

Genetic Relationships of American Alligator Populations Distributed Across Different Ecological and Geographic Scales

WADE A. RYBERG,^{1*} LEE A. FITZGERALD,¹ RODNEY L. HONEYCUTT,¹
AND JAMES C. CATHEY²

¹The Department of Wildlife and Fisheries Sciences, Texas A&M University,
College Station, Texas 77843-2258

²Texas Parks and Wildlife Department, Gus Engeling Wildlife Management
Area, Tennessee Colony, Texas 75861

ABSTRACT Although much work has been conducted on coastal populations of the American alligator (*Alligator mississippiensis*), less is known about the population dynamics and genetic structure of populations of alligators confined to inland habitats. DNA microsatellite loci, derived from the American alligator, were used to investigate patterns of genetic variation within and between populations of alligators distributed at coastal and inland localities in Texas. These data were used to evaluate the genetic discreteness of different alligator stocks relative to their basic ecology at these sites. Observed mean heterozygosities across seven loci for both coastal and inland populations ranged from 0.50–0.61, with both inland and coastal populations revealing similar patterns of variation. Measures of F_{st} revealed significant population differentiation among all populations; however, analyses of molecular variance (AMOVAs) failed to demonstrate any apparent geographic pattern relative to the population differentiation indicated by F_{st} values. Each population contained unique alleles for at least one locus. Additionally, assignment tests based on the distribution of genotypes placed 76% of individuals to their source population. These genetic data suggest considerable subdivision among alligator populations, possibly influenced by demographic and life history differences as well as barriers to dispersal. These results have clear implications for management. Rather than managing alligators in Texas as a single panmictic population, translocation programs and harvest quotas should consider the ecological and genetic distinctiveness of local alligator populations. *J. Exp. Zool. (Mol. Dev. Evol.)* 294:325–333, 2002. © 2002, Wiley-Liss, Inc.

Although a steady decline in populations of the American alligator was noted by naturalists as early as the 1900s, the species did not receive national attention until the late 1960s after huge population declines in response to years of excessive hunting pressure. Coupled with extreme hunting pressure, systematic exploration of oil and gas resources in the southeastern United States created a network of waterways that left remote alligator populations vulnerable to exploitation by man (McIlhenny, '35; Joanen and McNease, '87). The state of Louisiana was the first to pass legislation protecting the American alligator by establishing controls on seasonal harvests, and subsequent to this legislation, federal protection was added (Joanen and McNease, '87). Today, the American alligator is the most well studied crocodylian, and its successful recovery and management are the result of basic research on alligator populations. Most research programs on

the American alligator have focused on coastal populations (Thompson and Gidden, '72; Gartside et al., '77; Adams et al., '80; Taylor and Neal, '84; Joanen and McNease, '87; Taylor et al., '91; Glenn et al., '98). Considerably less information is available for alligators restricted to inland habitats. Coastal and inland habitats used by alligators are extremely different. Coastal habitats are more homogeneous, characterized by swamps, slow moving water, and marshy areas that tend to support relatively dense populations of alligators (Ross, '89). In contrast, inland habitats are more heterogeneous, composed of streams and creeks interspersed with ponds and lakes where water

*Correspondence to: Wade Ryberg, The Department of Wildlife and Fisheries Sciences, Texas A&M University, 2258 TAMU, 210 Nagle Hall, College Station, Texas 77843-2258.

E-mail: wryberg@neo.tamu.edu

Received 24 January 2002; Accepted 13 September 2001

Published online in Wiley InterScience (www.interscience.wiley.com). DOI:10.1002/jez.10207

levels fluctuate regularly and crocodylian populations become fragmented (Ouboter and Nanhoe, '88). Based on these differences between coastal and inland habitats, one would expect alligators residing in these two areas to display different life history strategies and population structures. Prior to the establishment of sustained harvest strategies for alligators, these differences need to be examined in detail.

Recent ecological and population studies of genetic variation in species of special concern have employed DNA microsatellite loci for evaluating patterns of genetic variation within and between populations (Dever et al., personal communication; Friar et al., 2001; Garnier et al., 2001; Lee et al., 2001; Sundqvist et al., 2001). Most conservation plans for these species have interpreted estimates of genetic diversity as a means of ensuring the maintenance of adaptive diversity and evolutionary potential in populations. Recently, 11 DNA microsatellite loci were characterized for the American alligator and used to examine genetic variation within and between single populations of alligators in Louisiana and Florida (Glenn et al., '98). These genetic markers for alligators are of interest to ecologists and population biologists because they provide levels of variation higher than that previously detected with allozymes (Gartside et al., '77; Menzies et al., '79; Adams et al., '80). A subset of these markers was used to examine geographic variation in alligators from Louisiana, Alabama, and South Carolina (Davis et al., 2000). This study (Davis et al., 2000) revealed geographic patterns of genetic variation and population differentiation similar to those found by Glenn et al. ('98), with coastal populations displaying extensive gene flow. Comparisons between coastal and inland populations, however, displayed more restricted gene flow patterns. Davis et al. (2000) stated that the inland population of alligators was isolated from large aquatic habitats where gene flow is high, thus reducing the number of migrants from other populations. If this statement is true, then alligator populations in coastal and inland habitats should show genetic differentiation from each other and perhaps different ecologies. Nevertheless, given the American alligator's amphibious lifestyle and the fact that many coastal and inland alligator populations are interconnected by river drainages, genetic differences may be restricted to populations associated with different river drainages. An evaluation of genetic variation between and among coastal and inland alligator popula-

