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Microsatellite DNA Analyses Support an East-West Phylogeographic Split of American Alligator Populations

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ABSTRACT We examined the population genetic structure of American alligators *(Alligator mississippiensis)* sampled from 12 localities across the southeastern United States. The primary goal of this study was to determine the extent of population differentiation among alligators from four Florida lakes using eight microsatellite loci and compare the results to additional sites located at varying distances from them. Analyses of population structure revealed little differentiation (F_{ST}=0.039; Rho=0.012) among the four Florida lakes, Apopka, Griffin, Orange and Woodruff, which are all located in the St. John's River watershed in north-central Florida. Further, there was little differentiation among these samples and samples collected from the Everglades National Park (F_{ST}=0.044; Rho=0.009) and south Georgia (F_{ST}=0.045; Rho=0.032). Therefore, these six samples were pooled together as a ''FL/sGA group.'' Similarly, samples collected in the western extent of the range, Anahuac National Wildlife Refuge in Texas and Salvador Wildlife Management Area, Marsh Island Wildlife Refuge and Rockefeller Wildlife Refuge in Louisiana, also lacked population structure (F_{ST}=0.024; R_{ST}=0.040). These four populations were pooled into the ''TX/LA group.'' Comparisons of these two groups with samples taken from the Santee Coastal Reserve in South Carolina and Mobile, Alabama yielded three to four times more differentiation among groups (F_{ST}=0.131; Rho=0.187). These and other analyses support the hypothesis of an east-west phylogeographic split in American alligator populations and are consistent with studies of many freshwater fish and aquatic and terrestrial turtles distributed throughout this same geographic region. *J. Exp. Zool. (Mol. Dev. Evol.) 294:352–372, 2002.*

INTRODUCTION

The American alligator (*Alligator mississippiensis*), together with its only congener, the Chinese alligator (*Alligator sinensis*), are the sole extant temperate-dwelling crocodilian species (Buffetaut, '89). While the ultimate fate of the Chinese alligator in the wild remains unknown (Thorbjarnarson et al., 2000), American alligators have recovered from tremendous exploitation that occurred during the first half of the last century

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and now thrive in many of the wetland ecosystems of the southeastern United States. The rise in alligator numbers and human encroachment into alligator habitat has resulted in an increase in human-alligator interactions (Zoroya, 2001). State agencies have responded by implementing nuisance programs which must address the real or perceived threats of such interactions. Additionally, state-regulated hunts, which monitor the taking of limited numbers of animals from specific harvest areas, have been implemented in many states within the alligator's range (Joanen and McNease, '87). Such practices require informed management decisions.

One area of critical concern in the management of healthy wild populations is the maintenance of genetic diversity (Thorbjarnarson et al., '92; Haig, '98). Concomitantly, a subject of immediate interest to many researchers is the elucidation of the genetic structure of populations (Avise, '94; Haig, '98). Molecular techniques have been employed to answer questions about underlying genetic variation (Villarreal et al., '96; Gibbs et al., '97; Newman and Squire, 2001), examine fine-scale reproductive dynamics (Davis et al., 2001b; Neff, 2001) and develop markers for species and population identification (Norman et al., '94; Baker et al., '96; FitzSimmons et al., 2001). Many previously unstudied populations of crocodilian species are being newly investigated (Dever and Densmore, 2001; Pontillas, 2001). The American alligator, however, has been the subject of numerous population genetic studies. Allozyme analyses revealed low genetic variation within Florida, Louisiana and South Carolina alligator populations (Gartside et al., '77; Menzies et al., '79; Adams et al., '80), and limited variation among populations (Adams et al., '80). Other studies utilizing microsatellite markers revealed much higher amounts of variation within and among populations from Florida, South Carolina, Alabama and Louisiana (Glenn et al., '98; Davis et al., 2001a).

There are many questions about the spatial scale at which genetic makers have the power to discriminate and the extent to which they can detect gene flow among populations. One study of mitochondrial DNA in alligator snapping turtles (*Macroclemys temminckii*) revealed drainage specific haplotypes (Roman et al., '99). Microsatellite studies of wood frog (*Rana sylvatica*) populations showed evidence of genetic subdivision at fine scales (i.e., < 5km) (Newman and Squire, 2001) and analyses of the eastern massasagua rattlesnake (*Sistrurus c. catenatus)* also suggested fine-scale population structure, even across continuous populations (Gibbs et al., '97). The application of such analyses to wildlife-forensic and law enforcement issues have been helpful in a growing number of cases (Roman et al., '99; Primmer et al., 2000). For the alligator and other crocodilian species, the ability to distinguish among different populations or regions could enhance the identification of illegally taken hides and meat by law enforcement personnel (FitzSimmons et al., 2001). A study of American alligators that compared coastal and inland populations in Texas reported closer genetic relationships among individuals within drainages than among individuals from different river systems (Ryberg et al., 2003). While other studies of American alligators have shown the potential for discriminating among individuals from larger geographic distances, assignment tests failed to provide precise assignments of alligators to their correct population of origin in two Louisiana populations that were 50 km apart (Davis et al., 2001a).

The goal of this study was to ascertain the population genetic structure of American alligators using eight microsatellite markers. Twelve sample sites were chosen from across the species' range (Fig. 1). Of particular interest was the evaluation of the power of these markers for detecting gene flow among adjacent populations. Two groups were analyzed, one from the eastern and one from the western extreme of the range. Lakes Apopka, Griffin, Orange and the Lake Woodruff National Wildlife Refuge (Woodruff) from north-central Florida formed the eastern group. Anahuac National Wildlife Refuge (Anahuac), Texas and Rockefeller Wildlife Refuge (Rockefeller), Marsh Island Wildlife Refuge (Marsh Island) and Salvador Wildlife Management

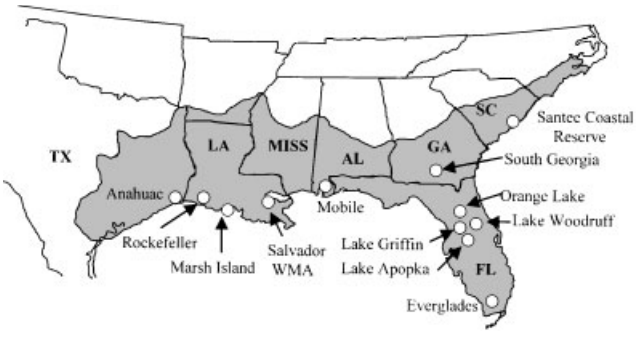

Fig. 1. Distribution of the American alligator (shaded, adapted from Coulson and Hernandez, '83) with sampling sites indicated (open circles).

Area (Salvador WMA) in Louisiana formed the western group. These populations and groups of populations were compared to additional sampling sites to discern the power of these markers for detecting population differentiation across varying distances. One of these additional populations, Mobile, Alabama, was of special interest due to its intermediate geographic position among the eastern and western groups, as well as its lack of representation in previous alligator genetic studies in the literature.

MATERIALS AND METHODS

Samples (Appendix I) and sample sites (Fig. 1)

North-central Florida

Lakes Woodruff, Apopka, Orange, and Griffin are located in north-central Florida and are part of the St. John's River watershed. Each is connected to one another via streams, marshes, or swamps (A. Woodward, personal communicatoin). Lake Woodruff is a slightly eutrophic lake that has been used as a reference site for a number of alligator studies (Guillette et al, '97; Crain et al., '98; Milnes et al., 2001). Lake Apopka is a hypereutrophic lake located 3 km downstream from an EPA Superfund site where an industrial spill of dicofol occurred in 1980. Alligators from Lake Apopka have been the subject of numerous studies of reduced egg viability and endocrine disruption resulting from such contaminants (Guillette et al., 2000). Orange Lake is considered to be intermediate in terms of human impact, but has contaminant impacts similar to those of Lake Woodruff (Milnes et al., 2001). The alligator population of Orange Lake has been subject to commercial and public harvest since 1981. Lake Griffin is a highly eutrophic lake similar to Lake Apopka, but has a lower contaminant level. This lake has been the site of intense agricultural activity development over the last two decades.

These lakes were not chosen originally for contaminant work but were picked by the FL Fish and Wildlife because they represented 'average' lakes in FL. This agency wanted basic information on life history—we only learned after several years of study that several of these lakes (Apopka and Okeechobee) had areas of high contamination. The effects of contaminants on alligators from some of these populations may introduce biases relative to sampling lakes chosen at random or chosen from only the least impacted populations. Our expectation a priori was that contaminants had not impacted these populations sufficiently to produce significant effects but that any biases would be toward reduced intra-population variation (due to bottlenecks or selection) and increased differentiation among populations.

For this study, adult and sub-adult alligators were sampled from these four lakes in three different years. Methods of capture and sample collection were described by Guillette et al. ('99). A total of 59 alligators were sampled from Lake Woodruff, with 28 sampled in 1997 and 31 in 1999. Forty-one alligators were sampled from Lake Apopka in 1999. The Orange Lake sample consisted of 14 alligators collected in 1997 and 12 collected in 2001. Thirty-four alligators were sampled in 1999 on Lake Griffin. For each sample, blood was collected and placed in storage buffer and frozen at $-20^{\circ}C$ until DNA extraction. All samples were collected under the authority of the Florida Department of Natural Resources (DNR) by DNR personnel or those authorized by such personnel according to approved animal use protocols.

Everglades, Florida

The Everglades National Park of south Florida has been highly impacted by human manipulation (Morea et al., 2000). It is believed to be a harsh environment for alligators due in part to the low food availability and high temperatures imposed by its subtropical climate (Jacobsen and Kushlan, '89; Barr, '97). Predominant vegetation was described by Howarter et al. (2000). For this study, samples of blood were collected from twenty-four adult alligators under the authority of the National Parks Service in June 1977.

