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Genetic Delineation of Management Units for the Desert Tortoise, *Gopherus agassizii*, in Northeastern Mojave Desert

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Blood samples from 236 desert tortoises, *Gopherus agassizii*, representing 15 sites in northeastern Mojave Desert (Nevada, Utah), were assayed for variation in allozymes and mitochondrial DNA restriction sites. Morphometric measurements from 257 tortoises from the same locations were subjected to sheared principal component analysis. The 15 localities were partitioned into five management units. Geographic concordance between the five management units delineated in this study and proposed Desert Wildlife Management Areas (DWMAs), delineated ecologically, was assessed. Suggestions for improving placement and boundaries of DWMAs are discussed.

THE desert tortoise, *Gopherus agassizii*, is one of four extant species of testudinid tortoises found in desert and semitropical regions of North America. Its range includes the Mojave Desert of southern Nevada, California, and Utah and the Sonoran Desert of western Arizona, western Sonora, Mexico, and northern Sinaloa, Mexico (Lamb et al., 1989).

Beginning in the early 1970s, declines of from 3–59% in desert tortoise populations were noted over much of its range in the United States (USFWS Recovery Plan, 1994, unpubl.). Declines were attributed to direct impacts, human population growth, and habitat loss, degradation, and fragmentation (USFWS Recovery Plan, 1994, unpubl.). Mojave Desert populations of *G. agassizii* were listed as threatened in April 1990, bringing federal protection to tortoises in the Mojave Desert, an area defined administratively as west and north of the Colorado River, including the southern tip of Nevada, the extreme southwestern corner of Utah, and southeastern California. Among criteria for delisting the species is formation of several Desert Wildlife Management Areas (DWMAs) that eventually will provide "reserve level" protection for populations of *G. agassizii* within the area (USFWS Recovery Plan, 1994, unpubl.).

Moritz (1994a, 1994b) provided a useful dichotomy between Evolutionarily Significant Units (ESUs) and Management Units (MUs) in recent reviews of the application of genetic data to conservation questions. Generally, population segments within a species' geographic range are considered ESUs if they possess two criteria: (1) "substantial" reproductive isolation from other such segments; and (2) evolutionarily important genetic uniqueness (Waples, 1991). MUs can be distinguished by significant nuclear or mitochondrial gene frequency differences (Moritz, 1994b). Populations with divergent gene frequencies likely exchange so few migrants that they exhibit substantive demographic independence and should be managed appropriately (Moritz, 1994b).

Previous studies revealed that informative genetic variability can be detected in desert tortoise populations. Lamb et al. (1989) used whole mitochondrial DNA (mtDNA) restriction length polymorphism (RFLP) analysis and found two restriction-site changes among 12 desert tortoises from southern Nevada and Utah. Rainboth et al. (1989) detected polymorphism at eight allozyme loci in desert tortoises from southern California. Genetic studies of *G. agassizii* in the northeastern Mojave Desert of Nevada and Utah were undertaken to document genetic variability and to identify genetically distinct populations or MUs (Moritz, 1994a, 1994b). Variation at presumed allozyme loci, mtDNA, and morphometry was employed in the analysis. One goal of the research was to assess geographic concordance between genetically distinct populations and proposed DWMAs.

MATERIALS AND METHODS

Whole blood was taken from 236 individuals at 14 sites in Nevada and from 14 individuals at five sites on the Beaver Dam Slope, Utah (Fig. 1). Samples from Utah sites were pooled in all analyses. Blood was drawn via standard methods (Jacobson et al., 1992) and transported on ice until frozen at −80 C.

