GENETIC DIVERSITY AND POPULATION STRUCTURE OF THE TEXAS TORTOISE (*GOPHERUS BERLANDIERI*): IMPLICATIONS FOR CONSERVATION

THESIS

Presented to the Graduate Council of
Texas State University-San Marcos
in Partial Fulfillment
of the Requirements

for the Degree

Master of SCIENCE

by

Akiko Fujii, B.S.

San Marcos, Texas
December 2008

ACKNOWLEDGEMENTS

I would like to thank Dr. Michael R. J. Forstner for being my major advisor, providing instruction for my thesis project. I also would like to thank Drs. Chris C. Nice, Francis L. Rose, and Thomas R. Simpson for their advice in developing ideas, formulating questions, collecting samples, and analyzing the data.

I would like to express my special thanks to the Desert Tortoise Council who directly supported my research through the David Morafka Memorial Award. I am grateful for Taylor Edwards of Arizona Research Laboratories at the University of Arizona for generating molecular data for me.

I like to thank Jonathan Scalise for being my field partner in sample collections. My research would not have been possible without the people and organizations who provided me all of the opportunities to sample their properties: Chaparral Wildlife Management Area (CWMA) of Texas Parks and Wildlife Department (TPWD), the Southmost Preserve of the Texas Nature Conservancy, and many private landowners who shared an interest to conserve and manage the wildlife on their land.

This manuscript was submitted on October 28, 2008.

TABLE OF CONTENTS

Page

LIST OF TABLES

Tables **Page**

LIST OF FIGURES

Figures **Page**

ABSTRACT

GENETIC DIVERSITY AND POPULATION STRUCTURE OF THE TEXAS TORTOISE (*GOPHERUS BERLANDIERI*): IMPLICATIONS FOR CONSERVATION

by

Akiko Fujii, B.S.

Texas State University-San Marcos

December 2008

SUPERVISING PROFESSOR: MICHAEL R. J. FORSTNER

The Texas tortoise (*Gopherus berlandieri*) is a state threatened species in Texas. Expanding agricultural practices and urban development are major causes of habitat degradation for *G. berlandieri*. In order to provide genetic data that can inform conservation planning for this species, genetic variation, population structure and its underlying processes were examined in the U.S. populations of *G. berlandieri*. An *a priori* hypothesis for geographic pattern in its population structure as shaped by the Nueces River basin was tested. A total of 127 individuals representing nine sampling areas were genotyped for 11 microsatellite loci. Assignment tests, *F*-statistics, and

analysis of molecular variance (AMOVA) indicate that *G. berlandieri* forms weak population differentiation into northern and southern populations with a boundary at southern Duval County. A test of isolation by distance and indirect estimation of *Nm* suggest recent gene flow between two populations. Estimation of the extent of recent migration appears to be complicated by human translocation of the tortoises. A lack of concordance between the detected population structure boundary and the Nueces River basin did not support the *a priori* hypothesis. *Gopherus berlandieri* is weakly differentiated due to ongoing migration as evidenced by a pattern of isolation by distance. Given the limited population structure and continuous habitat degradation, designation of two management units may not be warranted. Conservation efforts rather should emphasize connectivity between the populations to maintain genetic diversity in both populations.

CHAPTER I

INTRODUCTION

The Texas tortoise, *Gopherus berlandieri* (Agassiz 1857), is endemic to the Tamaulipan thorn scrub ecosystem occurring in southern Texas and northeastern Mexico (Rose and Judd 1982; Rose and Judd 1989). *Gopherus berlandieri* is listed as a threatened species in Texas (Rose and Judd 1982) and internationally under CITES II (Groombridge 1982). Recent agricultural expansion and development of human infrastructure have substantially reduced habitat for the tortoise and jeopardized the continued existence of this species. Unsuitable land practices for the tortoise such as road construction, deer fencing, intensive grazing, and the introduction of exotic buffelgrass (*Pennisetum ciliare*) disturb tortoise movement, exacerbate vehicle mortality, and create population fragmentation (Judd and Rose 2000; Kazmaier et al. 2001a; Kazmaier et al. 2001b). In addition, upper respiratory tract disease (URTD) has been reported with increasing frequency in *G. berlandieri* (Judd and Rose 2000). *Gopherus berlandieri* has not yet been considered a species of immediate concern; however, the severity of anthropogenic impacts on this species remains apparent.

Despite the current threats to *G. berlandieri* and continuing habitat deterioration, this species remains the only member of the genus *Gopherus* without a conservation plan. Other species of *Gopherus* (the desert tortoise, *G. agassizii*, the gopher tortoise, *G.*

polyphemus, and the Bolson tortoise, *G. flavomarginatus*) all receive federal protection that promotes research and management actions. Substantial time and monetary investments are required to initiate conservation programs as illustrated by the recovery plans for *G. agassizii* and *G.polyphemus* (USFWS 1990; USFWS 1994; USFWS 2008). Furthermore, conservation of testudine species is confounded by life history traits which are typical of long-lived organisms (Klemens 2002). Slow sexual maturation, low recruitment, and high juvenile mortality (Bury and Smith 1986; Congdon et al. 1993) account for slow responses to conservation measures (Congdon et al. 1993; Gibbs and Amato 2000). Delay in initiating active planning will negatively affect the efficacy of conservation efforts and consequently the probability of recovery (Gibbs and Amato 2000). In order to develop a sound management plan for *G. berlandieri*, it is important to obtain greater understanding of this species, including knowledge of the geographic distribution of genetic variation.

One of the principal goals of species conservation is maintenance of intraspecific genetic diversity that seeks to preserve the evolutionary potential of the species (Vogler and DeSalle 1994; Frankham 1996; Frankham et al. 2002; Funk et al. 2002). Small populations tend to suffer from decreasing genetic diversity with increasing genetic drift; hence, these populations have reduced ability to cope with environmental changes (Franklin 1980; Frankel and Soulé 1981; Frankham et al. 2002). Assessment of genetic diversity therefore can provide an estimate of the future viability of the species.

Identifying the major evolutionary segments and their underlying processes are important considerations when prioritizing populations for conservation (Avise 1989; Moritz 1999; Moritz 2002). Molecular population genetics and phylogeography have

been widely applied to threatened and endangered testudine species (e.g. Fitzsimmons et al. 1995; Caccone et al. 2002; Cunningham et al. 2002; Engstrom et al. 2002; Beheregaray et al. 2003; Leuteritz et al. 2005). Many of these studies documented the geographic structure in genetic diversity and assessed implications for conservation (e.g. Lenk et al. 1999; Walker and Avise 1998; Engstrom et al. 2002; Souza et al. 2002; Fritz et al. 2005). Studies of *G. agassizii* in the Mojave Desert employed multiple molecular markers and unveiled fine-scale geographic population structure which was shaped by the Colorado River and mountainous landscape (Lamb et al. 1989; Rainboth et al. 1989; Britten et al. 1997). *Gopherus polyphemus* also displayed genetic assemblages that were historically isolated by the Apalachicola River basin (Osentoski and Lamb 1995). These studies suggest that distinctive populations can be genetically identified and assigned as important segments for protection.

Managing groups of individuals that share a common evolutionary history (Volger and DeSalle 1994) is a basic principle of evolutionary significant units (ESUs) (Moritz 1994; Volger and DeSalle 1994; Waples 1995) or management units (MUs) within an ESU (Moritz 1994; Moritz 2002). Genetic delineation of ESUs or MUs has been performed in several testudine species (e.g. Britten et al. 1997; Mockford et al. 2007; Murphy et al. 2007; Paquette et al. 2007). For instance, MUs of the Mojave populations of *G. agassizii* were genetically assessed and confirmed in relation to the recovery units that were originally described based on morphological, behavioral, and ecological characteristics (USFWS 1994; Britten et al. 1997; Murphy et al. 2007). Although sole reliance on molecular data is not recommended in defining conservation

units (Murphy et al. 2007), it is a vital tool for species such as *G. berlandieri* whose underlying population structure has never been studied.

Genetic diversity within *G. berlandieri* and its possible relationship to local geographic history has never been examined. Because few studies have been conducted to investigate biogeographic events in southern Texas, little evidence is available to infer the history of *G. berlandieri*. Investigating genetic diversity and population structure in relation to landscape features might provide further data that will enhance conservation efforts for this species. Herein, the objectives of this study are to assess genetic variation, population structure, and the processes that would explain the observed pattern of population structure in *G. berlandieri* within the U.S. portion of the species' range. An *a priori* geographic pattern of genetic structure is hypothesized to occur at a river bisecting its distribution in Texas. The Nueces River Basin, a major drainage system in Texas, bisects southern Texas in a northwest to southeast orientation. The Nueces River probably maintained a wider flow during the late Pleistocene than it does at present (Aslan and Blum 1999). Therefore, this river could have served as a geographic barrier to dispersal and limited gene flow in *G. berlandieri*, isolating its populations to the north and south of the river. Investigating gene flow relative to this geographic feature may provide insight into the extent and nature of dispersal for these tortoises. The use of genetic information in helping to guide future management options for this species is also discussed.

CHAPTER II

METHODS

Sample collection

Blood samples were collected from live tortoises at nine areas in southern Texas: San Antonio, Frio County, eastern Zavala County, western Zavala County, Chaparral Wildlife Management Area (CWMA), Duval County, upper Rio Grande Valley (RGV), Starr County, and Brownsville (Fig. 1). A small aliquot of blood (~1 ml) was drawn from the femoral vein and placed into blood storage buffer (100 mM Tris, 100 mM EDTA, 2 % SDS). Muscle tissues were also collected from dead tortoises found killed by automobiles on roads. Muscle tissue was cut from the least exposed area of the carcasses and placed into 95 % ethanol. A total of 144 individuals were collected across the nine sampling areas.

