Final Report: Movements and Barriers to Movement of the Desert Tortoise (*Gopherus agassizii*).

Project Number: 2005-UNR-576-P

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EXECUTIVE SUMMARY

The Mojave desert tortoise is listed as a threatened species under the U.S. Endangered Species Act. It has a large geographic range, long generation time, and a cryptic life style. We used hyper-variable microsatellite markers to identify population structure, movements, and biological boundaries for subpopulations of the Mojave desert tortoise. Despite discovering low levels of population differentiation across the range, we were able to detect hierarchical structuring of the population using Bayesian assignment tests. Three basal groups were identified that correspond to the mitochondrial DNA haplotypes identified two decades previously. Additional structure was evident within each basal unit, and they were loosely concordant with major geographic barriers. Our conclusions about these three basal groups, and the substructure within those groups, required sampling tortoises from the entire geographic range, and sampling tortoises uniformly across the entire range in order to adhere to restrictions imposed individual based population genetic analyses. Another recent study differently concluded more fine-scale structure of desert tortoise subpopulations, but the investigators did not sample all parts of the species range, nor did they sample uniformly. Thus, their conclusions of greater genetic structure are based upon sampling for traditional population genetic analyses. Our conclusions about population structure translate into new recommendations for altering the boundaries for recovery units for the Mojave desert tortoise. That translation indicates that approximately the same number of recovery units as prescribed in the Recovery Plan of 1994, but with very different boundaries, especially in Nevada.

INTRODUCTION

Understanding population structure within a species, and understanding the microevolutionary processes shaping those patterns are important for basic (e.g. understanding population dynamics) and applied ecology (e.g. delineating conservation units). Indeed, designating boundaries is critical to the DCP and called for in the MSHCP. Natural populations devolve from a complicated mixture of historical and present demographic processes, which result in a cumulative genetic signature. These processes are intimately tied to geographic, landscape, and habitat features. Because of these processes, the underlying genetic population structure can provide a summation of how individuals move within the landscape, if those movements result in dispersal and establishment in a new area (i.e., gene flow among the populations occurs). Additionally, genetic population structure can provide an indirect assessment of physical, ecological, and biological factors influencing movement (Manel et al. 2003, Storfer et al. 2007, Waples and Gaggiotti 2006). A rich literature in population genetic theory indicates that gene flow can be a powerful force in evolution, constantly reducing genetic differentiation (though potentially enhancing polymorphism) in the face of mutation, selection, and genetic drift (Hartl and Clark 1997, Lowe et al. 2004). Ultimately, patterns of gene flow dictate the level of differentiation a population or group of populations exhibits.

Delineating population boundaries can be complex for at least two reasons: natural borders can be unapparent, and some physical borders may not correspond with biological

differentiation among populations. In many cases, population structure can be cryptic because a species' range is large and continuous without definitive geographic boundaries or concomitant genetic differentiation. The number of species exhibiting this phenomenon is increasing in the published literature, particularly within highly mobile carnivores (e.g. McRae et al. 2005, Pilot et al. 2006, Rueness et al. 2003). Geographic distance often explains a portion of the genetic variation among populations, especially in the absence of known barriers (Slatkin 1993). In these cases, levels of genetic differentiation are inversely related to the geographic proximity of populations (Wright 1943, Slatkin 1993). The influence of isolation-by-distance is mainly dependent on the dispersal ability of a species (Epperson 2003). Moreover, geographic, ecological, and behavioral barriers can influence gene flow beyond the simple process of "isolation-by–distance" (Lowe et al. 2004, Storfer et al. 2007). Typically, these unclear barriers complicate delineating population boundaries.