tions is needed at a finer scale to gain a better understanding of population differences. This kind of information can be used to improve conservation and management strategies for American alligators throughout their range.

We used seven DNA microsatellite markers to examine genetic relationships among American alligator populations from both coastal and inland localities in Texas. These populations demonstrate varying scales of geographic and ecological separation along the Texas Gulf Coast and among several river drainages. The coastal alligator populations studied occupy expansive swamps and marshes and maintain large population sizes, while the inland alligator populations occupy smaller, fragmented, aquatic ecosystems and maintain smaller population sizes. This network of populations overlaying different habitat types and river drainages allows the following predictions to be examined: (1) populations of alligators in distinct coastal and inland habitats should exhibit different patterns of genetic structure depending upon the degree of isolation and population size; (2) distinct river drainages harbor genetically distinct populations of alligators.

Materials and Methods

Genetic samples and laboratory methods

A total of 191 individuals from the following six populations were sampled (Fig. 1): Guadalupe Delta Wildlife Management Area (GD, $n=17$); Mad Island Wildlife Management Area (MI, $n=10$); J. D. Murphree Wildlife Management Area (JD, $n=67$); Dam B Wildlife Management Area (DB, $n=29$); Gus Engeling Wildlife Management Area (GE, $n=44$); and Coon Creek Club (CC, $n=24$). The GD, MI, and JD alligator populations form a coastal grouping, while the DB, GE, and CC populations form an inland grouping. In addition, the DB and JD, GE and CC, MI, and GD populations are located on the Neches, Trinity, Colorado, and Guadalupe Rivers, respectively (Fig. 1), forming distinct population groupings within separate river drainages.

DNA was isolated from tissue biopsies collected from live (captured and released) and harvested alligators between 1999 and 2001. These tissues were derived from dorsal scutes along the tail, which were notched as a means of identifying individuals. Tissues were digested over-night with Proteinase K (20mg/ml) at 55°C in an incubated shaker, and DNA was extracted using the spin columns, buffers, and protocol provided in DNeasy

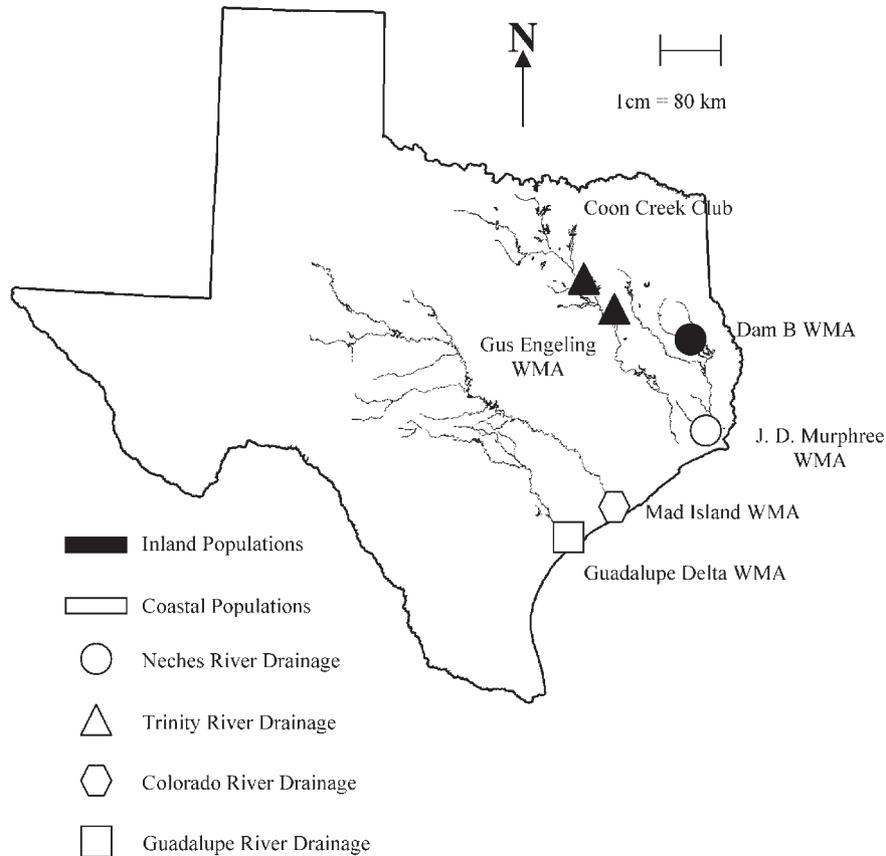


Fig. 1. Map of Texas showing the locations of six alligator populations where genetic samples were obtained.