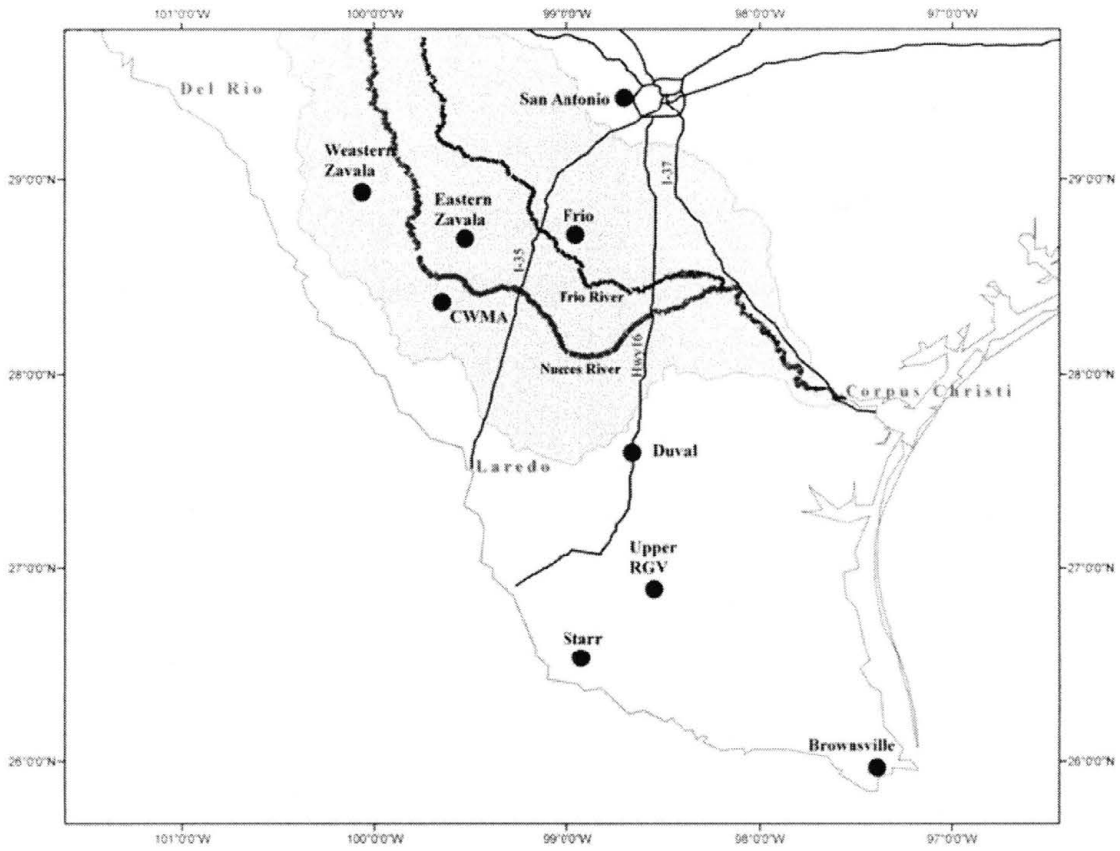

FIGURE 1. The U.S. species range of the Texas tortoise (*Gopherus berlandieri*) in southern Texas and the sampling areas within which the tortoises were collected for the study. The map shows southern Texas with the major highways (interstate highways I-35, I-37, and state highway 16) and the major rivers (Nueces River and Frio River). Circles represent the approximate centers of the nine sampling areas: San Antonio, Frio County, eastern Zavala County, western Zavala County, Chaparral Wildlife Management Area (CWMA) located in Dimmit and La Salle County, Duval County, upper RGV (Rio Grande Valley) in Jim Hogg, Hidalgo, and Brooks County, Starr County, and Brownsville. The shaded area represents the Nueces River basin.

Microsatellite genotyping

Total genomic DNA was extracted from blood and muscle samples using QIAGEN DNeasy Blood & Tissue Kit according to the manufacture's instruction. Each individual was genotyped at 16 microsatellite loci (Goag3, Goag4, Goag5, Goag6, Goag7, Goag32, GP15, GP19, GP26, GP30, GP55, GP61, GP81, GP96, GP102, and Cm58). Primers for Goag, GP and Cm were previously characterized for *Gopherus agassizi*, *Gopherus polyphemus* and *Chelonia mydas* by Edwards et al. (2003), Schwartz et al. (2003), and FitzSimmons et al. (1995) respectively. The polymerase chain reaction (PCR) amplification was carried out in two multiplex reactions for each locus with the PCR conditions described in Edwards et al. (2004) and Murphy et al. (2007). Fragment analysis was conducted on ABI Prism 3730 DNA Analyzer (PE Biosystems).

Statistical analyses

Among successfully amplified loci, those showing two or more alleles were used for analyses. Individuals for which fewer than three loci amplified were eliminated from the analyses.

Because natural population boundaries were not clearly defined for *G. berlandieri*, clustering analyses were carried out before estimating standard population parameters that requires *a priori* knowledge of population boundaries. Assignment of individuals to populations was performed using two clustering methods. First, the number of genetic clusters (K) was estimated using STRUCTURE 2.2 (Pritchard et al. 2000). STRUCTURE is a clustering software program based on a Bayesian approach with the Markov Chain Monte Carlo (MCMC) estimation. Because shared ancestry was expected in *G.*

berlandieri, the admixture model with correlated allele frequency was used. A range of populations (K) from one to seven was tested 10 times with 5×10^5 iterations and 5×10^4 burn-in at each K. The number of clusters (K) with the highest mean log-likelihood was selected as the best description of population structure. Second, a spatial Bayesian clustering program, GENELAND 3.1.2 (Guillot et al. 2008), was used to incorporate geographic locations of samples in inferring the number of genetic clusters. The number of clusters (K) was varied between one to five, using the maximum rate of Poisson process at 100, the maximum number of nuclei at 300, and the Dirichlet model for allele frequency with 5×10^5 MCMC iterations and 100 thinning. This process was replicated five times to assess the consistency of the modal K. The same operation was carried out at the fixed K inferred by the first part of GENELAND analysis with 10^4 iterations and 10 thinning. Posterior probability of population membership was plotted using 50 pixels on X and Y axis. Consistency of the result was verified by iterating the analysis 10 times.

Traditional methods to estimate population parameters were performed for the inferred populations determined by the clustering methods described above. Observed and expected heterozygosity per locus was calculated for each population using FSTAT 2.9.3 (Goudet 1995). Significant departure from Hardy-Weinberg equilibrium (HWE) for each locus and presence of linkage disequilibrium between loci in each population were tested using GENEPOP 4.0 (Rousset 2008). Exact tests for HWE (Guo and Thompson 1992) and G-based likelihood tests for linkage disequilibrium using the Markov Chain algorithm were carried out with 10^5 iterations with a burn-in of 10^4 iterations to estimate statistical significance. Allelic richness, the average number of alleles per locus corrected for sample size, was calculated using FSTAT. The loci that significantly deviated from

HWE after sequential Bonferroni correction (Rice 1989) were tested for null alleles using MICRO-CHECKER (van Oosterhout et al. 2004), and genotypes were adjusted based on the estimated allele frequencies by the Brookfield algorithm (Brookfield 1996).

A genetic distance-based analysis was used to assess the degree of population structure. Pairwise ρ (Goodman 1997) corrected for sample size and allele variance of R_{ST} (Slatkin 1995) and F_{ST} (Weir and Cockerham 1984) values were calculated using RSTCALC 2.2 (Goodman 1997) and FSTAT 2.9.3 (Goudet 1995), respectively with 10,000 permutations. R_{ST} estimates take into account the variance in a repeat number under the stepwise mutation model (SMM), whereas F_{ST} estimates the variance in allele frequency under the infinite alleles model (IAM) (Slatkin 1995; Gaggiotti et al. 1999). Given the limited sample size and the number of loci as well as possible deviations from SMM, F_{ST}, which is recommended as more conservative estimate (Gaggiotti et al. 1999), was also calculated.

Hierarchical partitioning of a total genetic variation was conducted using analysis of molecular variance (AMOVA) implemented by ARLEQUIN (Excoffier et al. 2005). The amount of genetic variation accounted for by the difference between the two populations, among sampling areas within populations, and among individuals within sampling areas were estimated with F_{ST} and R_{ST}. Statistical significance was determined by 10000 permutations.

Genetic variation and the degree of population structure were compared between *G. berlandieri* and *G. agassizii* in the Sonoran Desert, Arizona (n = 154 from nine sampling areas) (Edwards et al. 2004). The comparable sample size and study range of the sister species of *G. berlandieri* make this data set from *G. agassizii* an ideal

benchmark to assess genetic diversity in *G. berlandieri*. Allelic richness and expected heterozygosity in the entire sample of *G. berlandieri* were compared to those of *G. agassizii*. Assuming the nine sampling areas as putative populations as described by Edwards et al. (2004), the degree of population structure was estimated with F_{ST} and ρ as described above. STRUCTURE analysis was conducted with the same parameters described previously in order to detect larger groups of the populations.

