A Genetic Assessment of the Recovery Units for the Mojave Population of the Desert Tortoise, *Gopherus agassizii*

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ABSTRACT. – In the 1994 Recovery Plan for the Mojave population of the desert tortoise, *Gopherus agassizii*, the US Fish and Wildlife Service established 6 recovery units by using the best available data on habitat use, behavior, morphology, and genetics. To further assess the validity of the recovery units, we analyzed genetic data by using mitochondrial deoxyribonucleic acid (mtDNA) sequences and nuclear DNA microsatellites. In total, 125 desert tortoises were sampled for mtDNA and 628 for microsatellites from 31 study sites, representing all recovery units and desert regions throughout the Mojave Desert in California and Utah, and the Colorado Desert of California. The mtDNA revealed a great divergence between the Mojave populations west of the Colorado River and those occurring east of the river in the Sonoran Desert of Arizona. Some divergence also occurred between northern and southern populations within the Mojave population. The microsatellites indicated a low frequency of private alleles and a significant correlation between genetic and geographic distance among 31 sample sites, which was consistent with an isolation-by-distance population structure. Regional genetic differentiation was complementary to the recovery units in the Recovery Plan. Most allelic frequencies in the recovery units differed. An assignment test correctly placed most individuals to their recovery unit of origin. Of the 6 recovery units, the Northeastern and the Upper Virgin River units showed the greatest differentiation; these units may have been relatively more isolated than other areas and should be managed accordingly. The Western Mojave Recovery Unit, by using the new genetic data, was redefined along regional boundaries into the Western Mojave, Central Mojave, and Southern Mojave recovery units. Large-scale translocations of tortoises and habitat disturbance throughout the 20th century may have contributed to the observed patterns of regional similarity.

KEY WORDS. – Reptilia; Testudines; Testudinidae; *Gopherus agassizii*; tortoise; conservation genetics; distinctive population segment; evolutionary significant unit; management units; microsatellites; mitochondrial DNA; Mojave Desert; USA

The desert tortoise (*Gopherus agassizii*) is a widespread species (or possible species complex) occurring in the southwestern United States and northwestern Mexico (Fritts and Jennings 1994; Berry et al. 2002; Stebbins 2003). The US Fish and Wildlife Service (USFWS) federally listed the species as threatened under the Endangered Species Act, as amended, in the northern one third of its geographic range, specifically, populations living north and west of the Colorado River in the Mojave and Colorado deserts (USFWS 1990; Fig. 1). The listing occurred primarily because of population declines and habitat loss and deterioration, which were attributed to human activities. In recognition of the distinctiveness of the threatened populations, the USFWS developed the *Desert Tortoise (Mojave Population) Recovery Plan* (referred to herein as *Recovery Plan*) (USFWS 1994) and designated 26,087 km^2 of critical habitat (Berry 1997).

About 83% of the critical habitat is on land managed by government agencies.

The federal listing of the desert tortoise as a threatened species brought about a redirection of government efforts to recover the species within its 4 southwestern states (California, Arizona, Nevada, and Utah). Several government agencies prepared new long-term management plans or amended older land-use plans to support recovery efforts (Berry 1997), a process that required more than 16 years. The extent of landscape affected by these efforts was significant and included parts of the Mojave Desert and the Colorado Desert (also called western Sonoran Desert). For convenience, the USFWS termed the populations within critical habitat as the "Mojave" population, when in fact they occur in both the Mojave and Colorado deserts. Herein, we follow this terminology. For populations in the Sonoran Desert of Arizona, we use "Sonoran" populations.
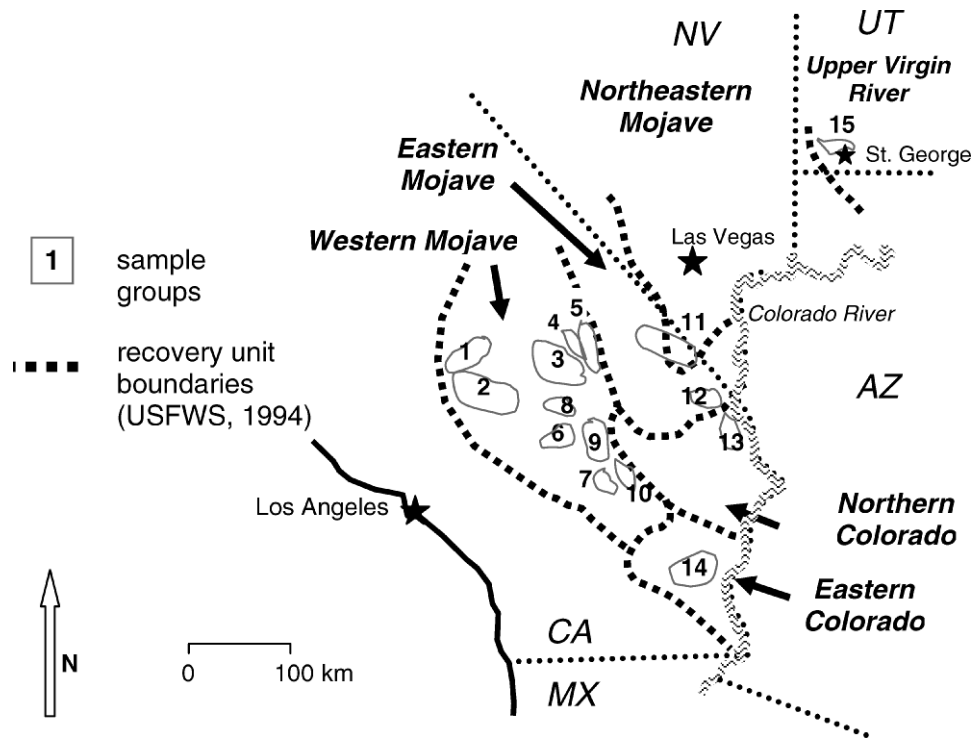
Figure 1. Sample groups and recovery unit boundaries for *Gopherus agassizii* as described in the *Desert Tortoise (Mojave Population) Recovery Plan* (USFWS 1994) and sample sites for this study. Because of their geographic proximity, 3 tortoises from the Eastern Mojave Recovery Unit were combined with 57 tortoises from the Northeastern Mojave Recovery Unit to form sample group 11.

Desert tortoises exhibit substantial differences in morphology (Weinstein and Berry 1987; Germano 1993), physiology (Turner et al. 1986; Wallis et al. 1999; Averill-Murray 2002; Averill-Murray et al. 2002a, 2002b; McLuckie and Fridell 2002), behavior (e.g., Woodbury and Hardy 1948; Burge 1977; Averill-Murray et al. 2002b; Jennings 2002), and genetics (Lamb et al. 1989; Lamb and Lydeard 1994; McLuckie et al. 1999; Lamb and McLuckie 2002) throughout the geographic range in the United States. This variation occurs within and between the Mojave and Sonoran populations.

The authors of the *Recovery Plan* recommended protection of 6 evolutionarily significant units (ESUs) or distinct population segments (DPSs) in 6 "recovery units" (Ryder 1986; Waples 1991, 1998; US Department of the Interior and US Department of Commerce 1996). They noted that the ESUs (or DPSs) consisted of "populations or groups of populations that show significant differentiation in genetics, morphology, ecology or behavior . . . and thus are important components of the evolutionary legacy of *Gopherus agassizii*" (USFWS 1994). They stated that the conservation of all ESUs would help to ensure that "the dynamic process of evolution [in this species] will not be unduly constrained in the future [Waples 1991]" (USFWS 1994). It is important to note that the authors used the phrases ESUs, DPSs, and recovery units synonymously, and their intent was to draw on multiple criteria to delineate units (after Waples 1991, and similar to Crandall et al. 2000). The USFWS also recommended that concepts in the *Recovery Plan* be subjected to

hypothesis-testing. In the case of genetics, the limited available mitochondrial deoxyribonucleic acid (mtDNA) data suggested that *G. agassizii* might be composed of more than 1 species, with the Colorado River acting as a boundary in the northern part of the geographic range (Lamb et al. 1989; summarized in Berry et al. 2002).

Since the *Recovery Plan* (USFWS 1994) was published, the fields of population and conservation genetics have advanced rapidly. Numerous new, powerful techniques are now available for processing, statistically analyzing, and interpreting genetic samples (e.g., DeSalle and Amato 2004; Pearse and Crandall 2004; Manel et al. 2005; Allendorf and Luikart 2007). In 1996, the federal government further clarified the Endangered Species policy on DPSs for vertebrates (US Department of the Interior and US Department of Commerce 1996). The academic dialog on the definitions and applicabilities of ESUs, DPSs, and other related concepts, such as management units (MUs), Canadian designatable units (DUs), and adaptive evolutionary conservation has continued to be rigorous and brisk (Crandall et al. 2000; Fraser and Bernatchez 2001; Pearman 2001; Moritz 2002; Green 2005). However, distinct infraspecific populations of American vertebrates, except for salmonid fishes, can currently only receive legal protection as DPSs, not as ESUs.

A factor complicating the genetic study of desert tortoise populations has been human-mediated translocation. The tortoise has received much well-intended attention by governmental agencies and concerned citizens

since the 1930s (California Code of Regulations 2007). Thousands of tortoises have been taken into captivity and then released. Still others have been translocated from one area to another in the desert. Commercial harvesting and interstate transportation have been significant.

Our objectives are to contribute to recovery efforts for this species by: 1) characterizing genetic differences in the Mojave populations to determine whether the existing 6 recovery units are genetically distinguishable and, if so, to what extent; 2) evaluating the potential effects of numerous releases and translocations of tortoises on genetic structure; and 3) placing the genetic data in the context of ecological and behavioral differences in desert tortoises to support the conservation of ecological and evolutionary processes.

METHODS

Sample Collection

We salvaged blood from desert tortoises used in research projects on health, disease, and physiology, and through collaboration with other scientists (Henen et al. 1997; Brown et al. 1999; Christopher et al. 1999, 2003; Edwards 2003). Desert tortoises were captured by hand in the field by following federal and state protocols (Averill-Murray 2000; Berry and Christopher 2001). Samples were collected from tortoises ($n = 628$) at 31 study sites that occur within the geographic range where the tortoise is federally listed (USFWS 1990) (Table 1; Fig. 1). We did not include sites from Nevada or the Beaver Dam Slope, Utah. Study sites were in remote areas as well as < 2 km from towns or human habitation. We also obtained mtDNA sequences from 4 *G. agassizii* from the Sonoran Desert of Arizona (Edwards et al. 2003), 1 sample of the bolson tortoise (*Gopherus flavomarginatus*) from a private collection, and 1 sample of the Texas tortoise (*Gopherus berlandieri*) from the Department of Animal Care and Technologies at Arizona State University, Tempe (J. Badman).

About 1 ml whole blood was collected via brachial, jugular, or subcarapacial venipuncture, and the samples were stored on ice or dry ice in (ethylenediamine tetraacetic acid [EDTA]), lithium heparin, or 95% ethanol. Most samples (from health and disease studies) were centrifuged first, the plasma was removed, and the red blood cells were retained and frozen for DNA extraction.

Molecular Techniques

Molecular procedures were conducted at the Genomic Analysis and Technology Core, University of Arizona. Genomic DNA was isolated 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. 2003). The DNA was resuspended in low TE (10 mM Tris-pH 8.0, 0.1 mM EDTA) and diluted to a 5 ng/μL working stock for polymerase chain reaction (PCR) amplifications.