Louisiana

Rockefeller Wildlife Refuge is a 30,750 hectare (ha) coastal refuge located in southwest Louisiana. Refuge boundaries and predominant vegetation have been described previously by Joanen ('69). Thirty-four adult alligators were sampled from Rockefeller in 1997 according to Davis et al. (2001b). Twenty-four and 27 adult and sub-adult alligators were harvested from Marsh Island Wildlife Refuge (30,000 ha) and Salvador Wildlife Management Area (12,400 ha), respectively, in 1997 as part of experimental harvests conducted by the Louisiana Department of Wildlife and

Fisheries. Harvested alligators were brought by trappers to a check station where blood was drawn from the cervical sinus into a heparinized syringe. The blood was then placed into DNA lysis/storage buffer (1:1 by volume) and stored at −20°C until further analysis.

Anahuac, Texas

Anahuac National Wildlife Refuge is a 13,765 ha refuge located on the coast of south-central Texas, approximately 40 km north of Galveston. Blood samples were collected from 33 adult alligators on Anahuac Refuge in September 1997 under the authority of park officials.

Mobile, Alabama

Twenty-three samples collected from Mobile consisted of blood or muscle tissue taken from adult and sub-adult nuisance animals captured in 1997 and 1998. Animals originated from Mobile and Baldwin counties and were collected by state approved nuisance trappers.

Santee Coastal Reserve, South Carolina

Santee Coastal Reserve (Santee) is a 10,118 ha area on the north-central coast of South Carolina. Habitat characteristics and sampling methods were described by Rhodes and Lang ('95, '96). One hatchling per clutch was sampled from 19 nests in 1997 and from 12 nests in 1999. All samples were collected under the authority of the South Carolina Department of Natural Resources. Tissue samples included either a tail tip or blood drawn from the anterior sinus. Samples were placed in DNA lysis/storage buffer (1:1 by volume for blood) and frozen at −20°C until DNA extraction.

South Georgia

Samples collected from south Georgia consisted of muscle tissue or hides in brine solution from nuisance animals taken primarily in 1997 (Appendix I). Animals were removed by state approved nuisance trappers and originated from a nine-county area (Colquitt, Crisp, Dooley, Grady, Lee, Mitchell, Sumter, Thomas and Worth).

DNA isolation, amplification and ABI 377 gel electrophoresis

DNA was isolated according to Glenn et al. ('98) or Davis et al. (2001b) following the methods of Carter and Milton ('93). Briefly, blood, muscle or hide tissue was digested overnight in proteinase K (10 mg/ml) and TENS (12 mM Tris pH 8.0, 12 mM EDTA, 120 mM NaCl, 1% SDS). Digests were added to a diatomaceous earth (MUD) and guanidine thiocyanate mixture, vortexed and incubated from 15 minutes to overnight. Pelleted MUD was washed with 70% ethanol and dried, after which 125 ul of TLE (10 mMTris pH 8.0, 0.1 mM EDTA) was added to re-suspend the DNA. Following incubation and centrifugation, the DNA-containing supernatant was removed and checked for quantity and quality by ethidium bromide staining of DNA subjected to electrophoresis in a 1% agarose gel. Eight microsatellite loci were utilized in this study including, Amiμ-6, Amiμ-8, Amiμ-15, Amiμ-17d, Amiμ-18, and Amiμ-20 (Glenn et al., '98). All loci were chosen for their polymorphism and ease of amplification. All loci have dinucleotide (AC) microsatellites with >10 uninterrupted repeats. Amiμ-17d is a compound microsatellite locus consisting of 2 dinucleotide and 2 tetranucleotide repeats [$(AC)_n(AT)_n$ $(ATGT)_n$ $(AGAT)_n$]. It should be noted that for this study primer b of Amiμ-17d was modified from Davis et al. (2001a,b) in attempt to eliminate the occurrence of null alleles. The modification consisted of a 5 base pair extension of the 5'end (5'GATTT-) and a shortening of the 3' end by 7 base pairs (Amiμ-17a: 5'-GCTGACCTTGGTTG-GAAACTCTA; Amiμ-17d: 5'-GATTTCCTGTT-CTTGCATAAA). Therefore, all Amiμ-17d fragments are five base pairs longer than in previous studies (Glenn et al., '98; Davis et al., 2001a,b). Two additional loci containing only tetranucleotide repeats, Amiμ-202 and Amiμ-203, were recently developed and have not been published previously (N. Schable, personal communication). The primer sequences are: Amiμ-202a: 5'-GAA-TAGGAATGTTGGCACTA, Amiμ-202b: 5'-AACT-TATGGCAAACAGATAGAG, (60°C annealing temp); Amiμ-203a: 5'-GTGCTCCTTGCCTGATATGC, Amiμ-203b: 5'-ACCTGGGCTACATGCTATCT, (60°C annealing temp).

One primer from each pair was labeled with a fluorescent dye for detection on an ABI Prism 377 automated DNA sequencer (Applied Biosystems, Inc., Foster City, CA) according to Davis et al. (2001b). Primer Amiμ-202a was labeled with HEX and primer Amiμ-203b was labeled with TET (Applied Biosystems, Inc.). Multiplex PCR reactions were possible using Amiμ-6, Amiμ-8, Amiμ-15, and Amiμ-18 together, though the remaining loci had to be amplified individually.

Amplifications were carried out using a Techne Genius thermal cycler in either 25 or 50 ul reaction volumes according to the following parameters: 95°C for 3 minutes followed by 32–35 cycles of 95°C denaturing for 30 seconds, 55°C or 60°C (depending upon the primers being used) annealing for 15 seconds and 72°C extension for 30 seconds. A 72°C extension for 5 minutes was included as a final step. In most cases Promega Taq DNA polymerase (Promega Corp., Madison, WI) performed sufficiently well to obtain reliable amplicons. However, Sigma JumpStart Taq (Sigma, St. Louis, MO) or Gibco Taq DNA polymerase (Life Technologies, Gaithersburg, MD) enabled amplification in cases where Promega Taq failed. Following amplification, PCR products were inspected via ethidium bromide staining in a 1.2–1.5% agarose gel alongside a 100 base pair ladder (Promega Corp.) to estimate PCR product yield. For loading onto the ABI Prism 377, a cocktail of 3.0 ul Dextran/formamide, 0.6 ul Promega CXR fluorescent ladder and approximately 10 ng of each PCR product was prepared. This cocktail was denatured by incubation at 95°C for 5 minutes and placed on ice. Of this, 0.8–1.5 ul was loaded into the wells of a 0.2mm thick 4.5% polyacrylamide gel (12cm well-to-read length) and the amplicons separated over a 1.5-hour period. GeneScan and Genotyper programs (Applied Biosystems, Inc.) were then used to identify microsatellite fragments/genotypes. All peaks were inspected visually for confidence in genotyping.

Statistical methods

As the loci Amiμ-202 and Amiμ-203 were new markers and had not been tested previously, three populations were arbitrarily selected and tested separately for non-random association of genotypes for these loci—Lake Apopka, Rockefeller and the 1999 Lake Woodruff sample. Additionally, while previous studies found no significant linkage disequilibrium among Amiμ-6, -8, -15, -17d, -18 and -20 (Glenn et al., '98), tests among these and Amiμ-202 and Amiμ-203 were performed to check for non-random association of genotypes among all loci.

Tests of Hardy-Weinberg equilibrium were performed to test for the random union of gametes under the alternative hypothesis of heterozygote deficiency (GenePop 3.2a, updated from Raymond and Rousset, '95). This alternative hypothesis was chosen due to the tendency for microsatellite loci to contain null alleles, which would result in an apparent excess of homozygotes. Tests for linkage disequilibrium by pair-wise locus comparisons for all populations were performed and allele frequencies, observed heterozygosity (H_O) and expected heterozygosity (H_E) were calculated using GenePop 3.2a.

Several statistical analyses were employed to detect population differentiation. Genic and genotypic differentiation were tested among all pairs of populations for all loci (GenePop 3.2a). F_{ST} values of population sub-divison were also calculated using GenePop3.2a according to Weir and Cockerham ('84). Tests of genotypic differentiation, based on the G-based exact tests of Goudet et al. ('96), were also performed using this program. Rho values were determined using RstCalc (Goodman, '97). These values are estimates of Slatkin's R_{ST} using transformed allele sizes for calculating variance components over loci. Arlequin 2.000 (Schneider et al., 2000) was used to conduct analyses of molecular variance (AMOVA) (Excoffier et al., '92) to determine how genetic variation was partitioned within and among populations. One AMOVA was performed under the null hypothesis of random mating among all populations. A second AMOVA examined population structure among four sub-divided populations or groups of populations to test for geographic substructuring among them.

A measure of genetic distance among populations, delta mu squared ($\delta\mu^2$), was calculated with R_{ST}Calc (Goodman, '97) according to the method of Goldstein et al. ('95). This distance is based on the squared differences in mean allele sizes averaged over all loci and has been shown to be a superior measure of genetic distance for microsatellites because it increases linearly with time of separation between populations (Goodman, '97). Delta mu squared genetic distances assume a stepwise mutation model which is consistent with observed mutations at these loci (Davis et al. 2001b). Delta mu squared genetic distances were plotted against geographic distances and tested for an isolation-by-distance effect. Differences in average $\delta\mu^2$ among populations and groups were tested using two-sample T-tests (MINITAB, release 12). Lastly, GeneClass (Cornuet et al., '99) was used to provide probabilities of assigning individuals to their population of origin. These assignments were based on Cavalli-Sforza genetic distance measurements (Cavalli-Sforza and Edwards, '67) and assigned individuals to the

populations they were most closely related to genetically.