Contemporary gene flow was analyzed by assessing isolation by distance that tests a correlation between genetic distance and geographic distances. Pairwise genetic distance and geographic distance were compared among sampling areas across the inferred populations and within populations using the Mantel test with 10,000 permutations for significance implemented by GENEPOP 4.0 (Rousset 2008). Migration rate and its direction between the populations were estimated using three methods, each with different assumptions. First, indirect estimate of gene flow (*Nm*) using the private allele method (Slatkin and Barton 1989) was calculated by GENEPOP. The indirect estimate of *Nm* assumes equilibrium populations with constant sizes connected by symmetrical migration (Wright 1969). Because natural populations do not always satisfy these assumptions (Paetkau et al. 2004), migration rates were also estimated by the more flexible method implemented by BAYESASS 1.3 (Wilson and Rannala 2003). BAYESASS is a Bayesian software program that estimates contemporary migration rates with MCMC approximation. Its approach is less restrictive than the estimate of *Nm* because the assumption of HWE is not required. In addition, BAYESASS estimates more contemporary migration (past few generations) than the *Nm* and asymmetrical migration rates between populations. Migration rates were estimated with 3×10^6 iterations, 10^5

burn-in and thinning of 1,000. Default parameters were used for delta values for allele frequency, migration rate, and inbreeding coefficient. Lastly, detection of recent migrants for the last few generations was performed with GENECLASS 2 (Piry et al. 2004). The frequency-based method (Paetkau and Strobeck 1995) and the Bayesian method (Rannala and Mountain 1997) were employed to assess the consistency in their estimation of migrants. The ratio of the likelihood of an individual assigned to the population where it was sampled to the maximum likelihood assigned to any population was simulated 10,000 times by Monte-Carlo resampling (Paetkau et al. 2004).

CHAPTER III

RESULTS

Microsatellite genotyping

Out of 144 individuals tested for microsatellite genotyping, 17 individuals for which fewer than three loci amplified were eliminated, making up a total of 127 individuals used for further analyses (Table 1). Of 16 microsatellite loci tested, 11 loci were polymorphic (Goag4, Goag6, Goag7, Goag32, GP15, GP30, GP55, GP61, GP81, GP102, and Cm58) (Table 2). One locus did not amplify (Goag3), and four loci were monomorphic (Goag5, GP19, GP26, and GP96). These five loci were excluded from further analyses. Scoring the loci that exhibited the highest polymorphism (GP55, GP61, and GP102) was difficult as their stutter peak patterns were inconsistent, and larger alleles amplified weakly. Because two of these loci (GP55 and GP61) were especially at issue for these potential problems, subsequent analyses were conducted with and without these two loci, hereafter called the complete data set and the reduced data set, respectively.

TABLE 1. The nine sampling areas, the geographic location for each area, the number of individuals of the Texas tortoise (*Gopherus berlandieri*) collected within each area, and the inferred population to which each sampling area belongs. The two populations were estimated with the microsatellite data set using STRUCTURE 2.2 (Pritchard et al. 2000).

Sampling area	Area location	Number of individuals	Population
San Antonio	-98.58226, 29.49303	12	North
Frio County	-98.91500, 28.72000	10	North
Eastern Zavala County	-99.57000, 28.74000	24	North
Western Zavala County	-100.1000, 28.93600	6	North
CWMA	-99.67000, 28.54000	35	North
Duval County	-98.65000, 27.67000	5	North
Upper RGV	-98.40000, 26.94000	6	South
Starr County	-98.89000, 26.53000	25	South
Brownsville	-97.48000, 25.93000	4	South

CWMA - Chaparral Wildlife Management Area and its vicinity, RGV - Rio Grande Valley.
Some samples collected on roadsides were included in the closest sampling areas.
Geographic coordinates for each area are approximate centers of the sampling areas.
The individuals collected within each area represent the number after eliminating the individuals for which fewer than three microsatellite loci were amplified.

TABLE 2. Comparison of the microsatellite motifs observed in the samples of Texas tortoise (*Gopherus berlandieri*) in this study with those described in the original species from which the microsatellite markers were isolated: the desert tortoise (*Gopherus agassizii*) (Edwards et al. 2003), the gopher tortoise (*Gopherus polyphemus*) (Schwartz et al. 2003), and the green sea turtle (*Chelonia mydas*) (Fitzsimmons et al. 1995).

Locus	Original species	Original motif	Motif in *G. berlandieri*	Allele range
Goag4	*G. agassizii*	$(CAA)_{24}$	$(CAA)_8(TAA)(CAA)$	8-11
Goag6	*G. agassizii*	$(TC)_8(AC)_{11}$	$(TC)_6(CC)(TC)_5(AC)_9$	20-26
Goag7	*G. agassizii*	$(AC)_3(GC)_5(AC)_{11}$	$(AT)(GC)_3(AC)_{11}$	15-23
Goag32	*G. agassizii*	$(AC)_6$	$(AC)_6$	6-8
GP15	*G. polyphemus*	$(GA)_{15}(GT)_8$	$(CA)_2(GA)_4(GT)_5(AT)(GT)_3$	14-33
GP30	*G. polyphemus*	$(GT)_{13}$	$(GT)_8$	8-11
GP55	*G. polyphemus*	$(GT)_9$	$(GT)_{22}$	10-38
GP61	*G. polyphemus*	$(GT)_{12}$	$(GT)_8(AA), (GT)_{30}$	9-43
GP81	*G. polyphemus*	$(GT)_{11}(GA)_{10}$	$(GT)_8(GA)(CA)(GA)_{11}$	17-20
GP102	*G. polyphemus*	$(GT)_5(CT)_{13}(CA)_5$	$(GT)_{12}(CT)_5(CA)_3$	13-43
Cm58	*Chelonia mydas*	$(CA)_{13}$	$(TA)_4(GA)_4(GC)(GA)_3$	12-13

Individual assignment tests

The assignment tests implemented by STRUCTURE reached the highest mean likelihood with the least variance at $K = 2$ in the complete data set (Fig. 2A) and at $K = 1$ in the reduced data set (Fig. 2B). When locus GP102 was excluded from the reduced data set because of its potential ambiguity described above, STRUCTURE recovered the highest mean likelihood at $K = 2$. Visualization of the estimated proportion of genotype assigned to either cluster with the sampling localities arranged from north to south suggested the population boundary that weakly separates the Duval County sampling area to the north and the rest to the south (Figs. 3A, 3B). The northern clustering was less uniform than the southern clustering. The northern cluster contained a quite few individuals that showed greater proportion of genotype assigned to the southern cluster rather than the northern cluster. The first procedure implemented by GENELAND to infer the number of populations and population boundary provided the highest posterior probability of population membership at $K = 2$. The second procedure of 10 runs at $K = 2$ produced consistent results. The population boundary was estimated at just south of the Duval sampling area, separating the northern and southern cluster (Fig. 4). This result was consistent in both the complete and reduced data set. The population structure estimates performed by STRUCTURE and GENELAND generally agreed upon two populations with the weakly defined boundary at southern Duval County. Therefore, the following analyses treated a cluster that contains San Antonio, Frio County, eastern Zavala County, western Zavala County, CWMA and Duval County sampling areas as the northern population, and a cluster that contains Upper RGV, Starr and Brownsville as the southern population.

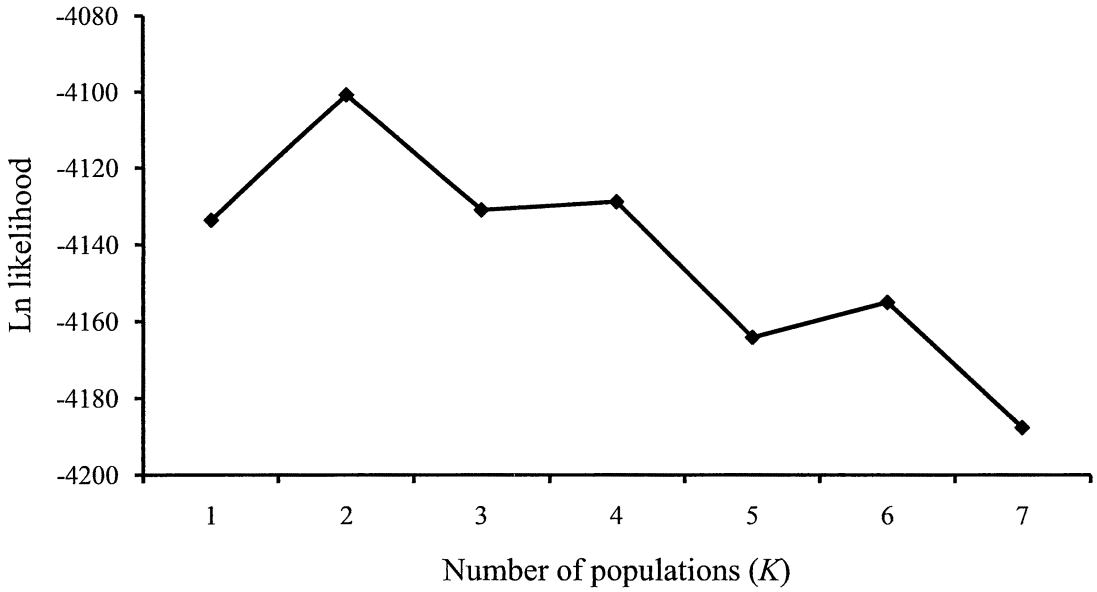

FIGURE 2A. The mean log likelihood of the individual assignment tests at each number of populations (*K*) tested by STRUCTURE 2.2 (Pritchard et al. 2000) using the complete data set for the Texas tortoise (*Gopherus berlandieri*) sampled for this study. The individual assignment into one to seven populations was tested 10 times by STRUCTURE. The mean likelihood over 10 runs at each *K* was determined to approximate the number of populations in *Gopherus berlandieri*. The highest mean likelihood was obtained at $K = 2$ in the complete data set.