MtDNA Sequencing. — We amplified an ca.1500–base-pair (bp) portion of the nicotinamide adenine dinucleotide dehydrogenase subunit (ND)3, arginine transfer RNA (tRNA) ND4L, and part of the ND4 genes by using primers Nap2 and New Gly (Arévalo et al. 1994; Britten et al. 1997; Edwards 2003). PCR followed Edwards (2003), and the PCR products were purified by using the QIAquick PCR purification kit (Qiagen, Valencia, CA) and were sequenced on an ABI Prism 3700 DNA Analyzer (PE Biosystems, Foster, CA). Internal primers were designed by using Oligo Primer Analysis Software 6.68 (Molecular Biology Insights, Inc, Cascade, CO): Nap2IN 5'AGGCGGTCAATAATGC-TAATC3' and NewGIN 5'TAATAAAACCAGACAAT-GAAAAAC3'. These primers amplified an 1109-bp portion of ND3/ND4, which was aligned and evaluated by using Sequence Navigator 1.0.1 (Applied Biosystems, Inc, Foster, CA).

Nuclear DNA Assessment. — Data gathering was carried out on an ABI Prism 3730 DNA Analyzer (PE Biosystems). All samples were tested for 16 microsatellite loci (Table 2). The loci were PCR amplified in 6 separate multiplex reactions by using 5' fluorescently labeled forward primers. We sequenced selected products for all loci to verify repeat motifs. Repeat motifs were identified by using Sequence Navigator 1.0.1 (Applied Biosystems, Inc). Reliably scored, variable loci were used for analysis.

Analysis

Grouping of Samples. — Sample sizes from each of the 31 study sites ranged from 3 to 74 (Table 1). Study sites were assigned to 1 of 15 sample groups based on location, proximity to nearby sites (≤ 60 km), potential topographic or geographic barriers to movement of tortoises, region of the desert, recovery unit as described in the *Recovery Plan* (Fig. 1), and the need to maintain a minimum sample size for statistical analyses. Thus, the 15 sample groups contained 18–83 tortoises (Table 1). Sample group 11 combined individuals from Ivanpah, California ($n = 57$), which belong to the Northeastern Mojave Recovery Unit, with 3 tortoises from Shadow Valley in the Eastern Mojave Recovery Unit owing to close geographic proximity of the localities. We assigned groups to regions of the Mojave and Colorado deserts by using boundaries similar to those described in Rowlands et al. (1982), and the boundary between the Mojave and Colorado deserts as described in Jaeger (1957), Benson and Darrow (1981), Rowlands et al. (1982), and Turner et al. (1995). For boundaries delineating the northern and eastern regions within the Colorado Desert, we followed the *Recovery Plan* (USFWS 1994; Rowlands 1995a, 1995b).

MtDNA. — We selected 125 tortoises representing all recovery units, including 47 samples from the Northeast-

Table 1. Desert tortoise study sites and sample groupings representing 8 regions for the Mojave population.

Desert region/recovery unit	Study site	No. samples	Group	No. samples in group
Western Mojave	Desert Tortoise Research Natural Area	58	1	62
	Fremont-Valley	4	1	
	Hinkley	12	2	83
	Kramer	3	2	
	Edwards Air Force Base	57	2	
	Fremont-Kramer	11	2	
"Central Mojave"	Superior-Cronese	10	3	19
	Fort Irwin (Goldstone)	9	3	
	Fort Irwin (Tiefort)	31	4	31
	Fort Irwin (Soda Mtns.)	33	5	47
	Fort Irwin (Eastgate 2)	14	5	
"Southern Mojave"	Lucerne Valley	12	6	26
	Ord-Rodman	14	6	
	MCAGCC[a] (Emerson)	9	7	71
	MCAGCC (Sand Hill)	62	7	
	Daggett	74	8	74
	MCAGCC (Lavic Lake)	8	9	27
	MCAGCC (Maumee Mine)	7	9	
	MCAGCC (Sunshine Peak)	12	9	
	MCAGCC (Bullion)	16	10	19
	MCAGCC (Lava)	3	10	
Northeastern Mojave	Ivanpah	34	11	60
	Ivanpah (site 14)	23	11	
	Shadow Valley[b]	3	11	
Eastern Mojave	Fenner	4	12	31
	Goffs	27	12	
Northern Colorado	Chemhuevi	7	13	18
	Upper Ward Valley	11	13	
Eastern Colorado	Chuckwalla	18	14	37
	Chocolate Mtns.	19	14	
Upper Virgin River	near St. George, UT	23	15	23

[a] MCAGCC = Marine Corps Air Ground Combat Center.
[b] Population occurring in the Eastern Mojave Recovery Unit assigned to the Northeastern Mojave sample group for purposes of data analysis owing to geographic proximity.

Table 2. Observed microsatellite motifs in Mojave desert tortoises, *Gopherus agassizii*, compared with that of the originally described species or population.

Locus	Species originally described	Original repeat motif	Observed motif in Mojave population	Range of Mojave alleles	Range of Sonoran alleles
Edwards et al. 2003					
Goag3	*G. agassizii* (Sonoran)	$(CAA)_6$	$(CAA)_6$	6–7	6–9
Goag4	*G. agassizii* (Sonoran)	$(CAA)_{24}$	$CAA)_{24}$	12–32	7–30
Goag5	*G. agassizii* (Sonoran)	$(GAT)_8$	$GACGAA(GAT)_2GACGAA$	null	6–38
Goag6	*G. agassizii* (Sonoran)	$(TC)_8(AC)_{11}$	$(TC)_8(AC)_{11}$	17--67	15–52
Goag7	*G. agassizii* (Sonoran)	$(AC)_3(GC)_5(AC)_{11}$	$(AC)_8(AT)_2GC(AC)_3(GC)_3(AC)_9$	13–28	12–28
Goag32	*G. agassizii* (Sonoran)	$(AC)_6$	$(AC)_6$	6	5–6
Schwartz et al. 2003					
GP26	*Gopherus polyphemus*	$(GT)_{12}$	$(GT)_7$	7	6–9
GP55	*G. polyphemus*	$(GT)_9$	$(GT)_7$	7–30	7–34
GP102	*G. polyphemus*	$(GT)_5(CT)_{13}(CA)_5$	$(TC)_2(TG)_2CG\ [(TG)_8(TC)_{14}]^a$	19–42	19–36
GP15	*G. polyphemus*	$(GA)_{15}(GT)_8$	$(GA)_{14}(GT)_{20}$	13–52	13–56
GP19	*G. polyphemus*	$(GT)_9/(GT)_3(GA)_6$	Allele 1; $(GT)_3/(GT)_2GAAA(GA)_4$	11 and 21	6, 11, and 21
			Allele 2; $(GT)_7ATGTATGT/(GT)_2GAAA(GA)_5$		
GP30	*G. polyphemus*	$(GT)_{13}$	$(GT)_5(CT)(GT)_4$	10–17	5–29
GP81	*G. polyphemus*	$(GT)_{11}(GA)_{10}$	$(GT)_9GACA(GA)_8$	16–28	18–22
GP61	*G. polyphemus*	$(GT)_{12}$	$(GT)_4AT(GT)_6$ & $(GT)_{16}$	11–38	9–43
GP96	*G. polyphemus*	$(GA)_{11}$	$(GA)_7$	7	7
FitzSimmons et al. 1995					
Cm58	*Chelonia mydas*	$(CA)_{13}$	$(TA)_5(GA)_3GC(GT)_3$	12	12–13

[a] Complex repeat; unable to obtain entire sequence.

ern Recovery Unit, and sequenced their mtDNA for a total evidence analysis (Kluge 1989; Ernisse and Kluge 1993) of unique haplotypes only. Unweighted maximum parsimony analyses were performed on potentially informative characters by using PAUP* 4.0b10 (Swofford 2002). Most parsimonious trees were obtained by using the heuristic tree search algorithm with random addition of individuals, 10,000 replicates while retaining minimal trees only and holding 10 trees at each replicate, tree bisection-reconnection branch swapping with the steepest descent, and collapsed zero-length branches. All multistate characters were evaluated as nonadditive (unordered). Nodal consistency was assessed by using nonparametric bootstrap proportions (Felsenstein 1985) and decay analysis (Bremer 1994) performed in PAUP*. Relative nodal support was assessed by using bootstrapping with 10,000 random pseudoreplicates of the data, with each pseudoreplicate being replicated twice.

Bayesian inference was also used to hypothesize matriarchal history (Huelsenbeck and Ronquist 2001; Buckley et al. 2002; Nylander et al. 2004; Ronquist 2004). MrModeltest 2.2 (Nylander 2004) was used to select the best evolutionary model based on the Akaike Information Criterion (Akaike 1974, 1979). Hierarchical likelihood ratio tests (Goldman 1993) compared log-likelihood scores of 56 models. Bayesian inference, conducted by using MRBAYES 3.1.2 (Huelsenbeck and Ronquist 2001), started with random trees. Six Markov chains were used, and the data set was run for 3×10^6 generations. Trees were sampled every 100 generations. Two independent analyses with different starting trees were run and the fluctuating values of likelihood were graphically monitored (Huelsenbeck and Bollback 2001). Log-likelihood scores of sample points were plotted against generation time to establish stationarity (Huelsenbeck and Ronquist 2001). The analysis was a priori required to achieve a split frequency standard deviation of ≤ 0.005. After discarding 25% of the sampled trees as burn-in, the remaining trees were used to generate a 50% majority rule consensus tree.

Nuclear DNA. — We used several methods of analyses to assess gene flow and population differentiation. Each of the methods had different assumptions and relied on different properties of the data, as noted below.

Population Structure. — We used 1) traditional techniques that a priori defined sample groups and 2) an a posteriori genotypic clustering method to analyze population structure. Individuals for which more than 3 loci did not amplify were discarded. Allelic frequency distributions for unique (study site or region restricted) and private alleles ($> 5\%$ in a sample group or region) were examined. Loci that exhibited more than 7 alleles were examined by using the log-likelihood-based (G-based) exact test (Goudet et al. 1996) in GENEPOP 3.1 (Raymond and Rousset 1995). A triangular contingency table and a modified version of the Markov-chain random walk algorithm (Guo and Thompson 1992) were used in

ARLEQUIN 2.0 (Schneider et al. 2000) to detect significant departures from the Hardy-Weinberg equilibrium (H-W). The multiple tests were not Bonferroni corrected because we looked for trends only and not a precise application of statistical tests. The trends would have remained with a Bonferroni correction but the levels of significance (p-values) would have been raised, possibly to the extent of no significance. Default parameters in GENEPOP and ARLEQUIN were used for all Markov-chain tests and permutations.

Linkage equilibrium is assumed by some statistical tests and, thus, was necessary to confirm. GENEPOP tested for linkage disequilibrium (nonrandom association between loci) among all pairs of loci in the entire sample and within each group by using the method of Garnier-Gere and Dillmann (1992).

Population genetic structure was assessed under nonequilibrium conditions (Pearse and Crandall 2004; Manel et al. 2005). We used STRUCTURE 2.1 (Pritchard et al. 2000) to a priori define cohesive genetic units. Because it does not provide a good measure of genetic structuring in populations that exhibit nonlinear patterns of isolation-by-distance (IBD; Kimura and Weiss 1964; Pritchard et al. 2000), as do Mojave desert tortoises, STRUCTURE was used as a guideline only. An extension to the program by Falush et al. (2003) accounts for correlations between linked loci that arise in admixed populations. We evaluated the 15 sample groups (K populations) with 4 simulations of 500,000 iterations for each K by using the default parameters for an admixture model with a prior mean Φ_{ST} (F_{ST} sensu Weir and Cockerham 1984) of 0.06 (0.05 SD), based on the mean generated from our data set. (We initially also tried the analysis with a lower number of runs by using prior mean Φ_{ST} of 0.01, without a noticeable difference in the outcome.) The best model had the smallest value of K and the largest likelihood values.