For allozyme analysis, samples of whole blood were thawed prior to use and applied to 14% horizontal starch gels using filter paper wicks. Methods used and loci assayed were similar to those of Rainboth et al. (1989). General histo-

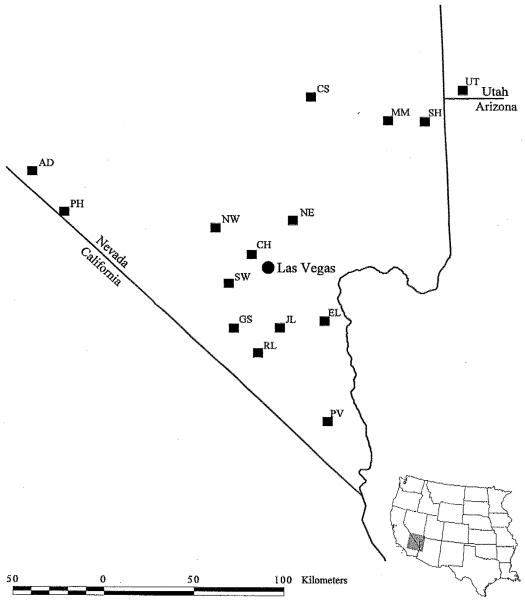

Fig. 1. Map of sample localities in northeastern Mojave Desert: PV = Piute Valley; SW = southwestern Las Vegas Valley; NE = northeastern Las Vegas Valley; NW = northwestern Las Vegas Valley; MM = Upper Mormon Mesa; CH = Charleston West; AD = Amargosa Desert; PH = Pahrump Valley; GS = Goodsprings; EL = Eldorado; RL = Roach Lake; CS = Coyote Spring; SH = Sand Hollow; JL = Jean Dry Lake; and UT = Beaver Dam Slope.

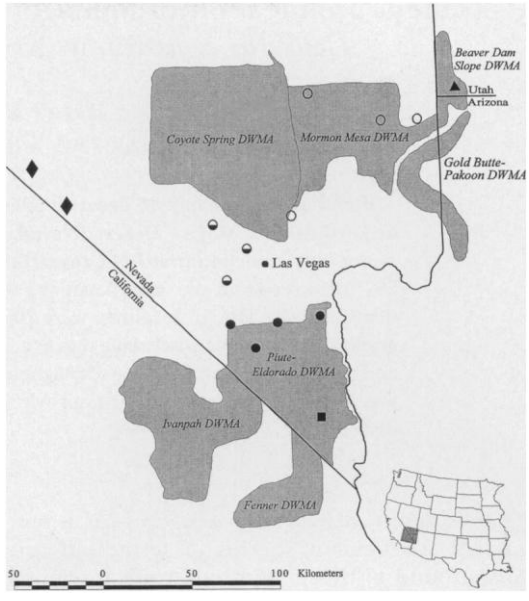

Fig. 2. Proposed Desert Wildlife Management Areas (DWMAs) and genetically delineated management units (MUs) for desert tortoises in northeastern Mojave Desert. MUs are Piute Valley (filled square), southern Las Vegas Valley (filled circles), northern Las Vegas Valley (open circles), Beaver Dam Slope (filled triangle), and Amargosa Desert/Pahrump Valley (filled diamonds). Half-filled circles represent transitional samples between the northern and southern Las Vegas Valley MUs.

chemical staining procedures followed Selander et al. (1971). Protein loci, abbreviations, and enzyme commission numbers for the enzymes assayed are given in Appendix 1. Genotype frequencies were obtained from direct counts of the electrophoretic phenotypes on gels. Conformance to Hardy-Weinberg expectations and estimates of genetic variability were obtained using BIOSYS-1 (Swofford and Selander, 1981). Homogeneity of allele frequencies among samples was tested using log-likelihood (G) tests (Sokal and Rohlf, 1981).

For mtDNA analysis, total genomic DNA was extracted from frozen, whole-blood samples by using a proteinase K-phenol-chloroform isolation protocol (Hillis et al., 1990). Two adjacent portions of the mtDNA molecule were amplified using polymerase chain reaction (PCR) by employing premixed sets of primers. One primer set, "New Gly" and "Nap2" (Arevalo et al., 1994), facilitated amplification of the ND3, arginine tRNA, ND4L, and part of the ND4 genes (1200 bp); a second primer set, "ND4" and "Leu" (Arevalo et al., 1994), amplified the remainder of ND4, and the histidine, serine, and leucine tRNA genes (900 bp). Hereafter, the first fragment is designated ND3, the second ND4.

