DESERT TORTOISE CONSERVATION GENETICS

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DEDICATION

This work is dedicated to my grandmother, Nellie Chafey, who saw the intrinsic value in all creatures, great and small.

TABLE OF CONTENTS

LIST OF TABLES.....7

LIST OF FIGURES.....9

ABSTRACT.....10

CHAPTER 1 - COMPARISON OF PHYLOGEOGRAPHIC PATTERNS BETWEEN MOJAVE AND SONORAN POPULATIONS OF THE DESERT TORTOISE, *GOPHERUS AGASSIZII*.....11

ABSTRACT.....11

INTRODUCTION.....12

MATERIALS AND METHODS.....15

RESULTS.....21

DISCUSSION.....27

CHAPTER 2 - IMPLICATIONS OF ANTHROPOGENIC LANDSCAPE CHANGE ON INTER-POPULATION MOVEMENTS OF SONORAN DESERT TORTOISES USING MICROSATELLITE DNA ANALYSIS.....34

ABSTRACT.....34

INTRODUCTION.....35

MATERIALS AND METHODS.....37

Data Collection.....37

Molecular Techniques.....37

Molecular Analysis.....39

TABLE OF CONTENTS – *Continued*

Radiotelemetry and Spatial Analysis.....41

RESULTS.....43

Microsatellite DNA Results.....43

Population Differentiation.....50

Within-Population Structure.....55

DISCUSSION.....57

Phylogeography.....57

Gene Flow.....58

Movement Barriers.....60

Population Viability.....61

Management and Conservation Implications.....62

APPENDIX A - PCR PRIMERS FOR MICROSATELLITE LOCI IN DESERT TORTOISE (*GOPHERUS AGASSIZII*, TESTUDINIDAE).....65

APPENDIX B – NATURAY HISTORY NOTE. *GOPHERUS AGASSIZII* (DESERT TORTOISE). MOVEMENT.....73

LITERATURE CITED.....78

LIST OF TABLES

TABLE 1.1 Descriptive statistics for mtDNA sequences from Mojave and Sonoran populations of *G. agassizii*.....22

TABLE 1.2 Haplotype distribution of desert tortoise study sites from Mojave and Sonoran populations.....24

TABLE 1.3 Results of the nested cladistic analysis of geographical distance for mtDNA haplotypes of Mojave and Sonoran populations of *G. agassizii*.....26

TABLE 2.1 Diversity indices for 7 microsatellite loci in 9 populations of desert tortoises.....44

TABLE 2.2 Kolmogorov-Smirnov test of difference between allele frequency distributions between all population pairs and between each population and the total sample for 4 loci.....47

TABLE 2.3 Distribution of unique and private alleles in 9 populations of desert tortoises in southeastern Arizona 48

TABLE 2.4 Probability of excess or deficit of heterozygosity across 7 desert tortoise microsatellite loci.....49

TABLE 2.5 Average percentage of intermediate allelic states (M) for 7 microsatellite loci in 9 desert tortoise populations in southern Arizona.....49

TABLE 2.6 Slatkin's $\hat{M}$ (absolute number of migrants exchanged per generation between populations) calculated among 9 desert tortoise populations in southern Arizona.....51

LIST OF TABLES - *Continued*

TABLE 2.7 Population pairwise F_{st} values and geographic distances among 9 desert tortoise populations in southern Arizona.....51

TABLE 2.8 Radiotelemetry data for 34 tortoises from Saguaro National Park, Rincon Mountain District.....56

TABLE A.1 Forward (F) and reverse (R) primers, repeat motif, annealing temperature (T_a), and $MgCl_2$ concentrations for PCR amplification of 9 microsatellite loci the desert tortoise.....71

TABLE A.2 Hardy-Weinberg proportions and fixation indices for microsatellite loci in Sonoran and Mojave populations of desert tortoises.....72

LIST OF FIGURES

FIGURE 1.1 Location of and number of tortoises sampled from 4 desert tortoise study sites from the Western Mojave Recovery Unit of the Mojave population in California.....16

FIGURE 1.2 Location and number of tortoises sampled from 8 study sites from the Sonoran population in southern Arizona.....17

FIGURE 1.3 Evolutionary network of desert tortoise haplotypes from Mojave and Sonoran populations.....23

FIGURE 2.1 Location and number of tortoises sampled from 9 mountain ranges containing desert tortoise populations in southern Arizona.....38

FIGURE 2.2 Two study sites within the desert tortoise population at Saguaro National Park (Rincon Mountain District), east of Tucson, Arizona.....42

FIGURE 2.3 Genetic distance (pairwise F_{st}) vs. geographic distance (km) among 9 desert tortoise populations in the Sonoran Desert.....52

FIGURE 2.4 Distribution of interstates and major roads in southern Arizona that may obstruct tortoise movement between populations.....53

FIGURE 2.5 Distribution of urbanized areas in southern Arizona that may obstruct tortoise movement between populations.....54

ABSTRACT

Managing for the long-term survival of a species requires an understanding of its population genetics. As habitat becomes increasingly fragmented, the ability of animals to move among different populations and share genetic material can become hindered. The desert tortoise, *Gopherus agassizii,* inhabits the Mojave and Sonoran deserts of North America. Desert tortoises face many threats to their continued survival, including habitat loss and fragmentation. I used microsatellite DNA markers in Sonoran desert tortoises and found little genetic differentiation among populations, indicating that gene flow occurred historically among disjunct populations. I used radiotelemetry to document an inter-population movement made by a desert tortoise and conclude that the urban topography of the modern landscape makes movement between localities virtually impossible. Using mitochondrial sequences, I compared the genetic structure of desert tortoise populations in the Sonoran and Mojave deserts and found that despite differences in habitat selection and landscape heterogeneity, populations in both deserts exhibit similar patterns of population genetic structure. My data indicate that effective population sizes of desert tortoises are likely small and that dispersal events probably play an important role in the long-term maintenance of populations. I also assess the potential long-term effects of anthropogenic landscape change on desert tortoise population viability. Understanding the historical connectivity between and within the Mojave and Sonoran populations of desert tortoises will help facilitate the conservation of this species.

CHAPTER 1 - COMPARISON OF PHYLOGEOGRAPHIC PATTERNS BETWEEN MOJAVE AND SONORAN POPULATIONS OF THE DESERT TORTOISE, *GOPHERUS AGASSIZII*

ABSTRACT

The desert tortoise, *Gopherus agassizii*, is native to the North American desert southwest and is recognized as having a Sonoran population and a threatened Mojave population. Tortoises from these two populations are separated by the Colorado River and differ in morphology, mtDNA, reproductive ecology, and habitat selection. I used 981 base pairs of mitochondrial DNA sequence data to estimate genetic variation between and within the Mojave and Sonoran populations. Within each population, I investigated genetic structure among study sites within relatively small geographic areas (<100 km radius). From the Mojave population I sampled 36 individuals from 4 study sites in the Western Mojave Recovery Unit in California and from the Sonoran population I sampled 40 individuals from 8 study sites in southern Arizona. My results are consistent with previous studies that have shown that Mojave and Sonoran populations exhibit strongly divergent maternal lineages, and that gene flow has not occurred between the two deserts for approximately 6 million years. Tortoises at sites in the Western Mojave Recovery Unit, where habitat is fairly continuous, exhibit a population genetic structure characteristic of isolation by distance. Although study sites in southern Arizona appear more isolated by landscape heterogeneity, I also found little genetic differentiation among the majority of study sites, indicating that gene flow

occurred, until the recent proliferation of anthropogenic barriers, among these Sonoran population study sites as well. My results suggest that geographic features are not necessarily a good predictor of what constitutes an evolutionarily significant unit for the desert tortoise. Understanding the landscape connectivity within and between the Mojave and Sonoran populations of desert tortoises will help facilitate the conservation of this species.

INTRODUCTION

The desert tortoise, *Gopherus agassizii*, is native to the southwestern deserts of North America and is recognized as having distinct Mojave and Sonoran populations. The Mojave population (defined as all tortoises north and west of the Colorado River) was federally listed as threatened by the U.S. Fish and Wildlife Service in 1990 (USFWS 1990). The Sonoran population (all tortoises south and east of the Colorado River) is not federally listed but is currently considered a Species of Special Concern by the Arizona Game and Fish Department (AGFD 1996). Threats to desert tortoises include habitat loss from human development, habitat alteration by off-road vehicles and grazing, habitat fragmentation, illegal collecting, road mortality, and disease (USFWS 1994, AIDTT 1996, AIDTT 2000). In 1994, the USFWS established six recovery units within the Mojave population (USFWS 1994). Recovery units were identified based on evolutionarily significant units (ESUs) using published and unpublished data on genetic variability, morphology, and behavior patterns of populations as well as ecosystem types (Berry 1997). Genetic studies have contributed to the designation of ESUs by helping to define demes of desert tortoises that appear to be on independent evolutionary

trajectories. However, genetic structure within ESUs is still insufficiently understood. Investigation into the patterns of gene flow that occur within ESUs and whether those patterns differ among ESUs relative to geographic features, can help facilitate conservation efforts for this species (Berry et al. 2002).

The Mojave and Sonoran populations differ in morphology, seasonal activity, reproductive ecology, habitat selection, and mitochondrial DNA divergence (Lamb et al. 1989, Germano 1992, Germano 1993, Germano 1994a, Germano 1994b, Germano et al. 1994, McLuckie et al. 1999, Van Devender 2002). I sampled desert tortoises from the Mojave population in the Western Mojave Recovery Unit (western Mojave) in California and from the Sonoran population in southern Arizona. These areas represent two very different types of desert tortoise habitat. Tortoises in the western Mojave primarily occur in valleys, alluvial fans, and bajadas in Mojave desertscrub (creosote bush, *Larrea tridentata*; white bursage *Ambrosia dumosa*; USFWS 1994). In this environment, tortoises excavate deep burrows in washes and surrounding desert flats. In contrast, tortoises in southern Arizona generally inhabit areas of rocky foothills associated with leguminous trees and mixed cactus (foothill paloverde, *Parkinsonia microphylla*; saguaro, *Carnegiea gigantea*; prickly pear and cholla, *Opuntia* spp.) characteristic of Arizona Upland Sonoran desertscrub (Turner and Brown 1982). Tortoises in southern Arizona den in rocky outcrops and caliche caves in habitat patches separated by low desert valleys (Barrett 1990). Tortoises in the western Mojave do not appear to have the same shelter site requirements as those in southern Arizona (Germano et al. 1994), and this may contribute to the Mojave population reaching much higher densities in the past

and covering larger geographic areas (Van Devender 2002). The widespread and continuous tortoise habitat in the western Mojave suggests a greater potential there for gene flow than in the Tucson area (Van Devender 2002).

In this study, I used mtDNA sequence data to estimate genetic variation between and within the Mojave and Sonoran populations of the desert tortoise. Previous desert tortoise genetic studies have examined intraspecific phylogeny, genetic variability, and population structure of desert tortoises using allozyme and restriction fragment surveys of mtDNA (e.g., Lamb et al 1989, Rainboth et al. 1989, Britten et al. 1997, McLuckie et al. 1999). The only studies to date that have used mtDNA sequencing have examined phylogenetic relationships over large geographic areas (Lamb and Lydeard 1994, Osentoski and Lamb 1995). Here, I examine genetic structure of desert tortoise populations on a fine geographic scale and compare patterns of gene flow across continuous and divided patches of habitat. Because anthropogenic landscape changes are relatively recent events (Lovich and Bainbridge 1999) with respect to the long generation time of the desert tortoise (estimated at 25 years; USFWS 1994), my estimates of population structure based on mtDNA sequence analysis are sensitive to past patterns of gene flow. Knowledge of the evolutionary history and genetic structure of desert tortoise populations within ESUs can provide data to design translocation strategies, determine the necessity of migration corridors, and set conservation priorities specific to each recovery unit.

MATERIALS AND METHODS

From the Mojave population, I sampled 36 desert tortoises from 4 study sites northeast and southeast of Barstow in the Western Mojave Recovery Unit in San Bernardino County, California (Figure 1.1). The maximum distance between any 2 sites was 157 km (SH - FIC) and the minimum distance 52 km (SH - OR). In Arizona, I sampled desert tortoises from 7 study sites in Pima and Pinal counties in the Tucson vicinity and from 1 site northeast of Phoenix in Maricopa County for a total of 40 tortoises from the Sonoran population (Figure 2.2). The maximum distance between the 7 Tucson area sites was 128 km (SNP – FL) and the minimum distance 16 km (DP - PM). The single site in Maricopa County (Sugarloaf) is 59 km north of the closest study site in the Tucson area.

I hand-captured and processed tortoises between March-October 2000 and 2001 using standard methods and following U.S. Fish and Wildlife Service and Arizona Interagency Desert Tortoise Team guidelines (Averill-Murray 2000, Berry and Christopher 2001). I collected less than 1cc of blood by brachial venipuncture and stored it on ice in EDTA or lithium heparin buffer.

I isolated total genomic DNA from red blood cells by overnight lysis with proteinase K at 55 °C, followed by a phenol/chloroform extraction and isopropanol/sodium acetate precipitation (Goldberg et al. 2003). I resuspended the purified DNA in low TE (10mMTris-pH 8.0, 0.1mM EDTA) and diluted to 5 ng/μl. I used polymerase chain reaction (PCR) to amplify the ND3, arginine tRNA, ND4L, and

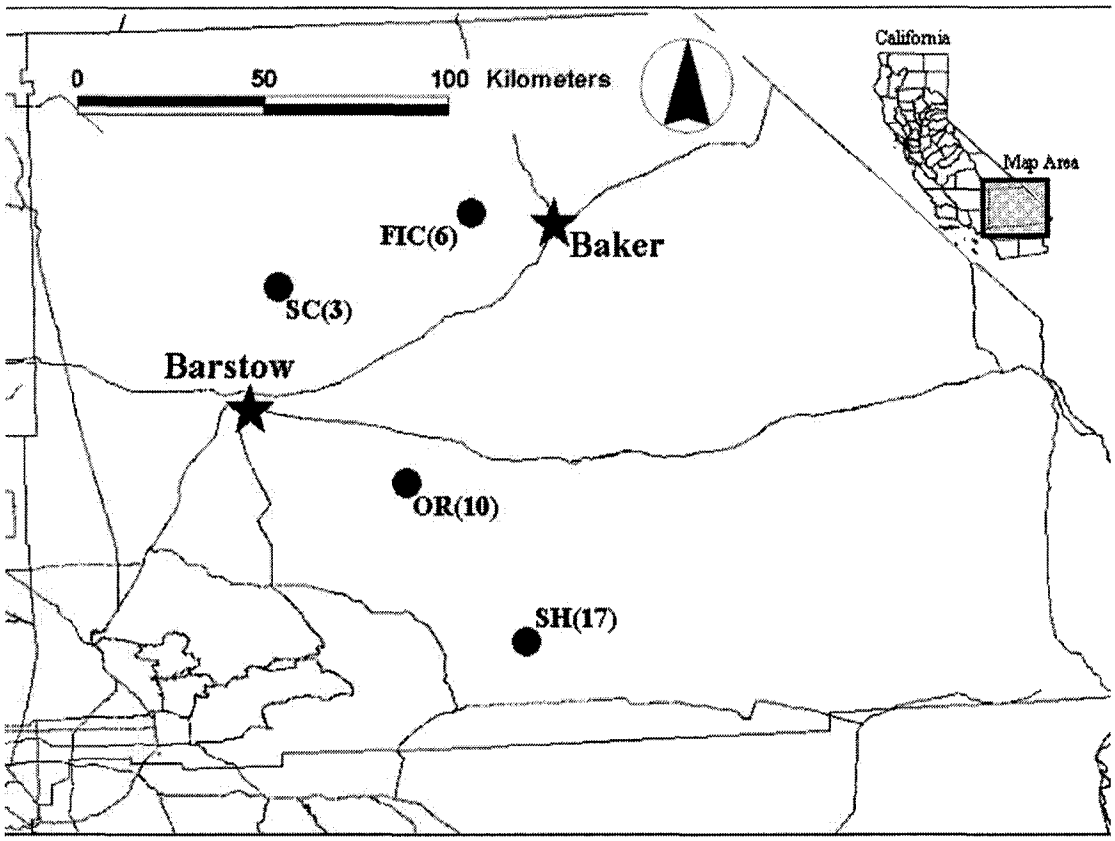

FIGURE 1.1 Location of and number of tortoises sampled from 4 desert tortoise study sites from the Western Mojave Recovery Unit of the Mojave population in California: Fort Irwin Control (FIC), Superior-Cronese (SC), Ord-Rodman (OR), and the Sand Hill Training Area (SH) of the Marine Corps Air Ground Combat Center.