Tissue Kits (Qiagen, Valencia, CA). DNA concentration and purity were estimated by comparison to size standards on agarose gels stained with ethidium bromide. Only samples from presumably unrelated adults were used in estimates of population genetic statistics.

Seven pairs of oligonucleotide primers were used to amplify six microsatellite loci with a pure dinucleotide repeat of $(AC)_n$ and one locus with an interrupted $(AC)_n$ repeat. All primer pairs were described previously by Glenn et al. ('98) but were fluorescently labeled for automated genotyping (Table 1). Amplifications employing the polymerase chain reaction (PCR) were performed in an Omn-E thermocycler (Promega, Madison, WI). Reactions were performed in 25 μ L volumes with a final concentration of 100 μ g/mL BSA, 150 μ M of each dNTP, 2.5 units of non-commercial *Taq*, 2.5 μ L of 10x reaction buffer (Promega, Madison, WI), 0.6-3 :M each primer (Table 1), and 100ng of DNA. All reactions were overlaid with oil and included the following PCR parameters: denaturation 94°C for 2 min, followed by 35 cycles of 94°C for 1 min, annealing temperature for 1 min, and

72°C for 1 min. A 72°C extension for 20 min was added as a final step. DNA concentrations and PCR products were determined in a 0.8% agarose gel stained with ethidium bromide. PCR products were combined in a cocktail according to fragment size overlap, the fluorescent label used for specific primer pairs, and ability to multiplex loci in a single reaction (Table 1). Genotypes were determined with an ABI 310 automated DNA sequencer (Applied Biosystems, Foster City, CA) and the Genescan software. Genotypes were scored twice by different investigators.

Statistical methods

Standard statistics of genetic variability, including observed heterozygosity (H_O), expected heterozygosity (H_E), allele frequencies, and number of alleles per locus, were estimated using GENEPOP v. 3.1 (Raymond and Rousset, '95). Exact tests for conformity to Hardy-Weinberg equilibrium (HWE) at each locus and across loci and tests for linkage disequilibrium were performed

TABLE 1. Characteristics of PCR primers used to amplify microsatellite loci from American alligators in Texas

Locus	Primer Sequences (5' to 3')	Repeat ¹	T ²	L ³	C ⁴	PCR ⁵
3a	ACTGTTGCTTTTCCTAATCTGTTT	(AC) ₁₆	49	HEX	A	2.4
3b	CATAAAATTGTGCTAATGTCCC					2.4
6a	TTCTTTCCCAGATACACACTT	(AC) ₂₀	54	FAM	B	3.0
6b	AGTAGAAGGGGACAGGTTATT					3.0
15a	CACGTACAAATCCATGCTTTC	(AC) ₁₆	54	TET	B	3.0
15b	GGGAGGGTTCAGTAAGAGACA					3.0
16a	TTTGGGCTGTGAAACAAGTATT	(AC) ₂₇	54	TET	B	3.0
16b	TCCCCTGATAGTCTTCTATAAAC					3.0
17a	GCTGACCTTGGTTGGAAACTCTA	(AC) ₂₂ (AT) ₇ (ATGT)	49	FAM	A	2.4
17b	CCTGTTCTTGATAAANCTGATA	¹² (AGAT) ₉				2.4
18a	ATCTCCGAGGGGAAAAATACA	(AC) ₂₃	55	TET	A	0.6
18b	AATAGATGGAGTGATGTTATAGTCAG					0.6
20a	TTTTTCTTCTTCTCCATTCTA	(AC) ₂₅	55	HEX	A	3.0
20b	GATCCAGGAAGCTTAAATACAT					3.0

¹repeat motif.

²optimal annealing temperature (°C) for PCR.

³ABI fluorescent label used for each primer pair.

⁴Cocktail label.

⁵final concentration (µM) of primer used in PCR amplification.

using the Markov Chain Method described by Guo and Thompson ('92).

Tests for genotypic and genic differentiation among populations were performed following Goudet et al. ('96) and Raymond and Rousset ('95), respectively. Weir and Cockeram's θ ('84), an estimator of Wright's ('31) F_{st} , was calculated using Genepop v. 3.1 (Raymond and Rousset, '95) and FSTAT 2.7 (Goudet, '95) to assess population differentiation. Levels of gene flow were computed from θ following calculations from Hartl and Clark ('97).