We used 12 and 10 restriction enzymes to screen for variation in the ND3 and ND4 fragments, respectively. All restriction enzymes used had four base-pair recognition sequences. Single digestions of 5 μl of each PCR amplification followed manufacturers' protocols. DNA fragments were electrophoresed through 0.8% agarose gels and visualized with ethidium bromide. MtDNA haplotype diversity within each population was estimated after Nei (1987). Homogeneity of haplotype frequencies among samples was tested using Monte Carlo simulations (Roff and Bentzen, 1989) with 2000 randomizations.

The simultaneous test procedure (STP, Sokal and Rohlf, 1981) was used to test the homogeneity of clusters of populations within or near DWMAs (Fig. 2) based on GPI-1 allele and ND4 haplotype frequencies. Constant critical values of $\chi^2_{0.05[28]} = 41.337$ and $\chi^2_{0.05,[14]} = 23.685$ were used for the STP heterogeneity tests of GPI-1 and ND4 frequencies, respectively (Sokal and Rohlf, 1981). STP groupwide type-one error

rates were controlled for both markers using the sequential Bonferroni method (Rice, 1989).

For morphometric analysis, eight standard carapace measurements were taken from 111 males and 105 females from Nevada and from 20 males and 21 females from Beaver Dam Slope of Utah (Appendix 2). Measurements were log base$_e$-transformed. Principal component analysis (PCA) with shearing of second and third principal components (PCs) was performed following Humphries et al. (1981) and Rohlf and Bookstein (1987). Shearing removes effects of intersite size differences from all but PC1 (Rohlf and Bookstein, 1987). Scores for PC2 and PC3 were plotted to visualize groupings of tortoises with similar shape. Morphometric analyses were carried out separately for each sex because of known size and shape dimorphism (M. Weinstein and K. Berry, 1988, BLM Report, unpubl.).

RESULTS

Sample sizes for allozyme assays ranged from seven to 34 individuals; polymorphism was detected at four of 15 allozyme loci (Appendix 1). Variability at IDH, sMDH, and PGM was limited to rare alleles at single localities (Appendix 3). GPI was polymorphic at all localities (Appendix 3). Observed heterozygosity ranged from 0.010 in the sample from Utah to 0.057 in the sample from Sand Hollow (Appendix 3). All samples conformed to Hardy-Weinberg expectations at all polymorphic loci.

A G-test revealed that allele frequencies at GPI differed significantly among localities ($G = 171.0$, $df = 28$, $P < 0.01$). A homogeneous cluster of southern populations ($G = 25.76$, $P > 0.99$) consisting of Goodsprings, Jean Dry Lake, Eldorado, Roach Lake, and Piute Valley was found within the Piute-Eldorado DWMA (Fig. 2). Similarly, the Las Vegas Valley NE, Coyote Spring, Upper Mormon Mesa, Sand Hollow, and Beaver Dam Slope sites constituted a homogeneous cluster ($G = 10.62$, $P > 0.99$) of northern populations associated with the Coyote Spring, Mormon Mesa, and Beaver Dam Slope DWMAs (Fig. 2). The northern and southern clusters of populations differed significantly in GPI allele frequencies ($G = 330.2$, $P < 0.001$). The three sites (Las Vegas Valley NW, SW, and Charleston West) intermediate geographically to these two groupings formed homogeneous groupings with both southern ($G = 31.36$, $P > 0.99$) and northern ($G = 25.7$, $P > 0.99$) population clusters. Finally, the Amargosa Desert and Pahrump Valley sites formed a homogeneous cluster ($G = 1.215$, $P > 0.99$) that differed significantly in GPI allele frequencies from northern ($G = 75.55$, $P = 0.005$), southern ($G = 117.91$, $P < 0.001$), and intermediate ($G = 70.48$, $P < 0.001$) population clusters.