part of the ND4 genes of the tortoise mitochondrial genome (1500 bp) using the primers Nap2 and New Gly (Arévalo et al. 1994). These primers have been used successfully in previous desert tortoise research (Britten et al. 1997). I performed PCR in 50-µl volumes with 10 mM Tris-HCl pH 8.3, 3.5 mM $MgCl_2$, 50 mM KCl, 2 units of Taq Polymerase (Sigma-Aldrich), 0.2 mM of each dNTP, and 20 pmol of each primer. I cycled the PCR

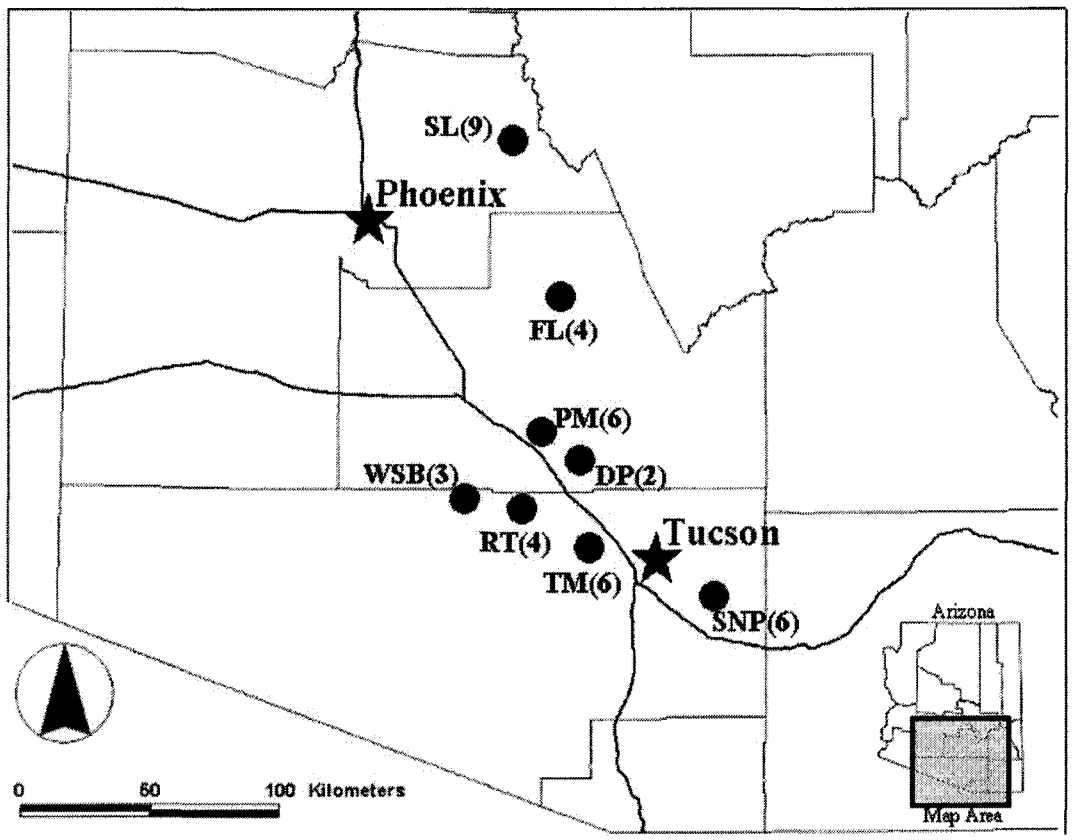

FIGURE 1.2 Location of and number of tortoises sampled from 8 study sites from the Sonoran population in southern Arizona: Desert Peak (DP), Florence Military Reservation (FL), Picacho Mountains (PM), Ragged Top (RT), Rincon Mountains (Saguaro National Park; SNP), Sugarloaf (SL), Tucson Mountains (TM), and West Silver Bell Mountains (WSB).

reactions in a Mastercycler Gradient (Eppendorf) with a 5-minute 94 °C initial denaturation, followed by 35 cycles of 1 min at 94 °C, 2 min at 50.6 °C, 2 min at 72 °C,and a final 6 min extension at 72 °C. I purified the PCR products using the QIAquick PCR purification kit (Qiagen) and sequenced them on an ABI Prism® 3700 DNA Analyzer (PE Biosystems). I used Oligo Primer Analysis Software version 6.68

(Molecular Biology Insights, Inc.) to design internal primers (Nap2IN 5'AGGCGGTCAATAATGCTAATC3' and NewGIN 5'TAATAAAACCAGACA ATGAAAAAC3'), and sequenced a 981 base pair portion of the ND3/ND4 amplicon for analysis. I aligned and evaluated concatenated mtDNA sequences using Sequence Navigator version 1.0.1 (Applied Biosystems, Inc.).

I estimated nucleotide diversity and polymorphism for each population and for the entire sample using ARLEQUIN version 2.0 (Schneider et al. 2000). Nucleotide diversity (π) is a measure of the amount of genetic variability in a sample. The parameter θ (nucleotide polymorphism) is associated with effective population size (N_e). For haploid data (mtDNA), $\theta = 2N_e u$, where u is the mutation rate (Watterson 1975). I tested for selective neutrality using Tajima's D (Tajima 1989), which examines the relationship between π and θ and is sensitive to processes that reduce or increase heterozygosity in a population. Under neutrality, in a panmictic, infinitely large population, π and θ should be equal and therefore D is zero. A positive value for D indicates an excess of heterozygosity, resulting from processes such as a reduction in population size, long-term population subdivision, or balancing selection. A negative value of D indicates a reduction of heterozygosity that could be the result of an expansion event, positive directional selection, or the presence of weakly deleterious alleles (Tajima 1989). I used ARLEQUIN to compute the mismatch distribution for each population. The mismatch distribution is the distribution of the observed differences between all pairs of haplotypes (Rogers and Harpending 1992). The mismatch distribution is usually

multimodal in samples drawn from populations at demographic equilibrium and is unimodal in populations having experienced demographic expansion. I estimated sequence divergence between haplotypes in ARLEQUIN using the two-parameter method of Kimura (1980). I used GENETREE (version 9.0 distributed by R.C. Griffiths, University of Oxford, Oxford) to estimate coalescence times for each of the populations and for the entire sample. The coalescent is the time to which all alleles trace back to the most recent common ancestor (TMRCA). GENETREE uses a Markov chain simulation to produce likelihood estimates under the infinite sites model (Griffiths and Tavaré 1994). I estimated the mutation rate (u) by calculating sequence divergence for the entire sample, divided by 2, and then divided by 5.5 x10^6, the estimated time of divergence between the Mojave and Sonoran populations (Avise et al. 1992; Osentoski and Lamb 1995). I estimated the effective population size (N_e) by dividing the estimate of θ by 2, and then again by u. I then multiplied the GENETREE TMRCA parameter by N_e to estimate the time to coalescence. I estimated the degree of population differentiation within and among the Mojave and Sonoran populations using AMOVA (analysis of molecular variance) in ARLEQUIN. AMOVA examines the variance in gene frequencies while also taking into account the number of mutations between haplotypes (Excoffier et al. 1992). ARLEQUIN uses Wright's F-coefficients to determine how genetic variation is partitioned among populations within a region and among individuals within populations (Wright 1951).

To distinguish patterns of recurrent gene flow from that of range expansion or recent common ancestry, I used the nested cladistic analysis (NCA) of the geographical

distribution of genetic haplotypes in GeoDis version 2.0 (Posada et al. 2000). This technique uses the haplotype tree to define a series of nested branches (clades), thereby allowing an evolutionary analysis of the spatial distribution of genetic variation, following the assumption that younger haplotypes will be more geographically restricted than ancestral types (Templeton 1998). The program GeoDis uses several tests to examine geographical association. First, an exact permutational contingency test is performed and a chi-square statistic is calculated. This NCA treats the sampling locations as categorical variables but does not incorporate any geographical distance information. GeoDis also performs a NCA using geographical distances. Two main statistics are calculated: the clade distance (D_c), which measures the geographical spread of a clade, and the nested clade distance (D_n), which measures how a clade is geographically distributed relative to other clades in the same higher-level nesting category. An interior-tip statistic is estimated within each nested category as the average interior distance minus the average tip distance. Templeton (1998) provides a key for the interpretation of these results in biological terms. I used straight-line distance as a measure of geographic distance because evidence suggests that long-distance movements of tortoises and other reptiles do not follow natural geographic forms but are essentially linear in nature (Barrett et al. 1990, King and Duvall 1990, Reinert and Rupert 1999, Appendix B).

RESULTS

I sequenced 981 base pairs of mtDNA from 76 tortoises (36 Mojave and 40 Sonoran). I observed 44 fixed differences in the mtDNA sequences between Mojave and Sonoran populations of desert tortoises. Divergence between these non-sympatric maternal lineages averaged 0.048. Tajima's D for the entire sample of 76 tortoises was significantly positive, a consequence of pooling populations with fixed differences (Table 1.1). Sequence divergence estimates between haplotypes within each population ranged from 0.001 to 0.002. In the sample of 36 individuals from 4 Mojave locations, I identified only 2 variable mtDNA sites that give rise to 3 haplotypes, each of which is characterized by a single base-pair difference from the Moj1 haplotype (Figure 1.3). All study sites from the western Mojave shared haplotype Moj1 (Table 1.2). One individual at Sand Hill had a unique haplotype (Moj2) and 2 individuals at the Ord-Rodman site shared a third haplotype (Moj3). Nucleotide diversity and polymorphism were both very low for the 36 western Mojave tortoises and Tajima's D was negative (Table 1.1). The comparison of differences between all pairs of haplotypes for both the western Mojave and the southern Arizona samples produced a unimodel, L-shaped distribution. Estimates of expansion time could not be calculated because the variance of the mismatch was smaller than the mean for these samples (Rogers 1995). Using these sequence data, the estimate of the mutation rate (u; 4.0×10^{-9} per base pair per year) was equal to that proposed by Avise and collaborators (1992) for other turtle species. TMRCA between the Mojave and Sonoran populations (Table 1.1) was also consistent with other studies

TABLE 1.1 Descriptive statistics for mtDNA sequences from Mojave and Sonoran populations of *G. agassizii*: nucleotide diversity (π), polymorphism (θ), Tajima's D, Fst, effective population size (N_e), estimated time to most recent common ancestor (TMRCA) in years. Tucson area does not include the Sugarloaf site. Parenthetic values are the standard deviation.

	n	π	θ (per gene)	Tajima's D	p	Fst	N_e	TMRCA
Mojave	36	0.00017 (0.00026)	0.4823 (0.4)	-1.28 (0.90)	0.05	0.041	60,287	98,268 (48,832)
Sonoran	40	0.00049 (0.00048)	0.9404 (0.5)	-1.17 (0.95)	0.12	0.459	117,550	170,448 (76,407)
Tucson area	31	0.00013 (0.00023)	0.5006 (0.4)	-1.51 (0.89)	0.06	-0.089	62,575	96,991 (48,182)
Sonoran and Mojave	76	0.02301 (0.01135)	10.2013 (2.9)	3.97 (0.91)	<0.001	0.996	1,275,162	5,955,007 (510,064)

(Avise et al. 1992, Osentoski and Lamb 1995, McLuckie et al. 1999). Coalescent times within regions were similar between the western Mojave and Tucson area samples (Table 1.1). The AMOVA detected little differentiation among sites in the western Mojave, suggesting that gene flow occurs or has occurred in the recent past (Table 1.1). In the sample of 40 individuals from 8 study sites in southern Arizona, I identified 4 variable

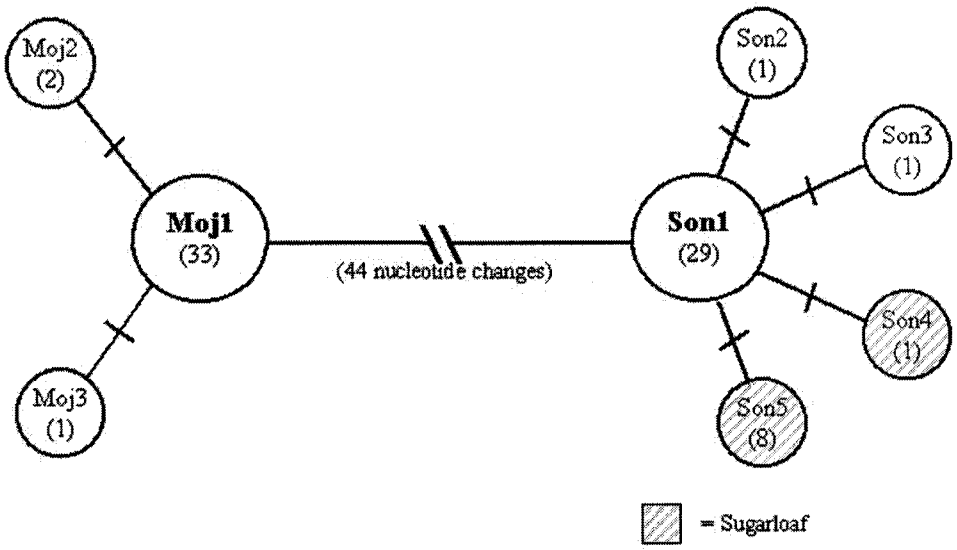

FIGURE 1.3. Network of desert tortoise haplotypes from Mojave and Sonoran populations. Each haplotype is characterized by a single base pair novel mutation. Parenthetic values indicate number of individuals exhibiting the haplotype.

mtDNA sites that give rise to 5 haplotypes, each of which is characterized by a single base pair difference from the Son1 haplotype (Figure 1.3). All sites, with the exception

of the most geographically distant site (Sugarloaf), shared haplotype Son1 (Table 1.2). One individual in the Picacho Mountains had a unique haplotype (Son2) and 1 individual in the Tucson Mountains had a unique haplotype (Son3). All of the individuals sequenced from Sugarloaf were characterized by 2 haplotypes unique to this site. One individual at Sugarloaf had haplotype Son4 and 8 individuals had haplotype Son5.

Nucleotide

TABLE 1.2 Haplotype distribution of desert tortoise study sites from Mojave and Sonoran populations.