RESULTS

Equilibrium

Tests of linkage disequilibrium among loci within Apopka, Rockefeller and the 1999 Woodruff sample resulted in two significant pair-wise comparisons each in Apopka and Rockefeller and none in Woodruff after sequential Bonferroni correction. Tests of genotypic disequilibrium over all populations yielded a total of nine significant pair-wise tests. Among these, the pairs Amiμ-8/Amiμ-202 and Amiμ-202/Amiμ-203 were significant in two populations each. However, the total number of significant pair-wise tests was far less than the number expected by chance alone (18.2) for 364 tests. Also, given that the number of loci used here is small relative to the number of chromosomes in the alligator genome, it is unlikely that these three are linked, particularly since there was no significant linkage disequilibrium among the third pair, Amiμ-8/Amiμ-203. Thus, these results are not considered biologically significant and the loci are treated as unlinked.

Tests of random union of gametes revealed that all populations except south Georgia and Mobile conform to Hardy-Weinberg (HW) equilibrium. South Georgia showed a violation of HW, probably due to these samples originating from a large area (nine counties). Mobile deviated from HW primarily because of locus Amiμ-17d. Locus Amiμ-17d. did not conform to HW expectations of panmixia ($P < 0.05$) in Mobile and Salvador WMA. This finding is consistent with results from previous studies which have also detected a deviation from HW expectations at this locus (Davis et al., 2001a), as well as the presence of null alleles in low frequency (0.06) in the Rockefeller population (Davis et al., 2001b). This locus is, however, still one of the most polymorphic loci in this study. As null alleles result in more conservative estimates of variation and differentiation, Amiμ-17d was included in all further analyses.

Genetic variation

Allele sizes, corresponding numbers of repeat motifs and allele frequencies are given in Appendix II. Summary statistics for all populations (Table 1) show that Santee has the lowest observed heterozygosity (H_O=0.547) and the lowest mean number of alleles (4.1) across all loci. This can be attributed to the lack of diversity in the two loci that provide the most for other populations—Amiμ-17d and Amiμ-202. Mobile had the second lowest H_O (0.587) and mean number of alleles (7.0), undoubtedly due in part to the presence of null alleles in this population. Marsh Island had the highest H_O (0.786), though nearly all of the remaining populations (other than Santee and Mobile) had mean allele numbers that were higher. These results are identical to the results in a study by Davis et al. (2001a) that examined many of these same populations using only 5 loci. In this study, the mean H_O across all populations and loci (0.694) was only slightly higher than in Davis et al. (2001a, 0.637).

Unique (private) alleles were found in all populations except Santee and in all loci except Amiμ-203 (Appendix II). Most of these occurred in frequencies of less than 5%, although allele 165 at locus Amiμ-15 occurred at a frequency of 16.7% in the Everglades population and allele 184 in Amiμ-18 was represented 10% of the time in the sGA population.

Population structure

Allele frequency distributions (Appendix II and Fig. 2) indicated differentiation among western and eastern populations. The western population group consisted of Texas and all Louisiana populations, hereafter called the ''TX/LA group.'' The eastern group included all Florida samples and the south Georgia sample, hereafter called the ''FL/sGA group.'' Locus Amiμ-6 showed very disparate patterns of allele distributions among the Florida populations and the TX/LA group for alleles 110, 122, 124 and 128. Similar patterns were found in locus Amiμ-8 (alleles 134 and 136), locus Amiμ-15 (alleles 149 and 159) and locus Amiμ-18 (alleles 164, 172, 188). Differences in allele frequency distribution in Amiμ-17d and Amiμ-20 were characterized by differences in frequencies among clusters of alleles. For example, in locus Amiμ-20 the alleles that tended to occur in the highest frequency in the TX/LA group were alleles 138-144 whereas allele 160 occurred in the highest frequency in most populations of the FL/sGA group. Amiμ-202 and Amiμ-203 had similar allele distributions among groups. Allele distributions for the Mobile population were equivocal with regard to which group this population was more closely related. Santee allele distributions appeared to be very different from all other populations or groups of populations.

TABLE 1. Summary statistics for all loci across all populations of American alligators

	Everglades, FL				Lake Woodruff, FL				Orange Lake, FL				Lake Griffin, FL			
Locus	N[1]	A[2]	H_o[3]	H_E[4]	N[1]	A[2]	H_o[3]	H_E[4]	N[1]	A[2]	H_o[3]	H_E[4]	N[1]	A[2]	H_o[3]	H_E[4]
Amiμ-6	24	6	0.500	0.536	59	8	0.729	0.663	23	7	0.739	0.790	34	6	0.618	0.582
Amiμ-8	24	7	0.750	0.693	59	6	0.611	0.663	24	4	0.417	0.463	34	5	0.588	0.575
Amiμ-15	24	9	0.583	0.813	59	7	0.797	0.756	24	4	0.708	0.733	34	6	0.559	0.635
Amiμ-17d	24	8	0.792	0.781	59	12	0.813	0.852	22	10	0.727	0.784	34	11	0.765	0.839
Amiμ-18	24	7	0.667	0.654	59	5	0.542	0.546	24	6	0.708	0.694	34	6	0.765	0.707
Amiμ-20	24	8	0.792	0.790	58	8	0.690	0.664	24	9	0.917	0.833	33	6	0.697	0.716
Amiμ-202	24	19	1.000	0.941	56	20	0.964	0.903	22	16	0.773	0.925	31	19	0.903	0.879
Amiμ-203	23	7	0.869	0.830	58	7	0.707	0.652	23	9	0.826	0.808	33	7	0.697	0.793
Mean	24	8.9	0.741	0.736	58	9.1	0.738	0.712	23	8.1	0.727	0.749	33	8.3	0.699	0.716

	Lake Apopka, FL				South Georgia				Santee, SC				Mobile, AL			
	N[1]	A[2]	H_o[3]	H_E[4]	N[1]	A[2]	H_o[3]	H_E[4]	N[1]	A[2]	H_o[3]	H_E[4]	N[1]	A[2]	H_o[3]	H_E[4]
Amiμ-6	38	6	0.465	0.526	32	7	0.469	0.651	31	3	0.226	0.209	22	4	0.227	0.213
Amiμ-8	41	6	0.830	0.734	31	7	0.484	0.621	31	4	0.646	0.690	23	6	0.870	0.769
Amiμ-15	41	6	0.683	0.695	32	7	0.750	0.768	31	3	0.677	0.600	23	5	0.609	0.567
Amiu-17d	41	12	0.775	0.694	31	14	0.710	0.891	31	4	0.258	0.362	21	8	0.524	0.862
Amiμ-18	40	6	0.853	0.853	30	5	0.667	0.642	30	6	0.667	0.673	23	6	0.783	0.798
Amiμ-20	40	6	0.800	0.721	32	7	0.750	0.768	31	4	0.613	0.684	23	6	0.435	0.472
Amiμ-202	39	18	0.872	0.900	32	19	0.844	0.913	27	6	0.704	0.699	22	15	0.864	0.925
Amiμ-203	39	7	0.769	0.733	32	9	0.781	0.812	31	3	0.581	0.647	21	6	0.667	0.692
Mean	40	8.4	0.736	0.708	32	9.4	0.649	0.758	30	4.1	0.547	0.546	22	7	0.587	0.662

	Salvador, LA				Marsh Island, LA				Rockefeller, LA				Anahuac, TX				mean across populations			
	N[1]	A[2]	H_o[3]	H_E[4]	N[1]	A[2]	H_o[3]	H_E[4]	N[1]	A[2]	H_o[3]	H_E[4]	N[1]	A[2]	H_o[3]	H_E[4]	N[1]	A[2]	H_o[3]	H_E[4]
Amiμ-6	27	7	0.815	0.804	24	5	0.958	0.738	34	7	0.676	0.746	33	5	0.576	0.658	31.8	5.7	0.591	0.575
Amiμ-8	27	10	0.740	0.711	24	11	0.750	0.832	34	8	0.765	0.688	32	8	0.875	0.786	32.1	6.8	0.627	0.685
Amiμ-15	27	5	0.481	0.504	24	4	0.708	0.628	34	5	0.559	0.540	33	5	0.426	0.394	32.2	5.5	0.617	0.649
Amiμ-17d	27	17	0.741	0.926	22	14	0.727	0.925	34	16	0.765	0.886	32	16	0.786	0.872	31.5	11.8	0.698	0.806
Amiμ-18	27	5	0.667	0.693	24	7	0.625	0.785	34	7	0.853	0.789	33	7	0.848	0.820	31.8	6.1	0.720	0.712
Amiμ-20	27	7	0.815	0.776	22	7	0.773	0.744	33	6	0.757	0.781	33	6	0.757	0.736	31.7	6.7	0.725	0.718
Amiμ-202	26	15	0.846	0.909	24	11	0.875	0.881	34	15	0.971	0.895	30	14	0.833	0.889	30.6	15.6	0.859	0.887
Amiμ-203	26	10	0.769	0.793	24	6	0.833	0.783	32	8	0.687	0.779	32	6	0.770	0.812	31.2	7.1	0.744	0.755
Mean	27	9.5	0.733	0.765	24	8.1	0.786	0.79	34	9	0.754	0.795	32	8.4	0.729	0.735	31.6	8.51	0.694	0.723

[1]Number of individuals sampled; [2]number of alleles per locus; [3]observed heterozygosity; [4]expected heterozygosity.