Relatively new analyses for populations using genetic data (e.g. Bayesian assignment tests; Pritchard et al. 2000, Manel et al. 2005) allow delineating genetic populations. Some analyses fit a model of genetic data based upon assumptions about patterns of genetic markers in populations mating at random (i.e. at Hardy-Weinberg equilibrium, Pritchard et al. 2000). Actual populations are neither truly panmictic nor completely isolated in most cases, and maintain varying levels of gene flow (Waples and Gaggiotti 2006). Most models can be used to test hypotheses of departure from panmixia among groups (Waples and Gaggiotti 2006, Palsboll et al. 2006). However, as levels of genetic connectivity increase (towards panmixia), any boundaries among putative subpopulations become less distinct. The blurring of boundaries among populations is most pronounced when the main barrier to gene flow, and genetic differentiation among subpopulations, is geographic distance (Waples and Gaggiotti 2006). Bayesian methods to assign individuals to subpopulations are not ideal for analysis of subpopulations that are largely isolated by distance rather than by relatively impermeable geographic barriers. Bayesian methods tend to be more helpful in analysis of populations that have distinct boundaries to gene flow and large concomitant genetic differentiation (Evanno et al. 2005, Pritchard et al. 2000, Pritchard et al. 2007, Waples and Gaggiotti 2006). Nevertheless, Bayesian methods can distinguish among populations with dispersal patterns differing from Wright's island model, and with varying amounts of gene flow (Evanno et al. 2005, Rowe and Beebee 2007, Waples and Gaggiotti 2006, Latch et al. 2007). Also, models that incorporate spatial configuration of populations have the potential to improve the capacity of analyses to distinguish among subpopulations with varying levels of differentiation (Coulon et al. 2006, Dunaloup et al. 2002, Guillot et al. 2005a). Due to potential inability of these methods to uncover biological processes of gene flow, multiple analytical methods should be combined to infer the biology of gene flow (Rowe and Beebee 2007).

In the research reported here, we address complexities in identifying population boundaries, and the associated conservation implications, in the widely-distributed, threatened Mojave desert tortoise (*Gopherus agassizii*). The desert tortoise is distributed in the deserts of the southwestern United States and northwestern Mexico. *G. agassizii* occupies both the Sonoran and Sinaloan Deserts, south and east of the Colorado River, and the Mojave and Colorado Deserts, north and east of the Colorado River (Germano 1994); however, only the Mojave "distinct population segment" (DPS) is listed as threatened under the U.S. Endangered Species Act of 1973. Within the range of the Mojave desert tortoise, habitat is extremely diverse, but relatively continuous from southwestern Utah to southwestern California. Pronounced population declines have been associated with several threats to population persistence, mainly attributed to

increased human impacts within and on the Mojave Desert (Lovich and Bainbridge 1999, Edwards et al. 2004, Tracy et al. 2004, USFWS 1994).

As a long-lived species, with a long generation time (10-25 yrs), and cryptic lifestyle, the desert tortoise is an ideal candidate to infer population processes from genetic data. Relatively little is known about population dynamics or dispersal patterns for this species, which can be partially attributed to its sparse population densities and cryptic morphology and behavior. Desert tortoises, particularly hatchlings and juveniles, spend the majority of their time in retreats below ground (Hillard 1996, Morafka 1994, Nagy and Medica 1986, Tracy et al. 2004, Wilson and Morafka 1999). These characteristics make it difficult to collect adequate data, and population research on such a high-longevity species generally requires long-term studies, which are logistically complicated (USFWS 1994). At the very least, population genetic data can provide some insight into population dynamics not generally possible from field studies.

Inferring population boundaries and translating those inferences into conservation planning can profoundly influence how management is implemented for such a species. The desert tortoise has a wide distribution, and its natural geographic distribution largely differs from political boundaries. The Mojave desert tortoise's range traverses four states (Utah, Arizona, Nevada, and California), and that range is currently divided into six recovery units (a management unit associated with a species' recovery plan; USFWS 1994). Preserving genetic and ecological diversity among populations continues to be a primary objective in desert tortoise conservation. The original recovery units reflected the best available scientific data at the time of listing (USFWS 1994), and they were delineated to preserve considerable variation in morphology (Weinstein and Berry 1987), ecology (Germano et al. 1994), and genetics (Lamb et al. 1989, Lamb and Lydehard 1994, Rainboth et al. 1989). However, new data offer the opportunity to evaluate and revise those recovery units.

The main objective of our research is to evaluate the population structure of the Mojave desert tortoise, using hyper-variable microsatellite genetic markers. We hypothesized that the Mojave desert tortoise would exhibit genetic differentiation following a pattern of isolation-by-distance, which is occasionally interrupted by natural geographic barriers, such as large mountain ranges. Additionally, our goal was to evaluate the original recovery units, make recommendations for potential revisions of their boundaries, and to compare our recommendations to those outlined in another recent study for this species (Murphy et al. 2007). We compared and contrasted individual-based methods for identifying genetic populations, which do not a priori require subjective groupings. Specifically, we inferred population structure in the Mojave desert tortoise using Program STRUCTURE (Pritchard et al. 2000) and GENELAND (Guillot et al. 2005). Additionally, we addressed the impacts and importance of sampling design for population genetic studies, and how sampling schemes can limit inferences made from these genetic markers.