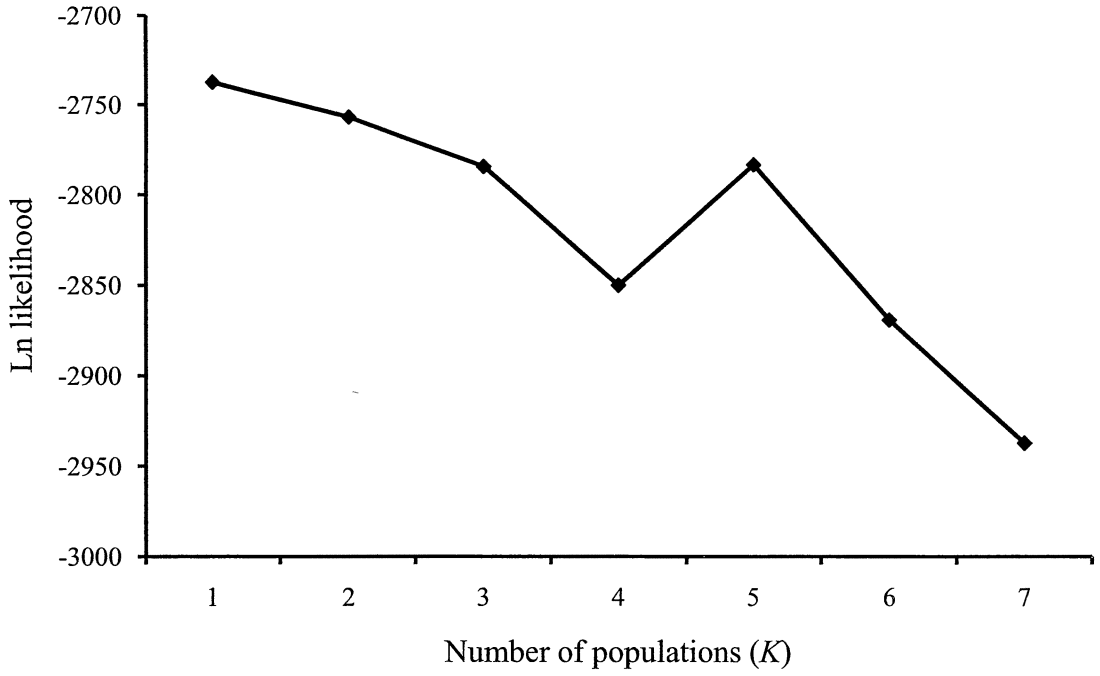

FIGURE 2B. The mean log likelihood of the individual assignment tests at each number of populations (*K*) tested by STRUCTURE 2.2 (Pritchard et al. 2000) using the reduced data set for the Texas tortoise (*Gopherus berlandieri*) sampled for this study. The individual assignment into one to seven populations was tested 10 times by STRUCTURE. The mean likelihood over 10 runs at each *K* was determined to approximate the number of populations in *Gopherus berlandieri*. The highest mean likelihood was obtained at $K = 1$ in the reduced data set that excluded two loci (GP55 and GP61).

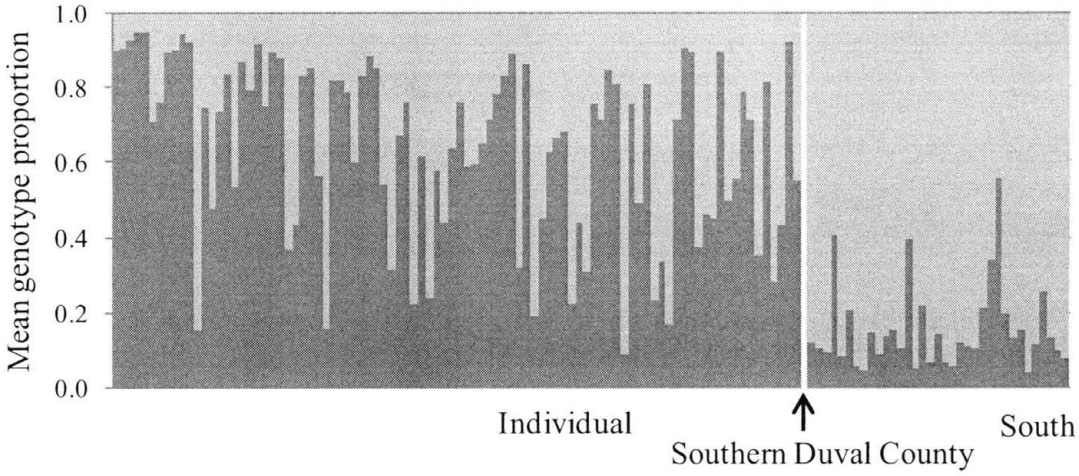

FIGURE 3A. The mean genotype proportion for each individual assigned into two populations by STRUCTURE 2.2 (Pritchard et al. 2000) using the complete data set for the Texas tortoise (*Gopherus berlandieri*) sampled for this study. The mean genotype proportion for each individual into two populations ($K = 2$) was estimated for all individuals ($n = 127$) by STRUCTURE using the complete data set. Dark colored bars represent the mean genotype proportions into the northern population, and light colored bars represent the mean genotype proportions into southern population.

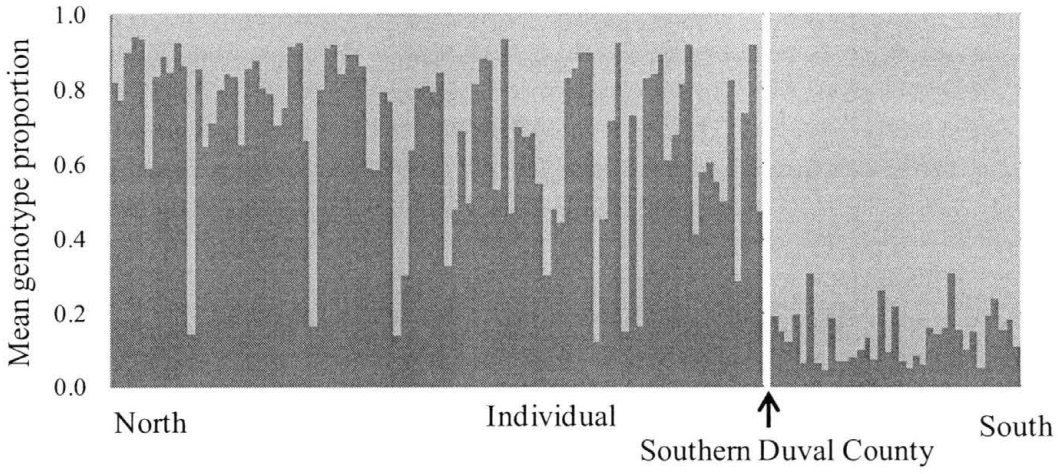

FIGURE 3B. The mean genotype proportion for each individual assigned into two populations by STRUCTURE 2.2 (Pritchard et al. 2000) using the reduced data set for the Texas tortoise (*Gopherus berlandieri*) sampled for this study. The mean genotype proportions for each individual into two populations ($K = 2$) were estimated for all individuals ($n = 127$) by STRUCTURE using the reduced data set that excluded two loci (GP55 and P61). Dark colored bars represent the mean genotype proportions into the northern population, and light colored bars represent the mean genotype proportions into southern population.

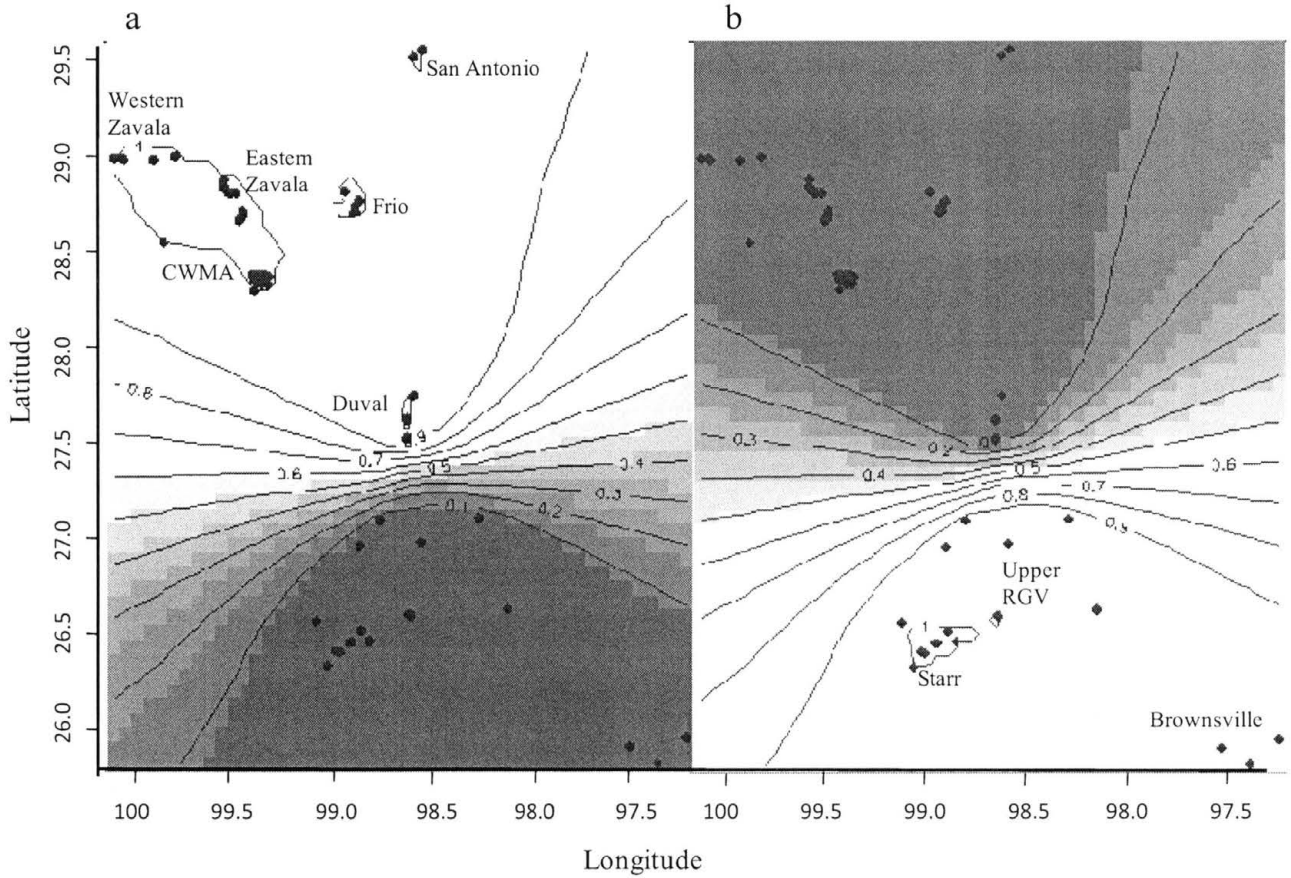

FIGURE 4. Geographic assignment of individuals of the Texas tortoise (*Gopherus berlandieri*) into two populations implemented by GENELAND 3.1.2 (Guillot et al. 2008). Membership probabilities into two population ($K = 2$) were estimated incorporating geographic locations of individual samples ($n = 127$) by GENELAND. Membership probabilities were denoted on contours, and increase with lighter color. The left figure represents clustering of the northern population, and the right figure represents clustering of the southern population. The estimated population boundary is located at southern Duval County.