Three analyses of molecular variance were performed using AMOVA (Excoffier et al., '92) to identify natural groupings of the six populations that account for the most variation. The first AMOVA was performed with no subdivision of the six populations. The second subdivided the six populations by habitat type: coastal (GD, MI, and JD) and inland (DB, GE, and CC). The third subdivided the six populations according to river drainages. The eastern most subgroup was the Neches River drainage (JD and DB) followed by the Trinity River drainage (GE and CC), and the western most subgroup included the GD and MI populations.

Genetic distances between populations and between pairs of individuals using the proportion of shared alleles summed over all loci were calculated according to Bowcock et al. ('94). Genetic distances between populations were plotted against geographic distances to test for an isolation by distance effect using a Mantel test.

We also applied two techniques that differ conceptually from the standard population genetic statistics. First, using the genetic distances between populations and between pairs of individuals, we determined relationships of either localities or individuals using Neighbor-Joining (NJ) trees in PAUP version 4.0d63 (Swofford, '98). Second, we used an assignment test (Paetkau et al., '95) to obtain observed allele distributions among predefined populations and assign each individual to the population for which its expected genotype frequency was highest.

RESULTS

Microsatellite diversity

Based on tests of heterozygote deficiency, only the MI population showed no significant deviation from HWE ($P > 0.05$) at all loci (Table 2). The remaining five populations contained at least two loci significantly out of equilibrium ($P < 0.05$), but no population showed significant deviation from HWE at all loci (Table 2). Locus 16 showed significant deviation from HWE in all populations except MI, which has the smallest sample size. This deviation was probably due to the presence of null alleles in those populations. Of the 126 pair-wise comparisons made, 10 showed significant ($P < 0.002$) linkage disequilibrium. No loci were consistently in linkage disequilibrium across all populations.

Levels of genetic polymorphism were similar for all six populations (Table 2). Observed hetero-

TABLE 2. Summary statistics for all microsatellite loci across all alligator populations in Texas

Locus ¹	GD				MI				JD				DB				GE				CC				X ⁶
	N ²	A ³	Ho ⁴	He ⁵	N ²	A ³	Ho ⁴	He ⁵	N ²	A ³	Ho ⁴	He ⁵	N ²	A ³	Ho ⁴	He ⁵	N ²	A ³	Ho ⁴	He ⁵	N ²	A ³	Ho ⁴	He ⁵	
3	17	3	0.41	0.56	10	4	0.50	0.50	67	3	0.49	0.51	29	3	0.52	0.63	44	3	0.52	0.55	24	4	0.50	0.49	3.3
6	17	2	0.65	0.52	10	3	0.60	0.53	66	9	0.74*	0.81	29	4	0.69	0.61	44	5	0.23*	0.61	24	6	0.33*	0.67	4.8
15	17	2	0.06	0.06	10	4	0.40	0.36	67	7	0.52*	0.65	29	3	0.41	0.54	44	5	0.52*	0.77	24	7	0.50*	0.76	4.7
16	17	4	0.54*	0.69	10	2	0.40	0.51	67	9	0.43*	0.70	29	4	0.34*	0.53	44	7	0.43*	0.70	24	8	0.61*	0.68	5.7
17	17	10	0.88*	0.88	10	7	0.80	0.86	67	28	0.70*	0.93	29	13	0.83*	0.91	44	12	0.75*	0.74	24	8	0.63	0.75	13
18	17	5	0.47*	0.68	10	3	0.70	0.65	67	13	0.73*	0.88	29	5	0.59	0.60	44	7	0.59*	0.63	24	6	0.67*	0.62	6.5
20	17	4	0.47*	0.60	10	6	0.80	0.73	66	7	0.68	0.76	29	5	0.66	0.62	44	4	0.70	0.64	24	6	0.77	0.73	5.3
Mean	17	4	0.50	0.57	10	4	0.60	0.59	67	11	0.61	0.75	29	5	0.58	0.63	44	6	0.53	0.66	24	6	0.57	0.67	6.2

Locus¹ designation of loci following Glenn et al. ('98).

N² number of individuals.

A³ number of alleles.

Ho⁴ observed heterozygosity.

He⁵ expected heterozygosity.

X⁶ average number of alleles/locus across all populations.

*Denotes population loci showing deviations from HWE ($P < 0.05$).

zygosity (H_O) ranged from 0.50 in the GD population to 0.61 in the JD population with an average of 0.57 across all populations. The mean number of alleles per locus for each population was similar for five of the six populations, ranging from four to eleven with an average of six across all populations. The JD population contained nearly twice the number of alleles found in the rest of the populations. The difference in allele numbers between JD and the other populations was probably attributable to differences in sample size.

Allele frequencies and distributions (Fig. 2) revealed several trends. Across all seven loci, 33 private alleles were observed. Every population studied had at least one private allele, but only three of the populations lacked an allele that was shared in the remaining five populations. The JD population had the largest number of private alleles (19). The GD, MI, and DB populations each contained one private allele, and only the GD and MI populations lacked an allele that was shared in the remaining five populations. The GE and CC populations had three and eight private alleles, respectively, and neither lacked an allele that was shared in the remaining five populations. All private alleles occurred in low frequency, except for allele E of locus 6 (11.4%) and allele I of locus 16 (14.9%) in the JD population.