To reduce the strongest effects of multilinear IBD, we performed an analysis on the Western Mojave Recovery Unit but first removed the northern- and southernmost samples. The analysis included sample groups 1–10 and used 1,000,000 iterations with a prior mean of Φ_{ST} at 0.01.

Population differentiation was also assessed by using WHICHRUN 4.1 (Banks and Eichert 2000), which calculates the likelihood of a given individual originating from either of 2 or more candidate populations. If the groups identified by STRUCTURE and/or the 6 units hypothesized in the Recovery Plan were distinct and not interconnected by frequent gene flow, then WHICHRUN should assign an individual to its source population with a high likelihood score and assign it to other populations with low scores. Stringency for population allocation used a selection criterion of the log of the odds ratio (LOD) for the 2 most likely source populations. The chance of error is equal to the inverse of this ratio; assignments with a LOD of at least 2 had a ≤ 0.01 chance of error.

Traditional equilibrium-based F-statistics, using analysis of molecular variance (AMOVA) in GENEPOP, were also employed to infer population structure. Inbreeding coefficients (Φ_{IS}; F_{IS} sensu Weir and Cockerham 1984) were calculated for each locus in each sample group. Genetic distances based on pairwise Φ_{ST} were calculated among groups and individuals by using GENEPOP and were visually assessed by producing a multidimensional monotonic scaling plot (MDS) that used the program NTSYS (Exeter Software, NTSYS pc 2.1, Setauket, NY). Goodness of fit was measured by using the Stress test (Kruskal and Wish 1978). Mantel tests obtained from NTSYS assessed correlations between genetic and geographic distances among sample groups. The Φ_{ST} values estimated population structure and gene flow by assuming mutation-drift or migration-drift equilibrium with symmetric migration in both directions for all pairwise combinations of populations. The Φ_{ST} values also assumed an island model that may not be met in desert tortoises, especially because they have experienced recent demographic declines (see Whitlock and McCauley 1999).

Demographic History. — Two very different models assessed historical changes in population density. First, BOTTLENECK (Piry et al. 1999) was used to test for evidence of historical changes in effective population sizes and deviations from equilibrium conditions for each of the sample groups, regions, and the entire population. Populations with recent reductions in effective population size should show an excess of heterozygosity (Cornuet and Luikart 1996; Spencer et al. 2000). Significance of the observed deviations, assuming the infinite alleles model, was determined by the Wilcoxon test as well as the Sign test method of Piry et al. (1999). Second, the M-ratio test of Garza and Williamson (2001) was used to investigate changes in population density and to evaluate bottlenecking, where M is the ratio of the total number of alleles (k) to the overall range in allele size (r). When rare alleles are lost during a population bottleneck, the number of allele size classes is reduced to a greater extent than the range in allele size. Value M is reduced in populations known to have declined in size. In total, 20 populations had the required number of individuals for applying this test. Bottlenecking was assumed to have occurred if M was above the critical value M_C (Garza and Williamson 2001). Congruent findings from the 2 tests would suggest that the results were not biased for any single method or set of assumptions.

Human-Mediated Translocations. — We compiled published and unpublished data and interviewed biologists in state and federal wildlife and land management agencies, then mapped localities of releases or escapes of captive tortoises and translocations of wild tortoises. The results of WHICHRUN assessed the source of an individual tortoise and assignments or misassignments to specific populations. BOTTLENECK, G-based exact tests in GENEPOP, and estimates of inbreeding values (Φ_{IS}) provided information on population trends. Significant

deviations from H-W, estimates of recent gene flow and distributions of haplotypes from previously described analyses also provided valuable information.

RESULTS

MtDNA Evaluation. — Estimations of maternal history and population structure were based on *G. agassizii* from the Mojave population and the outgroup taxa (Table 3). All sequences were deposited in GenBank (Accession no. DQ649394–DQ649409).

Seven haplotypes were observed among the 125 *G. agassizii* from the Mojave population (Table 3). Five localities had a single haplotype, and 1 region, the Northeastern Mojave, had 3 sympatric haplotypes, likely a result of the greater extent of sampling at this locality. One haplotype, MOJ-A01, occurred in all but the Northeastern Recovery Unit. Similarly, haplotype MOJ-B01 was common in the Northeastern and Upper Virgin River recovery units but also occurred in low frequency in the Western Mojave and Eastern Colorado recovery units (Table 3). Haplotype MOJ-A02 occurred in 2 nearby localities in the Southern Mojave. MOJ-A03 was found in the nearby Western Mojave and Southern Mojave recovery units. In contrast, haplotypes MOJ-A04 and -B02 occurred at single locations only. Haplotypes within the Mojave population differed at most by 4–5 bp, or only 0.6%, and haplotypes MOJ-B01–03 differed from one another by 1–2 bp only, as did MOJ-A01–04.

Maternal History. — The phylogenetic evaluation was based upon 60 potentially cladistically informative nucleotide positions. In total, 842 nucleotide positions did not vary between the outgroup and ingroup taxa. Autapomorphies occurred at 22 nucleotide sites. The cladistic analysis of the sequences yielded 2 most parsimonious solutions (length = 77 steps, CI = 0.81, RI = 0.95, RC = 0.76). By using *G. flavomarginatus* as the primary outgroup, *G. berlandieri* was resolved as the sister group to all maternal lineages of *G. agassizii*. The consensus trees (Fig. 2) had 2 strongly supported lineages at the base of the tree, one containing Sonoran samples and the other containing samples from the Mojave population. Within the Mojave population, 2 major sublineages were resolved: Haplogroup A, "broadly distributed," and Haplogroup B, Northeastern Mojave. Both lineages contained 1 haplotype that was relatively broadly distributed (Table 3), along with alternative haplotypes. The 2 most basal nodes for *G. agassizii* were strongly supported having bootstrap proportions of 100% and decay indices of 9–10 steps for the Sonoran and Mojave lineages, respectively (Fig. 2). Within the Mojave, Haploclades A and B were only weakly supported; bootstrap proportions = 53%–65% and decay values were 1–2 steps.

When using MRMODELTEST, the general time reversal plus invariant sites (GTR + G) model was selected for use in the Bayesian inference analysis (–lnL = 2111.7654; K = 9; AIC = 4241.5308). Bayesian inference resulted in

Table 3. The distribution of mitochondrial deoxyribonucleic acid haplotypes from the Mojave desert tortoise, *Gopherus agassizii*.

Desert region/ recovery unit[a]	Group	Haplogroup A				Haplogroup B			Total
		MOJ-A01	MOJ-A02	MOJ-A03	MOJ-A04	MOJ-B01	MOJ-B02	MOJ-B03	
Western Mojave	1	2				1			3
	2	10		1					11
Central Mojave	3	6							6
	5	2							2
Southern Mojave	6	6	2						8
	7	7		1					8
	8	3							3
	9	5	1						6
	10	6							6
Northeastern Mojave	11					40	1	6	47
Eastern Mojave	12	8							8
Northern Colorado	13	3			1				4
Eastern Colorado	14	6				1			7
Upper Virgin River	15	1				4		1	6
Total		65	3	2	1	46	1	7	125

[a] Within the Mojave Desert, 2 major sublineages were resolved: Haplogroup A "broadly distributed", and Haplogroup B, Northeastern Mojave (Fig. 2). The greater relative sampling in the Northeastern Mojave (group 11) reflected an attempt to locate a haplotype from Haplogroup A.

a tree that was identical to the maximum parsimony consensus trees. The Bayesian posterior probabilities were higher than the bootstrap proportions (Fig. 2).

Microsatellite Evaluation. — Of the 16 loci surveyed in 628 desert tortoises (Table 1), 11 were highly variable and informative: Goag03, Goag04, Goag06, Goag07,
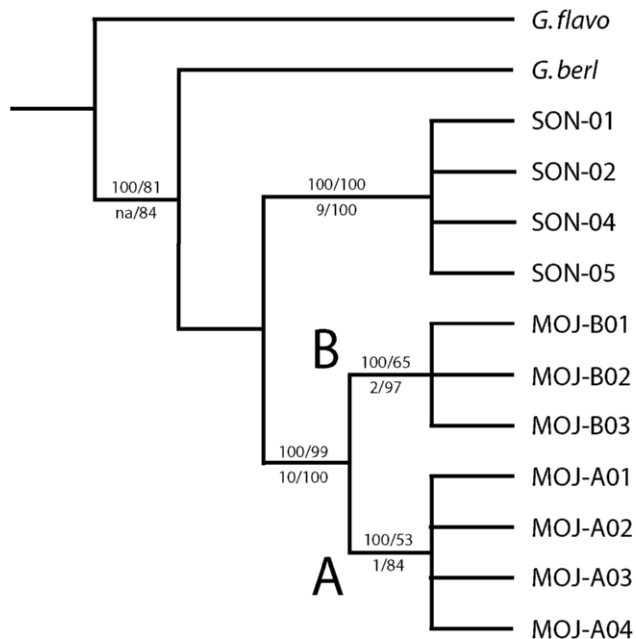

Figure 2. A 50% majority rule consensus tree based on maximum parsimony and Bayesian inference evaluations of the mitochondrial deoxyribonucleic acid sequence data from tortoises, genus *Gopherus*. SON = Sonoran and MOJ = Mojave populations of the desert tortoise (*Gopherus agassizii*) and outgroups G. berl (*G. berlandieri*) and G. flav (*G. flavomarginatus*). Numbers above the branches are given as frequency of resolution in the maximum parsimony evaluation/bootstrap proportions, and below as Bremer support/Bayesian posterior probabilities. Na = not applicable, and letters at nodes denote haplogroup lineages of Mojave populations discussed in text.

GP15, GP19, GP30, GP55, GP61, GP81, and GP102. Five loci showed insufficient variation and were excluded from our analyses: GP26, GP96, Cm58, Goag05, and Goag32. For locus Goag03, only 2 study sites exhibited variation: groups 11 and 15 (Northeastern Mojave and the Upper Virgin River recovery units, respectively). For all microsatellite loci used in this study, individual genotypes were summarized by regional groups and are available from the Internet home page of RWM (www.zoo.utoronto.ca/drbob/publications).

Major differences occurred between repeat motifs at some microsatellite loci in *G. agassizii* when compared with species or the population for which the locus was originally isolated, including GP19, GP30, GP61, GP81, and GP102 (Table 2). We were not able to precisely determine the motif for GP102 in *G. agassizii*. Homozygous amplicons were vague in the middle of the sequences, suggesting that 2 alleles were present. Fragment analysis did not allow determination of a heterozygous state (difference in repeat motifs) when amplicon lengths were equal. We did not clone these products to determine the competing sequences but rather made an arbitrary assignment of repeat numbers. Consequently, data for GP102 were not necessarily reflective of all possible heterozygous states.

Locus GP61 exhibited 2 different motif states; alleles having more than 16 repeats had a simple dinucleotide motif, $(GT)_{16+}$. However, alleles scoring in the range of 10–12 repeats had a compound motif, $(GT)_4AT(GT)_6$. As in the Sonoran population (Edwards et al. 2004), heterozygous individuals had both motifs. The simple motif had a greater range of allelic states than the compound motif.