Ten restriction enzymes were used to screen 100 individuals for polymorphism in the ND4 fragment. No sites were detected with *Bsp*1286I, *Bst*UI, *Hha*I, or *Sau*96I; 11 monomorphic sites were detected with the remaining six enzymes. One polymorphism at a single *Hin*fI restriction site was detected, diagnosing two mtDNA haplotypes designated ND4-A and ND4-B. Only ND4-A and ND4-B fragment patterns were detected in an additional 53 individuals digested with *Hin*fI. Twelve restriction enzymes were used to screen 22 individuals for polymorphism in the ND3 fragment. No sites were detected with *Bsp*1286I, *Bst*UI, or *Rsa*I; 14 monomorphic sites were detected with the remaining eight enzymes. These eight enzymes were used to screen an additional 37 individuals, and a single polymorphic *Hae*III site was detected. Between one and seven enzymes (including *Hae*III) were used to screen 16 more individuals, but no polymorphism was detected. Because the *Hae*III variant in the ND3 fragment was found in only three individuals (Table 1), all of which were ND4-A, and because the three individuals were not clustered geographically, further analysis was based only on variation detected in the ND4 fragment.

The geographic distribution of *Hin*fI variants in the ND4 fragment is given in Table 1. Overall, ND4-A is far more common in the study area than ND4-B. However, the frequency of each form within and among populations is decidedly nonrandom ($\chi^2 = 72.77$; $P < 0.001$; Table 1). STP revealed overall heterogeneity in ND4 frequencies across the total sample ($G = 88.98$, $P < 0.001$). This cluster of populations was only marginally heterogeneous with the exclusion of the Piute Valley sample ($G = 32.417$, $P = 0.045$). Piute Valley formed heterogeneous clusters with the other samples from the Piute-Eldorado DWMA (Goodsprings, Eldorado, Roach Lake, and Jean Dry Lake; $G = 36.84$, $P = 0.011$) and samples from Coyote Springs, Mormon Mesa, and Beaver Dam Slope DWMAs (Las Vegas Valley NE, Coyote Springs, Mormon Mesa, Sand Hollow, and Beaver Dam Slope; $G = 65.58$, $P < 0.001$). Piute Valley formed a homogeneous cluster with the intermediate samples (Las Vegas Valley NW, SW, and Charleston; $G = 31.35$, $P = 0.06$) and the western samples (Amargosa Desert and Pahrump; $G = 39.99$, $P = 0.45$).

TABLE 1. SUMMARY OF MTDNA HAPLOTYPES IDENTIFIED FROM *HINFI* AND *HAEIII*. Digestions of the 900bp (ND4) and 1200bp (ND3) fragments, respectively. Restriction fragment sizes from enzymes that diagnose different haplotypes are given in base-pairs. Haplotype diversity ($\hat{h}$) is given for ND4 haplotypes.

	ND4			ND3	
	A	B	$\hat{h}$	C	D
	*Hin*fI 400 340 160	*Hin*fI 500 400		*Hae*III 650 320 280	*Hae*III 650 600
Populations					
Piute Valley	1	22	0.08	10	0
Las Vegas Valley SW	4	2	0.53	4	1
Las Vegas Valley NE	6	0	0.00	6	0
Las Vegas Valley NW	5	1	0.34	5	0
Upper Mormon Mesa	4	0	0.00	3	1
Charleston West	5	0	0.00	6	0
Amargosa Desert	9	0	0.00	4	1
Pahrump Valley	12	4	0.40	5	0
Goodsprings	4	3	0.57	5	0
Eldorado	5	4	0.55	2	0
Roach Lake	10	6	0.50	4	0
Coyote Springs	12	0	0.00	2	0
Sand Hollow	8	0	0.00	5	0
Jean Dry Lake	9	0	0.00	6	0
Beaver Dam Slope	13	4	0.39	0	0