		Mojave haplotypes		
Location	Sample size	Moj1	Moj2	Moj3
Sand Hill	17	16		1
Ord-Rodman	10	8	2	
Superior-Cronese	3	3		
Fort Irwin Control	6	6		
Total	36	33	2	1

		Sonoran haplotypes				
Location	Sample Size	Son1	Son2	Son3	Son4	Son5
Rincon Mountains	6	6				
Tucson Mountains	6	5		1		
Ragged Top	4	4				
West Silver Bells	3	3				
Desert Peak	2	2				
Picacho Mountains	6	5	1			
Florence	4	4				
Sugarloaf	9				1	8
Total	40	29	1	1	1	8

diversity and polymorphism were both very low for the southern Arizona sites and Tajima's D was negative but not significant (Table 1.1). The AMOVA showed considerable genetic differentiation among sites in southern Arizona due to the fact that Sugarloaf does not share haplotypes with the other 7 sites. However, when Sugarloaf

was removed from the analysis, there was no differentiation detected among the 7 Tucson area study sites (Table 1.1).

The NCA exact contingency test based on 1,000 permutations for the western Mojave samples generated an observed X^2 value that was not different from random (X^2 = 6.55, p = 0.376). However, when performed on the clade containing all 8 sites from southern Arizona, the observed X^2 generated by the NCA exact contingency test was significantly greater than expected (X^2 = 51.03, p<0.005), indicating that strong associations exist between clades and geographical location. Applying results of the NCA of geographical distance in Table 1.3 to the inference key developed by Templeton (1998), the chain of inference for the western Mojave data suggests restricted gene flow with isolation by distance. The inference chain for the southern Arizona data indicates that the sampling design is inadequate to discriminate between isolation by distance versus long distance dispersal. The X^2 from the NCA exact contingency test for the 7 Tucson area sites (not including Sugarloaf) was not significant (X^2 = 8.55, p = 1.0). The NCA of geographical distance run for the 7 Tucson area sites did not produce any significantly small of large distance measures (D_c, D_n, I-T). The chain of inference therefore concludes that there is no geographical association of haplotypes among sites in the Tucson area, a result of panmixia, inadequate geographical sampling, or small sample size.

TABLE 1.3. Results of the nested cladistic analysis of geographical distance for mtDNA haplotypes of Mojave and Sonoran populations of *G. agassizii*. Each desert population was defined as a higher-level clade. All tip haplotypes were one-step from the interior haplotypes. Clade distance (D_c) measures how geographically widespread are the individuals that bear a particular haplotype. Nested clade distance (D_n) measures how far individuals bearing a particular haplotype are from individuals bearing a different haplotype within the same clade. $(Int\text{-}Tip)_c$ and $(Int\text{-}Tip)_n$ gives the average differences between the interior haplotype and the tip haplotypes within the nested group for the clade distances and the nested clade distances, respectively.

Geographic distance analysis

Clade	type	distance	*prob. <=*	*prob. >=*
Moj1 (Interior)				
	D_c	77.056	0.979	0.105
	D_n	74.401	0.959	0.125
Moj2 (Tip)				
	D_c	0.000	0.327	1.000
	D_n	50.009	0.335	0.734
Moj3 (Tip)				
	D_c	0.000	1.000	1.000
	D_n	50.297	0.729	0.752
Son1 (Interior)				
	D_c	54.365	0.001	1.000
	D_n	72.849	0.001	0.999
Son2 (Tip)				
	D_c	0.000	1.000	1.000
	D_n	55.359	0.144	1.000
Son3 (Tip)				
	D_c	0.000	1.000	1.000
	D_n	65.721	0.544	0.624
Son3 (Tip)				
	D_c	0.000	1.000	1.000
	D_n	106.436	1.000	0.216
Son4 (Tip)				
	D_c	0.000	0.001	1.000
	D_n	116.930	1.000	0.001

Test of interior vs. tip clades

Clade	type	distance	*prob. <=*	*prob. >=*
Mojave				
	$(int.\text{-}tip)_c$	77.056	0.979	0.046
	$(int.\text{-}tip)_n$	24.296	0.920	0.105
Sonoran				
	$(int.\text{-}tip)_c$	54.365	0.969	0.031
	$(int.\text{-}tip)_n$	-32.874	0.001	1.000

DISCUSSION

The 0.048 sequence divergence I estimated between non-sympatric maternal lineages of the desert tortoise suggests that there have been long-term barriers to gene flow between the Mojave and Sonoran populations. In comparison, the average sequence divergence was 0.192 between these desert tortoise samples and a green seaturtle (*Chelonia mydas*; GenBank accession number AB012104). Using the same two-parameter method of Kimura (1980), Lamb and Lynard (1994) estimated the range of sequence divergence among species of the genus *Gopherus* to be between 0.031 (*G. flavomarginatus* vs. *G. polyphemus*) and 0.074 (*G. berlandieri* vs. *G. polyphemus*). Avise et al. (1992) proposed that turtle mitochondria have a slower rate of mtDNA evolution than that observed for mtDNA in birds and mammals. Using this slower rate (0.4%/Myr), these data support previous calculations that divergence between Mojave and Sonoran desert populations of desert tortoises coincides with the formation of the Bouse Embayment in the Lower Colorado River Valley between California and Arizona approximately 5 million years ago (Lamb et al. 1989, Avise et al. 1992, Osentoski and Lamb 1995). Although my samples were collected from the east and west extremes of the range of the desert tortoise, my results are consistent with those of previous studies (Lamb et al. 1989, Avise et al. 1992, Osentoski and Lamb 1995). However, my estimates of divergence are limited to the interpretation of mtDNA data. Nuclear gene flow has been documented in multiple species that also show substantial mtDNA divergence (R.W. Murphy, *pers. comm.*). In addition, McLuckie et al. (1999) demonstrated Mojave haplotypes, and therefore the potential for genetic exchange, at a single site east of the

Colorado River. Further research utilizing autosomal markers, especially along the boundary of the two populations, would help clarify the extent of this separation.

Survey data suggest that the Mojave population of the desert tortoise had much higher densities in the past than the Sonoran population (Berry and Medica 1995, Averill-Murray et al. 2002). However, from a genetic standpoint, both areas in this study demonstrate a history of maintaining small effective population size. The low degree of nucleotide diversity and polymorphism among mtDNA sequences within both the western Mojave and southern Arizona (Table 1.1) is most likely an attribute of desert tortoises maintaining a long-term, small effective population size. Consistent with these findings, Rainboth et al. (1989) concluded that the low degree of variation observed among allozymes was also a result of small population size in the western and eastern Mojave Desert, (the site termed "eastern" Mojave was actually in the Colorado or western Sonoran Desert). The 2 unique haplotypes (Son4 and Son5) observed at the Sugarloaf site are each only 1 mutation step from the Son1 haplotype shared by sites in the Tucson area. The rise of these younger haplotypes to such high frequencies at this site gives further support to the hypothesis that tortoises maintain small effective population size because a frequency change like this would most likely be a result of genetic drift in a small population.

The negative values calculated for Tajima's D within each study area (Table 1.1) indicate that there has been a demographic or selective event that reduced diversity, such as a population bottleneck followed by a population expansion. Population expansion preserves the genetic signature of a population at the time of expansion. The unimodal,

L-shaped mismatch distribution also supports a pattern of population growth in both the western Mojave and southern Arizona (Rogers and Harpending 1992). The slower mutation rate of mtDNA in tortoises, coupled with long-term maintenance of small population sizes, may reflect a prehistoric reduction of genetic diversity in tortoise populations that coincides with the most recent major glacial-interglacial climate change, approximately 22,000+ years ago (Van Devender et al. 1976, Morafka 1977, Morafka and Berry 2002). Fossil evidence documents the occurrence of the desert tortoise in both of these regions during the late Wisconsin glacial period (see summary in; Morafka and Berry 2002), so I do not believe this pattern reflects a new expansion into these areas. This pattern could also reflect a more recent bottleneck caused by anthropogenic extirpation, coinciding with the North American megafauna extinctions of the glaciopluvial transition 12,500-10,000 years ago (Van Devender et al. 1976, Morafka 1977, Morafka and Berry 2002). Several North American turtle species went extinct during this period (*Geochelone* sp., *Terrrapene c. putnami*, *Gopherus laticauda*) and pre-Columbian human-induced extirpation has been well documented for the extirpation of the Bolson tortoise, *Gopherus flavomarginatus* (Bury et al. 1988, Morafka 1988, Morafka and Berry 2002). My TMRCA estimates are compatible with population growth occurring within this time frame. Coalescence traces the history of genes and not populations, so the coalescence time of the gene should precede the expansion event. In addition, estimates of θ are increased when there is population structure, so my calculated N_e is likely smaller than estimated. The similar but independent coalescence times calculated for the western Mojave and Tucson area suggest that the environmental

conditions resulting in a population expansion were geographically widespread.

The phylogeographic pattern of desert tortoise mtDNA haplotypes for both the 4 western Mojave and the 7 Tucson area sites is characteristic of intermediate gene flow in a species not subdivided by long-term zoogeographic barriers (Avise et al. 1987). Thus, I observed shared haplotypes across sites, but not all haplotypes are shared. The structure of the gene tree, the ANOVA, and the nested cladistic analysis all support the supposition that the pattern of genetic structure observed within both the western Mojave and the 7 Tucson area sites is a result of gene flow and not recent common ancestry or range expansion. The data suggest that the genetic relationship among desert tortoise sites is characteristic of isolation-by-distance (IBD; Kimura and Weiss 1964). While the outcome of the NCA was inconclusive for the Tucson area sites, the AMOVA and the evolutionary network of haplotypes support a structure characteristic of IBD. Research on these same Sonoran samples using microsatellite markers also supports an IBD model (Chapter 2). In a study of tortoises in the northeastern Mojave Desert, Britten and collaborators (1997) also found patterns of gene flow consistent with IBD using allozyme and mtDNA data. The desert tortoise is perhaps the ideal organism for the IBD model in that it is distributed across the landscape in patches and for which the primary difficulty of dispersal is geographic distance. Geographic distance separating tortoise locations appears to be the major limitation to gene flow among locations.

Unique haplotypes observed in the most geographically distant site in southern Arizona suggests that Sugarloaf is most likely isolated from sites in the Tucson area. This could result from formidable geographic barriers separating the Sugarloaf site from

the Tucson area sites. Male tortoises north of the Gila River in Arizona (which separates both the Sugarloaf and the Florence sites from the Tucson area sites) reach larger averages sizes than those south of the Gila (Averill-Murray et al. 2002) and this morphological difference may be evidence of a north/south division in the Sonoran population. However, mtDNA data only address the movements of females. Males do not pass on their mtDNA to offspring, so this marker only details female genealogies. The addition of autosomal loci and a sampling scheme that included samples collected from sites between the Sugarloaf site and sites in the Tucson area could discriminate between population fragmentation and isolation-by-distance.

My results suggest that heterogeneity of the landscape does not necessarily dictate what constitutes an ESU for the desert tortoise. Despite differences in tortoise reproductive ecology and habitat selection, the genetic structure observed within areas of comparable geographic size in the western Mojave and the Tucson area is similar. The landscape heterogeneity in the Tucson area does not appear to limit the ability of tortoises to move between habitat patches. While natural geographic barriers certainly play a role in limiting the ability of tortoises to move long-distances, our understanding of desert tortoise dispersal is incomplete. Some barriers may be of long-term consequence, such as the Colorado River maintaining the genetic separation of Mojave and Sonoran populations, or, similarly, the Gila River that more recently may have separated the Sugarloaf site from sites in the Tucson area. The differentiation of the Sugarloaf population highlights the need for additional analyses of northern populations in Arizona. Other potential natural barriers that may be sources of divergence include the Baker sink

and Soda Dry Lake area which separates the central and eastern Mojave Desert, the southern Ward and Cadiz valleys which separates the southern Mojave from the Chemehuevi/Ward area, and the Moapa and Virgin rivers in the northeastern Mojave. Application of molecular analyses throughout the range of the desert tortoise would help delineate ESUs in the Sonoran population of the desert tortoise in Arizona and Sonora, Mexico and help resolve the extent of gene flow among existing ESUs in the Mojave population. Additional molecular research is also necessary to resolve the identity of species or subspecies from the current definition of *Gopherus agassizii* (Berry et al. 2002).

Gene flow among habitat patches is part of the evolutionary history of the desert tortoise. These estimates of population structure based on mtDNA sequence analysis reflect the movement patterns of desert tortoises prior to anthropogenic habitat fragmentation. Currently, it is unlikely that successful long-distance dispersal events could occur over much of the range of the desert tortoise due to recent proliferation of major human development (Appendix B). As tortoise localities become increasingly isolated from areas which they historically exchanged migrants, they become more vulnerable to stochastic events. It is important that management strategies be designed to facilitate natural patterns of inter-population movement that occurred historically within ESUs. Connectivity of the landscape should be maintained wherever possible among tortoise localities that constitute ESUs. ESUs that experience increased habitat fragmentation will be more vulnerable to stochastic processes that could lead to local extinction. Translocation of tortoises from neighboring localities should be evaluated as

a potential management strategy to recover declining sites that are isolated by anthropogenic barriers. However, before tortoise translocation strategies are implemented, the effects of translocation on the survivorship of relocated individuals and the populations into which they are introduced need to be evaluated and the potential for disease transmission from one population to another needs to be assessed (Dodd and Seigel 1991, Jacobson 1993, Cunningham 1996, Seigel and Dodd 2002). In addition, translocation will not likely be a sustainable strategy unless threats are also identified and alleviated. Management strategies that reflect the evolutionary history of gene flow specific to each ESU will better facilitate the long-term conservation of this species.

CHAPTER 2 - IMPLICATIONS OF ANTHROPOGENIC LANDSCAPE CHANGE ON INTER-POPULATION MOVEMENTS OF SONORAN DESERT TORTOISES USING MICROSATELLITE DNA ANALYSIS

ABSTRACT

In the Sonoran Desert of North America, populations of the desert tortoise (*Gopherus agassizii*) occur in rocky foothills throughout southwestern Arizona and northwestern Mexico. Although tortoise populations appear to be isolated from each other by low desert valleys, individuals occasionally move long distances between populations. Increasingly, these movements are hindered by habitat fragmentation due to anthropogenic landscape changes. I used molecular techniques and radiotelemetry to examine movement patterns of desert tortoises in southern Arizona. I collected blood samples from 170 individuals in 9 mountain ranges and analyzed variability in 7 microsatellite loci to determine genetic differentiation among populations. Gene flow estimates between populations indicate that populations exchanged individuals historically at a rate greater than one migrant per generation, and positive correlation between genetic and geographic distance of population pairs suggests that the limiting factor for gene flow among populations is isolation by distance. Life history traits of the desert tortoise, a long-lived species with delayed sexual maturity, may severely constrain the ability of small populations to respond to disturbances that increase adult mortality. Historic gene flow estimates among populations suggests that recovery of declining populations may rely heavily on the immigration of new individuals from adjacent mountain ranges. Management strategies compatible with the evolutionary history of

gene flow among disjunct populations will help ensure the long-term persistence of Sonoran desert tortoise populations.

INTRODUCTION

The desert tortoise, *Gopherus agassizii*, is native to the southwestern deserts of North America and is recognized as having distinct Mojave and Sonoran populations. The Mojave population (defined as all tortoises north and west of the Colorado River) was federally listed as threatened by the U.S. Fish and Wildlife Service in 1990 (USFWS 1990). The Sonoran population (all tortoises south and east of the Colorado River) is not federally listed but is considered a Species of Special Concern by the Arizona Game and Fish Department (AGFD 1996). Although a number of threats to tortoises have been identified, loss of habitat currently represents the greatest threat to Sonoran populations near rapidly growing communities such as Phoenix and Tucson (AIDTT 2000). In the Tucson area, many thousands of acres of tortoise habitat have been recently lost to large residential developments in the foothills of the Santa Catalina, Tortolita, Rincon, and Tucson Mountains. Development reduces the size of populations and isolates them by creating barriers such as highways and canals. There is a strong management need to identify important connections between tortoise populations before the opportunity to preserve these connections is gone (AIDTT 1996).