Genetic diversity

A wide range of microsatellite polymorphism was observed across loci in both the northern and southern population. Observed heterozygosity per locus ranged from 0.143 to 0.793 with the mean of 0.528 in the northern population, and 0.173 to 0.824 with the mean of 0.606 in the southern population (Table 3). The reduced data set showed slightly a smaller mean heterozygosity than the complete data set in both populations (Table 3). Allelic richness also ranged widely from 2.0 to 16.5 in the northern population, and 2.0 to 18.7 in the southern population. The observed and expected heterozygosities, and allelic richness were significantly greater in the southern population than in the northern population for both data sets ($P < 0.05$). Private alleles accounted for 22 % of the total alleles observed in both populations in the complete data set, and 18 % and 22 % in the reduced data set in the northern population and the southern population respectively (Table 3).

TABLE 3. Summary statistics of genetic diversity: the observed number of alleles, allelic richness, observed heterozygosity, and expected heterozygosity for each microsatellite locus for the northern and southern population of the Texas tortoise (*Gopherus berlandieri*).

Locus	Northern population (n = 92)				Southern population (n = 35)			
	A	*Ar*	*Ho*	*He*	*A*	*Ar*	*Ho*	*He*
Goag4	3 (0)	2.74	0.33	0.35	3 (0)	2.94	0.20	0.19
Goag6	5 (0)	4.58	**0.52**	0.54	7 (2)	7.00	0.69	0.76
Goag7	7 (2)	5.80	0.61	0.60	6 (1)	5.94	0.82	0.71
Goag32	3 (0)	3.00	0.66	0.60	3 (0)	3.00	0.80	0.65
Cm58	2 (0)	2.00	0.14	0.15	2 (0)	2.00	0.18	0.26
GP15	8 (1)	6.67	0.79	0.80	13 (6)	12.80	0.86	0.84
GP30	4 (0)	3.98	**0.38**	0.50	4 (0)	4.00	0.40	0.54
GP55	19 (4)	14.60	**0.56**	0.91	19 (4)	18.70	**0.77**	0.94
GP61	19 (6)	16.50	**0.78**	0.93	17 (4)	16.90	**0.74**	0.94
GP81	4 (0)	3.99	0.39	0.67	4 (0)	4.00	0.39	0.66
GP102	19 (7)	14.80	0.64	0.68	16 (4)	16.00	0.82	0.86
Mean	8.45 (1.82)	7.15	0.53	0.61	8.55 (1.9)	8.48	0.61	0.67
Mean*	6.11 (1.11)	5.28	0.50	0.54	6.44 (1.4)	6.41	0.57	0.61

A = observed number of alleles, *Ar* = allelic richness, *Ho* = observed heterozygosity, *He* = expected heterozygosity.
Bold font represent significant deviation from Hardy-Weinberg equilibrium after Bonferroni correction (P = 0.01).
Allele numbers in parenthesis are observed number of private alleles.
*denote mean values excluding loci GP55 and GP61.

The tests of Hardy-Weinberg equilibrium (HWE) revealed that two loci (GP61 and GP55) did not conform to HWE in either population. Two other loci (Goag 6 and GP30) in the northern population were also significantly deviated from HWE after sequential Bonferroni correction (Rice 1989). These four loci exhibited significant heterozygote deficits in both populations. Significant deficit in observed heterozygosity for two loci (GP55 and GP61) were unlikely due to conservative allele scoring because relaxed scoring that counted all possible peaks also returned the same result. Linkage disequilibrium was detected in three pairs of loci in the northern population and four different pairs in the southern population, but none of them were significant after sequential Bonferroni correction.

Population structure – F-statistics and AMOVA

Genetic distance-based estimates of population structure yielded F_{ST} values of 0.062 and 0.082, and ρ values of 0.064 and 0.078 in the complete and the reduced data set respectively with statistical significance ($P < 0.001$ for all). The F_{ST} based AMOVA estimated that 5.5 % and 7.8 % of the total genetic variation was accounted for between populations and 92 % and 90.6 % within population in the complete and the reduced data set, respectively. The R_{ST} based AMOVA showed 0 % of the total genetic variation between populations for both data sets. However, when locus GP102 was additionally excluded from the reduced data set and reanalyzed, 7.9 % of between-population variation was recovered. The absence of between-population variation when locus GP102 was included could be attributed to the large allelic size variance in the three loci having little structure (GP55, GP61, and GP102). Because R_{ST} takes into account allelic size

differences, the difference revealed by the frequency-based estimate might have been overcome.

Comparison between Gopherus berlandieri and Gopherus agassizii

Comparison of the data set of *G. berlandieri* and that of Sonoran *G. agassizii* populations (Edwards et al. 2004) showed similar overall expected heterozygosity and allelic richness (0.62 and 0.67 for heterozygosity and 10.1 and 12.1 for allelic richness respectively) with some differences in individual loci (Fig. 5). Estimate of population structure for *G. agassizii* showed F_{ST} value of 0.031 and ρ value of 0.033. The STRUCTURE analysis generated the highest mean likelihood with the smallest variance at $K=1$.

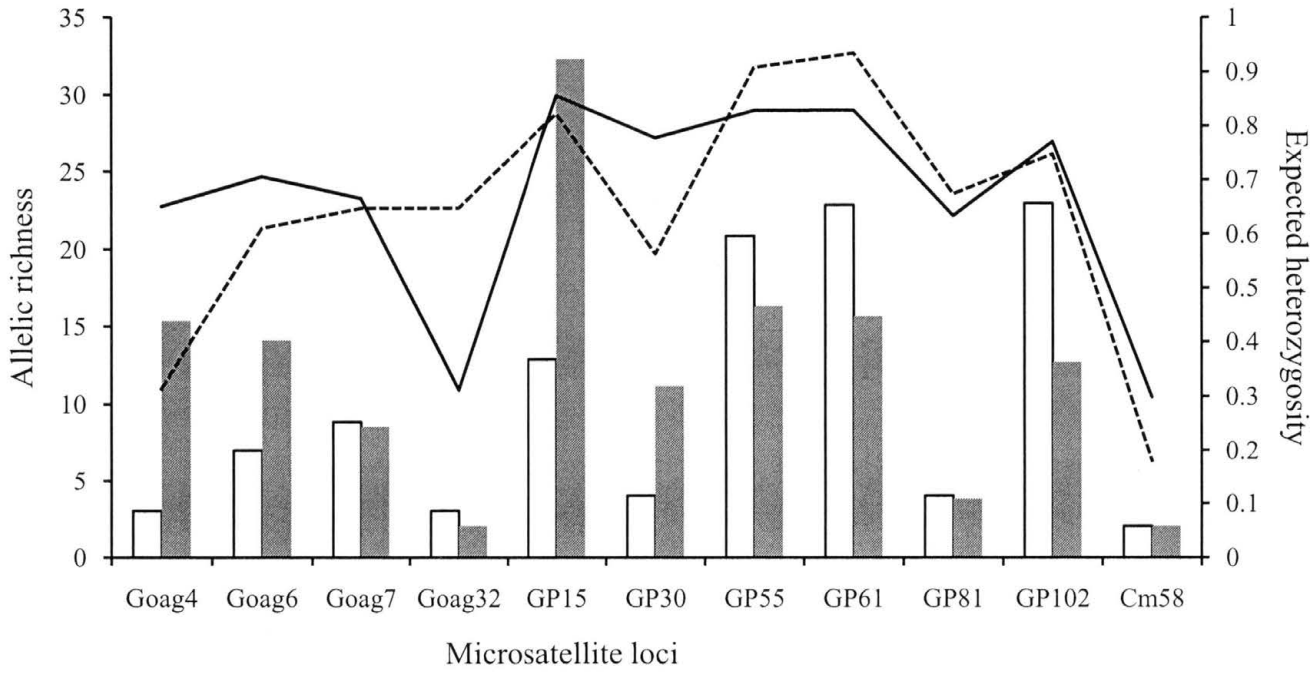

FIGURE 5. Comparison of genetic diversity (allelic richness and expected heterozygosity) between the Texas tortoise (*Gopherus berlandieri*) in the study and the Sonoran populations of the desert tortoise (*Gopherus agassizii*) in the published study (Edwards et al. 2004). Allelic richness and expected heterozygosity were compared between *G. berlandieri* (n = 127) and the Sonoran populations of *G. agassizii* (n = 154) in Arizona (Edwards et al. 2004). Hallow bars and filled bars represent allelic richness of *G. berlandieri* and *G. agassizii* respectively. Dashed line and solid line represent expected heterozygosity of *G. berlandieri* and *G. agassizii* respectively.