Morphometric variation.—PC1 accounted for 84.2% of the variance in eight carapace measurements for male desert tortoises, whereas sheared PCs two and three accounted for 9.1% and 3.1% of the variance, respectively (Appendix 2). In males, width was the most heavily loaded variable on size-dependant PC1; plastron length between gular and anal notches and greatest width were the most heavily loaded variables on PC2; and plastron length between gular and anal tips and notches were the most heavily loaded measurements on PC3 (Appendix 2). Males from the Beaver Dam Slope, Utah, formed a cluster discrete from Nevada tortoises when sheared principal component scores were plotted on PC2 and PC3 (Fig. 3). Males from the same locality in Nevada did not form clusters on the plots of these two PCs. PC1 accounted for 81.6% of the variance in carapace measurements among female desert tortoises, whereas sheared PCs two and three accounted for 9.6% and 3.8% of the variance, respectively (Appendix 2). Females had highest loadings for the same variables on PC2 as males, although signs were reversed (Appendix 2). Measurements heavily loaded on PC3 for females were plastron length from gular to anal tips and height at third vertebral (Appendix 2). As with males, there was a tendency for females from Utah to separate from Nevada tortoises on the plots of PCs two and three; whereas no clustering by locality was observed for Nevada tortoises (Fig. 3).

DISCUSSION

Geographic distributions of mtDNA haplotypes and allozyme genotypes, in combination with morphometric analyses, identify five homogeneous clusters of northeastern Mojave Desert tortoise populations (Fig. 2). A southern cluster, designated southern Las Vegas Valley MU (Goodsprings, Jean Dry Lake, Roach Lake, and Eldorado), is distinguished by high frequencies of the GPI-d allele (Fig. 2). A northern cluster, the northern Las Vegas Valley MU (Las Vegas Valley NE, Coyote Spring, Sand Hollow, and Upper Mormon Mesa; Fig. 2), is defined by high frequencies of the GPI-c allele. Lying between the northern and southern Las Vegas Valley MUs is a cluster of transitional populations (Las Vegas Valley NW, SW, and Charleston West).

Geographic outliers among the sampled populations constitute three additional MUs. The Piute Valley population differed from all other populations in possessing a high frequency of the ND4-B haplotype. The Beaver Dam Slope population from Utah differed morphometrically from all other populations. Finally, tortoise populations sampled from Amargosa Desert and Pahrump Valley form another MU because

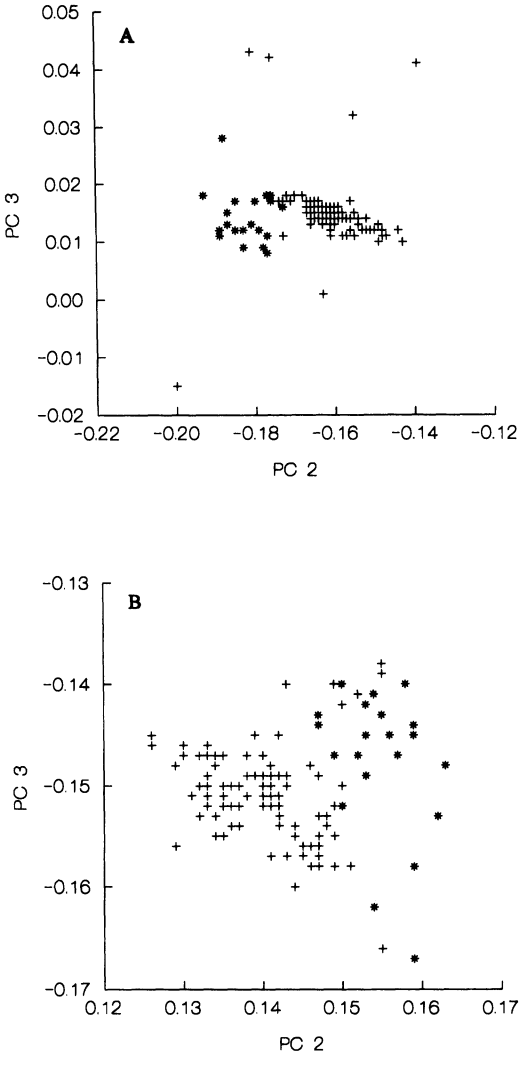

Fig. 3. Plot of sheared principal component scores based on eight carapace measurements for 15 samples of male (A) and female (B) desert tortoises from northeastern Mojave Desert. PC 2 and PC 3 refer to second and third principal components. Utah tortoises are designated by * and Nevada tortoises by +.

they possess the GPI-b allele at high frequencies.