Tortoises in the southern Arizona generally inhabit rocky foothills associated with leguminous tree (foothill paloverde, *Parkinsonia microphylla*; desert ironwood, *Olneya tesota*) and mixed cactus vegetation communities (saguaro cactus, *Carnegiea gigantea*; cholla, *Opuntia* spp.). Although foothill populations appear to be isolated by low desert

valleys, radiotelemetry data show that tortoises are capable of making long-distance movements between populations (Barrett et al. 1990, Averill-Murray and Klug 2000, Appendix B). However, the importance of these movements and whether they contribute to gene flow is unknown. Determination of the extent to which disjunct populations interact is an important aspect of desert tortoise conservation.

I used 7 microsatellite DNA markers to examine the genetic relationships of tortoises in 8 populations in the vicinity of Tucson and 1 population northeast of Phoenix. By comparing genetic distance (variation between populations) with geographic distance and calculating migration rates among these populations, I estimated historic rates of gene flow. I then used GIS data to denote human barriers that potentially obstruct tortoise movements. In addition, I evaluated genetic relatedness among individual desert tortoises within a single population located in the Rincon Mountain District of Saguaro National Park while simultaneously gathering information on movements and home ranges using radiotelemetry. For this population, I compared genetic differences among individuals to geographic distances between them to determine if gene flow within the population is random in regard to geographical location of individuals or if habitat features such as ridges and drainages influence population structure.

Because major human development is fairly recent (Lovich and Bainbridge 1999) with respect to the generation time of the desert tortoise (estimated at 25 years; USFWS 1994), the genetic structure of tortoise populations has not likely yet been affected by modern anthropogenic landscape changes. By measuring gene flow among populations, we can obtain a snapshot of the movement patterns of desert tortoises prior to

anthropogenic habitat fragmentation. The degree of relatedness of tortoises both within and among mountain ranges has implications for how sustainable small populations may be as they become increasingly isolated.

METHODS

Data Collection - I sampled desert tortoises from 8 sites in Pima and Pinal counties in the vicinity of Tucson and from 1 population northeast of Phoenix in Maricopa County (Figure 2.1). The maximum distance between any 2 populations was 186 km, the minimum distance 16 km. Between 8 and 38 tortoises were sampled from each population, depending on population size, for a total of 170 tortoises. I hand-captured and processed tortoises March-October 2000 and 2001 using standard methods and following Arizona Interagency Desert Tortoise Team guidelines (Averill-Murray 2000). I collected <3cc blood by brachial or jugular venipuncture and stored it on ice with an EDTA or lithium heparin buffer.

Molecular Techniques - I isolated total DNA from blood by overnight lysis with proteinase K at 55 °C, followed by a phenol/chloroform extraction and isopropanol/sodium acetate precipitation (Goldberg et al. 2002). I resuspended the DNA in low TE (10mMTris-pH 8.0, 0.1mM EDTA) and diluted to 5 ng/μl for polymerase chain reaction (PCR) amplifications. I prepared a microsatellite-enriched genomic library for tortoises based on the methods of Hamilton et al. (1999) and identified 6 novel

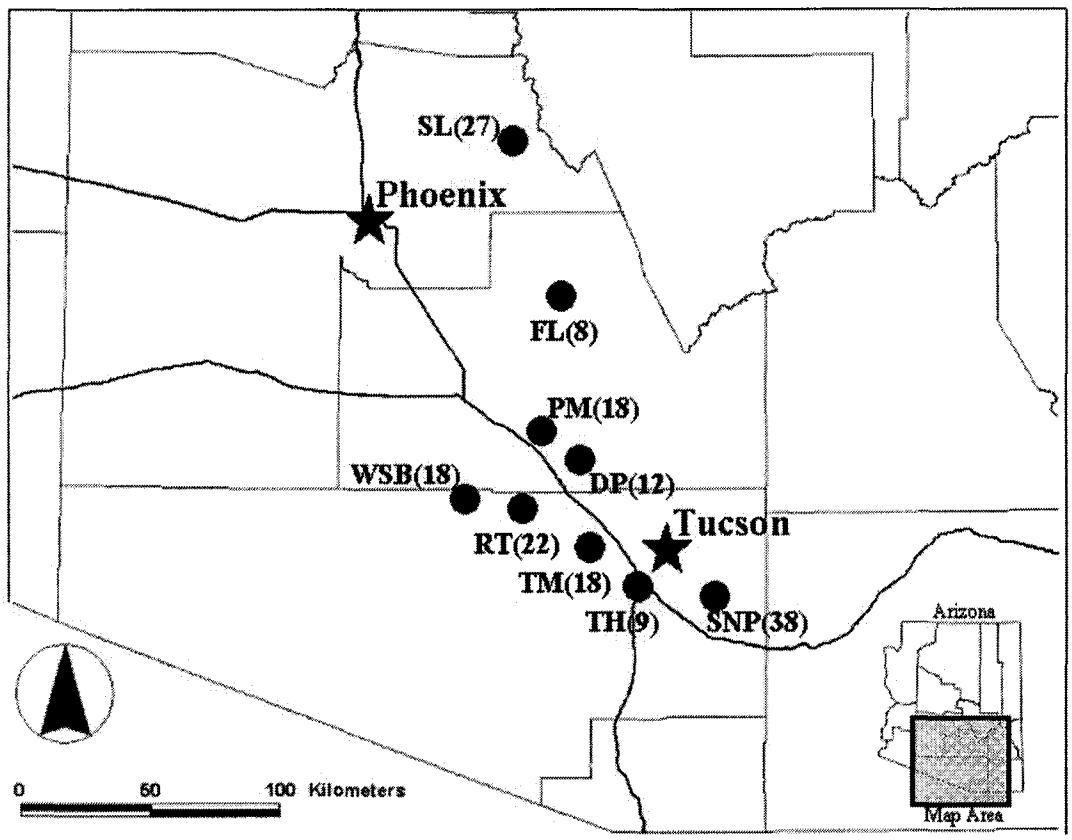

FIGURE 2.1 Location and number of tortoises sampled from 9 mountain ranges containing desert tortoise populations in southern Arizona; Desert Peak (DP), Florence Military Reservation (FL), Picacho Mountains (PM), Ragged Top (RT), Rincon Mountains (Saguaro National Park; SNP), Sugarloaf (SL), Tumamoc Hill (TH), Tucson Mountains (TM), and West Silver Bell Mountains (WSB).

microsatellite loci that exhibited variation in my sample set (3 tri-nucleotide loci, *Goag*3, *Goag*4, and *Goag*5; and 3 di-nucleotide loci *Goag*6, *Goag*7, and *Goag*32). In addition, I used primers for a microsatellite locus identified in *Chelonia mydas* (Cm58; FitzSimmons et al. 1995) that successfully amplified and proved variable in desert tortoises (Appendix A). I PCR-amplified loci and assessed variability using 5' fluorescently labeled forward primers as described in Appendix A.

Molecular Analysis - I calculated microsatellite allele frequencies for each locus in each population and examined frequency distributions for unique and private alleles. I compared allele frequency distributions between all population pairs and between each population and the total sample for all loci exhibiting >7 alleles using a Kolmogorov-Smirnov test (KS-test; Chakravarti et al. 1967). The KS-test is a non-parametric test that determines if two datasets differ significantly without making assumptions about the distribution of data. The test statistic (*D*) can be interpreted as the maximum difference between the cumulative distributions. I used ARLEQUIN (version 2.0, Schneider et al. 2000) to detect significant departure from Hardy-Weinberg equilibrium at each locus using a triangular contingency table and a modified version of the Markov-chain random walk algorithm (Guo and Thompson 1992). I tested for linkage disequilibrium (nonrandom association between loci) among all pairs of loci in the entire sample and within each population using a likelihood-ratio test with an empirical distribution obtained by permutation (Slatkin and Excoffier 1996). I used default parameters in ARLEQUIN for all Markov-chain tests and permutations. I determined the inbreeding coefficient (F_{IS}; Weir and Cockerham 1984) for each locus in each population using GENEPOP version 3.1 (Raymond and Rousset 1995).

I used BOTTLENECK (Piry et al. 1999) to identify recent bottlenecks in each population and in the entire sample. This test is based on the assumption that a bottlenecked population (one that has experienced recent reductions in effective population size) will show an excess of heterozygosity over that expected under mutation-drift equilibrium (Cornuet and Luikart 1996). In addition, I used the method of

Garza and Williamson (2001) to assess recent reductions in population size. This method examines the ratio of the total number of alleles to the overall range in allele size (M). M can be interpreted as the average percentage of intermediate allelic states in a population and its value will decrease when a population is reduced in size. I calculated M for each population and for the total region and then simulated M (10,000 replicates) based on the allelic frequencies of the sample populations using 3 parameters: theta ($4N_e\mu$), P_S (percentage of mutations that add or delete only one repeat), and $delta_g$ (mean size of larger mutations). Simulations generated a statistic M_C, which is the critical value at which 95% of the simulations of M in an equilibrium population are greater than M_C. A reduction in population size is suggested when $M<M_C$. I used 2 models; one recommended by Garza and Williamson (theta = 10, $P_S = 0.9$ and $delta_g = 3.5$) and a more conservative model based on microsatellite data sets from 20 natural populations (Garza and Williamson 2001; theta = 10, $P_S = 0.88$ and $delta_g = 2.8$). A theta value of 10 represents an effective population size of 5000 individuals (with mutation rate $\mu = 5x10^{-4}$).

I inferred population structure from microsatellite data using AMOVA (analysis of molecular variance) in ARLEQUIN. I used Wright's F_{ST} (Wright 1951) to determine how genetic variation was partitioned within the region, among populations, and among individuals within populations. I used FSTAT version 2.9.3.2 (Goudet 1995) to calculate bootstrap estimators for significance of F-statistics. As a comparison, I also calculated genetic variability using a stepwise mutation model using Slatkin's R_{ST} (Slatkin 1995). I calculated genetic distances among populations and individuals using ARLEQUIN using

pairwise F_{ST}. Negative F_{ST} and R_{ST} values were treated as zero. I estimated the number of migrants exchanged per generation between pairs of populations (2Nm) using Slatkin's $\hat{M}$ (Slatkin 1991) in ARLEQUIN as well as using the private allele method of Barton and Slatkin (1986) in GENEPOP. I used NTSYSpc (version 2.02h, Applied Biostatistics Inc.) to perform mantel tests to assess correlation between genetic distances and geographic distances among populations. If gene flow has been the cause of genetic similarity among populations and geographic distance between populations affects the dispersal of individuals between populations, then the correlation between the matrices should be significant (Slatkin and Maddison 1990).

Radiotelemetry and Spatial Analysis - I assessed within-population genetic structure at 2 established study sites in the Rincon Mountain district of Saguaro National Park (Figure 2.2). The first site (Mother's Day Fire) lies entirely within the park boundary. Collaborating researchers have radiotracked 9 tortoises here since 1996 (Esque et al. 1998). The second site (Rocking K) is approximately 6 km south of the Mother's Day Fire and is located along the park's south boundary. Collaborators and I have radiotracked 25 tortoises at this site since July 1999. We monitored tortoises, on average, twice weekly during the active season (March-October) and once weekly during winter.

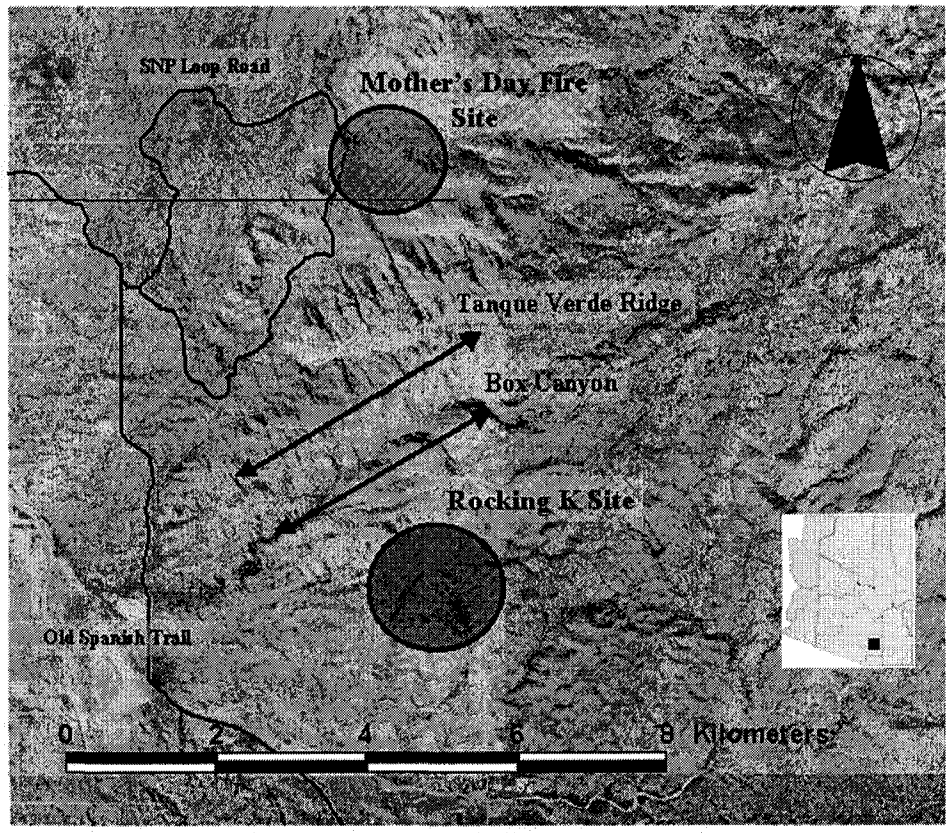

Figure 2.2 Two study sites within the desert tortoise population at Saguaro National Park (Rincon Mountain District), east of Tucson, Arizona. Geographic features (Box Canyon and Tanque Verde Ridge) potentially separate the sites, but otherwise, the sites are connected by continuous tortoise habitat.

I estimated tortoise home range size using the minimum convex polygon (MCP) method (White and Garrott 1990) including all point locations for each individual from all years for which telemetry data were available. I compared MCP home ranges between the two sites by multiple regression (Ramsey and Schafer 1997) with explanatory variables of sex, size (MCL), and number of point locations. Multiple regression was analyzed using JMP version 4.0.0 software (Sall and Lehman 1996).

I mapped potential human-constructed barriers to tortoise movement between mountain ranges in ArcView GIS (version 3.2, Environmental Systems Research Institute, Inc.) based on available GIS data. I used straight-line distance as a measure of geographic distance between populations because evidence suggests that long-distance movements of tortoises and other reptiles do not follow natural geographic forms but are essentially linear in nature (Barrett et al. 1990, King and Duvall 1990, Reinert and Rupert 1999, Appendix B). Within the Saguaro National Park radiotelemetry plots, tortoises were located to within approximately 5 meters using hand-held Global Positioning System (GPS) receivers (GARMIN International Inc.). I determined the arithmetic mean of all point locations for each individual from all years for which telemetry data were available using the Animal Movement Analyst Extension (version 1.1, Hooge and Eichenlaub 1997) in ArcView and used that point location to determine the geographic distances among the 34 individual tortoise home ranges. I used available base coverages from digital orthophoto quarter quads (DOQQs) to assess presence of physical features such as ridges and drainages between plots.