Tests for genic differentiation among each pair of populations over all loci were significant ($P < 0.05$) for all comparisons except for Salvador WMA and Marsh Island ($P = 0.175$). Genic differentiation was also tested among each of the two sample pools taken from Lake Woodruff ('97 and '99), Orange Lake ('97 and 2001) and Santee Coastal Reserve ('97 and '99). Santee showed strong support for identical allele distributions among years (P=0.914) whereas Lake Woodruff and Orange Lake did not ($P < 0.05$). Samples from the same locations were pooled for all subsequent analyses, however, because samples originated from large pools of individuals whose members represented the same breeding group and the level of variation detected among years within locations was lower than any between-location comparison.

F_{ST} and Rho values are given in Table 2. All pair-wise values of F_{ST} were significant ($P < 0.05$) except for Salvador WMA and Marsh Island, whereas Rho values suggested less structure within each of the FL/sGA and TX/LA groups (values in bold are not significant). Analyses of molecular variance (AMOVAs) were more consistent with the Rho values, however. For example, with all populations treated distinctly, among population variation was 11.63% (Table 3, panel A). When an AMOVA was performed with two populations and two groups of populations—1) TX/LA group, 2) FL/sGA group, 3) Mobile and 4) Santee—the among group variation was similar,

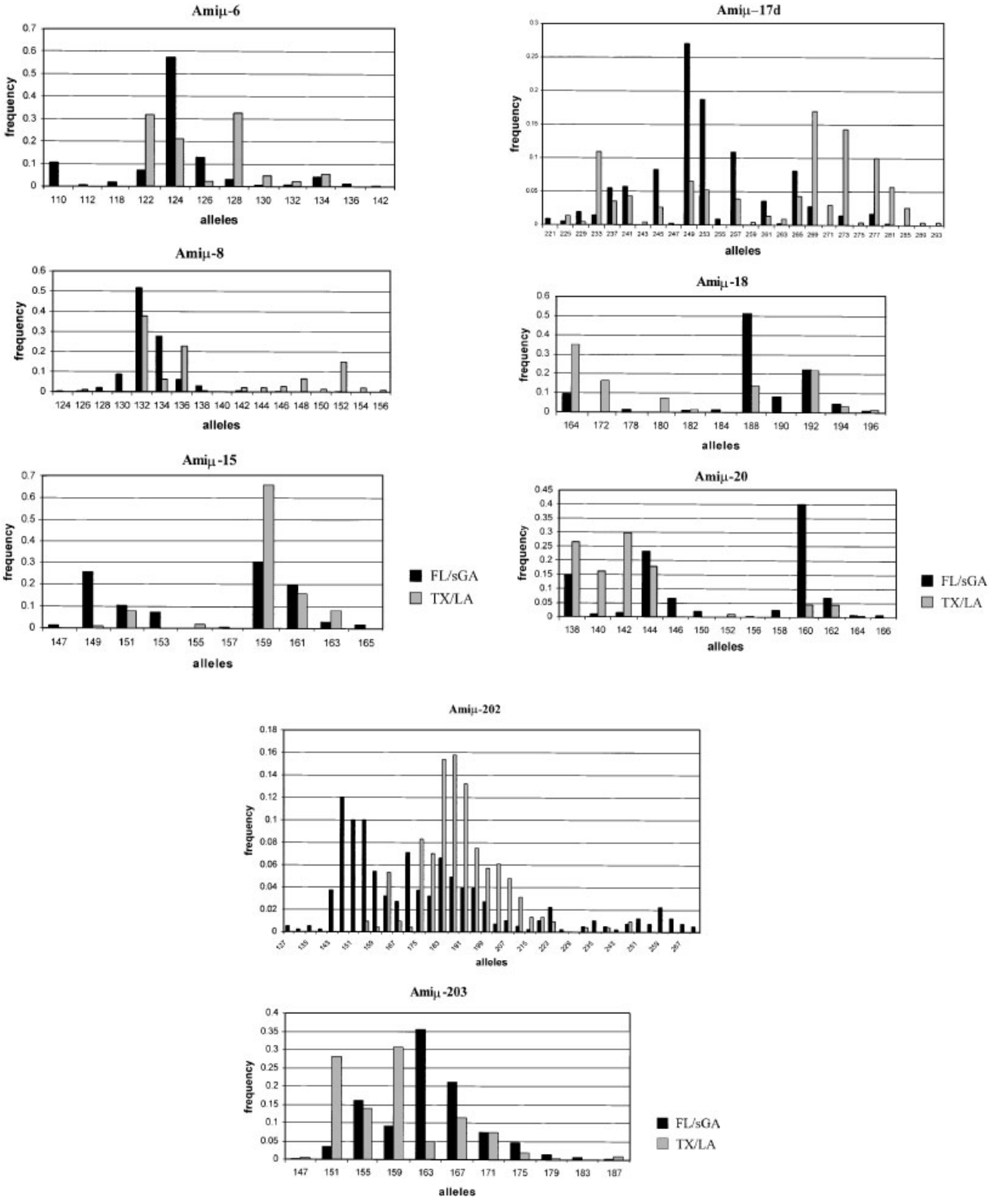

Fig. 2. Allele frequency distributions of eight microsatellite loci for groups of American alligator populations located in the eastern (FL/sGA) and western (TX/LA) extremes of its range. The FL/sGA group includes Everglades National Park and Lakes Apopka, Griffin, Orange and Woodruff. The TX/LA groups consists of Anahuac Wildlife Refuge, Texas and three Louisiana sites—Rockefeller Wildlife Refuge, Marsh Island Wildlife Refuge and Salvador Management Area. Alleles are given as total fragment sizes.

TABLE 2. F_{ST} (lower diagonal) and Rho (upper diagonal) values for American alligator populations. Rho values are averaged over loci. All values except those in bold are significant after sequential Bonferroni correction

	Everglades	Apopka	Griffin	Woodruff	Orange	S. Georgia	Santee	Mobile	Salvador	Marsh Is.	Rockefeller	Anahuac. TX
Everglades	X	**0.0233**	**0.0275**	0.0140	**−0.0070**	0.0654	0.1159	0.1194	0.3171	0.3164	0.2548	0.2200
Apopka	0.0558	X	**0.0174**	0.0590	**0.0108**	0.0666	0.1562	0.0870	0.2455	0.2527	0.1886	0.1647
Griffin	0.0473	0.0313	X	**0.0238**	**0.0072**	0.0840	0.1627	0.0947	0.2842	0.2906	0.2164	0.1977
Woodruff	0.0626	0.0502	0.0400	X	**0.0096**	0.0857	0.1718	0.1261	0.3135	0.3233	0.2498	0.2405
Orange	0.0555	0.0433	0.0278	0.0313	X	**0.0359**	0.1283	0.0984	0.2904	0.2973	0.2284	0.2095
S. Georgia	0.0559	0.0569	0.0372	0.0672	0.0435	X	0.1161	0.0792	0.2178	0.2187	0.1628	0.1560
Santee	0.2032	0.2142	0.2113	0.2148	0.1625	0.1705	X	0.1549	0.3503	0.3434	0.2960	0.2444
Mobile	0.1390	0.1036	0.1136	0.1487	0.1478	0.0941	0.2992	X	0.1647	0.1483	0.1072	0.0523
Salvador	0.1388	0.1337	0.1039	0.1535	0.1141	0.0928	0.2358	0.1188	X	**0.0083**	**0.0248**	0.0653
Marsh Is.	0.1245	0.1132	0.1021	0.1467	0.1060	0.0889	0.2196	0.1174	**−0.0007**	X	**0.0288**	**0.0350**
Rockefeller	0.1416	0.1368	0.1231	0.1629	0.1340	0.1141	0.2333	0.1218	0.0231	0.0144	X	**0.0306**
Anahuac. TX	0.1672	0.1540	0.1415	0.1794	0.1371	0.1130	0.2042	0.1231	0.0404	0.0318	0.0287	X

11.78% (Table 3, panel B). This result demonstrates that using these four groupings maintains the amount of variation found when all populations are examined distinctly. Concomitantly, the among group variation within these groups was only 3.60%, providing further evidence for low variation within the FL/sGA (F_{ST}=0.039; Rho=0.012) and TX/LA (F_{ST}=0.024; Rho=0.040) groups.

Genetic distance

Delta-mu genetic distances were calculated and used to test for relationships among populations and groups of populations. In a test for isolation by distance, a plot of $\delta\mu^2$ versus geographic distance produced a regression line with an R-squared value of 0.607 ($P<0.05$). Another test that utilized the natural log of the geographic distances versus Rho values also resulted in a significant isolation by distance effect ($P<0.05$). Not surprisingly, the lowest genetic distances were among the populations that are the closest to one another geographically. The largest genetic distances were among Santee and two Louisiana sites (Salvador WMA and Marsh Island). Genetic distance measurements also supported a TX/LA and FL/sGA split (Table 4). The average value of $\delta\mu^2$ among all pairwise comparisons within the FL/sGA group was 0.099. Within the TX/LA group, the average $\delta\mu^2$ was 0.104. Among these two groups, the average $\delta\mu^2$ was more than six times greater (0.689) than within these groups and this difference was significant (P=0.000, T=−13.02, df=23).