The geographic distribution of desert tortoises in the northeastern Mojave Desert is consistent with an isolation-by-distance effect and partial habitat isolation. Field surveys of desert tortoises in the study area suggest that tortoises usually are not found above 1200 m in elevation (Bury et al., 1994). Consequentially, desert tortoise habitat is relatively continuous between Roach Lake in the south Las Vegas Valley and Coyote Spring in the north Las Vegas Valley. Although a direct test of isolation-by-distance was not possible given the small number of polymorphic loci detected (Slatkin, 1993), the presence of transitional populations between the two Las Vegas Valley MUs is strongly suggestive of it.

The Piute Valley is partially isolated from the southern Las Vegas Valley by the McCullough Range to the west and north and the Colorado River (Lake Mead) to the east. Similarly, Amargosa Desert/Pahrump Valley is almost completely isolated from tortoise habitat to the east by the Spring Mountains and the Spotted and Specter Ranges. In both cases, drift and founder events may account for divergent allozyme and mtDNA haplotype frequencies.

Morphological distinctness of tortoises from Beaver Dam Slope has been recognized in the species recovery plan with the formation of a separate DWMA (USFWS Recovery Plan, 1994, unpubl.). The presence of a distinctive carapace shape in tortoises from Beaver Dam Slope may suggest that this population has been isolated in the recent past. However, both the species recovery plan (USFWS Recovery Plan, 1994, unpubl.) and Bury et al. (1994) note that adequate habitat for tortoises occurs to the south, east, and west of the Beaver Dam Mountains and that tortoises in this region are not genetically isolated from populations in Nevada or Arizona.

Delineation of DWMAs was based on both ecological and genetic considerations (USFWS Recovery Plan, 1994, unpubl.), and their locations and boundaries have not been finalized. One question that could be addressed by our study regards how effective proposed DWMAs are in preserving genetically delineated MUs among northeastern Mojave Desert tortoises. Four MUs (Beaver Dam Slope, northern and southern Las Vegas Valley, and Piute Valley) identified in this study are represented by proposed DWMAs. However, none of the three transitional samples between the north and south Las Vegas Valley were contained within a DWMA. Although these intermediate populations contained no unique genetic constituents, they undoubtedly provide important genetic and demographic links between the two Las Vegas Valley population segments. Because they exist in the vicinity of Las Vegas, one of the most rapidly growing urban areas in the United States, it is likely that these populations may be impacted significantly. Absence of dispersal corridors or other mechanisms that promote gene flow eventually may isolate population segments within the Las Vegas Valley. Similarly, populations in the Amargosa Desert and Pahrump Valley are not contained within any proposed DWMA. Further study of desert tortoise popu-

lations on the Nevada-California border, south of Beatty, Nevada, may clarify the relationship of Amargosa Desert/Pahrump Valley desert tortoises to other tortoises in the northeastern Mojave Desert.

The fairly high degree of concordance between locations of ecologically based DWMAs and genetically based MUs delineated here is encouraging, both for conservation of desert tortoises and for other species whose recovery planning has yet to take place. Agreement between different types of data serves to clarify directions that land managers should take in conservation of threatened and endangered biota.

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APPENDIX 1. ENZYMES AND OTHER PROTEINS, NUMBER OF LOCI, ENZYME COMMISSION NUMBERS, AND ELECTROPHORETIC CONDITIONS USED TO ASSAY ISOZYME VARIABILITY IN 15 SAMPLES OF DESERT TORTOISE POPULATIONS FROM THE NORTHEASTERN MOJAVE DESERT.