RESULTS

Microsatellite DNA Results - I amplified and sized 7 microsatellite loci for all 170 samples from 9 populations (Table 2.1). All 7 loci were polymorphic in all populations. Loci *Goag*3, *Goag*32, and Cm58 exhibited only marginal variability (2-3 alleles), but loci *Goag*4, *Goag*5, *Goag*6, and *Goag*7 were highly variable (8-27 alleles). The allelic distributions for these loci did not exhibit normality. The KS-test found

TABLE 2.1 Diversity indices for 7 microsatellite loci in 9 populations of desert tortoises. # = number of individuals genotyped; Size = the range of allele repeat lengths; H_{obs} = observed heterozygosity; H_{exp} = expected heterozygosity; S.D. = standard deviation of randomization tests for Hardy-Weinberg equilibrium; and F_{IS} = Weir and Cockerham's inbreeding estimator (1984).

Goag3

Population	#	Size	H_{obs}	H_{exp}	*p*	S.D.	F_{IS}	*p*
Desert Peak	12	6-8	0.3333	0.3696	1.000	<0.001	-0.114	1.000
Florence	8	6-8	0.6250	0.5750	1.000	<0.001	-0.296	1.000
Rincon Mountains (SNP)	38	6-8	0.4359	0.4612	0.761	0.004	0.056	0.757
Picacho Mountains	18	6-8	0.3333	0.4587	0.272	0.004	0.206	0.260
Ragged Top	22	6-8	0.3182	0.3541	0.376	0.004	0.104	0.384
Sugarloaf	27	6-8	0.2963	0.2998	1.000	<0.001	-0.106	1.000
Tumamoc Hill	9	6-8	0.3333	0.2941	1.000	<0.001	-0.143	1.000
Tucson Mountains	18	6-8	0.5556	0.4524	0.771	0.004	-0.236	0.755
West Silver Bell Mountains	18	6-8	0.1111	0.1619	1.000	<0.001	-0.015	1.000

Goag4

Population	#	Size	H_{obs}	H_{exp}	*p*	S.D.	F_{IS}	*p*
Desert Peak	12	10-23	0.2500	0.2355	1.000	<0.001	-0.065	1.000
Florence	8	10-15	0.6250	0.4583	0.487	0.005	-0.400	0.487
Rincon Mountains (SNP)	38	9-25	0.7692	0.7543	0.858	0.003	-0.020	0.861
Picacho Mountains	18	9-29	0.7778	0.7318	0.543	0.002	-0.065	0.558
Ragged Top	22	9-25	0.6818	0.7600	0.790	0.002	0.082	0.750
Sugarloaf	27	9-24	0.5185	0.5646	0.616	0.003	0.033	0.542
Tumamoc Hill	9	9-23	0.7778	0.7712	0.739	0.002	-0.009	0.702
Tucson Mountains	18	9-23	0.4444	0.4349	0.526	0.003	-0.023	0.571
West Silver Bell Mountains	18	7-24	0.7222	0.7794	0.308	0.001	0.043	0.225

Goag5

Population	#	Size	H_{obs}	H_{exp}	*p*	S.D.	F_{IS}	*p*
Desert Peak	12	9-34	1.0000	0.9348	0.894	0.001	-0.073	0.739
Florence	8	14-29	0.7500	0.8500	0.391	0.002	0.097	0.407
Rincon Mountains (SNP)	38	9-38	0.8205	0.9261	0.036	<0.001	0.115	0.001
Picacho Mountains	18	6-34	1.0000	0.9222	0.034	<0.001	-0.087	0.014
Ragged Top	22	9-35	0.9091	0.9345	0.761	0.001	0.028	0.909
Sugarloaf	27	9-27	0.8889	0.8595	0.068	0.001	-0.035	0.072
Tumamoc Hill	9	9-32	1.0000	0.8824	0.433	0.001	-0.143	0.422
Tucson Mountains	18	15-38	0.8889	0.9111	0.040	0.001	0.016	0.174
West Silver Bell Mountains	18	12-33	0.7778	0.9270	0.024	0.001	0.165	0.043

TABLE 2.1 - *Continued*

Goag6

Population	#	Size	H_{obs}	H_{exp}	*p*	S.D.	F_{IS}	*p*
Desert Peak	12	15-27	0.0833	0.6486	<0.001	<0.001	0.863	<0.001
Florence	8	15-25	0.3750	0.8250	0.130	0.004	0.506	0.115
Rincon Mountains (SNP)	38	15-26	0.1539	0.6936	<0.001	<0.001	0.773	<0.001
Picacho Mountains	18	15-29	0.4444	0.6810	0.066	0.001	0.322	0.079
Ragged Top	22	15-51	0.5000	0.7484	0.006	0.001	0.335	0.002
Sugarloaf	27	17-49	0.4444	0.7219	<0.001	<0.001	0.365	0.001
Tumamoc Hill	9	15-25	0.3333	0.6863	0.086	0.002	0.455	0.071
Tucson Mountains	18	15-25	0.3333	0.6825	<0.001	<0.001	0.485	<0.001
West Silver Bell Mountains	18	15-52	0.3889	0.6762	<0.001	<0.001	0.402	0.001

Goag7

Population	#	Size	H_{obs}	H_{exp}	*p*	S.D.	F_{IS}	*p*
Desert Peak	12	12-18	0.3333	0.5978	0.0378	0.002	0.385	0.029
Florence	8	14-22	0.5000	0.7333	0.241	0.009	0.253	0.262
Rincon Mountains (SNP)	38	12-22	0.3846	0.6201	<0.001	<0.001	0.376	0.002
Picacho Mountains	18	12-22	0.6111	0.6698	0.753	0.003	0.043	0.742
Ragged Top	22	12-19	0.5000	0.5867	0.378	0.004	0.151	0.326
Sugarloaf	27	14-22	0.6667	0.7505	0.206	0.004	0.097	0.200
Tumamoc Hill	9	12-18	0.2222	0.4706	0.366	0.004	0.439	0.341
Tucson Mountains	18	12-22	0.5000	0.6048	0.332	0.002	0.126	0.339
West Silver Bell Mountains	18	12-21	0.3889	0.7540	<0.001	<0.001	0.466	0.001

Goag32

Population	#	Size	H_{obs}	H_{exp}	*p*	S.D.	F_{IS}	*p*
Desert Peak	12	5-6	0.2500	0.3007	1.000	<0.001	-0.100	1.000
Florence	8	5-6	0.1250	0.2417	1.000	<0.001	0.000	-
Rincon Mountains (SNP)	38	5-6	0.2051	0.2488	0.493	0.005	0.095	0.483
Picacho Mountains	18	5-6	0.1111	0.2032	0.177	0.005	0.460	0.169
Ragged Top	22	5-6	0.4091	0.4577	1.000	<0.001	0.041	1.000
Sugarloaf	27	5-6	0.4815	0.4004	0.288	0.005	-0.300	0.283
Tumamoc Hill	9	5-6	0.2222	0.3072	1.000	<0.001	-0.067	1.000
Tucson Mountains	18	5-6	0.5000	0.4365	1.000	<0.001	-0.150	1.000
West Silver Bell Mountains	18	5-6	0.1667	0.2079	1.000	<0.001	-0.062	1.000

Cm58

Population	#	Size	H_{obs}	H_{exp}	*p*	S.D.	F_{IS}	*p*
Desert Peak	12	12-13	0.3333	0.3587	1.000	<0.001	-0.158	1.000
Florence	8	12-13	0.3750	0.5917	0.539	0.005	0.300	0.530
Rincon Mountains (SNP)	38	12-13	0.3333	0.3353	1.000	<0.001	-0.060	1.000
Picacho Mountains	18	12-13	0.1667	0.3667	0.085	0.002	0.490	0.085
Ragged Top	22	12-13	0.3182	0.2738	1.000	<0.001	-0.167	1.000
Sugarloaf	27	12-13	0.3333	0.3599	1.000	<0.001	-0.009	1.000
Tumamoc Hill	9	12-13	0.1111	0.2157	1.000	<0.001	0.000	-
Tucson Mountains	18	12-13	0.1667	0.1571	1.000	<0.001	-0.062	1.000
West Silver Bell Mountains	18	12-13	0.1667	0.2079	1.000	<0.001	-0.062	1.000

relatively few significant differences between distributions of allelic frequencies for the more variable loci, *Goag*4, *Goag*5, *Goag*6, and *Goag*7 (Table 2.2). There was no consistent pattern for any locus or population that would suggest that its allelic frequency distribution was different from the rest of the sample. Only 4 private alleles (frequency $\geq 5\%$) were detected, 1 in each of 4 populations (Table 2.3). No private alleles had frequencies greater than 7% in a population. The mean frequency of private alleles [P(1)] for the total sample was 0.034.

Three of the loci (*Goag*5, *Goag*6, and *Goag*7) deviated significantly from expected heterozygosities under Hardy-Weinberg proportions using exact probability testing (Appendix A, Table A.1) and the associated inbreeding estimator (F_{IS}) at these 3 loci was positive. F_{IS} over all loci for the entire sample was 0.161 (99% confidence interval for bootstrapping across loci: 0.016 to 0.376). Tests for linkage disequilibrium rejected the null hypothesis of independence of 4 of the 7 loci. However, analyses performed without three of the linked loci (*Goag*5, *Goag*7, and *Goag*32) did not affect the results of the AMOVA or the genetic distance calculations. I proceeded with analysis using the full set of loci, but also calculated descriptive statistics with the exclusion of the apparently linked loci for comparison.

I did not find significant excess or deficiency in heterozygosity when all loci in the sample set were examined together (Table 2.4). The entire sample and each individual population fit the expected beta distribution, suggesting that there have not been recent reductions in population size (Cornuet and Luikart 1996). Using the method of Garza and Williamson (2001), all values generated for the average percentage of

intermediate allelic states (M) for both models fell above the critical value M_C (Table 2.5).

TABLE 2.2 Kolmogorov-Smirnov test of difference between allele frequency distributions between all population pairs and between each population and the total sample for 4 loci. Compared populations; Desert Peak (DP), Florence Military Reservation (FL), Picacho Mountains (PM), Rincon Mountains (Saguaro National Park; SNP), Ragged Top (RT), Sugarloaf (SL), Tumamoc Hill (TH), Tucson Mountains (TM), and West Silver Bell Mountains (WSB). Total = pooled frequency distribution for all populations. * indicates significance level $p<0.05$.

A. Maximum difference between distributions (*D*) for Loci *Goag*4 (below diagional) and *Goag*5 (above diagional).

	DP	FL	PM	SNP	RT	SL	TH	TM	WSB	Total
DP		0.18	0.15	0.21	0.18	0.24	0.18	0.15	0.15	0.33
FL	0.04		0.18	*0.39	*0.36	0.12	0.06	0.15	0.18	*0.52
PM	0.30	0.35		0.21	0.18	0.24	0.12	0.03	0.03	*0.33
SNP	0.22	0.26	0.13		0.24	*0.33	*0.33	0.24	0.21	0.15
RT	0.22	0.26	0.22	0.09		0.27	0.30	0.21	0.18	0.15
SL	0.13	0.17	0.17	0.09	0.09		0.12	0.21	0.24	*0.42
TH	0.17	0.17	0.17	0.09	0.09	0.13		0.09	0.12	*0.46
TM	0.13	0.17	0.17	0.13	0.09	0.09	0.13		0.06	*0.36
WSB	0.35	*0.39	0.09	0.17	0.26	0.22	0.22	0.22		*0.33
Total	*0.39	*0.43	0.17	0.17	0.17	0.26	0.26	0.26	0.22	

B. Maximum difference between distributions (*D*) for Loci *Goag*6 (below diagional) and *Goag*7 (above diagional).

	DP	FL	PM	SNP	RT	SL	TH	TM	WSB	Total
DP		0.09	0.18	0.36	0.18	0.18	0.09	0.18	0.27	0.36
FL	0.03		0.27	0.36	0.18	0.18	0.18	0.18	0.27	0.36
PM	0.05	0.05		0.18	0.18	0.18	0.27	0.27	0.09	0.18
SNP	0.03	0.03	0.03		0.27	0.27	0.46	0.18	0.27	0.18
RT	0.11	0.11	0.05	0.08		0.18	0.18	0.18	0.27	0.27
SL	0.05	0.05	0.08	0.06	0.08		0.18	0.18	0.18	0.27
TH	0.03	0.05	0.05	0.03	0.11	0.05		0.27	0.36	0.46
TM	0.05	0.05	0.03	0.03	0.05	0.08	0.05		0.36	0.18
WSB	0.13	0.13	0.08	0.11	0.08	0.16	0.13	0.08		0.27
Total	0.21	0.21	0.16	0.18	0.11	0.16	0.21	0.16	0.13	

TABLE 2.3 Distribution of unique and private alleles in 9 populations of desert tortoise in southeastern Arizona. T = total number of alleles from a population; U = number of alleles unique to the population; parenthetic values are the number of unique alleles that occur at a frequency >5% (private alleles); % = (U/T)x100. No allele unique to a population occurred at a frequency >7% in that population.

	Desert Peak			Florence			Picacho Mtns.			Ragged Top			Rincon Mtns.			Sugarloaf			Tucson Mtns.			Tumamoc Hill			West Silverbell		
Locus	T	U	%	T	U	%	T	U	%	T	U	%	T	U	%	T	U	%	T	U	%	T	U	%	T	U	%
*Goag*3	3	0	0	3	0	0	3	0	0	3	0	0	3	0	0	3	0	0	3	0	0	2	0	0	3	0	0
*Goag*4	4	0	0	3	0	0	10	3(1)	30	8	0	0	7	0	0	6	0	0	6	0	0	6	0	0	11	3	27
*Goag*5	14	0	0	8	0	0	14	1	7	20	1	5	21	0	0	11	0	0	13	0	0	10	0	0	14	0	0
*Goag*6	4	0	0	4	0	0	6	0	0	8	2	25	5	1(1)	20	6	2	33	6	1(1)	17	4	0	0	8	2(1)	25
*Goag*7	4	0	0	4	0	0	6	0	0	5	0	0	8	0	0	5	0	0	6	0	0	3	0	0	6	0	0
*Goag*32	2	0	0	2	0	0	2	0	0	2	0	0	2	0	0	2	0	0	2	0	0	2	0	0	2	0	0
Cm58	2	0	0	2	0	0	2	0	0	2	0	0	2	0	0	2	0	0	2	0	0	2	0	0	2	0	0
Total	33	0	0	26	0	0	43	4(1)	37	48	3	30	48	1(1)	40	35	2	33	38	1(1)	17	29	0	0	46	5(1)	52

TABLE 2.4 Probability of excess or deficit of heterozygosity across 7 desert tortoise microsatellite loci. Sign test and Wilcoxon sign-rank test (two tails) for mutation-drift equilibrium using three mutation models; infinite alleles model (IAM), stepwise mutation model, (SMM), and two-phased model (TPM).

	Mutation Model		
	IAM	SSM	TPM
Wilcoxon Test	0.578	0.688	0.078
Sign Test	0.241	0.424	0.164

TABLE 2.5 Average percentage of intermediate allelic states (M) for 7 microsatellite loci in 9 desert tortoise populations in southern Arizona. Two models were used to generate M_C, the critical value at which 95% of 10,000 simulations of M in an equilibrium population are greater than M_C; one recommended by the authors (theta = 10, P_S = 0.9, $delta_g$ = 3.5) and a more conservative model (theta = 10, P_S = 0.88, $delta_g$ = 2.8) based on microsatellite data sets from 20 natural populations, (Garza and Williamson 2001).