TABLE 3. Analyses of Molecular Variance (AMOVA) of American alligator populations. Panel A shows the results of an AMOVA when all 12 populations are analyzed separately. In Panel B, the analyses were performed with the 4 groupings shown

A. 1 group: all populations separate

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among populations	12	294.529	0.36723 Va	11.63
Within populations	759	2117.528	2.78989 Vb	88.37
Total	771	2412.057	3.15712	

B. 4 groups: 1) Santee;2) Mobile;3) TX/LA4) FL/SGA

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among groups	3	205.183	0.38824 Va	11.78
Among populations within groups	9	89.346	0.11881 Vb	3.60
Within populations	759	2117.528	2.78989 Vc	84.62
Total	771	2412.057	3.29694	

TABLE 4. Matrix of genetic and geographic distances. Upper diagonal contains geographic distances among American alligator populations (kilometers). Lower diagonal contains delta mu squared ($\delta\mu^2$) genetic distances (Goodman,'97)

	Everglades	Apopka	Griffin	Woodruff	Orange	S. Georgia	Santee	Mobile	Salvador	Marsh Is.	Rockefeller	Anahuac, TX
Everglades	X	375	410	435	485	740	970	1125	1385	1465	1515	1635
Apopka	0.0945	X	35	60	110	365	595	750	1010	1090	1140	1260
Griffin	0.0718	0.0330	X	60	75	330	585	715	975	1055	1105	1225
Woodruff	0.0438	0.1330	0.0642	X	100	355	535	740	1000	1080	1130	1250
Orange	0.0367	0.0572	0.0340	0.0372	X	255	510	640	900	980	1030	1150
S. Georgia	0.2022	0.1719	0.1837	0.1892	0.1352	X	540	435	695	775	825	945
Santee	0.2481	0.3472	0.2697	0.2627	0.2283	0.2208	X	915	1235	1315	1365	1485
Mobile	0.3217	0.2338	0.2583	0.3515	0.3015	0.1774	0.3219	X	260	340	390	510
Salvador	0.9775	0.7469	0.8520	0.9281	0.9937	0.6963	1.0456	0.4455	X	80	130	250
Marsh Is.	0.9720	0.7168	0.8574	0.9843	0.9391	0.7039	0.9986	0.4474	0.0337	X	50	170
Rockefeller	0.6778	0.4680	0.5374	0.6275	0.6084	0.4081	0.7255	0.2652	0.0714	0.0964	X	120
Anahuac, TX	0.5705	0.3641	0.4610	0.6104	0.5364	0.3540	0.5838	0.1098	0.1697	0.1367	0.1154	X

However, the average genetic distance among the sGA population and all Florida populations, 0.176, was also significant (P=0.000, T=−7.57, df=10), indicating that there is some differentiation between them. The average genetic distance within all LA sites was not significantly different from the average $\delta\mu^2$ among these and the Anahuac sample (P=0.056, T=−3.04, df=3). When sGA was removed from the FL/sGA group and Anahuac was removed from the TX/LA samples, the average $\delta\mu^2$ within the Florida and Louisiana samples drops to 0.060 and 0.067, respectively (with $\delta\mu^2$ among them going up to 0.789). Mobile was genetically intermediate among the eastern and western groups with significant genetic distances among Mobile and the FL/sGA group ($\delta\mu^2$=0.317; P=0.000, T=5.69, df=9). Between Mobile and the TX/LA group, $\delta\mu^2$ (0.274) was not significant (P=0.084, T=2.55, df=3). Lastly, the average $\delta\mu^2$ among Santee and the FL/sGA samples was significantly different than the average $\delta\mu^2$ within the FL/sGA group (P=0.000, T=6.60, df=13) and the TX/LA group (P=0.002, T=6.74, df=4).

Assignment tests

Assignment tests of individuals to populations of origin revealed several interesting trends (Table 5). In every case, individuals were assigned to their population of origin more often than they were assigned to any other population. Yet, there was no case in which all individuals were assigned exclusively to their own population. Rather, the

TABLE 5. Assignments of individuals to population(s) of origin based on Cavali-Sforz genetic distances (Cornuet, GeneClass,'99) In parentheses are the frequency of individuals assigned exclusively to their population of origin. Bold numbers indicate highest frequency of assignments for each population

	Assigned Population(s)													
Pop. of Origin	Ever	Apopka	Griffin	Wood	Orange	sGA	Santee	Mobile	Salv	Marsh	Rock	TX	Other[1]	No. Assign.[2]
Everglades (.375)	**0.833**	0.292	0.333	0.375	0.375	0.375	0.0	0.0	0.0	0.0	0.0	0.0	0.042	0.088
Apopka (.049)	0.610	**0.951**	0.659	0.683	0.585	0.634	0.0	0.0	0.024	0.0	0.024	0.0	0.024	0.024
Griffin (0)	0.559	0.706	**0.824**	0.588	0.647	0.706	0.0	0.0	0.059	0.0	0.029	0.0	0.059	0.118
Woodruff (.017)	0.492	0.661	0.576	**0.898**	0.593	0.458	0.0	0.0	0.0	0.0	0.0	0.0	0.017	0.085
Orange (.167)	0.333	0.458	0.500	0.500	**0.833**	0.458	0.0	0.0	0.0	0.0	0.0	0.042	0.042	0.125
S. Georgia (.312)	0.156	0.188	0.375	0.219	0.125	**0.750**	0.0	0.0	0.0	0.0	0.0	0.0	0.063	0.188
Santee (.581)	0.065	0.032	0.032	0.097	0.226	0.194	**0.871**	0.0	0.0	0.0	0.0	0.0	0.000	0.129
Mobile (.609)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**0.739**	0.043	0.130	0.043	0.0	0.043	0.217
Salvador (.074)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**0.741**	0.556	0.704	0.370	0.037	0.250
Marsh (.333)	0.0	0.0	0.0	0.0	0.042	0.083	0.0	0.0	0.375	**0.792**	0.417	0.167	0.042	0.167
Rockefeller (.059)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.588	0.382	**0.765**	0.618	0.118	0.118
Anahuac, TX (.091)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.606	0.485	0.697	**0.818**	0.030	0.152

[1]frequency of individuals assigned to any population other than population of origin.
[2]frequency of individuals not assigned to any population.

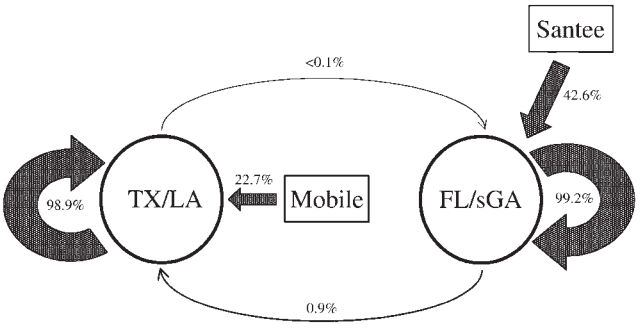

Fig. 3. Consolidation of the populations in Table 5 into four phylogeographic groups—FL/sGA, Santee, Mobile and TX/LA, and shows the percentage of assignments that are made within and among the groups.

percentage of correct, exclusive assignments to population of origin range from zero, for Lake Griffin, to 60.9% for Mobile (Table 5, in parentheses). Santee ranked second for exclusive, correct assignments with 58.1%. For all populations, some percentage of individuals were either not assigned to any population (range 2.4%–25%) due to rare alleles in the multi-locus genotype, or were assigned to populations other than their known population of origin (range 0–11.8%) due to some alleles being more common in other populations.

Assignments of individuals to populations supported the FL/sGA and TX/LA groupings. For example, individuals within each of these groups were assigned at some percentage to all other populations within their respective groups (Fig. 3). Overall, 98.91% of assignments of individuals within the TX/LA group were back to that group. Similarly, 99.17% of the assignments of individuals from the FL/sGA group were back to their own group. Only a very small fraction of individuals were assigned to a population outside their groups. Additionally, 22.73% of the individuals from Mobile were assigned to the TX/LA group, though no individuals from any other population were assigned back to Mobile. A similar trend was found with Santee, where 42.55% of the individuals were assigned to the FL/sGA group, and no individuals were assigned back to it.

DISCUSSION

Genetic structure

The results of this study demonstrate that the population genetic structure of American alligators is characterized by large-scale phylogeographic regions following an isolation by distance model. In the western extreme of the alligator's range, Texas and Louisiana populations lacked population structure and could not be distinguished from one another in assignment tests. In the eastern region, eight polymorphic microsatellite loci revealed very little genetic structure among lakes Apopka, Orange, Woodruff and Griffin in north-central Florida. While F_{ST} values were significant among populations within this group and even within the same populations across years (e.g., Woodruff and Orange), Rho values suggested genetic homogeneity among these lakes. Further, genetic distances among them were low compared to other populations and assignment tests could only assign individuals back to their group rather than to their correct lake of origin specifically. Given that these Florida lakes are all a part of the same drainage system, these results were not surprising. Interestingly though, a morphological study of hatchling alligators from Lakes Woodruff, Orange and Apopka indicated significant differences in some characters among Lakes Apopka and Orange, and Apopka and Woodruff (Milnes et al., 2001). That study suggested, our data concur, that many morphological differences among alligators may be the result of epigenetic and environmental effects rather than direct genetic inheritance.

While the central Florida lakes in this study formed a tight genetic unit, many analyses of population structure grouped the Everglades and south Georgia samples with them, with the Everglades being more closely related to the central lakes populations than south Georgia. This finding was somewhat surprising given that the Everglades is located 375 km to the south of the southern-most lake (Apopka) and was sampled approximately 20 years earlier. However, these results are consistent with the findings of Maxwell et al. ('95), which placed peninsular Florida and south Georgia into an ''ecoregion'' or ''aquatic ecological unit'' separate from the ecoregions farther north and west. Futher, this ecoregion correlates with a wide hybrid zone that extends from the eastern Florida panhandle through south Georgia and northern Florida to the Georgia coast (Remington, '68). The close genetic relationship among the Everglades and the northern Florida lakes populations also suggests that all Florida alligator populations share common ancestry relatively recently and that they were able to sustain high enough numbers historically to maintain genetic diversity.