Gene flow

The test of isolation by distance showed a significant correlation between genetic distance and geographic distance across populations in both data sets (Fig. 6). The reduced data set produced slightly higher correlation than the complete data set ($r = 0.42$, $P = 0.007$ and $r = 0.51$, $P = 0.004$ respectively). Isolation by distance among sampling areas within each population was not significant in either population. The indirect estimate of *Nm* was 3.73 and 3.18 migrants per generation in the complete and reduced data set, respectively. Contemporary migration rate estimated by the Bayesian approach was 0.068 per generation from the northern to the southern population (95 % *CI* = 0.008 – 0.149), and 0.010 from the southern to the northern population (95 % *CI* = 0.0004 – 0.036) in the complete data set. The reduced data set showed slightly lower values than the complete data set by about 0.008 and 0.002, respectively. Detection of recent migrants revealed two individuals: one from San Antonio, TX and another from Duval County that were statistically significant migrants in both data sets ($P < 0.01$). Another individual from CWMA was significant in the complete data set and nearly significant in the reduced data set ($P < 0.05$). Several other individuals from the northern population and fewer from the southern populations were also nearly significant ($P < 0.05$).

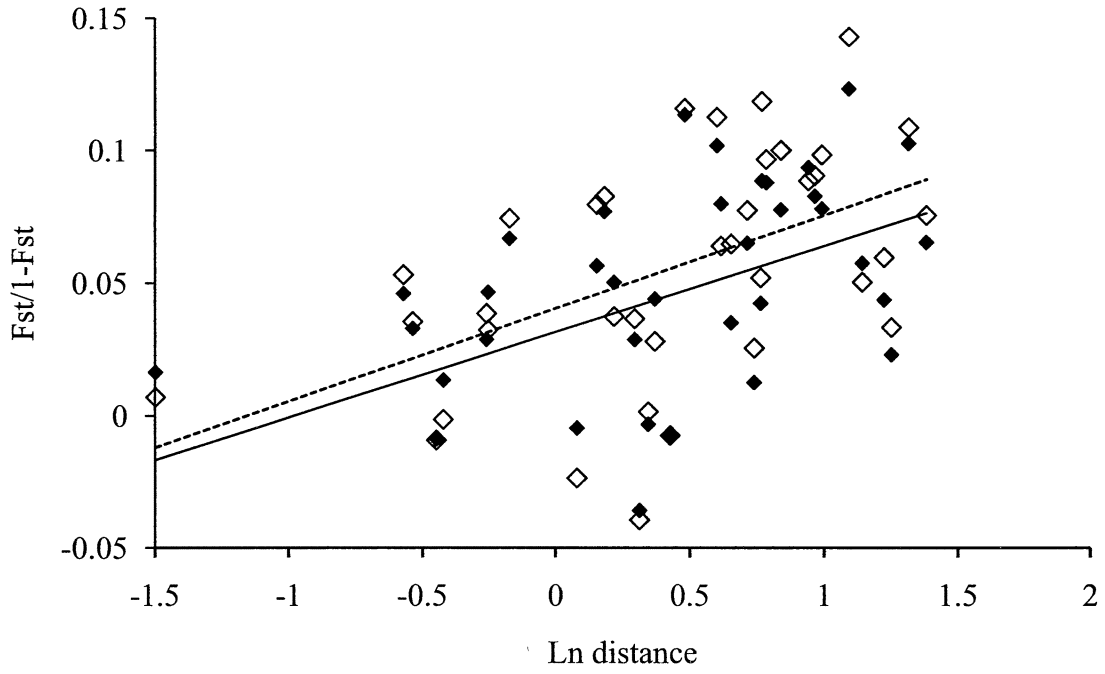

FIGURE 6. Isolation by distance among the nine sampling areas across two populations of the Texas tortoise (*Gopherus berlandieri*) included in the study as implemented by GENEPOP 4.0 (Rousset 2008). Geographic correlation of genetic distance was evaluated using the Mantel test implemented by GENEPOP. Analogs of genetic distance (F_{ST}/1-F_{ST}) were significantly correlated with logarithmic distance converted from geographic coordinates of the nine sampling areas across two populations in both the complete and reduced data set ($r = 0.42$, $P = 0.007$ and $r = 0.51$, $P = 0.004$ respectively). Filled markers and the solid line represent the complete data set, and hollow markers and the dashed line represent the reduced data set.

CHAPTER IV

DISCUSSION

All but one of the three species within the genus *Gopherus* are federally regulated, and those three also have extensive conservation planning and consequent resources directed toward preventing decline. However, *Gopherus berlandieri* while a state threatened species in Texas has not had the need to develop a conservation plan addressed. In order to better inform future conservation planning, genetic diversity, population structure, and the potential for a historical barrier at the Nueces River basin were examined for the U.S. populations of *G. berlandieri*.

Microsatellite amplification

The statistical analyses using the complete and reduced data sets emphasized the need for careful selection of microsatellite loci to minimize potential noise or bias without sacrificing the informative content. The microsatellite loci used in this study were originally designed for the sister species, *G. agassizii*, and the related species, *G. polyphemus* (Lamb et al. 1989; Lamb and Lydeard 1994; Murphy et al. 2007). The loci that displayed unusually wide allelic ranges, including large gaps in allelic sizes (GP55, GP61, and GP102) were originally designed for *G. polyphemus*. Of these three loci, two loci showed stuttering, weak amplification of large alleles, and significant deviation from HWE even after correction for null alleles. The cross-species amplification problems

involved in these loci showed an opposite pattern from typical ascertainment bias where shorter repeat lengths and lower heterozygosity are frequently observed in the species for which loci were not originally developed (Hutter et al. 1998; Vowles and Amos 2006). As demonstrated in this study, the use of multiple data sets is recommended to test consistency across analyses and identify any unusual behavior of the loci in question.

Genetic diversity

Moderate mean heterozygosity and a fair amount of private alleles in both populations demonstrate the appreciable amount of genetic variation present in *G. berlandieri*. The southern population appears more genetically diverse as it displayed higher allelic richness and heterozygosity than the northern population whose sample size was more than twice as large as that of from the southern population. Studies conducted within the range of southern population reported generally higher tortoise density than that of the northern population (e.g. Auffenberg and Weaver 1969; Kazmaier et al. 2001c). The southern population may have been better able to maintain a historically larger size across a more contiguous habitat, which probably facilitated preservation of greater genetic variation.

Numerous studies of testudine species including threatened and endangered species reported moderate to high genetic diversity for microsatellite loci (e.g. Sites et al. 1999; Ciofi et al. 2002; Cunningham et al. 2002; Edwards et al. 2004; Mockford et al. 2005; Tessier et al. 2005; Paquette et al. 2007). Although many authors have expressed a concern for population bottlenecks, the detection of the loss of genetic diversity as a result of modern anthropogenic impacts may be difficult to capture from genetic data

(Sumner et al. 2004; de Thoisy et al. 2006). This is especially true for tortoises as genetic drift proceeds slowly in populations of long-lived organisms (Tessier et al. 2005). Genetic diversity therefore may not serve as an efficient proxy for modern population decline in testudine species.

Population structure

The U.S. populations of *G. berlandieri* showed a limited amount of population structure, weakly resolving into northern and southern populations. Although the STRUCTURE analyses showed minor disagreement in estimating the number of populations, incorporation of geographic locations of the samples by GENELAND reinforced the presence of partially separated populations with a boundary at southern Duval County. Furthermore, *F*-statistics (F_{ST} and ρ) and AMOVA supported statistically significant but weak differentiation between two populations.

The analyses by STRUCTURE, using the reduced data set did not support any population structure, whereas two populations were inferred from the complete data set. This discrepancy observed between the two data sets is likely a consequence of two factors. It is possible that the limited sample sizes and geographic coverage may not capture sufficient signal for population structure that would otherwise be manifested by a more extensive geographic sampling. An alternative explanation could be that the extent of population structure that can be estimated from genetic data varies depending on number and choice of loci used. Because STRUCTURE assumes genetic equilibrium within a population, deviation from HWE due to apparent heterozygote deficiency caused by the presence of null alleles may be a confounding factor. The differing amount of information content in two data sets was also revealed by *F*-statistics and AMOVA.

Because the amount of population structure increased in the reduced dataset, the two loci excluded from the complete data set may form homogeneous allelic distribution across populations. The lack of structure in the two loci might have diluted the differentiation revealed by other loci.

The weakness in population differentiation was also revealed in the limited cohesion of clustering within populations. The northern population formed heterogeneous clustering in STRUCTURE, and the variability of genotype proportions was generally more pronounced in the individuals from southern part of this population (Fig. 3A, 3B). Additionally, in comparison to the southern population, the northern population included a greater number of loci with significantly deviation from HWE. Therefore, population admixture may be taking place more extensively in the southern part of the northern population. In contrast, the southern population formed a more homogeneous cluster. The unequal degree of ambiguity in clustering between two populations would support asymmetrical migration rates between two populations.

Phylogeography

This study hypothesized a historical role of the Nueces River basin in shaping genetic structure of *G. berlandieri*. The estimated population boundary at southern Duval County is outside of the modern Nueces River basin, and approximately 50 km south of the main river system. In addition, the northern population includes sampling areas just west and south of the Nueces River. Therefore, the current genetic evidence of population structure did not support historical geographic isolation by the Nueces River basin.

There is limited literature on the paleo river systems in southern Texas. Daub and Boothroyd (1978) hypothesized an association of the Nueces River with the Bordas

escarpment that runs in a northeast to southwest orientation in southern Texas. The paleo Nueces River could have crossed Duval County during the Quaternary before the escarpment became active (Daub and Boothroyd 1978). However, there is no clear evidence to support this hypothesis. A more recent study by Aslan and Blum (1999) proposed that the Nueces River had a wide channel during the late Pleistocene, and changed its course by reoccupying channels during the Holocene. Hence, the drainage basin of the Nueces River was probably stable with minor changes in recent history.