Population genetic structure

Overall patterns of population differentiation were examined using NJ analysis and proportion

of shared alleles among populations (Fig. 3). The resultant tree had long terminal branches, suggesting well differentiated populations. Tests of genic and genotypic differentiation supported these findings in that 182 of the 210 pairwise comparisons between populations were significantly differentiated ($P < 0.05$). All pairwise values for θ (Table 3) were significant ($P < 0.003$) even after Bonferroni correction, and all demonstrated significant population differentiation.

The three hypotheses of higher level structuring examined with AMOVA showed a slightly different pattern of population differentiation. Both the coastal versus inland subdivision and the river drainage subdivision showed no significant variance among groups ($P=0.90$ and $P=0.27$, respectively). However, the first AMOVA, performed with no subdivision of the six populations, partitioned the total variation into 10.24% among populations and 89.76% within populations. This analysis implies that these populations maintain a significantly high ($P < 0.05$) level of allelic variation, most of which is shared across populations (Fig. 2).

Correlation between genetic and geographic distances was significant using a Mantel test ($r=0.60$, $P=0.02$), indicating the populations follow an isolation by distance model. In another NJ tree based on the proportion of shared alleles among individuals, alligators generally clustered according to their source population, suggesting genetic subdivision (tree not shown). Seventy-six percent of all alligators were correctly assigned to their source population (Table 4), based upon the

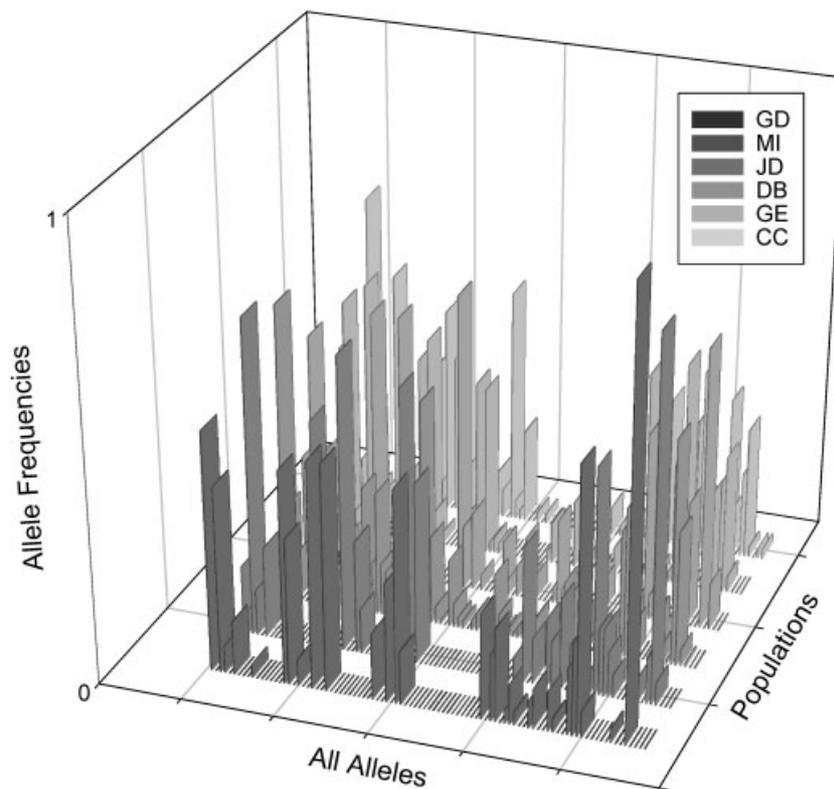


Fig. 2. Graph of allele frequencies at seven polymorphic loci in six Texas populations of the American alligator. All populations exhibit similar allelic distributions, but they differ in allele frequencies across the distributions.

assignment test of Paetkau et al. ('95). Five percent were assigned to geographically neighboring populations, possibly indicating the presence of recent immigrants. The assignment test generated perfect assignments for the MI and GD populations, possibly reflecting the isolation of these two populations in the extreme western portion of the species' range. The perfect assignments could also be an artifact of small sample size in these populations.

Pairwise θ values were used to estimate the number of individuals that migrate between each pair of sampling localities per generation (Nm ; Table 3). Estimates of Nm reflected the cross-assignments generated from the assignment test and suggested limited gene flow between most populations. Patterns of miss assignment could not be explained on the basis of sex or size of individuals.