The Santee population, which is the least variable of all those sampled, was significantly different from the FL/sGA and TX/LA groups. Genetic diversity is related to population size, mutation rate, and migration rate. The northern location of the Santee population may be correlated with a low long-term effective population size, which would reduce diversity. The colder temperatures may also reduce mutation rates, though this seems unlikely. The alligator population of the Santee Coastal Reserve is also believed to be comparatively unimpacted in terms of human manipulation. Historical translocation and hunting of alligators at this site was minimal, particularly in comparison with that of the other sites in this study. Thus the low genetic diversity of the Santee population may reflect the true gene diversity of alligator populations in the absence of human assisted gene flow (see further discussion below).

Several analyses put Mobile in an intermediate position among the FL/sGA and TX/LA groups, though differences in genetic distances placed Mobile closer to the latter group. This result was consistent with the Davis et al. (2001a) study that examined Mobile and two Louisiana sites using five microsatellites. The closer genetic distance of Mobile to the ''western group'' was also consistent with a large-scale study of the distribution of 241 freshwater fish species. In these analyses, a fundamental break among eastern and western species was found at the Apalachicola river, which forms a partial boundary between Alabama and Georgia (Swift et al., '86). Ross and Roberts ('79) reported similar findings for scale morphology in American alligators. Variation in some scale characteristics was found in alligator populations among Atlantic coastal/Florida populations and western populations including Louisiana, Texas and Arkansas. In their study, no populations were sampled in the intermediate areas of Mississippi and Alabama and it had been hypothesized that alligator populations east and west of the Mississippi River were distinct (Neill, '71). Our present study suggests that population genetic structure of American alligators is marked by four main phylogeographic areas: 1) a peninsular Florida and south Georgia region (FL/sGA); 2) a western region (TX/LA); 3) an intermediate region (Mobile); and 4) a north Atlantic coast region (Santee). Additionally, genetic distance measurements and allele frequency distributions of the Mobile sample indicates that the change seems to be clinal, rather than abrupt, across the east-west boundary. More samples from this and other geographically intermediate areas are needed to better discern the population dynamics across this zone.

Overall, the patterns of genetic variation observed in this study were consistent with those of Davis et al. (2001a). This was in contrast to a complete lack of genetic subdivision determined by mtDNA analyses (Glenn et al., 2002). This study sought to increase the number of polymorphic microsatellite loci from Davis et al. (2001a) and include additional populations to examine genetic structure at a finer geographic scale. One difference between these two studies was in the overall among population variation. Here the AMOVA resulted in a value that was less than half (11.63%) of that in Davis et al. (2001a) (26.46%). This difference was due to the effect of increasing sample sizes and including loci that have similar patterns of variation among groups (Amiμ-202 and Amiμ-203; Fig. 2). It should also be noted that a genotyping error was found in locus Amiμ-15 since the publication of Davis et al. (2001a) and the allele frequencies in this study reflect the correction. Yet, while the addition of three loci in this study still failed to provide fine-scale resolution among adjacent populations, the patterns of genetic variation among populations that were common to both studies remain the same.

Gene flow

The next issue is to characterize the cause of this genetic variation. Human intervention and exploitation have undoubtedly left an impact on the genetic structure of alligator populations throughout their range. Hunting pressure depleted many local alligator populations from Alabama and Mississippi (Ross and Roberts, '79), whereas population numbers in other areas remained high (Joanen and McNease, '87). Thousands of alligators were transplanted in the 1960s and 1970s by state, federal and wildlife organizations to restock depleted areas (Ross and Roberts, '79; A. Woodward, unpublished data). In Florida, translocated animals usually came from the same drainage system (A. Woodward, unpublished data). Thousands of alligators from Louisiana were transplanted to Arkansas in the 1970s and early 1980s and many were also released in Mississippi in the late 1970s (R. Elsey, unpublished data). Small numbers of alligators were also translocated in South Carolina, mostly within or

between nearby river drainages, but a high proportion returned to or near the original capture sites (W. Rhodes, unpublished data). Spotlight and nesting counts in these areas in the years following the transplantations reported substantial increases in alligator numbers, indicating that the relocations were apparently successful. Therefore, it is possible that present-day lack of population structure within the eastern and western regions is due, in part, to artificial human translocation of animals within regions. However, historical biogeography and ecosystem characteristics combined with life-history traits might yield similar genetic patterns. The close genetic relationship among the Everglades and north-central lakes populations could be the result of a southern expansion of alligators from the north as the ocean gradually receded during the late Pleistocene. Additionally, the Florida lakes samples in this study are all a part of the St. John's River watershed and are connected to one another through aquatic systems. Similarly, alligators from the TX/LA group inhabit fairly homogenous and continuous coastal marshes. These types of aquatic ecosystems facilitate (or at least do not limit) the movement of alligators, which are amphibious by nature. Additionally, mark and re-capture studies have shown that alligators can return as far as 20 miles to their sites of capture (Chabreck, '65) or can move 30 miles away from their initial capture site (R. Elsey, unpublished data). Therefore, fairly extensive non-human assisted gene flow within these large regional areas is also possible.

Further support for naturally high gene flow in American alligators is that the phylogeographic structure reported here is remarkably similar to studies of freshwater fishes and aquatic and terrestrial turtles of the southeastern United States (Walker and Avise, '98). In those studies, mtDNA analyses revealed a strong phylogenetic separation of populations in peninsular Florida and the Atlantic coast from Gulf coast populations. Composite faunal lists for these taxa were also concordant with these genetic data indicating a basal split between eastern and western regions (Walker and Avise, '98). In several turtle species, populations in peninsular Florida tended to be strongly differentiated from more northern and western species as well (Walker et al., '97; Walker and Avise, '98). Therefore, the geologic forces that shaped the landscapes and ecosystems of the southeastern United Sates since the last glacial period may have left lasting and detectable fingerprints on the population structure of a number of faunal species.

IMPLICATIONS AND FUTURE DIRECTIONS

Eight polymorphic microsatellite loci failed to provide fine-scale resolution among neighboring populations within eastern and western groups. Therefore, these markers show limited potential for assisting in wildlife law enforcement issues, which are likely to require more precise discrimination within regions. Developing and screening many additional markers may allow more fine-scale resolution or simply increase the confidence that fine scales can not be resolved. The low degree of fine-scale genetic structure among localities reported here was similar to studies of Morelet's crocodiles (*Crocodylus moreletii*) in Belize (Dever, 2001; Dever et al., 2002). In contrast, genetic differences have been found among populations of saltwater and freshwater crocodiles (FitzSimmons et al., 2001) and American alligators (Ryberg et al., 2003) inhabiting different drainage systems. In Ryberg's study, coastal and inland populations characterized by different habitat types were compared. Results indicated more gene flow along coastal populations than between these and inland sites. Similar findings were reported by Davis et al. (2001a) suggesting limited gene flow between inland and coastal populations in South Carolina. Therefore, sampling of additional inland sites in the northern extremes of the species boundary will provide insight into differences in genetic structure imposed by habitat differences. Together, these studies will make available information for the development of management plans by providing a genetic framework of conservation units that can be managed in ''real-world'' situations. When similar studies are implemented in the many threatened and endangered crocodilian species, such studies can only assist in the conservation and management of these species.

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APPENDIX I. Information about the samples used in this study including place where samples were collected, number of samples analyzed from each population, year sample was collected and from whom the sample was obtained

Location	# Samples	Year Collected	Source of Samples
FLORIDA			
Lake Apopka	41	1999	Lou Guillette
Lake Griffin	34	1999	Lou Guillette
Orange Lake	14	1997	Paul Moler and Allan Woodward
	12	2001	Lou Guillette
Lake Woodruff	28	1997	Paul Moler and Allan Woodward
	31	1999	Lou Guillette
Everglades	24	1977	BobMenzies and J. Kushlan
LOUISIANA			
Rockefeller Refuge	34	1997	Ruth Elsey
Marsh Island	24	1997	Ruth Elsey
Salvador	27	1997	Ruth Elsey
TEXAS			
Anahuac	33	1997	Tighe Teets
ALABAMA			
Mobile Bay	8	1997	Mike Casper and Gary Casper
	15	1998	Mike Casper and Gary Casper
SOUTH CAROLINA			
Santee Coastal	19	1997	Walt Rhodes
Reserve	12	1999	Walt Rhodes
GEORGIA			
South Georgia	1	1995	Tom Ethridge
	2	1996	Tom Ethridge
	31	1997	Bill McLean and Tom Ethridge

*APPENDIX II. Allele sizes and frequencies for eight microsatellite loci for all American alligator populations. Allele sizes are given as total fragment sizes and number of repeat motifs. The sizes of the original cloned fragments are in bold. Allele sample size is given under 2N. *The size of the cloned fragment for Amiμ-8 was ambiguous*