Schwartz et al. (2003) originally described the compound motif for GP19 in *Gopherus polyphemus* as $(GT)_9/(GT)_3(GA)_6$. We found a dramatically derived state
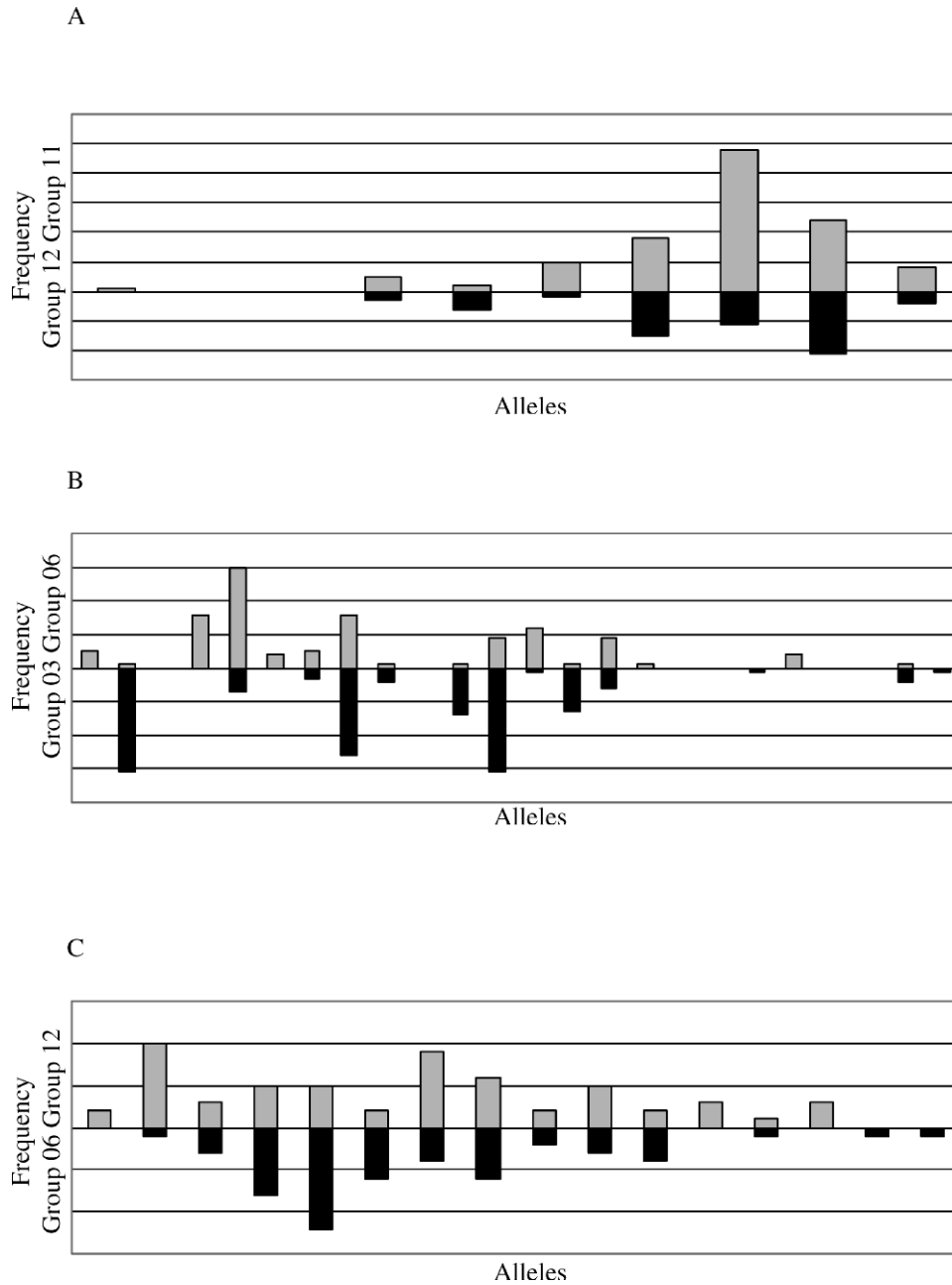
A

B

C

Figure 3. Comparison of allelic frequencies between sample groups of desert tortoises, *Gopherus agassizii*, from the Mojave population by using the G-based exact test for genotypic differentiation. Sample groups refer to Table 1. A: Locus GP81, $p = 0.024$, SE $= 0.002$; B: Locus GP102, $p < 0.001$, SE < 0.001; C: Locus Goag04, $p = 0.031$, SE $= 0.003$.

in our Mojave samples of *G. agassizii*, such that allele 11 sequenced as $(GT)_3/(GT)_2GAAA(GA)_4$ and allele 21 sequenced as $(GT)_7ATGTATGT/(GT)_2GAAA(GA)_5$. Consequently, we could not use analyses that required a stepwise mutation model, such as R_{ST} (Slatkin 1995).

Some dinucleotide loci exhibited imprecise pherograms (e.g., stutter peaks) when the number of repeats exceeded 25. A score of "35" could not be differentiated from "34" or "36". Consequently, pherograms were scored by using a standardized rule set for consistency with error on the conservative side. Loci GP15, GP61, GP102, and Goag06 may have reached the upper limits of our ability to detect repeat numbers, because larger

amplicons had very low intensity pherograms. Generally, alleles with more than 55 repeats were not scored, and, thus, we likely missed some alternative alleles.

The distributions of allele size classes for most loci were not normally distributed. Some were highly skewed, and others exhibited multiple peaks (Fig. 3). Unique and private alleles were detected in several sample groups at some of the more variable loci. In some cases, private alleles comprised a high proportion of the alleles observed within a population. For example, sample group 14 had 4 alleles at GP30; the private allele composed 25% of all alleles (Table 4) but it occurred at a frequency of < 5%.

Table 4. Distribution of unique and private alleles in 15 sample groups (summarized in Table 1) of desert tortoises from the Mojave population.[a]

Sample group	GP61 T	GP61 U	GP61 %	GP19 T	GP19 U	GP19 %	GP102 T	GP102 U	GP102 %	GP30 T	GP30 U	GP30 %	GP55 T	GP55 U	GP55 %	GP15 T	GP15 U	GP15 %	GP81 T	GP81 U	GP81 %	Goag4 T	Goag4 U	Goag4 %	Goag06 T	Goag06 U	Goag06 %	Goag7 T	Goag7 U	Goag7 %	Goag3 T	Goag3 U	Goag3 %
1	14			2			7			9	1	11	9			17	1(1)	4.2	7	2	29	16			26			5			1		
2	15			2			14			8	1	13	8	1	13	24			5			17	1	5.9	22			4			1		
3	9			2			9			5			8	1	13	15			5			13			16			4			1		
4	11			2			11			7			7			14			6			12			19			4			1		
5	13			2			11			7	1	14	12	1	8.3	16			5			15			21			5			1		
6	10			2			10			4			6			9			6			13			18			4			1		
7	16			2			13	1	7.7	8	2	25	7			21			7			12			21			4			1		
8	14			2			15			7			11			20	1	5	7	1	14	11			33			5			1		
9	11			2			12			6	1	17	7	1(1)	14	10			7			12			22			5			1		
10	13			2			7	1(1)	14.3	4			7			14	1	7.1	8	1	13	9			14			4			1		
11	15			2			16	1	6.3	8			6			19			7			16			19			6			2		
12	18			2			12			9			7			15			7			14			15	1	6.7	4			1		
13	13	1	7.7	2			12	1(1)	8.3	10	2(1)	20	5			14			7			11	1	9.1	14			5			1		
14	15	1(1)	6.7	2			12	1(1)	8.3	4	1	25	9			13			6			11	1(1)	9.1	21			5			1		
15	11			1			12			7			3			15			5			10			15			3			2		
Total	27			2			24			21			17			37			11			22			49			5			2		

[a] T = total number of allelic states observed in a sample group; U = number of alleles unique to the sample group; parenthetic values are the number of unique alleles that occur at a frequency > 5% (private alleles) in a sample group relative to the total number of alleles; % = the percentage of alleles that are unique in a sample group [(U/T) × 100]. No private alleles in a population occurred at a frequency > 8%.

The frequency of occurrence for the relatively rare, private allele was always ≤ 8%.

Most sample group pairwise comparisons between distributions of allelic frequencies (Fig. 3) were found to be significantly different by the G-based Exact test (Goudet et al. 1996). Three sample groups deviated from H-W in exhibiting a greater number of heterozygotes than expected (Table 5). By using a 5% cutoff, about 1 deviation is expected for each locus, except for Goag3. Three loci showed excessive deviations from expectations in the form of heterozygote deficiencies: GP30, G81, and Goag06. In total, 24.5% of the data points showed deviations from H-W, with 8.6% owing to Goag06 alone (Table 5).

Garnier-Gere's and Dillmann's (1992) test rejected the null hypothesis for linkage disequilibrium (equilibrium for locus pairs) for 45 (of 165) locus pairs within 15 sample groups. Nine sample groups had a percentage of total pairwise comparisons with p-values > 0.05 (range 0.0%–26.7%). However, locus pairs did not consistently exhibit disequilibrium among groups.

Bayesian likelihood values for all runs by using STRUCTURE typically stabilized after 50,000–100,000 iterations after burn-in. The analyses obtained the lowest average Ln for 6 subpopulations (Table 6). These subpopulations were concordant with the recommendations in the *Recovery Plan*. Because substantial differentiation was observed in the Western Mojave Recovery Unit, as revealed by Φ_{ST} values, we removed populations 11–15 and performed a new analysis to reduce the affects of IBD. This analysis suggested that the current Western Mojave Recovery Unit supported 4 subpopulations (Table 6): sample groups 1–2, 3–5, 8, and 6–7 plus 9–10 (Fig. 4).

A 2-dimensional, monotonic MDS plot displayed population differentiation among sample groups (Fig. 5). It had a stress of 1.39, a fair to good fit by Kruskal's and Wish's (1978) index. The 15 sample groups clustered complementary to their geographic proximities, as anticipated when assuming gene flow. Geographically distant sample groups 11 and 15 were noticeably separated from the other groups.

Population assignment tests correctly placed the majority of individuals back to their sample groups with high stringency (Table 7). Individuals not assigned to a sample group were frequently assigned to a geographically nearby group or to one within the same region. Geographically proximate groups 12 and 13 occurred near the boundary of 2 desert regions, the eastern Mojave Desert and northern Colorado Desert (Fig. 1). The population assignment evaluations had difficulty distinguishing individuals between these 2 recovery units. Whereas, 80% of the samples from group 11 were correctly assigned, only 48% of 31 samples from group 12 were correctly assigned. However, 87% of tortoises from group 12 were correctly assigned to groups 12 and 13 combined, indicating that, in this case, geographic proximity was a better predictor of genetic structuring

Table 5. Summary of deviation from Hardy-Weinberg expectations for 11 variable microsatellite loci and 15 sample groups of the desert tortoise, *Gopherus agassizii*. Sample groups refer to Table 1.

Locus	No. comparisons	No. heterozygote excess	No. heterozygote deficiency	Range in no. of repeats
GP61	15	0	2	11–38
GP19	14	0	0	11–21
GP102	15	1	1	19–42
GP30	15	0	7	10–17
GP55	15	0	3	7–30
GP15	15	0	2	13–52
GP81	15	0	6	16–28
Goag4	15	1	0	12–32
Goag06	15	0	13	17–67
Goag7	15	1	0	13–28
Goag3	2	0	0	6–7

than recovery unit. A similar trend was discovered for tortoises in group 13.