DISCUSSION

The panel of DNA microsatellite markers utilized in this study revealed moderate levels of polymorphism in populations of American alliga-

tors from the southeastern United States (Glenn et al., '98; Davis et al., 2000) and in populations from east Texas. In comparison to Davis et al.'s. (2000) geographic study, the six populations from localities in the southeastern United States exhibited more alleles at five of the seven loci than observed in the six Texas populations. Two loci used in our study were not included by Davis et al. (2000), but they were included in the analysis done by Glenn et al. ('98). The same seven loci studied by Glenn et al. ('98) revealed numbers of alleles similar to those seen in the six Texas populations. The only exception was locus 17, which exhibited four to five more alleles in the Texas populations than observed in the six southeastern populations. Levels of heterozygosity across populations reported from Davis et al. (2000) were slightly higher (0.52–0.76) than those found for Texas populations (0.50–0.61). Texas populations, however, showed slightly higher levels of heterozygosity than those reported from Glenn et al. ('98). At a finer geographic scale, the Texas populations demonstrated levels of polymorphism similar to those observed in alligator populations from other parts of the species' range.

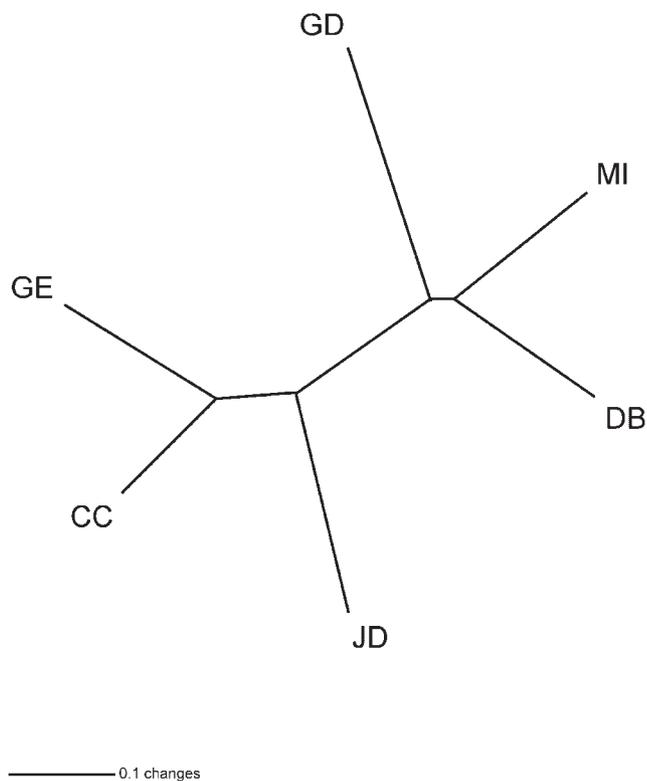


Fig. 3. Unrooted Neighbor-joining tree of six Texas populations of the American alligator based on the proportion of shared alleles. Long terminal branches indicate apparent population differentiation.

TABLE 3. Pairwise values for θ below diagonal and number of migrants per generation between populations (Nm) above diagonal for six alligator populations in Texas

	GD	MI	JD	DB	GE	CC
GD	–	1.74	1.07	1.80	0.73	0.86
MI	0.126*	–	1.46	5.48	0.98	1.33
JD	0.190*	0.146*	–	1.42	2.25	2.58
DB	0.122*	0.044*	0.150*	–	1.04	1.33
GE	0.254*	0.203*	0.100*	0.194*	–	4.05
CC	0.225*	0.159*	0.088*	0.159*	0.058*	–

*Significant following adjustment for multiple tests with the sequential Bonferroni method ($P < 0.0033$).

In addition to similarities in levels of polymorphism in Texas and southeastern populations of alligators, there were apparent similarities in population structure. Like Glenn et al. ('98) and Davis et al. (2000), our estimates of F_{st} were statistically greater than zero for each comparison, suggesting population subdivision. An evaluation of genetic and genotypic differentiation between populations proved significant, supporting population subdivision. Finally, the phyloge-

TABLE 4. Assignment of individual alligators to Texas populations and percentage of correct classifications

Source pop. (sample size)	Assigned to population:						Correct (%)
	GD	MI	JD	DB	GE	CC	
GD (17)	17	0	0	0	0	0	100
MI (10)	0	10	0	0	0	0	100
JD (67)	3	11	45	2	2	4	67
DB (29)	1	6	0	21	0	1	72
GE (44)	0	1	5	0	34	4	77
CC (24)	0	2	0	0	3	19	79

netic analysis grouped individuals into clades coinciding with the source populations, and the assignment tests provided similar results. Both of these analyses support population subdivision. The three hypotheses of higher-level structuring, tested using AMOVAs, provided no evidence of any underlying ecological or geographic component of population subdivision within Texas populations. Both the coastal versus inland and river drainage hypotheses failed to explain the variation observed among groups. Similar studies using genetic markers to estimate population subdivision in river otters (*Lutra canadensis*) and Morelet's crocodiles (*Crocodylus moreletii*) also failed to reveal population subdivision with respect to river drainages (Serfass et al., '98; Dever et al., personal communication). Like alligators, these species have amphibious lifestyles and the ability to disperse long distances. It has been shown that the overall similarity among river otter and Morelet's crocodile populations occupying different river drainages, and the minor differences among regional populations, are probably the result of immigration between regions. In our study, nearly all variation existed within rather than among populations, implying high levels of allelic variation shared among populations. In contrast, Davis et al. (2000) found that a significantly large portion of genetic variation existed among eastern populations as well as within them. This discrepancy makes sense in light of the fact that populations studied by Davis et al. (2000) were geographically more distant from each other than the Texas populations and probably had lower levels of gene flow, thus making them more susceptible to genetic drift.