The effects of geographic barriers on dispersal can be eased over the course of geologic and climatic events. When landscape features convert from barriers to filters, increasing migration will result in less pronounced genetic structure. In general, southern U.S. river drainages have likely decreased in magnitude substantially throughout the Holocene under the long-term drying trend for the continent. Even the Colorado River that isolated *G. agassizii* into eastern and western assemblages is no longer considered an effective barrier (Lamb et al. 1989). Breakdown of the barriers to dispersal was probably accelerated in smaller drainages such as the Nueces River. Even if the Nueces River acted as a partial barrier to gene flow in *G. berlandieri*, its effectiveness may have quickly become reduced in recent history. An inconsistent pattern of geographic distribution for genetic variation was also observed in *G. agassizii*. McLuckie et al. (1999) found a cryptic population of *G. agassizii* that exists east of the Colorado River, but genetically belongs to the western population. They suggested geologic, hydrologic, and anthropogenic factors that facilitated gene flow across the river. In order to clarify the population boundary in *G. berlandieri* in relation to the Nueces River basin, further sampling along the river and the estimated population boundary would be required.

Recent gene flow

The weak population structure indicates either recent divergence or persistent gene flow between two populations. Multiple analyses of migration generally agreed upon the dominant role of gene flow in the observed amount of structure. Significant isolation by distance indicates recent gene flow between the populations. More frequent gene exchange between populations in geographical proximity may explain the mixed proportions of genotypes observed in the individuals in the southern part of the northern population as depicted by the STRUCTURE analyses. The indirect estimate of *Nm* also indicates recent gene flow though the populations of *G. berlandieri* may not satisfy the assumptions for this estimate. Several tortoises were detected as recent migrants accounting for approximately 3 % of the total samples. Taken together the results collectively suggest that weak population structure is likely a consequence of recent gene flow that keeps the two populations from becoming differentiated.

Similar results were presented in the Sonoran populations of *G. agassizii* (Edwards et al. 2004). The comparative analyses of their data to *G. berlandieri* in this study exhibited similar genetic diversity in both species, and weaker population structure in *G. agassizii*. The STRUCTURE analysis of *G. agassizii* data also indicates a lack of significant population structure. Edwards et al. (2004) proposed that limited population structure was a result of ongoing gene flow by strong isolation by distance among populations. As similar population dynamics may be in effect in the populations of *G. berlandieri* that may be maintaining weak population structure via continued gene flow.

The direction of migration remains unresolved. The estimated migration rate from the northern population to the southern population was about six times greater than that in

the reverse direction. In contrast, the STRUCTURE analyses showed heterogeneous clustering in the northern population, indicating more frequent migration from the southern to northern population. Migration rates estimated by BAYESASS may be biased when migrants are largely from a small population to a large population, but the total proportion of migrants remains small in the recipient population (Wilson and Rannala 2003). In addition, estimated rates may not be reliable when populations are not sufficiently differentiated (Wilson and Rannala 2003).

Gene flow can be facilitated by both natural migration and translocation. For organisms with limited vagility, human translocation will easily surpass the distances that can be traveled by natural vagility. Varying degree of natural migration and human translocation of the tortoises may contribute to the complex population structure. *Gopherus berlandieri* may exhibit such a problem as anecdotal evidence suggests that tortoise translocations are common. Among the detected migrants, actual translocation is quite likely for at least one tortoise that was sampled from San Antonio, TX which is the northern extreme of the species range. Human mediated release was also addressed in studies of other testudinidae species (e.g. Schwartz and Karl 2005; Paquette et al. 2007). The impacts of translocated tortoises on homogenizing genetic differences between populations cannot be ignored (Schwartz and Karl 2005). However, quantifying its relative extent compared to natural migration is difficult except when tortoises were found in unusual places such as unsuitable habitats and outside the predicted species range.

Conservation of Gopherus berlandieri

Sound management strategies require understanding of cohesive forces, whether ecological or evolutionary, that preserve the integrity of a population. Populations may deserve ESU or MU status when significant genetic differentiation is observed. Defining conservation units can be difficult when populations are weakly differentiated, and the mechanism of migration is complicated by translocation. Further evidence from ecological and genetic data will be required in order to determine whether the northern and southern populations of *G. berlandieri* should be managed as MU. The two populations appear to have been experiencing gene flow in recent history, and this gene flow may be maintaining genetic variation in both populations. Edwards et al. (2004) pointed out the importance of migration that keeps the populations from declining especially when ongoing migration is disturbed by anthropogenic habitat modification. Translocation of the tortoises should be minimized in the management strategy for *G. berlandieri* not only to maintain the genetic differences observed between the populations, but also to avoid the potential spread of disease such as URTD. On the other hand, given continuing habitat alteration and fragmentation in southern Texas, the connectivity between the populations should not be ignored to assure the long-term persistence of *G. berlandieri*. Identifying inhospitable landscape features that constitute barriers to tortoise movement, such as highways, residential areas, and cropland, may help to locate the critical anthropogenic barriers to tortoise dispersal allowing focused management efforts.

REFERENCES

Agassiz L (1857) Contribution to the natural history of the United States of America Little, Brown and Co., Boston.

Aslan AM, Blum MD (1999) Contrasting styles of Holocene avulsion, Texas Gulf Coastal Plain, USA. In: Fluvial Sedimentology VI (eds. Smith ND and Rogers J), Spec Pubs Int Ass Sediment 28:193-209.

Auffenberg W, Weaver WG (1969) *Gopherus berlandieri* in southeastern Texas. Bull Fla State Mus Biol Sci 13:141-203.

Avise JC (1989) A role for molecular genetics in the recognition and conservation of endangered species. Trends Ecol Evol 4:279-281.

Beheregaray LB, Ciofi C, Caccone A, Gibbs JP, Powell JR (2003) Genetic divergence, phylogeography and conservation units of giant tortoises from Santa Cruz and Pinzon, Galapagos Islands. Cons Gen 4:31-46.

Britten HB, Riddle BR, Brussard PF, Marlow R, Lee TE (1997) Genetic delineation of management units for the desert tortoise, *Gopherus agassizii*, in Northeastern Mojave Desert. Copeia 3:523-530.

Brookfield JFY (1996) A simple new method for estimating null allele frequency from heterozygote deficiency. Mol Ecol 5:453-455.

Bury RB, Smith EL (1986) Aspects of the ecology and management of the tortoise *Gopherus berlandieri* at Laguna Atascosa, Texas. Southwest Nat 31:387-394.

Caccone A, Gentile G, Gibbs JP, Fritts TH, Snell HL, Betts J, Powell JR (2002) Phylogeography and history of giant Galapagos tortoises. Evolution 56:2052-2066.

Ciofi C, Milinkovitch MC, Gibbs JP, Caccone A, Powell JR (2002) Microsatellite analysis of genetic divergence among populations of giant Galapagos tortoises. Mol Ecol 11:2265-2283.

Congdon JD, Dunham AE, Sels RCV (1993) Delayed sexual maturity and demographics of Blanding turtles (*Emydoidea blandingii*) - implications for conservation and management of long-lived organisms. Conserv Biol 7:826-833.

Cunningham J, Baard EHW, Harley EH, O'Ryan C (2002) Investigation of genetic diversity in fragmented geometric tortoise (*Psammobates geometricus*) populations. Cons Gen 3:215-223.

Daub GJ, Boothroyd JC (1978) Stratigraphic interpretation of possible paleostream channel of ancient Nueces River, south Texas: Abstract. AAPG Bull 62.

de Thoisy B, Hrbek T, Farias IP, Vasconcelos WR, Lavergne A (2006) Genetic structure, population dynamics, and conservation of Black caiman (*Melanosuchus niger*). Biol Conserv 133:474-482.

Edwards T, Goldberg CS, Kaplan ME, Schwalbe CR, Swann DE (2003) PCR primers for microsatellite loci in the desert tortoise (*Gopherus agassizii*, Testudinidae). Mol Ecol Notes 3:589-591

Edwards T, Schwalbe CR, Swann DE, Goldberg CS (2004) Implications of anthropogenic landscape change on inter-population movements of the desert tortoise (*Gopherus agassizii*). Cons Gen 5:485-499.

Engstrom TN, Shaffer HB, McCord WP (2002) Phylogenetic diversity of endangered and critically endangered southeast Asian softshell turtles (Trionychidae: *Chitra*). Biol Conserv 104:173-179.

Excoffier LG, Schneider S (2005) Arelquin ver. 3.0: an integrated software package for population genetics data analysis. Evol Bioinform Online 1:47-50.

Fitzsimmons NN, Moritz C, Moore SS (1995) Conservation and dynamics of microsatellite loci over 300 million years of marine turtle evolution. Mol Biol Evol 12:432-440.

Frankel OH, Soulé ME (1981) Conservation and evolution. Cambridge University Press, Cambridge.

Frankham R (1996) Relationship of genetic variation to population size in wildlife. Conserv Biol 10:1500-1508.

Frankham R, Ballou JD, Briscoe DA (2002) Introduction to conservation genetics. Cambridge University Press, Cambridge.

Franklin IA (1980) Evolutionary change in small populations. In: Conservation biology: an evolutionary-ecological perspective (eds. Soule ME, Wilcox BA), pp. 135-184. Sinauer Associates, Sunderland, Massachusetts.

Fritz U, Cadi A, Cheylan M, Coic C, Detaint M, Olivier A, Rosecchi E, Guicking D, Lenk P, Joger U, Wink M (2005) Distribution of mtDNA haplotypes (cyt b) of *Emys orbicularis* in France and implications for postglacial recolonization. Amphibia-Reptilia 26:231-238.

Funk VA, Sakai AK, Richardson K (2002) Biodiversity: the interface between systematics and conservation. Syst Biol 51:235-237.

Gaggiotti OE, Lange O, Rassmann K, Gliddon C (1999) A comparison of two indirect methods for estimating average levels of gene flow using microsatellite data. Mol Ecol 8:1513-1520.