When sample groups were combined to reflect current recovery units, and when sample groups 12 and 13 were combined, assignment scores of $\geq 80\%$ were obtained (Table 7). For the Western Mojave Recovery Unit, we deleted geographically distant sample groups (1, 2, 11–15) and re(-)ran the assignment test. We combined samples 3–5 and samples 6–10, because they had higher proportions of misassigned individuals than all other units (Table 7). Although not given in Table 7, the percentage of individuals correctly assigned to the proposed Central Mojave (samples 3–5) and Southern Mojave (samples 6–10) recovery units combined was 52% each, with 24% being assigned to the combined unit as the second most likely assignment and 13% assigned to the adjacent Western Mojave Recovery Unit.

Finally, we combined the sample groups to reflect geographic regions, which reflected the current recovery units (Table 7). This treatment recognized variation within the Western Mojave Recovery Unit. In total, 8 regions were identified. Assignment scores ranged from 59.6% to 95.7%. The more fine-grained analyses, those that included a greater number of subdivisions, yielded lower assignment scores.

Geographic substructuring was further assessed by breaking and recombining specific units. The assignment tests produced 96%–98% accuracy when the distribution of tortoises was divided into 2 groups: Northeast (11, 15) and Central (1–10, 12–14), respectively. When geographically proximate groups were split and recombined, the assignment tests invariably decreased, some to less than 50% (sample groups 2, 6, and 8).

The hierarchical analysis of molecular variance indicated the absence of panmixia; significant genetic structuring was discovered. The AMOVA revealed that 93.9% ($p < 0.001$) of the observed variation was partitioned among individuals within sample groups ($\Phi_{IT} = 0.939$), whereas only 6.1% of the variation was among the sample groups ($\Phi_{ST} = 0.061$, $p < 0.001$). The positive significant correlations between genetic distance (pairwise Φ_{ST}) and geographic distance accounted for approximately 65% of the observed variation (Mantel test; $r^2 = 0.646$, $p = 0.002$).

By using BOTTLENECK, we detected a significant excess in heterozygosity in 2 sample groups, 11 and 15, the Northeastern Mojave and Upper Virgin River recovery units. The Wilcoxon Test with the (infinite alleles model [IAM]) detected an excess in both groups but the Sign Test (IAM) method of Piry et al. (1999) identified group 15 only. No deficit or excess in heterozygosity was detected when the data for all groups were combined. All sample sets fit the expected beta distribution (Cornuet and Luikart 1996), thus providing no evidence for bottlenecking. By using the method of Garza and Williamson (2001) to detect potential reduction in population size, all values of M fell above the critical value M_C. However, the results may not be reliable, because this test assumed stepwise mutation.

Human-Mediated Translocations. — Native Americans undoubtedly moved desert tortoises from one place to another (as implied in Schneider and Everson 1989). The distances were probably limited, except for annual gatherings for mourning ceremonies (i.e., Las Vegas Band, Southern Paiute: Kelly, no date) and the result may have been death for the tortoises.

Throughout the 20th century, tortoises were captured for domestic pets and were translocated for various purposes. Captive tortoises currently or formerly kept by residents of desert communities often escape or are deliberately released into adjacent desert lands. The sources of the captives may or may not be local relative to the point of escape or release. Escaped captives are so common that a publication gives actions to take when a former captive is found (Berry and Duck, 2006). Captives have been observed wandering within city limits or nearby in Ridgecrest, Barstow, Ft. Irwin, Victorville, and Twentynine Palms in the Western Mojave Recovery Unit; Needles in the Eastern Mojave Recovery Unit; Las Vegas in the Northeastern Mojave Recovery Unit; and St. George in the Upper Virgin River Recovery Unit. Tortoises are often taken to or released at protected areas such as parks and Natural Areas (Howland 1989; Ginn 1990; Jennings 1991; Connor and Kaur 2004).

Thousands of tortoises were released in the southwestern deserts by humane societies, California Department of Fish and Game, Nevada Department of Wildlife Resources, Utah Division of Wildlife Resources, State and National Park personnel, academicians and others (Fig. 6). Data are limited before the 1960s, but releases were documented for California and Utah (Hardy 1945; Woodbury and Hardy 1948; Jaeger 1950, 1955). Woodbury and Hardy (1948) surveyed Beaver Dam Slope, Utah (Northeastern Mojave Recovery Unit) for tortoises between 1936 and 1946. At least 6.1% of 281 tortoises found showed signs of previous captivity. Releases also occurred in the

Table 6. Inferred population structure obtained from the software program STRUCTURE 2.1 for all samples, and for a subset of samples from the current Western Mojave Recovery Unit (sample groups 1–10).[a]

All samples ($n = 628$)	Ln (variance below)				
K	Run 1	Run 2	Run 3	Run 4	Average Ln
1	–25,140.5	–25,144.0	–25,143.6	–25,143.3	–25,142.9
	99.7	106.1	106	105.8	
2	–24,362.2	–24,360.6	–24,360.8	–24,361.2	–24,361.2
	463.9	460.7	462.6	463.3	
3	–23,644.7	–23,646.2	–23,647.9	–23,648.6	–23,646.9
	568.4	570.5	572.8	574.9	
4	–23,283.3	–23,275.4	–23,269.5	–23,272.6	–23,275.2
	827.5	810.6	800.5	804.8	
5	–23,134.7	–23,038.1	–23,030.7	–23,042.5	–23,061.5
	1049.5	1056.0	1041.2	1062.6	
6	–22,881.4	–22,886.7	–22,883.4	–22,893.2	–22,886.2
	1249.2	1260.3	1251.2	1275.1	
7	–23,042.2	–22,840.3	–24,213.8	–24,745.5	–23,710.5
	1921.8	1521.7	4220.5	5220.9	
8	–22,901.4	–23,454.5	–23,144.8	–22,964.3	–23,116.3
	1712.3	3043.6	2204.3	1858.5	
9	–23,538.9	–24,007.6	–22,951.0	–23,041.1	–23,384.7
	3494.4	4412.3	2335.7	2230.9	
10	–22,857.7	–24,696.7	–22,900.7	–22,900.7	–23,339.0
	2208.1	5872.7	2262.5	2280.9	
11	–23,305.8	–24,272.3	–24,176.7	–24,377.2	–24,033.0
	3318.1	5406.3	5027.1	5490.7	
12	–23,236.8	–24,848.4	–23,590.5	–34,317.7	–26,498.4
	3426.8	6666.9	4129.0	25,502.9	
13	–24,346.5	–23,339.1	–34,657.2	–28,975.2	–27,829.5
	5879.4	3820.1	26,339.3	15,064.1	
14	–31,546.3	–560,553.8	–31,303.2	–24,971.2	–162,093.6
	20,362.5	1,077,674.6	19,809.4	7242.0	
15	–133,340.8	–28,256.8	–27,197.9	–41,616.9	–57,603.1
	223,973.3	13,936.0	11,869.1	40,664.7	

Western Mojave samples ($n = 459$)					
K	Run 1	Run 2	Run 3	Run 4	Average Ln
1	–17,343.6	–17,342.7	–17,338.4	–17,339.0	–17,340.9
	99.8	97.2	90.7	90.8	
2	–16,870.6	–16,871.0	–16,870.0	–16,873.2	–16,871.2
	405.0	406.7	405.5	411.5	
3	–16,968.7	–16,715.6	–16,722.3	–16,626.4	–16,758.3
	1218.3	693.6	847.8	657.2	
4	–16,438.7	–16,434.3	–16,432.9	–16,438.4	–16,436.1
	874.5	863.0	860.4	871.3	
5	–16,380.9	–16,404.5	–16,419.0	–18,206.9	–16,852.8
	1068.9	1114.4	1143.6	4629.7	
6	–16,742.5	–16,392.3	–16,418.5	–17,106.1	–16,664.9
	1876.6	1163.9	1217.5	2750.5	
7	–16,778.8	–17,811.3	–16,450.6	–18,021.6	–17,265.6
	2430.1	4440.4	1540.5	4871.7	
8	–16,343.7	–18,314.1	–18,520.9	–16,417.4	–17,399.0
	1837.0	5698.8	5924.8	1746.6	
9	–20,559.6	–17,456.7	–16,346.8	–19,067.6	–18,357.7
	10,289.0	4207.3	1842.1	7354.0	
10	–18,184.4	–406,665.0	–19,777.8	–21,971.6	–116,649.7
	5770.3	780,420.0	8955.7	13,321.4	

[a] K = the number of populations set as the a priori for the simulation; Ln = the log likelihood of the data averaged over all iterations after burn-in (with variance reported below); and the average Ln for all 4 runs for a given simulation. (For all simulations: 250,000 iterations per run with a burn-in of 5000).

vicinity of St. George and the Upper Virgin River Recovery Unit (Hardy 1945).

From the late 1960s to the mid 1970s, the California Department of Fish and Game sponsored numerous captive releases and kept records for > 800 individuals (Fig. 6). Their last official release was the rehabilitation experiment at the Quarterway and Halfway Houses in the Living Desert Reserve and Ft. Soda, respectively, in the late 1970s. Among 200 tortoises initially in the program, 30 survived, only to be moved to private lands in the Antelope Valley (Cook et al. 1978; Weber et al. 1979; Cook 1983).

In Nevada, the first documented releases of captive tortoises occurred on the Desert Game Range in 1973 (B.L. Burge, *pers. comm.*, December 2005; Fig. 6). In the late 1970s and early 1980s, employees of the Nevada
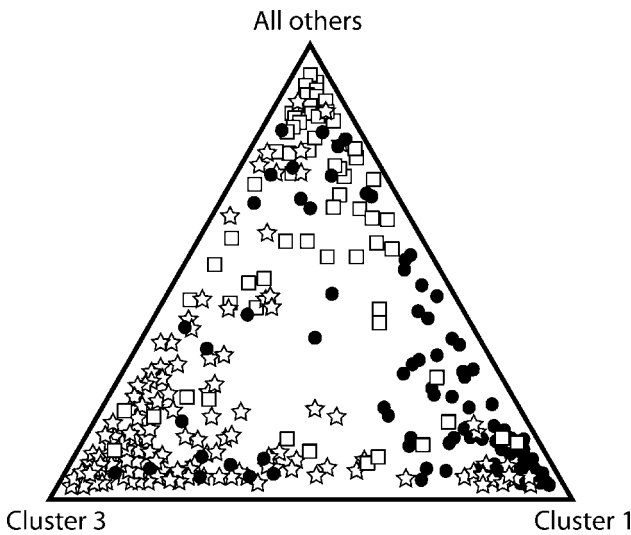
Figure 4. Triangle plot of the estimated membership coefficients for each individual in the Western Mojave Recovery Unit. Symbols correspond to sampling groups (given in Table 1) when the number of populations (K) is K = 3: circles = sample groups 1 and 2, squares = sample groups 3–5, stars = sample groups 6–10. Note the general clustering in the corners of each group and the overall pattern of admixture (gene flow). The cluster of stars in the circle samples depicts individuals mostly from Group 8, which is geographically the most proximate to the circle sample group.

Department of Wildlife Resources released hundreds of captive tortoises onto desert lands (R.J. Turner, *pers. comm.*, December 2005).

State and federal agencies approved the release of numerous captive and wild tortoises in 1997 at a long-term

release site in southern Nevada (Field 1999). Additional translocation projects occurred throughout Nevada between 1990 and 2005 (Corn 1991; Nussear 2004; Charles Le Bar, *pers. comm.*, December 2005).

Between 1973 and 1983, the Utah Division of Wildlife Resources released at least 195 captive tortoises on Beaver Dam Slope (Coffeen, *pers. comm.*, December 2005; Coffeen 1984, 1985). In 1980, a general survey conducted throughout 324 km^2 of the area revealed that 21.9% of 105 located tortoises were marked captives (Minden 1980). Tortoises were also released on the historical Woodbury and Hardy (1948) site; when the study site was surveyed in 1981, 23.3% of the 73 tortoises observed were marked captives (Minden and Keller 1981). In the mid to late 1980s, captive tortoises were released in the Upper Virgin River Recovery Unit at Grapevine Pass and Red Cliffs Recreation Area (Coffeen 1986); 71 captive tortoises were also released at Hurricane Cinder Knolls (McLuckie, unpubl. data, 2006).