MATERIALS AND METHODS

STUDY AREA

The Mojave desert tortoise is distributed within the Mojave and Colorado Deserts in California, southern Nevada, the southwest corner of Utah, and the northwest corner of Arizona (Fig 1). The Mojave and Colorado deserts (> 115,000 km^2) are heterogeneous in climate, geology, and topography (Berry et al. 2006), and vegetational associations (Rowlands et al. 1982). The range of geography and physiognomy of the desert tortoise distribution includes the lower reaches of the Colorado Plateau in Utah to physiographic Great Basin in Southern Nevada and California.

Each physiographic area has distinctive landforms and geological structure. A majority of the tortoise's distribution is encompassed by the larger Basin and Range Province (Hunt 1974, Trimble 1989). Although plains and alluvial fans cover 65% of the Mojave Desert, imposing mountain ranges, such as the Spring Mountains (3652 m) and the Providence Mountains (2148 m), provide commanding relief (Rowlands et al. 1982). Variation in elevation, slope, and soil type may be extremely important for habitat selection of this species (Andersen et al. 2000).

Abundance and seasonality of precipitation within the Mojave Desert is highly variable within and among years, but there is a consistent pattern of variation along a west-east gradient (Rowlands et al. 1982). Winter precipitation dominates in the Western Mojave, with greater than 75% of precipitation occurring between November and March, and less than 10% of precipitation occurring during the summer months of June - August (Germano et al. 1994). The percentage of summer and fall rainfall increases dramatically in the Eastern Mojave Desert (Germano et al. 1994). The phenology of annual vegetation, and the composition of the grass and forb flora is related to these differences. The majority of annual plants in the Western Mojave germinate during Fall and Winter months. Rainfall becomes more predictable in the southern portion of the Colorado Desert, which receives monsoonal precipitation typical of the Sonoran Desert (Burk 1977). Temperatures vary along a north-south gradient with the number of days below freezing, varying with both latitude and elevation. The number of freezing days decreases along a transect from southwestern Utah to the southern tip of the Colorado Desert in California (USFWS 1994).

Five major biotic regions occur in the Mojave Desert (Rowlands et al. 1982), and three regions occur in the Colorado Desert (USFWS 1994; Rowlands unpublished data). Vegetation is different in the Mojave and the Colorado Deserts. While many plant species overlap between these two deserts, the Colorado Desert contains some arboreal species that are sensitive to freezing (Burk 1977, Lovich and Bainbridge 1999). In many regions of the Mojave, creosote bush scrub, which is largely dominated by *Larrea tridentata* and *Ambrosia dumosa* covers up to 70% of the landscape (Germano et al. 1994, Rowlands et al. 1982, USFWS 1994). This association occurs below 1500 m on alluvial fans and bajadas. On the upper slopes, a succulent scrub association dominated by stem succulent species, including *Cactaceae* and *Yucca,* can be common (USFWS 1994). Different combinations of plant associations occur in each desert region and some unique plant communities occur in localized areas. For example, the Mojave saltbush – Allscale scrub community (dominated by *Atriplex spinifera* and *A. polycarpa)* only occurs in the Western Mojave Desert near Fremont Peak and Kramer Junction, CA (Rowlands et al. 1982, USFWS 1994). The Northern Mojave Desert is a transitional vegetation zone with a combination of plants common to the Mojave Desert and the Great Basin Desert (Rowlands et a. 1982). The Colorado Desert contains a unique combination of Sonoran Desert and Mojave Desert flora (Burk 1997).

Despite the variation in types of available habitat, desert tortoises are known to occur broadly throughout their range. Desert tortoises have been observed from below sea level to 2,225 m (Luckenbach 1982), though most tortoise observations are documented between 300m and 900m. In the Mojave and Colorado Deserts, desert tortoises most commonly occur in areas with gentle slopes, sufficient shrub cover, and friable soils to allow burrow construction (Bury et al. 1994, Andersen et al. 2000, USFWS 2007). Although desert tortoise habitat could be considered fairly contiguous in the Mojave and Colorado Deserts, the existence of large mountain ranges, such as the New York and Providence Mountains in California and the Spring Mountains in Nevada, low elevation playas, and a variety of other physical features, are

potentially formidable barriers to gene flow. Human-causes of habitat fragmentation, such as urban development and interstate freeways, are now widespread (Hunter et al. 2003, Lovich and Bainbridge 1999) and their role in changing population dynamics of this species will be addressed with more detail in the discussion.