LOCUS Amiμ-6

	Alleles												2N
frag. size	110	112	118	122	124	**126**	128	130	132	134	136	142	
#rpt motifs	12	13	16	18	19	20	21	22	23	24	25	29	
Everglades, FL	0.125	0.042	0.104	0.042	0.667	0.021	0.000	0.000	0.000	0.000	0.000	0.000	48
Apopka, FL	0.079	0.000	0.000	0.026	0.724	0.079	0.053	0.000	0.000	0.039	0.000	0.000	76
Griffin, FL	0.088	0.000	0.000	0.015	0.618	0.162	0.103	0.000	0.000	0.015	0.000	0.000	76
Woodruff, FL	0.144	0.000	0.008	0.042	0.508	0.246	0.008	0.017	0.025	0.000	0.000	0.000	118
Orange, FL	0.196	0.000	0.000	0.174	0.370	0.087	0.000	0.000	0.000	0.109	0.043	0.022	46
S. Georgia	0.000	0.000	0.016	0.188	0.547	0.047	0.016	0.000	0.000	0.141	0.047	0.000	64
Santee, SC	0.000	0.000	0.000	0.887	0.000	0.000	0.000	0.000	0.000	0.032	0.081	0.000	62
Mobile, AL	0.000	0.000	0.068	0.000	0.886	0.000	0.023	0.000	0.023	0.000	0.000	0.000	44
Salvador, LA	0.000	0.000	0.000	0.241	0.278	0.074	0.241	0.056	0.019	0.093	0.000	0.000	54
Marsh, LA	0.000	0.000	0.000	0.271	0.271	0.000	0.354	0.063	0.000	0.042	0.000	0.000	48
Rock., LA	0.000	0.000	0.000	0.250	0.221	0.015	0.382	0.074	0.029	0.029	0.000	0.000	68
Anahuac,TX	0.000	0.000	0.000	0.485	0.106	0.000	0.318	0.000	0.030	0.061	0.000	0.000	66

LOCUS Amiμ-8*

	Alleles																	2N
frag. size	124	126	128	130	132	134	136	138	140	142	144	146	148	150	152	154	156	
#rpt motifs, FL	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	
Everglades, FL	0.000	0.000	0.063	0.021	0.458	0.313	0.083	0.042	0.000	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.000	48
Apopka, FL	0.000	0.000	0.037	0.085	0.378	0.317	0.146	0.037	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	82
Griffin, FL	0.015	0.000	0.029	0.088	0.588	0.279	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	68
Woodruff, FL	0.000	0.000	0.000	0.153	0.500	0.254	0.068	0.017	0.000	0.008	0.000	0.000	0.000	0.000	0.000	0.000	0.000	118
Orange, FL	0.000	0.000	0.000	0.000	0.688	0.271	0.021	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	48
S. Georgia	0.000	0.016	0.000	0.081	0.565	0.242	0.000	0.065	0.000	0.000	0.000	0.016	0.000	0.000	0.016	0.000	0.000	62
Santee, SC	0.000	0.000	0.000	0.000	0.371	0.048	0.210	0.371	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	62
Mobile, AL	0.000	0.000	0.000	0.283	0.304	0.130	0.239	0.000	0.022	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.022	46
Salvador, LA	0.000	0.000	0.000	0.000	0.481	0.019	0.056	0.000	0.000	0.019	0.074	0.037	0.000	0.037	0.241	0.019	0.019	54
Marsh, LA	0.000	0.000	0.000	0.000	0.333	0.063	0.104	0.021	0.000	0.042	0.021	0.042	0.083	0.000	0.208	0.063	0.021	48
Rock., LA	0.000	0.000	0.000	0.000	0.338	0.015	0.441	0.000	0.000	0.015	0.000	0.029	0.059	0.000	0.088	0.015	0.000	68
Anahuac, TX	0.000	0.031	0.000	0.000	0.359	0.141	0.234	0.000	0.000	0.016	0.000	0.000	0.109	0.016	0.094	0.000	0.000	64

LOCUS Amiμ-15

	Alleles										2N
frag. size	147	149	151	153	155	157	**159**	161	163	165	
#rpt motifs	10	11	12	13	14	15	16	17	18	19	
Everglades, FL	0.000	0.104	0.458	0.063	0.000	0.000	0.083	0.000	0.125	0.167	48
Apopka,FL	0.000	0.171	0.037	0.037	0.000	0.000	0.256	0.463	0.037	0.000	82
Griffin, FL	0.000	0.206	0.044	0.059	0.000	0.000	0.559	0.118	0.015	0.000	68
Woodruff, FL	0.017	0.364	0.059	0.085	0.000	0.000	0.254	0.203	0.017	0.000	118
Orange, FL	0.000	0.375	0.000	0.125	0.000	0.000	0.271	0.229	0.000	0.000	48

S. Georgia	0.000	0.266	0.141	0.078	0.000	0.031	0.375	0.047	0.000	0.000	64	
Santee, SC	0.000	0.516	0.355	0.000	0.000	0.000	0.129	0.000	0.000	0.000	62	
Mobile, AL	0.000	0.261	0.065	0.000	0.000	0.000	0.609	0.022	0.043	0.000	46	
Salvador, LA	0.000	0.019	0.000	0.000	0.056	0.000	0.685	0.093	0.148	0.000	54	
Marsh, LA	0.000	0.000	0.042	0.000	0.000	0.000	0.542	0.250	0.167	0.000	48	
Rock., LA	0.000	0.000	0.162	0.000	0.015	0.000	0.647	0.147	0.029	0.000	68	
Anahuac, TX	0.000	0.015	0.076	0.000	0.000	0.000	0.742	0.152	0.015	0.000	66	

LOCUS Amiμ-18

	Alleles											2N
frag. size	164	172	178	180	182	184	**188**	190	192	194	196	
#rpt motifs	11	15	18	19	20	21	23	24	25	26	27	
Everglades, FL	0.042	0.000	0.021	0.000	0.000	0.000	0.542	0.167	0.188	0.021	0.021	48
Apopka,FL	0.050	0.000	0.038	0.000	0.000	0.000	0.463	0.063	0.287	0.100	0.000	80
Griffin, FL	0.147	0.000	0.015	0.000	0.000	0.000	0.471	0.088	0.221	0.059	0.000	68
Woodruff, FL	0.178	0.000	0.000	0.000	0.000	0.000	0.644	0.051	0.085	0.042	0.000	118
Orange, FL	0.063	0.000	0.000	0.000	0.000	0.000	0.500	0.188	0.167	0.042	0.042	48
S. Georgia	0.017	0.000	0.000	0.000	0.050	0.100	0.350	0.000	0.483	0.000	0.000	60
Santee, SC	0.117	0.050	0.000	0.000	0.000	0.000	0.167	0.000	0.533	0.083	0.050	60
Mobile, AL	0.261	0.109	0.000	0.022	0.000	0.000	0.283	0.000	0.217	0.109	0.000	46
Salvador, LA	0.463	0.241	0.000	0.000	0.019	0.000	0.074	0.000	0.204	0.000	0.000	54
Marsh, LA	0.375	0.083	0.000	0.104	0.042	0.000	0.167	0.000	0.208	0.021	0.000	48
Rock., LA	0.353	0.147	0.000	0.088	0.000	0.000	0.191	0.000	0.176	0.029	0.015	68
Anahuac, TX	0.242	0.182	0.000	0.091	0.000	0.000	0.106	0.000	0.273	0.076	0.030	66

LOCUS Amiμ-17d

	Alleles															
frag. size	221	225	229	233	**237**	241	243	245	247	249	253	255	257	259	261	263
#rpt motifs	42	44	46	48	50	52	53	54	55	56	58	59	60	61	62	63
Everglades, FL	0.000	0.042	0.000	0.000	0.000	0.000	0.000	0.146	0.000	0.396	0.188	0.000	0.063	0.000	0.125	0.021
Apopka, FL	0.049	0.000	0.000	0.012	0.049	0.098	0.000	0.049	0.000	0.280	0.207	0.012	0.098	0.000	0.012	0.000
Griffin, FL	0.000	0.000	0.088	0.000	0.029	0.000	0.000	0.162	0.015	0.309	0.176	0.000	0.059	0.000	0.074	0.000
Woodruff, FL	0.000	0.000	0.000	0.000	0.093	0.085	0.000	0.042	0.000	0.195	0.246	0.025	0.195	0.000	0.017	0.000
Orange, FL	0.000	0.000	0.023	0.091	0.023	0.045	0.000	0.023	0.000	0.409	0.205	0.000	0.045	0.000	0.000	0.000
S. Georgia	0.000	0.000	0.016	0.016	0.081	0.065	0.000	0.113	0.000	0.161	0.048	0.000	0.097	0.000	0.016	0.000
Santee, SC	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.790	0.016	0.000	0.113	0.081	0.000	0.000
Mobile, AL	0.000	0.000	0.000	0.071	0.238	0.119	0.000	0.071	0.000	0.000	0.000	0.000	0.000	0.000	0.095	0.000
Salvador, LA	0.000	0.037	0.000	0.019	0.019	0.056	0.000	0.019	0.000	0.111	0.074	0.000	0.037	0.019	0.000	0.000
Marsh, LA	0.000	0.000	0.000	0.068	0.023	0.068	0.000	0.023	0.000	0.136	0.091	0.000	0.000	0.000	0.000	0.000
Rock., LA	0.000	0.000	0.000	0.044	0.059	0.044	0.000	0.059	0.000	0.015	0.000	0.000	0.059	0.000	0.029	0.029
Anahuac, TX	0.000	0.016	0.016	0.281	0.031	0.016	0.016	0.000	0.000	0.031	0.063	0.000	0.047	0.000	0.016	0.000

APPENDIX II (Continued)

LOCUS Amiμ-17d (continued)