Calculations of the effective number of migrants (Nm), based on estimates of F_{st} , indicated low levels of gene flow between most Texas populations. Significant correlation between genetic and

geographic distances suggested populations differentiated in situ following an isolation by distance model. This correlation supports indications of low gene flow between most Texas populations. An analysis of individual genotypes, based on the proportion of shared alleles and the assignment test, estimated low interpopulation dispersal rates and identified few potential migrants. This observation is congruent with the low number of migrants based on F_{st} and the in situ differentiation of the isolation by distance model.

Although Davis et al. (2000) showed considerable gene flow among coastal alligator populations and limited gene flow between one inland and most coastal alligator populations at a large scale, and Dever et al. (personal communication) found considerable gene flow in Morelet's crocodile populations at a fine scale, our results indicate limited levels of gene flow between and among both coastal and inland populations of alligators in Texas. The difference between our study and those of Davis et al. (2000) and Dever et al. (personal communication) may be the result of scale and interpretation. Interpretations of gene flow probably differ according to the geographic distance between populations in question. We purposely selected alligator populations within the same river drainages and ecological habitat types in order to test hypotheses of population structure and gene flow at a finer geographic scale. The alligator populations studied by Davis et al. (2000) were dispersed hundreds of kilometers apart over a broader geographic scale. The Morelet's crocodile populations studied by Dever et al. were dispersed less than 40 kilometers apart at the finest geographic scale.

Implications for conservation

In our study, each population contained at least one private allele, and three of the six populations lacked an allele that was shared in the other five populations. The presence of these diagnostic alleles within a comprehensive analysis of genetic structure may have useful forensic applications, such as identifying the geographic origin of confiscated alligators or alligator products. The effectiveness of this marker panel in forensic situations was illustrated by the ability of the assignment tests to assign individuals to their source population with a high probability.

Despite historical population declines, genetic diversity in Texas populations of alligators is high across all populations and habitat types examined.

No apparent differences in genetic diversity were observed between expansive coastal and fragmented inland populations, and there were no apparent differences in diversity between large and small natural populations in Texas. These findings are surprising given that small populations of many animals result in declines of genetic diversity as a consequence of genetic drift (Hartl and Clark, '97; Crandall et al., 2000). Nevertheless, with the apparently small level of gene flow and significant population differentiation observed in this study, it would be prudent to manage alligator populations individually to preserve their unique genetic identities. Localized management of alligator populations ensures that the natural network of genetic diversity is conserved throughout the species' range, and the preservation of genetic diversity ensures that the functional and adaptive diversity of American alligator populations are not compromised.

Genetic differentiation aside, practically all harvested wildlife is managed based on demography and recruitment, and alligators are no different. Sadly, there is little data describing these characteristics for inland alligator populations, and Texas currently permits and regulates public harvest of inland populations. Our limited field observations indicate possible differences in demography, recruitment, nesting ecology, fecundity, and population size between inland alligators and their coastal counterparts. Therefore, we believe that future research on life history differences between coastal and inland alligator populations is necessary to establish successful management strategies for genetically different populations of alligators in Texas.

ACKNOWLEDGEMENTS

The authors would like to thank the following Texas Parks and Wildlife personnel for their assistance in trapping and sampling alligators: Dr. Todd Marindino (GD and MI), Amos Cooper (JD), Gary Calkins (DB), Dale Prochaska (GE), and Gary Rhodes (CC). We are grateful to Dr. Jim Derr and his laboratory staff at the Texas A&M University Veterinary School for providing access to the ABI 310. We also thank members of the Herpetology and Mammalian Genetics laboratories from Texas A&M University for their assistance and advice in various aspects of this research. Finally, we would like to thank the Texas Parks and Wildlife Department and Texas A&M University for funding this research. Work

by W. A. Ryberg was carried out as partial fulfillment of the requirements for a Masters degree at Texas A&M University.