Gibbs JP, Amato GD (2000) Genetics and demography in turtle conservation. In: Turtle Conservation (ed. Klemens MW), pp. 207-217. Smithsonian Institution Press, Washington D. C.

Goodman SJ (1997) R-ST Calc: a collection of computer programs for calculating estimates of genetic differentiation from microsatellite data and determining their significance. Mol Ecol 6:881-885.

Goudet J (1995) FSTAT (Version 1.2): a computer program to calculate *F*-statistics. J Hered 86:485-486.

Groombridge M (1982) The IUCN Amphibia-Reptilia Red Data Book Part 1: Testudines, Crocodylia, Rynchocephalia. p. 426. IUCN, Gland, Switzerland.

Guillot G, Santos F, Estoup A (2008) Analysing georeferenced population genetics data with Geneland: a new algorithm to deal with null alleles and a friendly graphical user interface. Bioinformatics 24:1406-1407.

Guo SW, Thompson EA (1992) A Monte-Carlo method for combined segregation and linkage analysis. Am J of Hum Genet 51:1111-1126.

Hutter CM, Schug MD, Aquadro CF (1998) Microsatellite variation in *Drosophila melanogaster* and *Drosophila simulans*: a reciprocal test of the ascertainment bias hypothesis. Mol Biol Evol 15:1620-1636.

Judd FW, Rose FR (2000) Conservation status of the Texas tortoise *Gopherus berlandieri*. Occas Pap Mus Tex Tech Univ 196.

Kayser M, Vowles EJ, Kappei D, Amos W (2006) Microsatellite length differences between humans and chimpanzees at autosomal loci are not found at equivalent haploid Y chromosomal loci. Genetics 173:2179-2186

Kazmaier RT, Hellgren EC, Ruthven DC (2001a) Habitat selection by the Texas tortoise in a managed thornscrub ecosystem. J Wildl Manag 65:653-660.

Kazmaier RT, Hellgren EC, Ruthven DC, Synatzske DR (2001b) Effects of grazing on the demography and growth of the Texas tortoise. Conserv Biol 15:1091-1101.

Kazmaier RT, Hellgren EC, Synatzske DR, Rutledge JC (2001c) Mark recapture analysis of population parameters in a Texas tortoise (*Gopherus berlandieri*) population in southern Texas. J Herpetol 35:410-417.

Lamb T, Lydeard C (1994) A molecular phylogeny of the gopher tortoises, with comments on familial relationships within the Testudines. Mol Phylogenet Evol 3:283-291.

Lamb T, Avise JC, Gibbons JW (1989) Phylogeographic patterns in mitochondrial DNA of the desert tortoise (*Xerobates agassizi*), and evolutionary relationships among the North American gopher tortoises. Evolution 43:76-87.

Lenk P, Fritz U, Joger U, Wink M (1999) Mitochondrial phylogeography of the European pond turtle, *Emys orbicularis* (Linnaeus 1758). Mol Ecol 8:1911-1922

Leuteritz TEJ, Lamb T, Limberaza JC (2005) Distribution, status, and conservation of radiated tortoises (*Geochelone radiata*) in Madagascar. Biol Conserv 124:451-461.

McLuckie AM, Lamb T, Schwalbe CR, McCord RD (1999) Genetic and morphometric assessment of an unusual tortoise (*Gopherus agassizii*) population in the Black Mountains of Arizona. J Herpetol 33:36-44.

Mockford SW, Herman TB, Snyder M, Wright JM (2007) Conservation genetics of Blanding's turtle and its application in the identification of evolutionarily significant units. Cons Gen 8:209-219.

Mockford SW, McEachern L, Herman TB, Synder M, Wright JM (2005) Population genetic structure of a disjunct population of Blanding's turtle (*Emydoidea blandingii*) in Nova Scotia, Canada. Biol Conserv 123:373-380.

Moritz C (1994) Defining Evolutionarily-Significant-Units for Conservation. Trends Ecol Evol 9: 373-375.

Moritz C (1999) Conservation units and translocations: strategies for conserving evolutionary processes. Hereditas 130:217-228.

Moritz C (2002) Strategies to protect biological diversity and the evolutionary processes that sustain it. Syst Biol 51:238-254.

Murphy RW, Berry KH, Edwards T, McLuckie AM (2007) A genetic assessment of the recovery units for the Mojave population of the desert tortoise, *Gopherus agassizii*. Chelonian Conserv Biol 6:229-251.

Osentoski MF, Lamb T (1995) Intraspecific phylogeography of the gopher tortoise, *Gopherus polyphemus*: RFLP analysis of amplified mtDNA segments. Mol Ecol 4:709-718.

Paetkau D, Slade R, Burden M, Estoup A (2004) Genetic assignment methods for the direct, real time estimation of migration rate: a simulation based exploration of accuracy and power. Mol Ecol 13:55-65.

Paetkau, D, Strobeck C (1995) The molecular basis and evolutionary history of a microsatellite null allele in bears. Mol Ecol 4:519-520.

Paquette SR, Behncke SM, O'Brien SH, Brenneman RA, Louis EE, Lapointe FJ (2007) Riverbeds demarcate distinct conservation units of the radiated tortoise (*Geochelone radiata*) in southern Madagascar. Cons Gen 8:797-807.

Piry S, Alapetite A, Cornuet JM, Paetkau D, Baudouin L, Estoup A (2004) GENECLASS2: a software for genetic assignment and first-generation migrant detection. J Hered 95:536-539.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945-959

Rainboth WJ, Buth DG, Turner FB (1989) Allozyme variation in Mojave populations of the desert tortoise, *Gopherus agassizi*. Copeia 1989:115-123.

Rannala B, Mountain JL (1997) Detecting immigration by using multilocus genotypes. Proc Nat Acad Sci U.S.A 94:9197-9201.

Raymond M, Rousset F (1995) An exact test for population differentiation. Evolution 49:1280-1283.

Rice WR (1989) Analyzing tables of statistical tests. Evolution 43:223-225.

Rose FL, Judd FW (1982) Biology and status of Berlandier's tortoise (*Gopherus berlandieri*). In: North American tortoises: conservation and ecology (ed. Bury RB), U.S.D.I. Fish and Wildlife Service Wildlife Research Report 12.

Rose FL, Judd FW (1989) *Gopherus berlandieri*. In: The conservation biology of tortoises (eds. Swingland IR, Klemens MW), Occasional paper of the IUCN Species Survival Commission 5, IUCN, Gland, Switzerland.

Rousset F (2008) GENEPOP ' 007: a complete re-implementation of the GENEPOP software for Windows and Linux. Mol Ecol Resources 8:103-106.

Schwartz TS, Karl SA (2005) Population and conservation genetics of the gopher tortoise (*Gopherus polyphemus*). Cons Gen 6:917-928.

Schwartz TS, Osentoski M, Lamb T, Karl SA (2003) Microsatellite loci for the North American tortoises (genus *Gopherus*) and their applicability to other turtle species. Mol Ecol Notes 3:283-286.

Sites JWJ, FitzSimmons NN, da Silva NJ Jr, Cantarelli VH (1999) Conservation genetics of the giant Amazon river turtle (*Podocnemis expansa*; Pelomedusidae) - inferences from two classes of molecular markers. Chelonian Conserv Biol 3:454-463.

Slatkin M (1995) A measure of population subdivision based on microsatellite allele frequencies. Genetics 139:457-462.

Slatkin M, Barton NH (1989) A comparison of 3 indirect methods for estimating average levels of gene flow. Evolution 43:1349-1368.

Souza FL, Cunha AF, Oliveira MA, Pereira GAG, dos Reis SF (2002) Estimating dispersal and gene flow in the neotropical freshwater turtle *Hydromedusa maximiliani* (Chelidae) by combining ecological and genetic methods. Genet Mol Biol 25:151-155.

Sumner J, Jessop T, Paetkau D, Moritz C (2004) Limited effect of anthropogenic habitat fragmentation on molecular diversity in a rain forest skink, *Gnypetoscincus queenslandiae*. Mol Ecol 13:259-269.

Tessier N, Paquette S, Lapointe FJ (2005) Conservation genetics of the wood turtle (*Glyptemys insculpta*) in Quebec, Canada. Can J Zool 83:765-772.

van Oosterhout C, Hutchinson WF, Wills DP, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535-538.

Vogler AP, Desalle R (1994) Diagnosing units of conservation management. Conserv Biol 8:354-363.

Vowles EJ, Amos W (2006) Quantifying ascertainment bias and species-specific length differences in human and chimpanzee microsatellites using genome sequences. Mol Biol Evol 23:598-607.

Walker D, Avise JC (1998) Principles of phylogeography as illustrated by freshwater and terrestrial turtles in the southeastern United States. Ann Rev Ecol Syst 29:23-58.

Waples RS (1995) Evolutionary significant units and the conservation of biological diversity under the Endangered Species Act. In: Evolution and the aquatic ecosystem: defining unique units in population conservation. Symposium 17 (ed. Nielsen JL), pp. 8-27. American Fisheries Society Bethesda, Maryland.

Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population structure. Evolution 38:1358-1370.

Wilson GA, Rannala B (2003) Bayesian inference of recent migration rates using multilocus genotypes. Genetics 163:1177-1191.

Wright S (1969) Evolution and genetics of populations: the theory of gene frequencies, Vol. 2. University of Chicago Press, Chicago.

VITA

Akiko Fujii was born in Tokyo, Japan, on February 14, 1983. After completing her work at high school, Japan in 2001, she entered Texas State University-San Marcos. She received the degree of Bachelor of Science from Texas State in December 2005. In September 2006, she entered the Graduate College of Texas State University-San Marcos.

Permanent Address: 2-28-4 Mizue

Edogawa-ku, Tokyo 132-0011, Japan

This thesis was typed by Akiko Fujii.