Population	M	M_C (recommended model)	M_C (conservative model)
Desert Peak	0.6617	0.5879	0.6345
Florence	0.6631	0.5487	0.5886
Rincon Mountains (SNP)	0.7592	0.6738	0.7297
Picacho Mountains	0.7006	0.6236	0.6718
Ragged Top	0.7218	0.6384	0.6904
Sugarloaf	0.6702	0.6518	0.7070
Tumamoc Hill	0.7189	0.6236	0.6718
Tucson Mountains	0.6108	0.5628	0.6033
West Silver Bell Mountains	0.7226	0.6236	0.6718
Total	0.8113	0.7249	0.7891

Population Differentiation - I detected only minimal genetic differences between pairs of desert tortoise populations from adjacent mountain ranges. For non-adjacent pairs, genetic distance was correlated with geographic distance. Hierarchical analysis of molecular variance of microsatellite data revealed that 96.3% ($p<0.001$) of the observed diversity was in individuals within populations ($F_{IT} = 0.963$), while only 3.7% ($p<0.001$) of the variation was among populations ($F_{ST} = 0.037$; 99% confidence interval for bootstrapping across loci: 0.017 to 0.053). Estimates using a stepwise mutation model also showed very weak differentiation among populations, with 96.8% ($p<0.004$) of genetic variation in individuals within populations (R_{IT}) and 3.2% ($p<0.001$) of variation among populations (R_{ST}). F-coeffecients calculated with the exclusion of the potentially linked loci did not differ sufficiently to change the interpretation of the data ($F_{ST} = 0.0355$, $p<0.001$; calculated for 4 loci). Estimates of the number of migrants per generation between populations using Slatkin's $\hat{M}$ (Table 2.6) ranged from 2.9 (Tumamoc Hill/Florence) to "infinite" (Ragged Top/Picacho Mountains). The estimate for effective number of migrants (corrected for population size) between populations using the private alleles method was 5.5 per generation.

Among the 9 Sonoran populations, there was a significant, positive correlation between genetic distance (pairwise F_{ST}) and geographic distance (Table 2.7). The correlation accounts for approximately 30% of the variation observed (Figure 2.3; Mantel test; $p = 0.030$). This correlation was maintained when pairwise R_{ST} was used as a measure of genetic distance ($r = 0.471$, $p = 0.015$). All of the populations I examined

TABLE 2.6 Slatkin's $\hat{M}$ (absolute number of migrants exchanged per generation between populations) calculated among 9 desert tortoise populations in southern Arizona. Estimates of $\hat{M}$ for populations with pairwise F_{ST} values ≤ 0 are considered to have an "infinite" number of migrants.

Population	#	DP	FL	SNP	PM	RT	SL	TH	TM
Desert Peak (DP)	12	-							
Florence (FL)	8	5.0	-						
Rincon Mountains (SNP)	38	16.5	6.0	-					
Picacho Mountains (PM)	18	129.7	7.6	393.3	-				
Ragged Top (RT)	22	16.3	5.0	113.2	Inf.	-			
Sugarloaf (SL)	27	8.4	10.2	7.6	11.3	11.6	-		
Tumamoc Hill (TH)	9	12.9	2.9	18.0	61.9	28.0	4.6	-	
Tucson Mountains (TM)	18	32.0	4.2	22.2	19.0	22.5	6.1	12.4	-
West Silver Bell Mountains	18	18.9	4.4	28.1	151.8	26.2	8.4	17.9	13.7

TABLE 2.7 Population pairwise F_{ST} values (below diagional) and geographic distances (above diagional; kilometers) among 9 desert tortoise populations in southern Arizona. * indicates significance level $p<0.05$

Population	#	DP	FL	SNP	PM	RT	SL	TH	TM	WSB
Desert Peak (DP)	12	-	66	72	16	27	123	48	31	41
Florence (FL)	8	*0.091	-	128	57	84	59	111	96	85
Rincon Mountains (SNP)	38	0.029	*0.076	-	88	81	186	30	48	99
Picacho Mountains (PM)	18	0.004	*0.062	0.001	-	27	112	63	44	33
Ragged Top (RT)	22	0.030	*0.090	0.004	0.000	-	138	52	33	18
Sugarloaf (SL)	27	*0.056	*0.047	*0.061	*0.042	*0.041	-	170	154	133
Tumamoc Hill (TH)	9	0.037	*0.148	0.027	0.008	0.018	*0.097	-	19	70
Tucson Mountains (TM)	18	0.015	*0.107	*0.022	*0.026	*0.022	*0.076	*0.039	-	51
W. Silver Bell Mountains (WSB)	18	0.026	*0.102	0.018	0.003	0.019	*0.056	0.027	*0.035	-

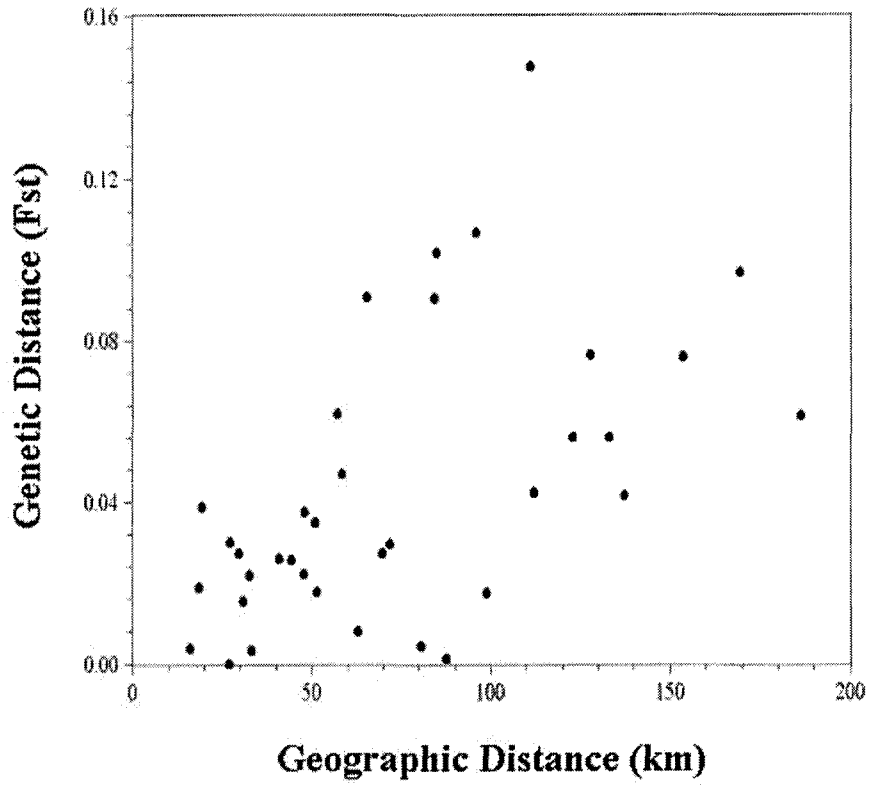

FIGURE 2.3 Genetic distance (pairwise F_{ST}) vs. geographic distance (km) among 9 desert tortoise populations in the Sonoran Desert. Mantel test ($r = 0.554$, $p = 0.030$)

have at least a dirt road separating them (Figures 2.4 and 2.5). The only population pairs in this sample set that could conceivably still exchange individuals at a natural frequency are Desert Peak/Picacho Mountains and Ragged Top/West Silver Bells. All other connections between populations have human barriers that would seriously obstruct natural tortoise movements.

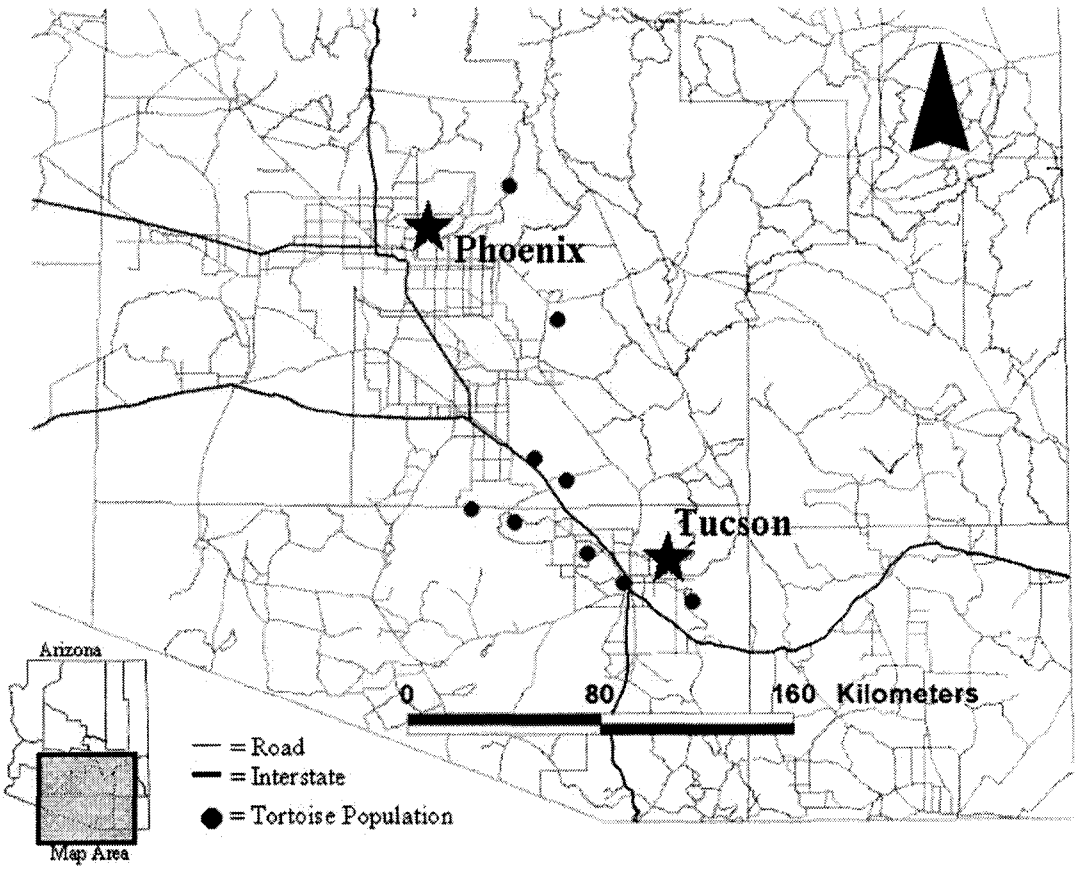

FIGURE 2.4 Distribution of interstates and major roads in southern Arizona that may obstruct tortoise movement between populations.

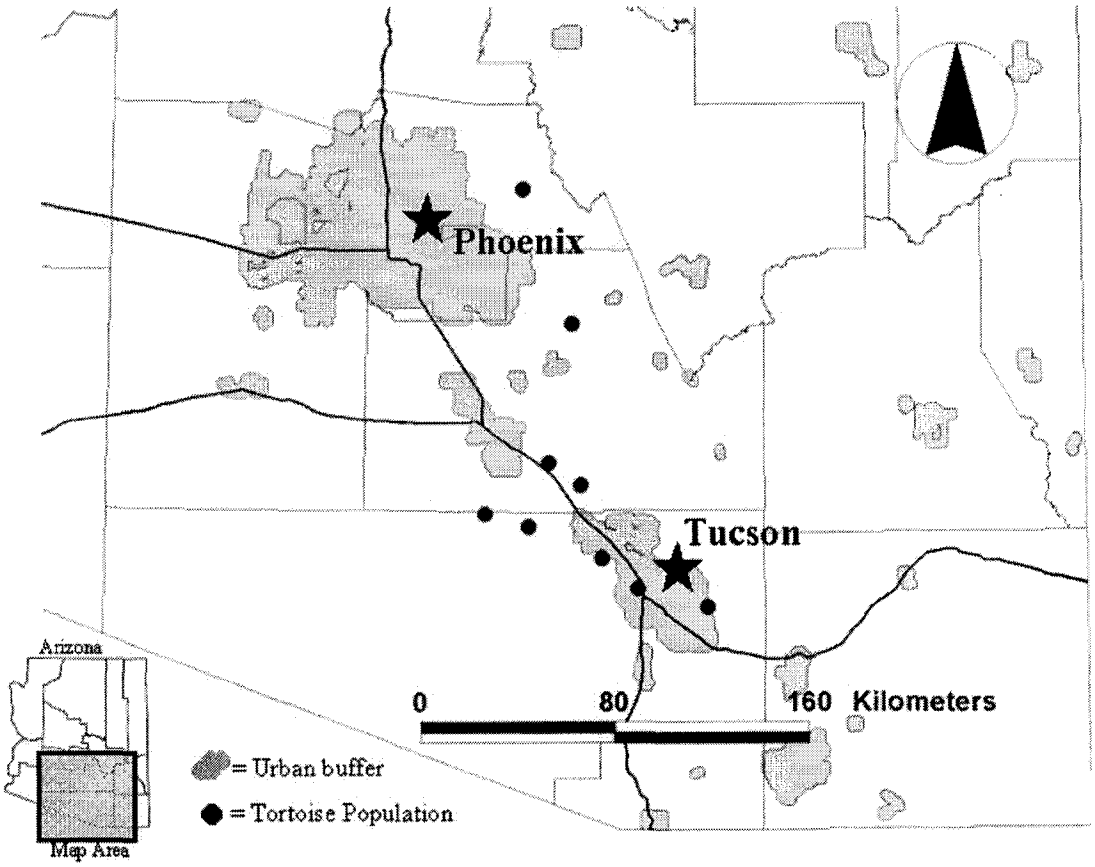

FIGURE 2.5 Distribution of urbanized areas in southern Arizona that may obstruct tortoise movement between populations.

Within-population Structure - Since initiation of desert tortoise radiotelemetry studies at Saguaro National Park in 1996, no radiotelemetered tortoises have moved between the Rocking K and the Mother's Day Fire sites. Home range size MCP estimates were calculated for 34 individuals (Table 2.8). Home range size for the total sample ranged from 0.33 ha to 81.58 ha ($\bar{x}$ = 18.01 ha, 95% C.I. 11.34 to 24.69 ha). Mean home range at the Rocking K site was 18.54 ha (95% C.I. 9.70 to 27.38 ha, $n = 25$) and mean home range at the Mother's Day Fire site was 16.55 ha (95% C.I. 7.77 to 25.34 ha, $n = 9$). After accounting for explanatory variables of sex, size (MCL), and number of point locations, multiple regression showed no significant difference between the mean home range size between the two sites ($F_{4,33} = 0.425$, $P>0.789$). During the study, a radiotelemetered adult female tortoise (RK459) made a long-distance movement of approximately 32 km out of the Park boundary to another mountain range (Appendix B). Because of this unusual behavior, the MCP home range size for RK459 (10,692 ha) was excluded from home range size comparisons. However, RK459 was included in genetic comparisons between the two sites.

Tortoise RK459 encountered several barriers that, without human facilitation, would likely have been insurmountable. A residential fence and an interstate highway (I-10) both required human assistance to cross. I believe a set of railroad tracks may also have acted as a barrier and that the tortoise followed them for some distance before encountering a place to cross. Lastly, I note that at least 4 residents collected the tortoise and contacted researchers. It is possible the tortoise would have become someone's illegal pet had its carapace not been affixed with an identifying label.

TABLE 2.8 Radiotelemetry data for 34 tortoises from Saguaro National Park, Rincon Mountain District. Average minimum convex polygon home range size (MCP) was not significantly different between the Mother's Day Fire and the Rocking K sites after accounting for sex, size (MCL; midline carapace length), and number of location points collected for each tortoise ($F_{4,33} = 0.425$, $P>0.789$). (MEAN: 18.01, 95% C.I. 11.34 to 24.69 ha). *Tortoise RK459 made long distance movement and was not included in home range size analysis.