LITERATURE CITED

- Adams EE, Smith MH, Baccus R. 1980. Biochemical variation in the American alligator. *Herpetologica* 36:289–296.
- Bowcock AM, Ruiz-Linares A, Tomfohrde J, Minch E, Kidd JR, Cavalli-Sforza LL. 1994. High resolution of human evolution with polymorphic microsatellites. *Nature* 368:455–457.
- Crandall KA, Bibida-Emonds ORP, Mace GM, Wayne RK. 2000. Considering evolutionary processes in conservation biology. *Trends in Ecology and Evolution* 15:290–295.
- Davis LM, Glenn TC, Elsey RM, Brisbin IL, Rhodes WE, Dessauer HC, Sawyer RH. 2000. Genetic structure of six populations of American alligators: a microsatellite analysis. In: Grigg GC, Seebacher F, Franklin CE, editors. *Crocodylian Biology and Evolution*. Chipping Norton, New South Wales, Australia: Surrey Beatty & Sons. p 38–50.
- Excoffier L, Smouse PE, Quattro JM. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: applications to human mitochondrial DNA restriction data. *Genetics* 131:479–491.
- Friar EA, Boose DL, LaDoux T, Roalson EH, Robichaux RH. 2001. Population structure in the endangered Mauna Loa silversword, *Argyroxiphium kauense* (Asteraceae), and its bearing on reintroduction. *Molecular Ecology* 10:1657–1663.
- Garnier JN, Bruford MW, Goossens B. 2001. Mating system and reproductive skew in the black rhinoceros. *Molecular Ecology* 10:2031–2041.
- Gartside DF, Dessauer HC, Joanen T. 1977. Genic homozygosity in an ancient reptile (*Alligator mississippiensis*). *Biochem Genet* 15:655–663.
- Glenn TC, Dessauer HC, Braun MJ. 1998. Characterization of microsatellite DNA loci in American alligators. *Copeia* 3:591–601.
- Goudet J. 1995. FSTAT (Version 1.2): a program to calculate F-statistics. *Journal of Heredity* 86:485–486.
- Goudet J, Raymond M, De Meeus T, Rousset F. 1996. Testing differentiation in diploid populations. *Genetics* 144:1933–1940.
- Guo SW, Thompson EA. 1992. Performing the exact test of Hardy-Weinberg proportions for multiple alleles. *Biometrics* 48:1933–1940.
- Hartl DL, Clark AG. 1997. *Principles of population genetics*, 3rd ed. Sunderland, MA: Sinauer Associates.
- Joanen T, McNease L. 1987. The management of alligators in Louisiana. In: Webb GJW, Manolis SC, Whitehead PJ, editors. *Wildlife management: crocodiles and alligators*. Chipping Norton, New South Wales, Australia: Surrey Beatty & Sons. p 33–42.
- Lee PLM, Bradbury RB, Wilson JD, Flanagan NS, Richardson L, Perkins AJ, Krebs JR. 2001. Microsatellite variation in the yellowhammer *Emberiza citrinella*: population structure of a declining farmland bird. *Molecular Ecology* 10:1633–1644.
- McIlhenny EA. 1935. *The Alligator's Life History*. Boston, MA: Christopher Publishing House.
- Menzies RA, Kushlan J, Dessauer HC. 1979. Low degree of genetic variability in the American alligator (*Alligator mississippiensis*). *Isozyme Bull* 12:61.
- Ouboter PE, Nanhoe LMR. 1988. Habitat selection and migration of *Caiman crocodylus* in a swamp and swamp-forest habitat in Northern Suriname. *Journal of Herpetology* 22:283–294.
- Paetkau D, Calvert W, Stirling I, Strobeck C. 1995. Microsatellite analysis of population structure in Canadian polar bears. *Molecular Ecology* 4:347–354.
- Raymond M, Rousset F. 1995. GENEPOP (Version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity* 86:248–249.
- Ross CA. 1989. *Crocodiles and Alligators*. New York, NY: Facts on File.
- Serfass TL, Brooks RP, Novak JM, Johns PE, Rhodes OE. 1998. Genetic variation among populations of river otters in North America: Considerations for reintroduction projects. *Journal of Mammalogy* 79:736–746.
- Sundqvist AK, Ellegren H, Olivier M, Vila C. 2001. Y chromosome haplotyping in Scandinavian wolves (*Canis lupus*) based on microsatellite markers. *Molecular Ecology* 10:1959–1966.
- Swofford DL. 1998. PAUP* Phylogenetic Analysis Using Parsimony (and Other Methods), version 4.0d63. Sunderland, MA: Sinauer Associates.
- Taylor D, Neal W. 1984. Management implications of size-class frequency-distributions in Louisiana alligator populations. *Wildlife Society Bulletin* 12:312–319.
- Taylor D, Kinler N, Linscombe G. 1991. Female alligator reproduction and associated population estimates. *Journal of Wildlife Management* 55:682–688.
- Thompson RL, Gidden CS. 1972. Territorial basking counts to estimate alligator populations. *Journal of Wildlife Management* 36:1081–1088.
- Weir BS, Cockerham CC. 1984. Estimating F-statistics for the analysis of population structure. *Evolution* 38:1358–1370.
- Wright S. 1931. Evolution in Mendelian populations. *Genetics* 16:97–159.