	Alleles										2N
frag. size	265	269	271	273	275	277	281	285	289	293	
#rpt motifs	64	66	67	68	69	70	72	74	76	78	
Everglades, FL	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	48
Apopka,FL	0.073	0.061	0.000	0.000	0.000	0.000	0.0000	0.000	0.000	0.000	82
Griffin, FL	0.029	0.015	0.000	0.044	0.000	0.000	0.000	0.000	0.000	0.000	68
Woodruff, FL	0.059	0.025	0.000	0.008	0.000	0.008	0.000	0.000	0.000	0.000	118
Orange, FL	0.091	0.045	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	44
S. Georgia	0.226	0.016	0.000	0.032	0.000	0.097	0.016	0.000	0.000	0.000	62
Santee, SC	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	62
Mobile, AL	0.143	0.214	0.000	0.000	0.000	0.048	0.000	0.000	0.000	0.000	42
Salvador, LA	0.056	0.130	0.037	0.167	0.019	0.056	0.111	0.037	0.000	0.000	54
Marsh, LA	0.045	0.091	0.068	0.159	0.000	0.114	0.068	0.023	0.000	0.023	44
Rock., LA	0.015	0.250	0.029	0.176	0.000	0.118	0.029	0.029	0.015	0.000	68
Anahuac, TX	0.063	0.172	0.000	0.078	0.000	0.109	0.031	0.016	0.000	0.000	64

LOCUS Amiμ-20

	Alleles													2N
frag. size	138	140	142	144	146	150	152	156	158	160	**162**	164	166	
#rpt motifs	13	14	15	16	17	19	20	22	23	24	25	26	27	
Everglades, FL	0.146	0.000	0.104	0.146	0.000	0.000	0.000	0.000	0.021	0.396	0.104	0.021	0.063	48
Apopka, FL	0.175	0.000	0.000	0.425	0.038	0.025	0.000	0.000	0.000	0.262	0.075	0.000	0.000	80
Griffin, FL	0.227	0.015	0.000	0.242	0.030	0.000	0.000	0.000	0.000	0.424	0.061	0.000	0.000	66
Woodruff, FL	0.043	0.000	0.000	0.190	0.095	0.000	0.000	0.009	0.034	0.534	0.086	0.009	0.000	116
Orange, FL	0.125	0.063	0.000	0.167	0.063	0.125	0.000	0.000	0.042	0.333	0.063	0.021	0.000	48
S. Georgia	0.250	0.000	0.016	0.172	0.141	0.000	0.000	0.000	0.047	0.359	0.016	0.000	0.000	64
Santee, SC	0.000	0.371	0.000	0.226	0.000	0.000	0.000	0.000	0.000	0.371	0.000	0.032	0.000	62
Mobile, AL	0.130	0.000	0.043	0.717	0.043	0.000	0.000	0.000	0.043	0.000	0.022	0.000	0.000	46
Salvador, LA	0.352	0.056	0.278	0.148	0.000	0.000	0.019	0.000	0.000	0.093	0.056	0.000	0.000	54
Marsh, LA	0.432	0.114	0.227	0.136	0.000	0.000	0.023	0.000	0.000	0.023	0.045	0.000	0.000	44
Rock., LA	0.273	0.121	0.333	0.152	0.000	0.000	0.000	0.000	0.000	0.045	0.076	0.000	0.000	66
Anahuac, TX	0.076	0.318	0.318	0.258	0.000	0.000	0.000	0.000	0.000	0.015	0.000	0.015	0.000	66

LOCUS Amiμ-202

	Alleles																
frag. size	127	131	135	139	143	147	151	155	159	163	167	171	175	179	183	187	191
#rpt motifs	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Everglades, FL	0.042	0.000	0.000	0.000	0.021	0.063	0.063	0.167	0.104	0.021	0.000	0.021	0.042	0.042	0.063	0.063	0.021
Apopka, FL	0.000	0.000	0.000	0.000	0.051	0.218	0.064	0.038	0.000	0.026	0.038	0.038	0.090	0.000	0.179	0.026	0.013
Griffin, FL	0.000	0.000	0.000	0.016	0.016	0.226	0.226	0.032	0.000	0.048	0.016	0.129	0.048	0.081	0.016	0.016	0.000
Woodruff, Fl	0.000	0.009	0.018	0.000	0.018	0.045	0.116	0.205	0.152	0.036	0.009	0.018	0.000	0.009	0.045	0.107	0.054

Orange, FL	0.000	0.000	0.000	0.000	0.159	0.182	0.091	0.068	0.000	0.023	0.023	0.000	0.045	0.045	0.000	0.045	0.045
S. Georgia	0.000	0.000	0.000	0.000	0.000	0.031	0.031	0.031	0.000	0.031	0.078	0.234	0.016	0.047	0.063	0.000	0.094
Santee, SC	0.000	0.037	0.000	0.000	0.000	0.500	0.093	0.000	0.000	0.000	0.000	0.000	0.000	0.130	0.056	0.000	0.185
Mobile, AL	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.023	0.068	0.068	0.068	0.023	0.159	0.091	0.045	0.159	0.114
Salvador, LA	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.019	0.000	0.019	0.019	0.019	0.096	0.154	0.115
Marsh, LA	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.021	0.000	0.000	0.083	0.083	0.104	0.167	0.250
Rock., LA	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.029	0.000	0.044	0.000	0.000	0.162	0.118	0.221	0.118	0.029
Anahuac, TX	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.119	0.033	0.000	0.050	0.050	0.167	0.200	0.167

LOCUS Amiμ-202 (continued)

	Alles														
frag. size	195	199	**203**	207	211	215	219	233	227	229	231	235	239	243	247
#rpt motifs	23	24	25	26	27	28	29	30	31	31.5	32	33	34	35	36
Everglades, FL	0.063	0.104	0.042	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.021
Apopka, FL	0.090	0.026	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.000	0.000	0.026	0.013	0.000	0.000
Griffin, FL	0.000	0.016	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.016	0.016	0.032
Woodruff, FL	0.018	0.000	0.009	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Orange, FL	0.000	0.000	0.000	0.091	0.023	0.000	0.000	0.045	0.000	0.000	0.000	0.045	0.000	0.000	0.000
S. Georgia	0.063	0.047	0.000	0.000	0.016	0.016	0.047	0.109	0.016	0.000	0.016	0.000	0.000	0.000	0.000
Santee, SC	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Mobile, AL	0.045	0.000	0.000	0.045	0.000	0.000	0.045	0.023	0.000	0.023	0.000	0.000	0.000	0.000	0.000
Salvador, LA	0.173	0.058	0.115	0.096	0.058	0.019	0.019	0.019	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Marsh, LA	0.083	0.021	0.083	0.083	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Rock., LA	0.044	0.059	0.059	0.029	0.029	0.000	0.000	0.000	0.000	0.000	0.015	0.000	0.015	0.000	0.029
Anahuac, TX	0.017	00.083	0.000	0.000	0.017	0.033	0.033	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000

LOCUS Amiμ-202 (continued)

	Alleles						2N
frag. Size	251	255	259	263	267	271	
#rpt motifs	37	38	39	40	41	42	
Everglades, FL	0.000	0.000	0.000	0.021	0.021	0.000	48
Apopka, FL	0.000	0.000	0.026	0.000	0.000	0.026	78
Griffin, FL	0.016	0.016	0.000	0.000	0.000	0.000	62
Woodruff, FL	0.036	0.018	0.054	0.027	0.000	0.000	112
Orange, FL	0.000	0.000	0.000	0.023	0.045	0.000	44
S. Georgia	0.000	0.000	0.016	0.000	0.000	0.000	64
Santee, SC	0.000	0.000	0.000	0.000	0.000	0.000	54
Mobile, AL	0.000	0.000	0.000	0.000	0.000	0.000	44
Salvador, LA	0.000	0.000	0.000	0.000	0.000	0.000	52
Marsh, LA	0.000	0.000	0.000	0.000	0.000	0.000	48
Rock., LA	0.000	0.000	0.000	0.000	0.000	0.000	68
Anahuac, TX	0.000	0.000	0.000	0.000	0.000	0.000	60

APPENDIX II (Continued)

LOCUS Amiμ-203

	Alleles											2N
frag. size	147	151	155	**159**	163	167	171	175	179	183	187	
# rpt motifs	10	11	12	13	14	15	16	17	18	19	20	
Everglades, FL	0.000	0.043	0.261	0.130	0.130	0.261	0.109	0.000	0.065	0.000	0.000	46
Apopka, FL	0.000	0.000	0.179	0.051	0.346	0.346	0.026	0.038	0.013	0.000	0.000	78
Griffin, FL	0.000	0.015	0.152	0.091	0.273	0.318	0.091	0.061	0.000	0.000	0.000	66
Woodruff, FL	0.000	0.000	0.069	0.060	0.552	0.164	0.112	0.034	0.009	0.000	0.000	116
Orange, FL	0.000	0.065	0.065	0.152	0.370	0.130	0.022	0.152	0.022	0.000	0.022	46
S. Georgia	0.016	0.125	0.313	0.125	0.250	0.047	0.063	0.016	0.000	0.047	0.000	64
Santee, SC	0.000	0.000	0.387	0.194	0.419	0.000	0.000	0.000	0.000	0.000	0.000	62
Mobile, AL	0.000	0.429	0.357	0.024	0.071	0.000	0.048	0.000	0.000	0.071	0.000	42
Salvador, LA	0.019	0.288	0.096	0.327	0.038	0.135	0.019	0.038	0.019	0.000	0.019	52
Marsh, LA	0.000	0.271	0.125	0.333	0.063	0.167	0.042	0.000	0.000	0.000	0.000	48
Rock., LA	0.000	0.203	0.078	0.391	0.031	0.125	0.125	0.031	0.000	0.000	0.016	64
Anahuac, TX	0.000	0.359	0.250	0.188	0.063	0.047	0.094	0.000	0.000	0.000	0.000	64