Site	Tort #	MCL (MM)	# Locations	Sex	MCP (ha)
Mother's Day Fire	MDB000	253	69	Male	4.61
Mother's Day Fire	MDB106	273	51	Male	16.02
Mother's Day Fire	MDB339	264	67	Male	2.81
Mother's Day Fire	MDB410	242	134	Female	22.6
Mother's Day Fire	MDB483	228	156	Female	10.9
Mother's Day Fire	MDB712	225	153	Male	19.6
Mother's Day Fire	MDB721	217	133	Male	6.88
Mother's Day Fire	MDB876	227	62	Female	35.6
Mother's Day Fire	MDB928	254	69	Male	29.94
Rocking K	RK103	231	15	Female	6.72
Rocking K	RK404	267	112	Male	14.29
Rocking K	RK411	222	68	Male	81.58
Rocking K	RK412	242	90	Male	4.75
Rocking K	RK413	249	43	Female	7.44
Rocking K	RK414	230	92	Male	9.86
Rocking K	RK416	227	42	Female	2.16
Rocking K	RK422	222	89	Female	6.41
Rocking K	RK429	242	77	Female	70.1
Rocking K	RK435	235	87	Male	24.38
Rocking K	*RK459	240	20	Female	10,692.2
Rocking K	RK468	220	24	Female	1.9
Rocking K	RK479	226	65	Female	21.65
Rocking K	RK480	249	67	Female	22
Rocking K	RK481	257	52	Male	30.56
Rocking K	RK482	262	51	Male	11.44
Rocking K	RK485	236	62	Female	6.73
Rocking K	RK486	234	64	Female	26.12
Rocking K	RK510	280	72	Female	58.04
Rocking K	RK511	253	60	Male	23.47
Rocking K	RK514	230	25	Female	0.334
Rocking K	RK515	245	31	Female	11.33
Rocking K	RK530	247	51	Male	5.34
Rocking K	RK532	267	49	Male	9.11
Rocking K	RK564	254	12	Female	0.33

Within the Rincon Mountain population at Saguaro National Park, little population genetic structure was observed between the 2 radiotelemetry sites despite geographic features (ridges and washes) that potentially separate them. I observed 76.9% ($p<0.001$) of genetic variation within the population, 20.7% ($p<0.001$) among individuals within each site, and only 2.4% ($p = 0.056$) between the 2 sites. Using a stepwise mutation model, R_{ST} between the two sites was 0.0 ($p<0.001$). There was no correlation between the genetic relationship among individuals (pairwise F_{ST}) and the geographic distance among their home ranges ($r = -0.072$, $p = 0.289$). I did not find evidence that within a single population, genetic variation between individuals was associated with behavior (home range) or habitat characteristics.

DISCUSSION

Phylogeography - The phylogeographic pattern of unique microsatellite alleles among desert tortoise populations (shared alleles across populations, but not all alleles shared) is indicative of intermediate gene flow in a species not subdivided by long-term zoogeographic barriers (Avise et al. 1987). The low frequency of private microsatellite alleles across populations and the significant correlation between genetic and geographic distance among populations suggests that the genetic relationship among desert tortoise populations is characteristic of isolation-by-distance (IBD; Kimura and Weiss 1964). The IBD model for Sonoran desert tortoise populations is also evident in mitochondrial DNA sequence data from a subset of these samples (Chapter 1). The desert tortoise is perhaps the ideal organism for the IBD model; one that is distributed across the landscape in isolated patches and for which the difficulty of dispersal is a function of geography.

Geographic distance between populations is the major limitation to panmixia. Within a continuously distributed population, however, topographic features do not appear to contribute significantly to within-population genetic structure, as exemplified in the Rincon Mountain population.

Gene Flow - Gene flow occurs, or occurred recently until the proliferation of anthropogenic barriers, among desert tortoise populations. The lack of differentiation among populations suggests that dispersal resulting in exchange of genetic material must have occurred in the past at a rate of at least one migrant per generation (OMPG) to alleviate differentiation resulting from mutation or genetic drift (Wright 1931). The results of the KS-test support that the variability shared among populations calculated using the AMOVA is representative of populations exhibiting similar distributions of allele frequencies. The distribution of low-frequency unique alleles detected across populations and the lack of evidence for a recent expansion using BOTTLENECK and the method of Garza and Williamson (2001) support the hypothesis that this lack of differentiation is a result of gene flow and not common ancestry. My estimates of migration using Slatkin's $\hat{M}$ show a minimum of 2.9 migrants per generation between population pairs, but gene flow can be variable and unpredictable among populations due to a wide array of demographic and environmental factors (Daly and Patton 1990) and estimates of absolute numbers of migrants are not reliable using microsatellite markers (Balloux and Lugon-Moulin 2002). Genetic variance among populations (F_{ST}) is only an indirect measure of gene flow and can be misleading when translated into dispersal rates

(Whitlock and McCauley 1999). The conditional average frequency of private alleles used to estimate gene flow (Nm = 5.5) also indicates gene flow above OMPG, but these measures should not be the only means used to draw inference to population structure. Therefore, I also rely on the natural history of tortoises and my observation of inter-population movement to draw conclusions. The most likely scenario for the desert tortoise is that gene flow occurs not at a regular rate, but with varying frequencies over time related to environmental fluctuations (Morafka 1994). Similar measures of gene flow, based on microsatellite data, were found in populations of geometric tortoises (*Psammobates geometricus*) in the western Cape Province of South Africa (F_{ST} = 0.031; Cunningham et al. 2002). This species shares a similar natural history with the desert tortoise in that the landscape contains physical barriers, such as mountains, that separate populations. In addition, the species is also long-lived and is faced with extreme habitat fragmentation due to human development.

The departure from Hardy-Weinberg equilibrium and associated positive inbreeding coefficient for some loci in the sample is most likely due to the population structure I observed for Sonoran desert tortoises (Table 2.1). Among tortoise populations, geographic distance is an isolating force that affects the probability of individuals mating and thus violates the assumption of panmixia. The structured distribution of low-frequency and intermediate alleles across populations (Table 2.3) and significant correlation between genetic and geographic distance among populations I observed in the study make Hardy-Weinberg equilibrium an unreasonable expectation for this species. Deviations from Hardy-Weinberg equilibrium can also result from non-

amplifying alleles. However, I believe the likelihood of this is small because all samples amplified for at least one allele, whereas I would expect some samples to not amplify at all (homozygotes) if null alleles were present in the population. The test of linkage disequilibrium assumes Hardy-Weinberg proportions, so linkage estimates may be incorrect due to the departure from Hardy-Weinberg equilibrium (Excoffier and Slatkin 1998). A structured population will exhibit allele associations as a consequence of non-random mating that are not a result of linkage within the genome. The possibility that some of these markers are linked may limit some of the conclusions that can be drawn from this analysis, but it did not impact the estimates of gene flow.

Movement Barriers - The modern landscape of southern Arizona contains many recently constructed anthropogenic barriers that may obstruct movements of tortoises between populations and disturb patterns of gene flow. During emigration of a radiotelemetered tortoise from the Rincon Mountains to the Santa Rita Mountains, researchers had to facilitate her movement across several anthropogenic barriers, such as fence lines, railroad tracks, and an interstate highway. She was also captured several times and temporarily adopted by private citizens (Appendix B). The genetic data confirm that such long-distance movements result in the exchange of genetic material among adjacent populations. Because tortoises exhibit extremely long generation times with respect to the recent proliferation of landscape barriers (Lovich and Bainbridge 1999), these estimates of gene flow predate anthropogenic habitat fragmentation and should not be taken as evidence that natural immigration/emigration still occurs. Documentation of this inter-population movement demonstrates that desert tortoises can

and sometimes are motivated to disperse great distances. However, the urban topography of the modern landscape makes such movements by tortoises virtually impossible without human assistance.

Population Viability - Tortoise populations confined to foothill habitats in southern Arizona are likely to be small. Three separate population viability analyses (PVAs) conducted on the Mojave population of the desert tortoise recommend that a minimum of 20,000 individuals is necessary for a 50% chance of persistence for 500 years (USFWS 1994). Estimated densities of tortoises in optimal areas in southern Arizona range from 23–56 adults/km^2 (Averill-Murray et al. 2002). Population levels in any given mountain range are far below that recommended by the PVAs generated for Mojave desert tortoises. I did not find evidence using these microsatellite loci indicative of a recent (<1000 generations) genetic bottleneck that would otherwise explain a small population size. The inference that tortoises maintain long-term, small effective population size is supported by mitochondrial DNA analysis (Chapter 1). Because effective population sizes of Sonoran desert tortoises are small, dispersal events probably play an important role in the long-term maintenance of these populations. Life history traits of the desert tortoise, a long-lived species with delayed sexual maturity, suggest that there are severe constraints on the ability of populations to respond to chronic disturbances (Congdon et al. 1993). Demographic modeling for tortoises indicates that adult females are the most crucial life stage for population longevity, such that even a small increase in their mortality rate could result in a population crash (Doak et al. 1994). It is unlikely that a closed population of desert tortoises experiencing a dramatic

reduction in adult survivorship would be able to offset that loss through compensatory increase in reproductive output. The high level of gene flow among populations suggests that if a population were to experience a catastrophic decline as a result of drought or other stochastic event, its recovery may rely heavily on the immigration of new individuals from adjacent mountain ranges for recovery.

Management and Conservation Implications - I demonstrate that tortoises in this study area historically dispersed between mountain ranges and that inter-population movements may be critical to the persistence of small tortoise populations. Because many historic dispersal routes are no longer available to desert tortoises as a result of anthropogenic landscape change, informed management strategies need to be in place to facilitate the long-term persistence of Sonoran desert tortoise populations. Many tortoise populations are becoming islands surrounded by human development. Encroachment of human development makes tortoise populations vulnerable to multiple threats, such as road mortality, illegal take, and exposure to disease from escaped or released domestic tortoises (AIDTT 2000).

The genetic data suggest that gene flow among populations is part of the evolutionary history of the desert tortoise and therefore inter-population movements may be critical to the long-term viability of populations. Assessing what constitutes a barrier to movement for tortoises is necessary for maintaining connectivity between populations. While a roadway may not be a barrier to a large ungulate, it may be impenetrable to a tortoise. Tortoises are able to cross some barriers and have been shown to use culverts (Ruby et al. 1994). Fencing or concrete barriers along highways may also help guide

tortoises toward appropriate crossing areas and prevent road mortality. Placement of culverts and corridors needs to specifically accommodate tortoises, as corridors designed for general wildlife use may not be effective (Barrett et al. 1990).

Microsatellite data can be used to design effective translocation strategies for wildlife by providing information on the rate of gene flow, the level of divergence between populations, genetic variability of populations, and the number of individuals for translocation (Maudet et al. 2002). Translocation of tortoises from nearest-neighbor populations should be evaluated as a potential management strategy to recover or maintain small populations isolated by anthropogenic barriers. Tortoises generally exhibit strong site tenacity (Barrett et al. 1990, Bailey 1992), and translocation studies of reptiles indicate that they generally fare poorly in unfamiliar areas (Barrett et al. 1990, Dodd and Seigel 1991, Reinert and Rupert 1999). However, preliminary studies in the Mojave Desert indicate that translocation may be an effective strategy for supplementing depauperate populations of desert tortoises (Tracy et al. 2000). Currently in Arizona, tortoises are sometimes relocated short distances during construction projects (AIDTT 1996). Before inter-population translocation of tortoises is implemented as a conservation strategy in the Sonoran Desert, effects of translocation on survivorship of relocated individuals and the populations into which they are introduced need to be evaluated and the potential for disease transmission from one population to another needs to be assessed (Dodd and Seigel 1991, Jacobson 1993, Cunningham 1996, Seigel and Dodd 2002). In addition, translocation will not likely be a sustainable strategy unless threats are also identified and alleviated. While it may be tempting to apply the OMPG

rule to isolated tortoise populations not declining, different schedules of supplementation may be appropriate depending on environmental and demographic conditions specific to each population (Mills and Allendorf 1996). Management strategies compatible with the evolutionary history of gene flow among disjunct populations will help ensure the long-term persistence of Sonoran desert tortoise populations.

APPENDIX A - PCR PRIMERS FOR MICROSATELLITE LOCI IN DESERT TORTOISE (*GOPHERUS AGASSIZII*, TESTUDINIDAE)

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ABSTRACT

The desert tortoise, *Gopherus agassizii*, is a threatened species native to the North American desert southwest and is recognized as having distinct Mojave and Sonoran populations. We identified six polymorphic microsatellite loci in the desert tortoise. All six loci were polymorphic in Sonoran samples. Five of the loci were variable in Mojave samples with varying degrees of amplification success. Two of the loci exhibited low allelic variation (2-3 alleles) while four were highly variable (8-27 alleles). These markers are useful for conservation genetic studies of the desert tortoise and may also be useful for studies of congeners in the United States and Mexico.

The desert tortoise, *Gopherus agassizii*, is native to the North American desert southwest and has distinct Mojave and Sonoran populations separated geographically by the Colorado River. The Mojave population is federally listed as threatened under the Endangered Species Act and the Sonoran population is fully protected under Mexican and United States state laws. We identified six novel microsatellite loci in samples of the Sonoran desert tortoise and successfully cross-amplified five of the markers in samples from the Mojave Desert. These markers may also be applicable to studies of congeners in the United States and Mexico, two of which (*G. flavomarginatus* and *G. polyphemus*) are species of concern.

We prepared a microsatellite-enriched genomic library for the tortoises using a protocol adapted from Hamilton et al. (1999). We isolated total genomic DNA from whole blood using a phenol/chloroform extraction protocol (Goldberg et al. 2003). We digested genomic DNA from a single individual using *Rsa*I (New England BioLabs, Inc.) and ligated SNX linkers onto both ends of the fragments (Hamilton et al. 1999). We probed a sample of ~100 ng of genomic DNA with a biotin-labeled oligo consisting of a trinucleotide motif (10 repeats) or a mixture of oligos with different motifs (CAA, CTT, ATC, AGT; 2 pmols of each) in a 100 µl volume of 5X SSC, 0.1% SDS, and 50% formamide. We performed the hybridization at 95 °C for 15 minutes, then stepped down the temperature 1 °C / minute to 60 °C, and incubated for 1 hour. We then added 300 µg of streptavidin-coated Dynabeads (M-280, Dynal) and incubated the samples at 43 °C with agitation for at least 5 hours. We washed the beads twice at room temperature with 2X SSC, 0.1% SDS, twice at 45 °C with 1X SSC, 0.1% SDS, and then twice at 65 °C in

1X SSC, 0.1% SDS for 5 minutes each. For hybridization with the CAA oligo only, we used a hybridization buffer of 12X SSC, 0.1% SDS, set the final step down and incubation temperature to 70 °C, and performed the third wash at 75 °C. We eluted the DNA from the beads in low TE (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA) at 95 °C for 10 minutes. We PCR amplified the eluate as described in Hamilton et al. (1999). In an alternative modification of this protocol, we digested the genomic DNA with *Alu*I, *Rsa*I, and *Nhe*I and used an oligo of 10 AGC repeats as a probe. We used a hybridization buffer of 12X SSC, 0.1% SDS, hybridized at 75 °C, and heated the last 2 washes to 80 °C.

We cloned the eluate amplicons using a TOPO TA cloning kit (Invitrogen) and amplified inserts from individual colonies using T7 and M13R universal primers. Sequences were obtained using an ABI Prism® 377 DNA Sequencer or ABI Prism® 3700 DNA Analyzer using ABI Big Dye terminator cycle sequencing methodology (PE Biosystems). We designed primers for amplification of microsatellites using Oligo version 6.68 (Molecular Biology Insights, Inc.).

