the Guadalupe River. However, the authors attributed this genetic structure not to the presence of reservoirs, but rather isolation by distance between the upper and lower stretches of the river. However, there is evidence that even small dams can be barriers to movement, with Killebrew et al. (2002) suggesting that a dam blocked upstream and downstream movement of *G. caglei*. Likewise, over 25 years of sampling above and below the Ross Barnett Reservoir, no marked *G. oculifera* were ever found to make cross reservoir movements (R. L. Jones, unpubl.). However, it should be noted that the distances between the study sites above and below the reservoir is roughly 42 km, and the farthest movements known for *G. flavimaculata* are about 5 km (Jones, 1996), with *G. flavimaculata* acting as a suitable surrogate species comparison for *G. oculifera*. So, although the impounding of rivers in recent decades may not currently influence levels of genetic diversity and produce genetic differentiation, these barriers seem to restrict dispersal and

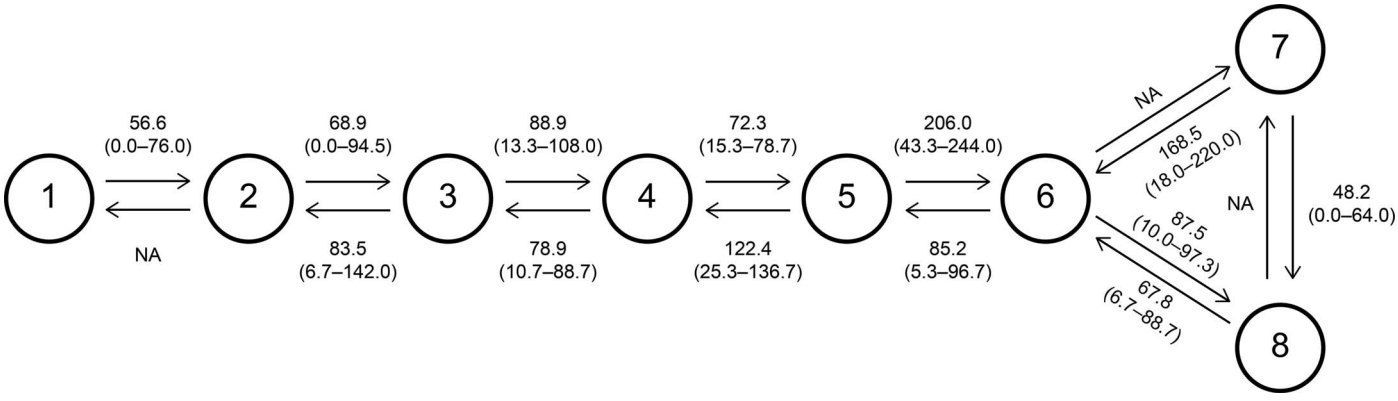

Fig. 3. The mutation scaled migration rates (M) with the 95% highest posterior density in parenthesis for each pair of sites of *G. oculifera* in the modified stepping stone model as calculated by Migrate-n. Parameters for which we were unable to recover a good posterior distribution are represented by NA. Site numbers correspond to Figure 1.

ultimately limit gene flow. *Graptemys oculifera* is considered a riverine species that prefers numerous sandbars and moderate to fast currents (Jones and Selman, 2009) which are not found within the reservoir. It is likely that there is no breeding population within the reservoir (Jones and Selman, 2009) and a roughly 42 km gap in suitable habitat between populations above and below the reservoir. Thus, future dispersal across this barrier—both the dam and reservoir—seems improbable. Therefore, we suggest that studies be undertaken on movements via radio telemetry or through mark–recapture studies with populations near dams and large reservoirs. These studies will provide valuable data in order to assess the current impediments of reservoirs and dams on the movements of species of *Graptemys*.

Conclusions.—The geographic formation of coastal river systems across the Gulf of Mexico inhabited by species of *Graptemys* could have played a significant role in shaping their genetic structure via different numbers of glacial refugia. The deeply dendritic Pascagoula likely contributed to greater genetic differentiation in *G. flavimaculata* due to multiple refugia, while the linear formation of the Pearl led to only one refugium with minimal genetic differentiation in *G. oculifera*. In our analyses, the Ross Barnett Reservoir does not seem to have an impact on the genetic structure of *G. oculifera*. However, we caution that this is likely the result of the long generation times of turtles and not necessary indicative of contemporary connectivity between upstream and downstream populations. Likewise, our analyses indicate that although there is not strong genetic structure in the Pearl River, there is also not total panmixia. Differentiation among sites follows a pattern of isolation by distance. Therefore, continued conservation of the Pearl River corridor should be considered by conservation planners, specifically when future riverine engineering projects are proposed. Lastly, subtle differences between populations should be considered when developing management plans and, if needed, reintroduction strategies for this species.

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