Evidence exists of a substantial transfer of tortoises from the western Mojave Desert in California to Utah. In April of 1970, 2 wardens arrested a commercial collector who claimed to have taken thousands of tortoises from the Western Mojave Recovery Unit of California between the 1960s and April 1970 and sold them commercially in Salt Lake City, Utah (Berry 1984). Some of these tortoises may have been released on the Beaver Dam Slope and north of St. George in the 1970s and early 1980s in what are now the Northeastern Mojave and Upper Virgin River recovery units.
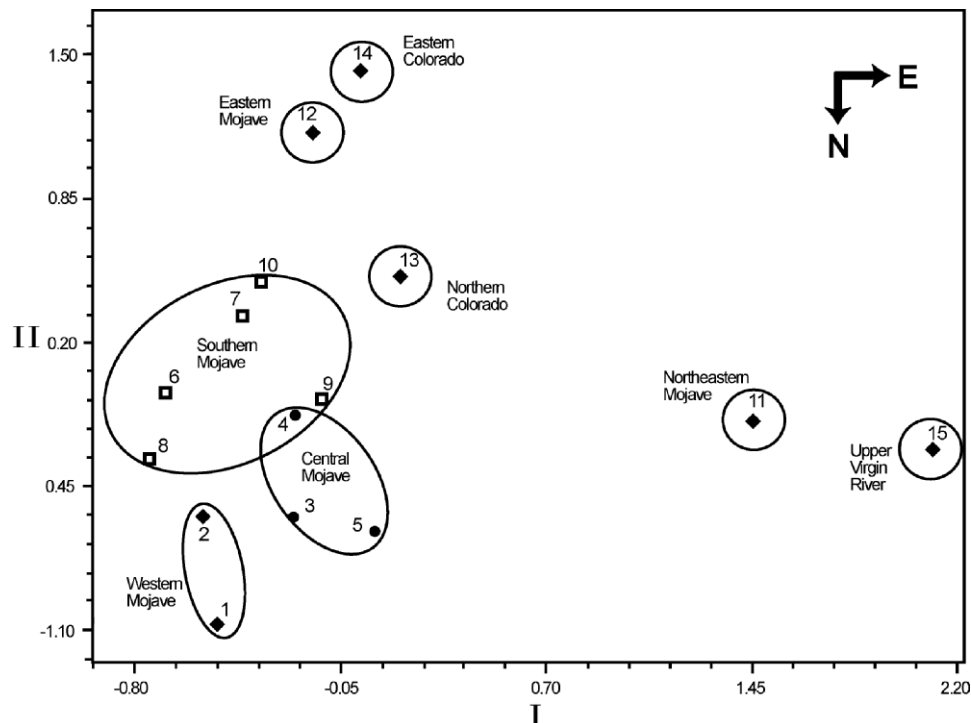

Figure 5. A 2-dimensional scaling plot of genetic distances (Φ_{ST}) for 15 sample groups of desert tortoises, *Gopherus agassizii*, from the Mojave population. Open squares and solid circles indicate samples from the southern and central Mojave Deserts, respectively.

Table 7. Population assignment tests for desert tortoises from the Mojave population and 8 desert regions or recovery units. The initial evaluation treated all 15 sample groups separately. The second treatment combined tortoises into units reflecting the recovery units recommended in the 1994 *Recovery Plan* except for combining sample groups 12 and 13. The third treatment considered populations on the basis of existing and proposed recovery units.

Sample group	No. samples	No. correctly assigned	% Correctly assigned	% With LOD > 2[a]	No. assigned to same region or neighboring group	% Assigned to same region
1	62	42	67.7	58.1	8	80.6
2	83	26	31.3	19.3	16	50.6
3	19	10	52.6	47.4	3	68.4
4	31	11	35.5	22.6	11	71.0
5	47	25	53.2	51.1	12	78.7
6	26	12	46.2	42.3	11	88.5
7	71	20	28.2	19.7	37	80.3
8	74	34	45.9	35.1	13	63.5
9	27	8	29.6	14.8	14	81.5
10	19	10	52.6	52.6	5	78.9
11	60	48	80.0	78.3	0	80.0
12	31	15	48.4	38.7	12 (to group 13)	87.1
13	18	10	55.6	27.8	3 (to group 12)	72.2
14	37	28	75.7	59.5	0	75.7
15	23	22	95.7	91.3	0	95.7
Combined groups						
15	23	23	100			
11	60	51	83.3	10		
12, 13	49	41	81.6	8.2		
14	37	35	91.9	5.4		
1–10	459	377	80	8.5		
Region						
Western Mojave	164	139	84.8			
Central Mojave	97	66	68.0			
Southern Mojave	198	118	59.6			
Northeastern Mojave	60	49	81.7			
Eastern Mojave	31	17	54.8			
Northern Colorado	18	13	72.2			
Eastern Colorado	37	33	89.2			
Upper Virgin River	23	22	95.7			

[a] LOD = log of the odds ratio.

DISCUSSION

Maternal History. — Two distinctive maternal lineages exist, one associated with the Sonoran population in Arizona and the other with the Mojave population. By using *G. flavomarginatus* as the outgroup, the sister group to *G. agassizii* was *G. berlandieri* (Fig. 2). This resolution differed from that of Lamb et al. (1989). Rooting with the same outgroup, they found that the Sonoran *G. agassizii* was the sister group of *G. berlandieri* and exclusive of the Mojave population. The difference could have resulted from several factors. Lamb et al. (1989) evaluated restriction fragment length polymorphisms, and we used more precise sequences. They also had greater taxonomic and geographic sampling. Although we might have reached a similar conclusion if we had used the same coverage, this was unlikely. The difference likely resulted from their use of presence/absence coding of nonhomologous fragment lengths.

Within Mojave population samples, little differentiation occurred among the 7 haplotypes (Fig. 2). Two primary maternal sublineages occur in the Mojave population, but the minor level of differentiation was not indicative of taxonomic differentiation. In contrast, the substantial sequence differentiation between Mojave and Sonoran (Arizona) populations is consistent with the hypothesis that *G. agassizii* consists of more than one species (Berry et al. 2002).

Descriptive Statistics of Microsatellite nuclear DNA (nDNA). — The motif differences in interspecies amplification of microsatellite loci indicated that evaluation of data required species-specific and even population-specific sequence information. Loci amplified between species (and within species too; Estoup et al. 2002.) did not necessarily follow assumptions of the stepwise mutation model.

Deviations from H-W could have several sources. Excess of homozygotes at some loci (e.g., Goag06) could have resulted from nonamplifying alleles, as a consequence of motif anomalies. Translocations of tortoises throughout the Mojave population also might have contributed to the excess of heterozygosity. For cases of heterozygotic deficit, ambiguities associated with high numbers of repeats might have artificially inflated the number of observed homozygotes or elevated Φ_{IS} values if translocated tortoises had very different allele frequencies
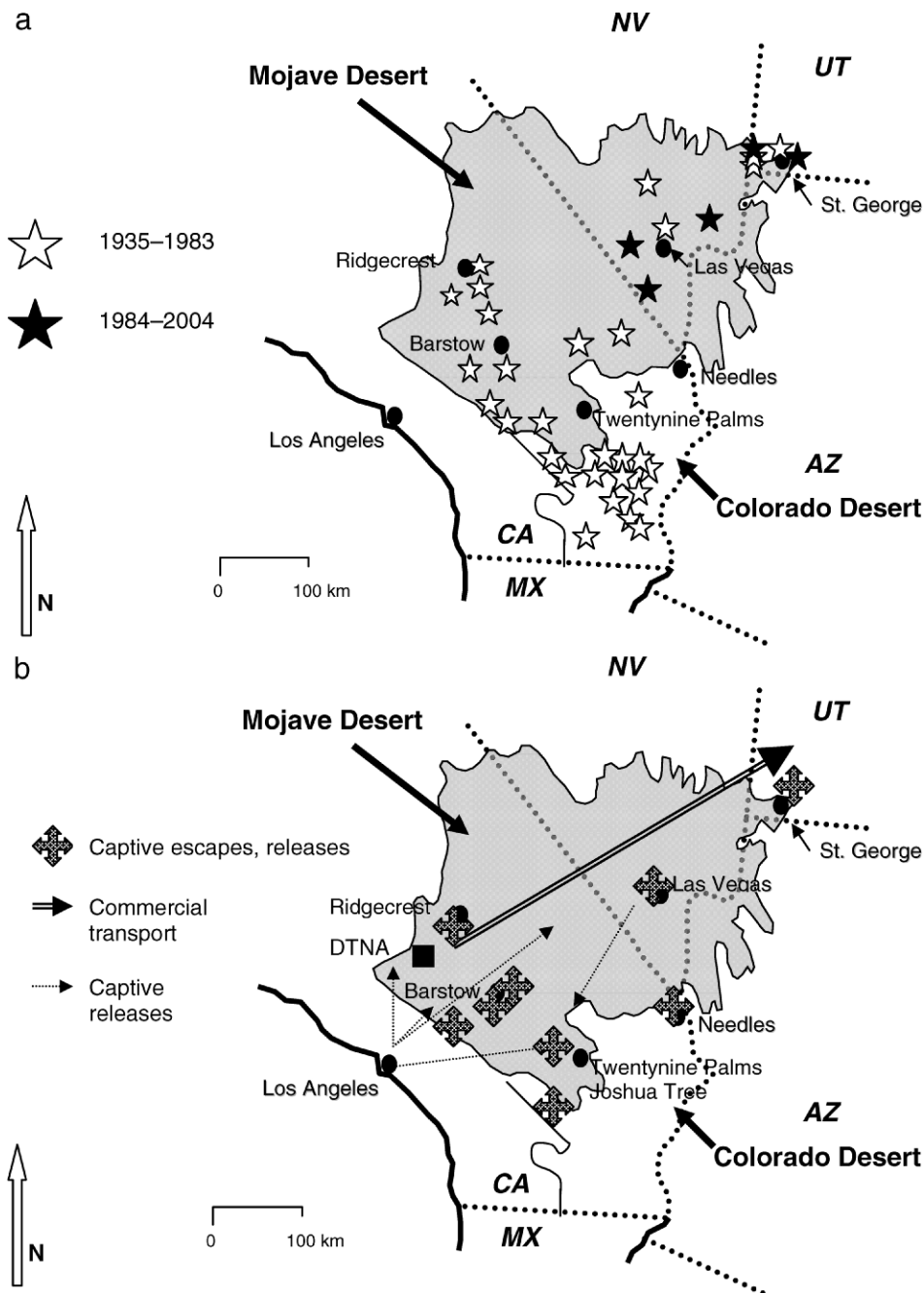
Figure 6. (a) Locations of captive desert tortoises, *Gopherus agassizii*, released by the California Department of Fish and Game, Nevada Department of Wildlife, Utah Division of Wildlife Resources or by others, as described in government reports and university theses and dissertations. The shaded area indicates the limit of the Mojave Desert. (b) Locations of areas where captives escaped or were released outside of desert towns. Tortoises were taken from the Los Angeles basin and released at places such as the Desert Tortoise Research Natural Area (DTNA) or Joshua Tree National Park. There were also large-scale commercial transfers of tortoises.