We performed PCR amplification with novel primers in 10 µl reaction volumes containing 0.2 µM of each primer, 10 mM Tris-HCl (pH 8.3), 0.25 mM of each dNTP, 0.4 units of Taq (Sigma-Aldrich), 50 mM KCl, 5 ng of genomic DNA template (2 ng for *Goag*6), and locus-specific amounts of $MgCl_2$ (Table A.1). PCR was performed with a PTC-100TM Thermocycler (MJ Research, Inc.) with an initial 5-min denaturation at 94 °C, followed by 35 cycles of 30 seconds at 94 °C, 30 seconds at the locus-specific annealing temperature (Table A.1), and 30 seconds at 72 °C (45 seconds for *Goag*5 and

*Goag*8), followed by a 6-min incubation at 72 °C. Addition of 2% formamide improved specificity of PCR product amplification for *Goag*5 and *Goag*6. Allelic lengths were determined on an ABI Prism® 3100 Genetic Analyzer and Genotyper® version 1.0 software (PE Biosystems) using 5' fluorescently labeled forward primers (Invitrogen).

The following repeat motifs and oligo combinations successfully enriched the library for microsatellites: CTT, CAA, and ATC combined; AGT alone; CAA alone; and AGC alone. We amplified 5 clones derived from the AGC hybridization; 2 had microsatellites. In 6 separate hybridizations using the 3-oligo hybridization, we screened a total of 63 clones and recovered 8 clones containing microsatellites (*Goag*4, *Goag*5; Table A.1). We screened 16 clones from the AGT oligo hybridization and found 2 microsatellite loci, both of which were dinucleotide repeats (*Goag*6, *Goag*7; Table A.1). The most efficient hybridization was with the CAA probe. Of a total of 150 clones sequenced, 71 contained microsatellites. Of these, many lacked sufficient flanking sequences for primer design and several clones appeared to contain the same microsatellite locus. Only 11 of the clones from the CAA oligo hybridization contained a trinucleotide repeat (*Goag*3). The remaining 60 were a variety of dinucleotide repeats, including *Goag*8 and *Goag*32. In total, we identified 53 unique microsatellite loci with sufficient flanking sequences to design primers. We were able to amplify a single amplicon for 7 loci. We assessed variability by testing the markers on 8 individuals from 8 Sonoran locations. Six loci exhibited variation and were scored for the entire sample.

We amplified and scored microsatellite loci for 170 individuals from 9 Sonoran sites in southern Arizona. All 6 loci were polymorphic in all populations. Loci *Goag*3

and *Goag*32 exhibited low variability (2-3 alleles), loci *Goag*4, *Goag*5, *Goag*6, and *Goag*7 were highly variable (8-27 alleles). Three loci (*Goag*5, *Goag*6, and *Goag*7) deviated from expected heterozygosities under Hardy-Weinberg proportions and had a significantly positive inbreeding coefficient (Table A.2). We attribute these deviations to population structure in tortoises, characterized by isolation by distance. We believe the likelihood of null alleles is small because all samples amplified for at least one allele.

We also examined these 6 primers on 20 to 40 individuals representing 4 locations in the Western Mojave Recovery Unit of the Mojave population (Table A.2). Locus *Goag*3 was monomorphic in the Mojave samples. Four loci, *Goag*4, *Goag*5, *Goag*6, and *Goag*32, expressed alleles outside of the range obtained for the Sonoran samples (Table A.1). Using the same PCR conditions optimized for the Sonoran samples, we were able to amplify only 4 of the Mojave samples using *Goag*5. Deviations from expected Hardy-Weinberg proportions at these loci (Table A.2) may indicate the presence of null alleles in Mojave samples. Additional work is necessary for more representative characterization of levels of polymorphism of these loci in Mojave samples.

In addition, we tested 11 microsatellite loci identified in several other chelonian species: *Chelonia mydas*, (Cm3, Cm58, Cm72, Cm84), *Caretta caretta* (Cc117, Cc7) *Eretmochelys imbricata*, (Ei8) and *Podocnemis expansa* (PE334, PE519, PE107), (FitzSimmons et al. 1995, FitzSimmons 1998, Sites et al. 1999). Of these, we successfully amplified 2 loci in the desert tortoise genome (Cm58, Cc7). Interestingly, the repeat array for both loci were dramatically different in the desert tortoise; for Cm58,

$(TA)_5(GA)_3GC(GT)_3$ instead of $(CA)_{13}$, and for Cc7, $(CA)_5(TC)_4$ instead of $(CA)_{14}$. However, without comparing flanking sequences we are not able to confirm if these were in fact the same loci from each species. In a test of 8 samples representing 8 Sonoran locations, Cc7 proved monomorphic in our sample of desert tortoises. Cm58 expressed 2 alleles in our sample set (Table A.1).

Understanding the population structure of desert tortoises in the Sonoran and Mojave deserts will assist conservation efforts for this species by allowing managers to delimit populations for monitoring and assess potential anthropogenic barriers to gene flow.

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TABLE A.1 Forward (F) and reverse (R) primers, repeat motif, annealing temperature (T_a), and $MgCl_2$ concentrations for PCR amplification of 9 microsatellite loci the desert tortoise. Amplicon size and observed number of alleles reported for both the Sonoran and Mojave populations (n = number of tortoises for which the PCR product was successfully amplified and scored).

					Sonoran samples			Mojave samples		
Locus	**Repeat Motif**	**Primer sequence (5' → 3')**	**PCR T_a (°C)**	**MgCl (mM)**	***n***	**Amplicon Size (bp)**	**Observed Alleles**	***n***	**Amplicon Size (bp)**	**Observed Alleles**
Goag3	$(CAA)_6$	F: CTG ATT GGT CTG ACT CCC T R: CCT GAT TGC TTC CTG ACA C	61	3.0	170	375-381	3	40	375	1
Goag4	$(CAA)_{24}$	F: CTC AAC AAA AGG TAA GTG ATG R: GCA TAA AAG TAA ACA GTA AAG TA	57	2.5	170	110-188	17	40	137-194	12
Goag5	$(GAT)_{17}$	F: AGG CAA GTG GGT GGT AAT G R: GCG ATT TTG AGG CTT CTT TC	65	3.5	170	257-365	27	4	245-248	2
Goag6	$(TC)_8(AC)_{11}$	F: TAA GGG CTA TGA GGA AGA AT R: GTA ATG GTG TGG GTG GGA	53	2.0	170	360-442	15	21	364-444	18
Goag7	$(AC)_3(GC)_5(AC)_{11}$	F: TCA ATC CAT TAG TCT TCA CCC R: TTT CTG TTT ATG CTC CGT ATT A	61	3.0	170	261-281	8	27	265-273	5
Goag8	$(CA)_{14}TA(CA)_3$	F: ATG CTG ACA ATA GAA CAA GA R: ACA TCT GGG GCT AAA GTG	57	2.5	8	192	1	-	-	-
Goag32	$(AC)_6$	F: GTG CTG CCT TGA TAA GTA A R: ATA GTT TTC TTT CCT ACA CAT	53	2.5	170	177-179	2	19	179-181	2
Cm58	$(TA)_5(GA)_3GC(GT)_3$	F: GCC TGC AGT ACA CTC GGT ATT TAT R: TCA ATG AAA GTG ACA GGA TGT ACC	56.5	3.0	170	131-133	2	-	-	-
Cc7	$(CA)_5(TC)_4$	F: TGC ATTGCT TGA CCA ATT AGT GAG R: ACA TGT ATA GTT GAG GAG CAA GTG	59	2.0	8	156	1	-	-	-

TABLE A.2 Hardy-Weinberg proportions and fixation indices for microsatellite loci in Sonoran and Mojave populations of desert tortoise. H_{obs} = observed heterozygosity and H_{exp} = expected heterozygosity, S.D. = standard deviation of randomization tests for Hardy-Weinberg equilibrium, F_{st} = Wright's fixation index, and. F_{IS} = Weir and Cockerham's inbreeding estimator.

Sonoran samples

Locus	H_{obs}	H_{exp}	*p*	*S.D.*	F_{ST}	*p*	F_{IS}	*p*
***Goag*3**	0.3626	0.3642	0.695	0.005	0.0117	0.127	-0.008	0.666
***Goag*4**	0.6374	0.6621	0.510	0.002	0.0494	<0.001	0.037	0.094
***Goag*5**	0.8830	0.9209	<0.001	<0.001	0.0167	0.002	0.041	0.021
***Goag*6**	0.3333	0.6973	<0.001	<0.001	0.0398	0.032	0.519	<0.001
***Goag*7**	0.4737	0.6686	<0.001	<0.001	0.0703	<0.001	0.288	<0.001
***Goag*32**				<0.001				
	0.2924	0.2950	1.000		0.0305	0.024	-0.008	1.000
Cm58	0.2690	0.2873	0.602	0.0048	0.0225	0.058	0.048	0.585

Mojave samples

Locus	H_{obs}	H_{exp}	*p*	*S.D.*	F_{ST}	*p*	F_{IS}	*p*
***Goag*4**	0.8000	0.8804	0.239	0.002	0.0264	0.123	0.064	0.254
***Goag*6**	0.6191	0.9280	<0.001	<0.001	0.1168	0.022	0.250	0.007
***Goag*7**	0.5926	0.7079	0.164	0.003	0.0163	0.405	0.145	0.154
***Goag*32**	0.0000	0.3926	<0.001	<0.001	0.1308	0.625	1.000	<0.001

APPENDIX B – LIFE HISTORY NOTES. *GOPHERUS AGASSIZII* (DESERT TORTOISE). MOVEMENT

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In the Sonoran Desert, *Gopherus agassizii* occurs in rocky foothills associated with saguaro cactus (*Carnegiea gigantea*) and foothill paloverde (*Parkinsonia microphylla*) characteristic of Upland Sonoran Desert Scrub plant community (Turner and Brown 1992. Pages 180-221 *in* Brown 1992. Desert Plants 4). Although these populations appear to be isolated by low desert valleys, radiotelemetry data have shown that tortoises are capable of making long distance movements between populations (Barrett et al. 1990. Final report, Bureau of Reclamation, Arizona Projects Office, Phoenix; Averill-Murray and Klug 2000. Technical Report 161, Arizona Game and Fish Dept., Phoenix). Long-distance movements between disjunct populations may facilitate genetic exchange (Britten et al. 1997. Copeia 1997:523-530) and be important for long-term maintenance of populations. Here we report an extraordinary movement by a female *G. agassizii* and the anthropogenic barriers encountered during this event. We

show that, while desert tortoises are capable and sometimes motivated to make inter-population movements, the urban topography of our modern landscape makes such movements increasingly difficult.

We affixed a radio transmitter (AVM G3, AVM Industries, Colfax, CA) to an adult female *G. agassizii* (238 mm MCL, 2700 g) on 14 August 2000, as part of an ongoing study in the Rincon Mountains at Saguaro National Park (SNP; 32°08'N, 110°41'W), Tucson, Arizona. At the time of transmitter attachment, the tortoise presented signs of upper respiratory tract disease (URTD; nasal discharge, wheezing, occluded nares, and exudate). Other tortoises at this study site have tested positive for *Mycoplasma agassizii*. We located the tortoise approximately every week. By 06 September, she had moved approximately 500 m southwest of the original capture location. We then lost contact with her until 18 September when a SNP volunteer observed the tortoise along a roadway ca. 1.5 km south of the original locality (32°06'N, 110°41'W). On 25 September, we found her approximately 8 km further south on a rocky slope surrounded by low-density housing (32°02'N, 110°40'W). The terrain between these locations is primarily flat ground dominated by creosote bush (*Larrea tridentata*) and is atypical of Sonoran desert tortoise habitat (Barrett 1990. Herpetologica 46:202-206). On 02 October, we found her on private property along a chain-link fence. We obtained permission and put her across. At this time, we affixed a note to the tortoise's carapace indicating she was part of a study at Saguaro National Park and included a contact phone number. We believe the tortoise over-wintered in Arizona

Upland Sonoran Desert Scrub on a large expanse of private land; however, we did not receive a signal from her between 02 October 2000 and late July, 2001.

On 31 July 2001 we were contacted by a resident who had found the tortoise in Vail, Arizona, in the middle of a paved street at a railroad crossing (approximately 15 km south of where she was first marked; 32°03'N, 110°42'W). We placed her south of the railroad tracks (within 0.5 km east of the crossing), oriented in the same general direction she was moving but away from residential housing. Over the next two months, we received 3 phone calls from residents who had found the tortoise and brought her home. Each time, we returned the tortoise to uninhabited areas in the vicinity. During this period she remained within 1.5 km north of Interstate 10 (a 4 lane freeway due south of Vail), and traversed an approximately 3-km east-west distance. We made an *a priori* decision to facilitate the tortoise's movement across Interstate 10 if she continued moving south.

On 29 August 2001, we located the tortoise on a frontage road beside I-10 (32°01'N, 110°42'W) and decided to transport her across the interstate. We placed the tortoise on a north-facing slope of the Santa Rita Mountains approximately 7 km south of the interstate, where we observed tortoise sign (31°55'N, 110°42'W). We decided the 7-km distance was necessary because medium density housing and many fences bisect land south of the interstate. The tortoise made several east-west movements along the foothill slopes at the new location, and on 18 September, 2001 we were contacted by a landowner who found her in the middle of a new residential development, 5 km west of the release point (31°54'N, 110°53'W). We collected her and returned her to the original release site

in the Santa Ritas. She spent the winter of 2000-2001 in the north end of the Santa Rita Mountains. We periodically found the tortoise at this same location until June 2002, when her transmitter failed prematurely. Not including the human-facilitated movement of ca. 7 km, this tortoise moved more than 30 km straight-line distance over the span of one year.

On 22 August 2002, we were contacted yet again by a family who found her on Interstate 10 under an overpass (32°01'N, 110°43'W), 7 km north of her over-wintering site, toward the original capture site. We changed transmitters and re-released her at the first point of capture, at the south end of Saguaro National Park. She has remained at this site through the winter of 2003. The tortoise currently presents signs of URTD, but did not do so consistently since the time of transmitter attachment.

It seems unlikely that the behavior exhibited by this tortoise was in response to stress caused by initial handling because we have placed transmitters on >70 tortoises since 1992 and no other tortoises have made long distance movements. Occasionally, within 24 hours of being handled, tortoises will move from the capture site, but less than 1 km. It is possible that humans other than researchers facilitated portions of the movements reported here, particularly during the initial 8 km movement during 18 – 25 September 2001, which crossed 2 roads. However, the tortoise had already begun an unusually long movement through an unpopulated area to reach the first road from the study site (1.5 km) and was in atypical habitat.

This tortoise encountered several barriers that, without human facilitation, would likely have been insurmountable. A residential fence and an interstate highway both

required human assistance to cross. We believe a set of railroad tracks may also have acted as a barrier and that the tortoise followed them for some distance before encountering a place to cross. Lastly, we note that at least four residents collected the tortoise and contacted us. It is possible the tortoise would have become someone's illegal pet if the identifying label had not been affixed to the carapace.

This project was funded by the Arizona Game and Fish Department Heritage Grant Program, the National Park Service, the Southwestern Parks and Monuments Association, and the US Geological Survey. Research was conducted under scientific research permits from the Arizona Game and Fish Department and the National Park Service. Tortoise handling protocols were approved by the University of Arizona (IACUC 00-084). We thank Kevin Bonine and Caren Goldberg for reviewing an earlier version of this note.

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