Locus	Enzyme or protein	No. of loci	Enzyme commission number	Buffer[a]
sAAT	Aspartate aminotransferase	1	2.6.1.1	4
ADA	Adenosine deaminase	1	3.5.4.4	TC-4
CK	Creatine kinase	1	2.7.3.2	4
GP	General protein	1	–.–.–.–	4
GPI	Glucosephosphate isomerase	1	5.3.1.9	4
HB	Hemoglobin	2	–.–.–.–	C
IDH ($NADP^+$)	Isocitrate dehydrogenase	1	1.1.1.42	4
LDH	L-Lactate dehydrogenase	2	1.1.1.27	4
sMDH	NAD Malate dehydrogenase	1	1.1.1.37	C
MPI	Mannose-6-phosphate isomerase	1	5.3.1.8	4
PEPA	Peptidase-A	1	3.4.–.–	C
PEPE	Peptidase-E	1	3.4.11.1	4
PGM	Phosphoglucomutase	1	5.4.2.2	TC-4

[a] C, an amine-citrate buffer after Clayton and Tretiak (1972) adjusted to pH 6.8; 4, a Tris-citrate buffer adjusted to pH 6.3 (Selander et al., 1971); TC-4, a Tris-citrate buffer adjusted to pH 6.9 after Whitt (1970).

APPENDIX 2. PRINCIPAL COMPONENT LOADINGS FOR EIGHT CARAPACE MEASUREMENTS (AFTER M. WEINSTEIN AND K. BERRY, 1988, BLM REPORT, UNPUBL.) IN MALE AND FEMALE DESERT TORTOISES. Loadings for the first three principal components (PCs) are given. PCs 2 and 3 were sheared. Percent of total variance in measurements accounted for by each PC is given.

Measurement	Male			Female		
	PC1	PC2	PC3	PC1	PC2	PC3
Midline carapace length	0.34	0.11	−0.23	0.36	−0.27	0.14
Width at third marginal	0.36	−0.29	0.09	0.32	0.25	−0.09
Width at fourth marginal	0.37	−0.31	0.09	0.35	0.33	−0.02
Width at seventh and eighth marginals	0.39	−0.38	0.05	0.36	0.42	0.03
Plastron length gulars to anals	0.36	0.36	−0.68	0.40	−0.31	0.35
Plastron length gular to anal notch	0.32	0.56	0.67	0.39	−0.43	0.17
Greatest width	0.41	−0.43	0.07	0.36	0.48	0.04
Height at third vertebral	0.26	0.12	−0.02	0.28	−0.24	−0.90
Percent of variance	84.2	9.1	3.1	81.6	9.6	3.8

APPENDIX 3. SAMPLE SIZES, ALLELE FREQUENCIES AT POLYMORPHIC LOCI, AND HETEROZYGOSITY (H) ESTIMATES FOR 15 SAMPLES OF DESERT TORTOISE FROM NORTHEASTERN MOJAVE DESERT.

Sample site[a] / Sample size		PV 34	SW 15	NE 13	NW 12	MM 16	CH 15	AD 11	PH 16	GS 18	EL 15	RL 16	CS 19	SH 7	JL 15	UT 14
Locus	Allele															
GPI	B	0.000	0.000	0.000	0.000	0.000	0.000	0.455	0.250	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	C	0.853	0.500	0.833	0.792	0.781	0.700	0.545	0.750	0.444	0.467	0.594	0.921	0.571	0.567	0.929
	D	0.147	0.500	0.167	0.208	0.219	0.300	0.000	0.000	0.556	0.533	0.406	0.079	0.429	0.433	0.071
IDH	C	0.984	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	D	0.016	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
sMDH	C	0.956	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	D	0.044	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
PGM	B	0.000	0.000	0.000	0.000	0.111	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	C	1.000	1.000	1.000	1.000	0.889	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	H	0.024	0.040	0.022	0.028	0.044	0.031	0.024	0.017	0.044	0.018	0.029	0.011	0.057	0.022	0.010

[a] Sample sites: PV = Piute Valley, SW = Las Vegas Valley SW, NE = Las Vegas Valley NE, NW = Las Vegas Valley NW, MM = Upper Mormon Mesa, CH = Charleston West, AD = Amargosa Desert, PH = Pahrump Valley, GS = Goodsprings, EL = Eldorado, RL = Roach Lake, CS = Coyote Spring, SH = Sand Hollow, JL = Jean Dry Lake, UT = Beaver Dam Slope, UT.

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