(a Wahlund effect, lower than expected heterozygosity owing to population substructuring). Technical difficulties of accurately scoring heterozygotes with high numbers of repeats surely contributed to the estimates of heterozygosity deficiencies at Goag06 and possibly at other loci (Table 5). Unfortunately, the proportions of misscored loci cannot be accurately partitioned from the data set to examine for a Wahlund effect (e.g., Chapuis and Estoup 2007).

In total, 24.5% of the data points showed deviations from H-W in the form of heterozygote deficiencies (Table 5). Such deviations may not significantly affect our conclusions. Dankin and Avise (2004) showed that 20% of the data points can deviate from H-W, without affecting the accurate determination of parentage. Empirically, the great correspondence between the results of the microsatellite analyses and ecological boundaries supports our

assumption of the utility of the data irrespective of their deviations from H-W expectations.

For tortoises, IBD (isolation-by-distance) affected the probability of individuals mating with one another and violated the assumption of panmixia for statistical tests. Significant pairwise associations of some loci (Table 5) may have reflected an absence of panmixia (i.e., a Wahlund effect), mating systems or problems in resolving alleles. However, because significant linkage disequilibrium was not observed in all groupings, this explanation was unlikely. The greater than expected deviations from H-W were strongly paralleled by Φ_{IS} values. Some deviations from H-W owed to technical constraints (e.g., Goag06), but this was unlikely for other loci (e.g., GP30, GP81). Some positive inbreeding coefficients and departures from H-W may have been because of population structure. However, inbreeding was unlikely to have occurred because most loci did not have significant Φ_{IS} values within a sample group.

Gene Flow. — Genetic structuring was strongly associated with geography (Slatkin and Maddison 1990), IBD, and the limited dispersal of individual tortoises (Mantel test; $r^2 = 0.646$, $p = 0.002$). The results of the AMOVA indicated the absence of panmixia. IBD was also reported by Britten et al. (1997) for allozyme and mtDNA data, and by Edwards et al. (2004) for Sonoran tortoises. Microsatellite variability was greater within than among sample groups, suggesting that the Mojave metapopulation was relatively homogeneous, i.e., the common alleles were broadly distributed. Gene flow likely occurred throughout populations in California, at least until the recent proliferation of anthropogenic barriers. The distribution of low-frequency, unique microsatellite alleles supported the hypothesis that the genetic structure resulted from gene flow and not common ancestry. Indeed, Edwards et al. (2004) noted that desert tortoises were ideal organisms for applying the IBD model, because they are distributed across the landscape in patches, and the difficulty of dispersion is a function of geography.

Bottlenecking. — The excess of heterozygosity in samples from the Northeastern Mojave and Upper Virgin River recovery units could have resulted from recent bottlenecking. However, this possibility was not supported by the ratio of the total number of alleles to the overall range in allele size. Population declines in the Northeastern Mojave and Upper Virgin River recovery units have been well documented in recent years (USFWS 1980; Minden and Keller 1981; Fridell and Coffeen 1993; McLuckie et al. 2004). Although other regions also experienced population declines (Berry and Medica 1995; Brown et al. 1999; Christopher et al. 2003), they did not show genetic evidence of bottlenecks. This inconsistency may have been because of at least 4 factors. First, our samples were collected over 10 years and this could have precluded the effects of recent declines. Second, the time frame for sampling may have been too short for observing a shift in heterozygosity for a long-

lived species with a long generation time. Garrigan and Hedrick (2003) reported that 5–10 generations were required to genetically detect bottlenecks. Moreover, Dinerstein and McCracken (1990) did not see bottleneck effects in the greater one-horned rhinoceros by using microsatellite DNA, despite well-documented evidence. Consequently, conclusions on the genetic structure of populations should not be based on molecular evidence alone but should accompany field observations. Third, polyandry, if common, and especially when combined with sperm storage, could have increased the effective population size (Sugg and Chesser 1994). Sperm storage for up to 3 years has been documented in the desert tortoise (Palmer et al. 1998) and anecdotal evidence suggests that it may occur for much longer. (One isolated captive female tortoise produced viable clutches for 15 years after her last known association with a male tortoise; P. Gould Glasco, *pers. comm.*, May 2006.) A controlled investigation of polyandry in the western Mojave Desert found that all females produced polyandrous clutches over a period of 2 years (Murphy, Edwards, Bratton, and Hagen, in prep.). And fourth, the observed increase in heterozygosity in the Northeastern Mojave and Upper Virgin River recovery units may also be a reflection of translocated tortoises. The translocation of gravid females or those that were storing sperm would serve to compound the possible explanations for excess heterozygosity.

Human-Mediated Translocations. — Translocations and releases of animals, especially if uninformed, can have negative genetic consequences (Allendorf and Luikart 2007). The historical releases and translocations of tortoises could have affected our results in the form of deviations from the H-W, increased heterozygosity and estimates of recent gene flow, anomalous distributions of some haplotypes, and increased Φ_{IS} values (through a Wahlund effect). The geographically disjunct occurrence of some haplotypes (MOJ-A01 with -B01 and -B03 in the Upper Virgin River; Table 3) could be caused by translocations. Because the widespread MOJ-A01 haplotype was absent in our initial survey of 7 tortoises in the Northeastern Recovery Unit only, we sequenced 40 additional samples: in total, 40 were MOJ-B01, 6 were MOJ-B03, and 1 was MOJ-B02. Because MOJ-A01 was absent from the Northeastern Recovery Unit, its presence in Upper Virgin River Recovery Unit was likely because of relocated tortoises. Our samples from the Northeastern Mojave were taken from relatively remote areas where the releases of captives were less likely.

Several other incidences of geographic mixing are evident: MOJ-B01 is geographically and genealogically associated with other members of Haplogroup B, but it also occurs in sympatry with Haplotypes MOJ-A01 (Haplogroup A) in the Western Mojave Recovery Unit (Table 3), specifically at the Interpretive Center at the Desert Tortoise Research Natural Area. This finding is concordant with documentation of multiple captive tortoise releases at the Natural Area (Howland 1989; Ginn

1990; Jennings 1991; Connor and Kaur 2004). Haplotype MOJ-B01 also occurs with MOJ-A01 in the Eastern Colorado Recovery Unit. Very long distance dispersion is the alternative explanation for the widespread occurrence of some haplotypes. Given the extent of documented translocations, the dispersion hypothesis is unlikely, particularly because our data lack other evidence of population expansion or recent ancestry.

Translocated tortoises could compromise the genetic integrity of a population by disruption to coadapted gene complexes in local environments or loss of fitness through outbreeding depression. In particular, Beaver Dam Slope, Utah, has a high frequency of released captive tortoises (Woodbury and Hardy 1948, Minden 1980, Minden and Keller 1981). Although we do not have genetic samples from this area, the excess of heterozygotes in the adjacent Upper Virgin River and Northeastern Mojave recovery units, in the absence of a decrease in the ratio of the total number of alleles to the overall range in allele size, could reflect first- or second-generation offspring from translocated tortoises. A similar problem may exist at the Desert Tortoise Research Natural Area and Joshua Tree National Park in the Western Mojave Recovery Unit. Outbreeding depression can lead to reduced fitness via disease in hybrid populations (Goldberg et al. 2005, Allendorf and Luikart 2007). The high levels of assignments of tortoises to the correct region (Table 7) indicate that, in some cases, survival rates of released tortoises may be low, e.g., the early California reintroduction experiments (Cook et al. 1978; Cook 1983; Weber et al. 1979).

Regional Differentiation. — The STRUCTURE analysis identified from 5 to 8 genetically structured units. These findings support the hypothesis of population structure in the *Recovery Plan* and the Desert Wildlife Management Units described in the Western Mojave Recovery Unit. When considering the close geographic proximity of some of our sample groups (e.g., groups 12 and 13), this result was consistent with our assumption that the Mojave population is genetically structured and that these genetic data were informative for designating recovery units. Sample group 8 may have the most admixture between the "Central" and "Southern" areas of the Western Mojave Recovery Unit. This subanalysis suggested that the Western Mojave Recovery Unit could be subdivided into at least 3 geographic groups. Although STRUCTURE is not a good measure of structure in populations that exhibit nonlinear patterns of IBD (Pritchard et al. 2000), the findings were congruent with the *Recovery Plan* and natural barriers to gene flow. Thus, we used these results as evidence for the assessment of recovery units.

The null hypothesis of a single, homogeneous, panmictic Mojave population was rejected. Although most alleles were broadly distributed, most sample groups significantly differed from one another in allelic frequencies (Table 7). Because the G-based exact test is sensitive to different sample sizes, as in our data, the imbalance in

samples might have accounted for the high number of significant differences. However, this does not appear to be true. Most individuals ($> 80\%$) were reassigned (Table 7) back to their sample group. The accuracy of the assignments implies genetic divergence.

The population assignment was viewed as a conservative result. Our data set was limited to 11 variable microsatellite loci only. Additional loci would have likely increased the accuracy of the assignments and the distinctiveness of each recovery unit.

Congruent patterns of genetic differentiation from different regions or taxa lend credence to conclusions. Comparatively, desert tortoises from Mojave and Sonoran populations had almost identical genetic structuring at local and regional levels. The AMOVA of microsatellites from the Sonoran population revealed that 96.3% ($p < 0.001$) of the diversity occurred in individuals within study sites ($\Phi_{IT} = 0.963$), whereas only 3.7% ($p < 0.001$) of the variation was among sites ($\Phi_{ST} = 0.037$) (Edwards et al. 2004). The same result occurred in a geographically equivalent sized subset of our data; $\Phi_{ST} = 0.037$ ($p < 0.001$). In both studies, a significant positive correlation occurred between genetic distance (pairwise Φ_{ST}) and geographic distance.

Recovery Units Revisited

The authors of the *Recovery Plan* proposed 6 recovery units to capture the known genetic, morphological, ecological, and behavioral diversity in desert tortoises as of 1993 (USFWS 1994). Their original objectives agree with the views of Crandall et al. (2000), specifically to preserve the options for adaptive diversity and evolutionary processes, maintain a network of populations, reduce the likelihood of further contraction of the geographic range, and minimize homogenization of the gene pool or pools by anthropogenic activities. The recovery units in the *Recovery Plan*, with some exceptions described below, appear to reflect natural, biological differences in populations and to fall within the DPSs described in government policy (US Department of the Interior and US Department of Commerce 1996).

We emphasize, however, that the genetic evidence presented here is not necessarily concordant with or related to morphological, ecological, and behavioral differences observed in the tortoise populations. Genetic evidence is only one factor among many that should be considered in managing desert tortoises (Crandall et al. 2000; DeSalle and Amato 2004; Green 2005). No direct evidence suggests that the mtDNA and microsatellite markers reflect the observed phenotypic differences and local adaptations, although the assumption is that identified genetic markers may serve as surrogates for these and other character traits (Pearman 2001). Behavioral differences between populations can be genetically linked, as in the case of garter snake food habits (Arnold 1981) and morphological variability in turtles can be heritable (Myers et al. 2006).

In the absence of data linking genotypic markers with specific phenotypic characters or adaptations in desert tortoises, we are confined to delineating recovery units based on available information, such as the differences in mtDNA and microsatellite markers described here, as well as differences in vegetative communities, physical attributes of the habitat, climate (e.g., mean number of freezing days annually, mean annual precipitation, amounts of precipitation occurring in summer), choice and availability of forage plants, cover sites (burrows, dens), and denning behavior.

The direct translation of molecular data into management units is subjective. On one extreme, it is possible to define 2 recovery units, based on the arbitrary subdivision of assignment values. However, the STRUCTURE analysis indicated the presence of at least 6 genetically cohesive units. Although this evaluation was compromised by multidimensional IBD, when we reduced the effects of IBD, 4 additional genetic units were identified in the Western Mojave Recovery Unit: sample groups 1–2, 3–5, 8, and 6–7 plus 9–10 (Fig. 4). Ultimately, the designation of recovery units must synthesize all relevant factors to achieve effective management.

Our analyses indicate that the Western Mojave Recovery Unit should be divided into 3 regions (western, southern, and central) and 3 corresponding recovery units: Western Mojave, Central Mojave, and Southern Mojave (Table 8, Fig. 7). Although the analysis by using STRUCTURE discovered 4 genetic units within the Western Mojave, the segregation of 1 site (8) would not facilitate effective management. Our proposed recovery units are similar to the 3 Desert Wildlife Management Areas described in the *Recovery Plan* and are concordant with the western, southern, and central regions of the Mojave Desert described by botanists and climatologists (Rowlands et al. 1982; Rowlands 1995a, 1995b). The western, central, and southern Mojave regions differ primarily in the amounts of summer rainfall, number of freezing days, and mean January minima and mean July maxima temperatures, as well as in species richness (vegetation) and types and composition of plant species with different metabolic pathways, e.g., C_3, C_4, and Crassulacean acid metabolism (CAM). The redefined Western Mojave Recovery Unit (Fig. 7) receives precipitation primarily in winter and < 10% of rainfall occurs in summer (Rowlands 1995a; Table 8). The summer flora is very limited, and tortoises rely heavily on the succulent green forbs and herbaceous perennial plants available in late winter and spring (Jennings 1993, 2002; Oftedal 2002; Oftedal et al. 2002). The proposed Central Mojave Recovery Unit is the hottest and driest of the 3 regions and is low in botanical diversity (Rowlands, 1995a). Of the 3 regions, the proposed Southern Mojave Recovery Unit has more summer precipitation and a higher richness of C_4 and CAM plant species (Rowlands 1995a). Until ca. 100 years ago, the Southern Mojave Recovery Unit was physically separated from the proposed Central Mojave

and Western Mojave recovery units by the Mojave River; human activities have since reduced or eliminated the flow along much of the river.

Climatic differences between all recovery units profoundly affect timing and availability of forage, as well as seasonal activities and very possibly depth of burrows and, thus, protection from freezing temperatures and the hot, dry summers. The existing eastern recovery units in the Mojave population have higher percentages of precipitation in the summer, thus supporting a more diverse and complex summer flora (Table 8; Rowlands 1995a, 1995b; Oftedal 2002). A winter flora is also available. Differences in the mean number of freezing days per annum contribute to seasonal activity periods and the types of winter hibernacula protecting the tortoises from freezing. The Northern and Eastern Colorado Desert recovery units are the warmest, with 1–16 freezing days/y compared with 29–127 freezing days/y in the Mojave. Northeastern recovery units are by far the coldest, possibly contributing to the well-developed dens and lengthy tunnels on Beaver Dam Slope (Woodbury and Hardy 1948) that are rarely observed outside the Northeastern Mojave and Upper Virgin River recovery units.

Genetic assignments do not support a separation between the Eastern Mojave and Northern Colorado recovery units, possibly because we only had 4 sample groups from these regions. The close geographic proximities of the sample groups (Fig. 7) are unlikely to reflect the potential diversity occurring along a 250 km north-south axis. Until more data are gathered along the north-south axis, we do not recommend treating the 2 recovery units as one, because of major differences in climate, forage availability, and seasonal activities. These distinctions may be exactly the kind of ecological/adaptive differences worthy of conservation management, independent of the units delimited by neutral molecular variation (Crandall et al. 2000; Allendorf and Luikart 2007). Significantly, unlike the genetically restricted and legally inapplicable ESU, the legal application of DPS allows for and promotes such protection (US Department of the Interior and US Department of Commerce 1996).

The Northeastern Recovery Unit (group 11) and the Upper Virgin River Recovery Unit (group 15) showed the strongest differentiation (MDS plot, assignment test, and unique matriarchal lineage). They may be more genetically isolated than other areas. Both potentially show evidence of recent population reductions. Additional sampling of these regions is encouraged for evaluation of current management strategies. Unfortunately, under current legislation these and perhaps other demes cannot be protected solely on the basis of the degree of threat alone, as recently advocated by Green (2005).

Recovery Actions. — Populations that have become disjunct or mixed as a result of recent anthropogenic activities may be suitable for restorative actions (Crandall et al. 2000; Allendorf and Luikart 2007). One restorative action would be to remove deliberately or inadvertently

Table 8. Physical and biological attributes of proposed recovery units for the Mojave population.

| Recovery unit	Mean annual precipitation (mm)	% Rainfall June–Sept	Mean no. freezing days annually	Mean July maximum temperature (°C)	Topography	Vegetation types
Western Mojave	90–150	3.1–9.9	33–84	35.4–37.4	Flats, valleys, alluvial fans, rolling hills, mountainous slopes	(1) Creosote Bush Scrub, (2) Mojave Saltbush-Allscale Scrub (endemic), (3) Indian Rice Grass Scrub-Steppe, (4) Hopsage scrub, (5) Cheesebush scrub (west Mojave type)
Central Mojave	109	18.3–20.7	57 +	39.1–42.9	Flats, valleys, alluvial fans, rolling hills, mountainous slopes, rock outcrops, badlands, sand dunes, lava flows	(1) Creosote Bush Scrub, (2) Big Galleta Scrub Steppe, (3) Hopsage Scrub, (4) Cheesebush scrub, (4) Desert Psammophytes
Southern Mojave	108	18.1–36.1	29–104	37.2–39.1	Flats, valleys, alluvial fans, rolling hills, mountainous slopes, rock outcrops, lava flows	(1) Creosote Bush Scrub, (2) Big Galleta Scrub Steppe, (3) Hopsage Scrub, (4) Cheesebush scrub, (4) Desert Psammophytes, (5) Blackbush Scrub
Eastern Mojave	112–208	27.5–37.7	34 +	34.8–36.1	Flats, valleys, alluvial fans, bajadas, rocky slopes	(1) Big Galleta-Scrub Steppe, (2) Succulent Scrub (*Yucca, Opuntia*), (3) Creosote Bush Scrub, (4) Cheesebush Scrub (eastern Mojave type), (5) Indian Rice Grass Scrub-Steppe
Northeastern Mojave	100–210	27.1–41.0	46–127	38.2–40.1	Flats, valleys, alluvial fans, rocky slopes, deeply cut washes	(1) Creosote Bush Scrub, (2) Big Galleta Scrub-Steppe, (3) Desert Needlegrass Scrub-Steppe, (4) Blackbush Scrub
Upper Virgin River	210	28.7	96 +	38.4	Rocks, caves, sandstone crevices, sand dunes	Transitional Vegetation: (1) Sagebrush Scrub, (2) Psammophytes, Great Basin (sand sage), (3) Blackbush Scrub
Northern Colorado	112–129	32.6–34.1	2–12	42.2–42.3	Flats, valleys, bajadas, rocky slopes, small washes	(1) Succulent Scrub (*Fouquieria, Opuntia, Yucca*), (2) Blue Palo Verde-Smoke Tree Woodland, (3) Creosote Bush Scrub (lava flows)
Eastern Colorado	96–100	32.3–34.4	1–16	40.5–42.2	Flats, valleys, alluvial fans, small washes, deeply dissected washes, rocky slopes	(1) Succulent Scrub (*Fouquieria, Opuntia, Yucca*), (2) Blue Palo Verde-Ironwood-Smoke Tree Woodland, (3) Creosote Bush Scrub (rocky slopes)
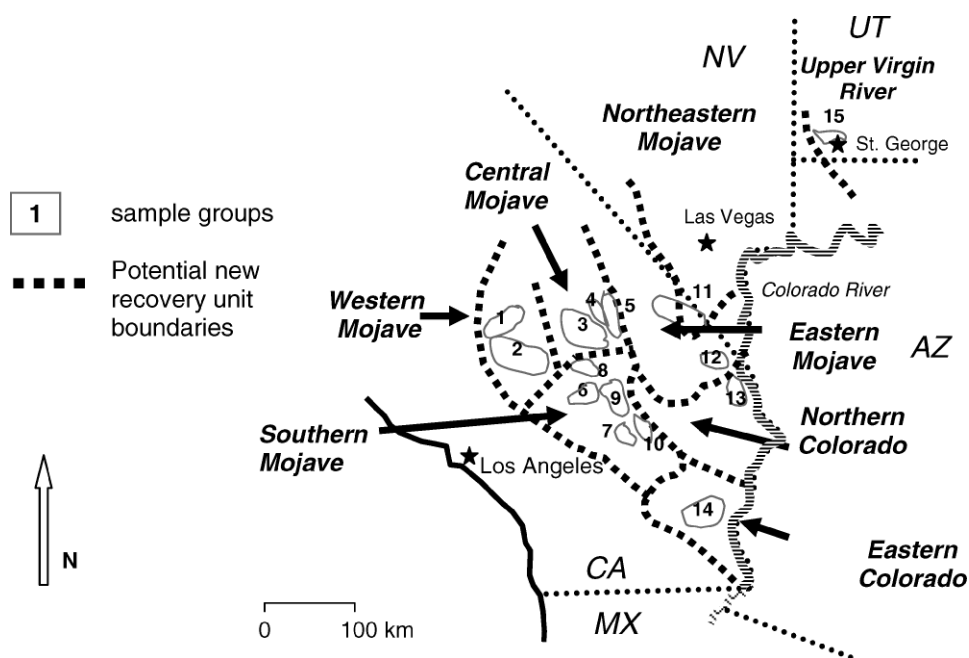
Figure 7. Sample groups of desert tortoises, *Gopherus agassizii*, shown with a new, preliminary alignment of recovery unit boundaries developed by using the mitochondrial deoxyribonucleic acid and microsatellite data presented in this study.

translocated tortoises from critical habitat. This strategy would be unreasonably difficult where populations are dense but may be a viable option where the area of interest and densities are limited, populations are declining, and most tortoises could be located and sampled. This strategy might be appropriate in the Upper Virgin River Recovery Unit and the Beaver Dam Slope Desert Wildlife Management Area (within the Northeastern Mojave Recovery Unit). Another restorative action would be to genetically test tortoises in the vicinity of frequently used recreation sites within national parks, research natural areas, and other protected areas: sites where visitors often release tortoises illegally, e.g., the Desert Tortoise Research Natural Area. The released tortoises from other populations could be identified and removed to a more appropriate place. In populations that have dropped below viable levels (e.g., Fremont-Kramer Desert Wildlife Management Area, Western Mojave Recovery Unit), informed and carefully planned augmentations or translocations could promote recovery, as has been done for a few other species (Allendorf and Luikart 2007). However, genetic planning is an essential part of such recovery efforts. Using tortoises within a well-defined recovery unit or local geographic area for headstarting or augmentation is far more desirable than translocating tortoises between recovery units. If local adaptations exist, then uninformed translocations of desert tortoises may do much more harm than good by introducing maladaptive genes into a locally adapted population.

Empirical studies need to be designed and tested to determine whether marker loci reflect specific adaptations with potential conservation value. For the Mojave population of the desert tortoise, the initial recovery units were defined on the basis of morphological, ecological, and behavioral differentiation, and the patterns of genetic variation parallel the earlier assessment in the *Recovery Plan*. Taken together, these 2 independent approaches strongly suggest the occurrence of local adaptation and evolutionary potential. Not only is it essential that this potential be conserved but also that underlying hypotheses be tested in the near future.

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