SAMPLING DESIGN

Our study was designed to sample putative intra- and inter-population genetic variability. However, boundaries to putative desert tortoise populations are not apparent, and sampling design is critically important as a means to identify population boundaries successfully. To study genetic diversity, gene flow, and population structure, it is important to collect samples from the entire geographic and ecological range of the species (Lowe, Harris, and Ashton 2004). For analyses, we assumed that there is no gene flow between the Mojave populations of the desert tortoise and Sonoran populations east and south of the Colorado River. These two populations apparently have been separated for 5.5 million years based upon analyses of mitochondrial DNA (Edwards et al. 2004, Lamb et al. 1986, Lamb and McLuckie 2002).

Sampling design differed based on land ownership and density of tortoises; however, efforts were made to sample evenly from all putative populations and to collect at least 30 samples from each geographic location where possible. Approximately half of DNA samples (46.8 %) used in this study were collected along randomly-placed transects during routine population monitoring conducted by the U.S. Fish and Wildlife Service (USFWS 2006). A small percentage of individuals (1.5%) were sampled opportunistically while technicians were en route to a transect. The remaining samples (51.7%) were collected from efforts not associated with population monitoring between 2004 and 2006. Some of these samples were collected along random transects within the Piute and Eldorado Valleys and from animals tracked with radio transmitters in those valleys, and other samples were collected from transects (4-12km) placed systematically to cover poorly sampled areas of the range. Many of these sampling transects were located outside of desert tortoise critical habitat to determine more effectively the locations of genetic boundaries for populations, and to sample any populations not located within currently-delineated critical habitat. Transect sampling was employed to minimize the probability of sampling within closely-related groups of tortoises (demes).

Overall, whole blood was collected from 748 desert tortoises throughout the Mojave and Colorado Deserts between 2004 and 2006 (Table 1, Fig. 1). These samples were grouped subjectively into 25 sampling locations that were considered to be a specific geographic area, often constituting one or two valleys, and reflecting geography and political boundaries (Table 1 provides a brief description of locations).

Sample collection, DNA isolation, and genotyping

Whole blood from 748 desert tortoises was dried onto dots of filter paper, and stored until DNA could be isolated from the samples. Total genomic DNA was extracted from up to three filter-paper dots using a dried blood protocol for QIAGEN DNeasy kits (Qiagen 2001). DNA was eluted in a TE buffer, quantified using a Labsystems Fluoroskan Ascent fluorometer, and diluted to concentrations between 5-10 ng/μl for amplification with microsatellite loci.

DNA was amplified using the polymerase chain reaction (PCR) and genotyped with 20 microsatellite markers. Six microsatellite primer sets (GP15, GP30, GP61, GOAG3, GOAG4, GOAG7) were obtained from previous studies of *Gopherus polyphemus* (Schwartz et al. 2003) and the Sonoran population of *Gopherus agassizii* (Edwards et al. 2003). An enriched

microsatellite library, developed by Genetic Identification Services, was used to identify 14 additional microsatellite primers sets (Hagerty et al. 2008). All microsatellite loci were amplified in six multiplex PCRs and two individual PCRs. All multiplex reactions contained ratios of primer concentrations that were determined by trial and error. Multiplex PCRs contained 7.5 μl Multiplex MM (QIAGEN), 3 μl PCR water, 1.5 μl primer cocktail (2μM) and 4 μl genomic DNA for a total PCR reaction volume of 16μl. Multiplex 1 (T_a = 57°C) contained primers GOAG7 and GOAG3. All multiplex PCR cycling was performed using a MBS Satellite 0.2G thermal cycler with the following profile: 1 cycle of 94°C for 15 min, 33 cycles of 94°C for 30s, appropriate annealing temperature for 90s, 72°C for 30s, and 1 cycle of 62°C for 30min. Multiplex 2 (T_a = 55°C) contained primers GP61, GP30, and GP15. PCR conditions were identical to Multiplex 1. The remaining multiplex reactions for GOA1, GOA2, GOA3, GOA4, GOA6, GOA8, GOA9, GOA11, GOA12, GOA13, GOA14, GOA22, and GOA23 were completed as described in Hagerty et al. (2008). GOAG4 and GOA17 were amplified as single PCRs. The 15-μl reactions contained 9.6 μl PCR water, 1.5 μl 10x Titanium taq PCR buffer (pH 8.0, 3.5mM $MgCl_2$) (CLONTECH Laboratories, Inc.), 0.3 primer (10μM), 0.2 μl titanium taq (QIAGEN), 0.4 dNTPs, and 4 μl genomic DNA. Cycling conditions were 1 cycle of 94°C for 1min, 33 cycles of 94°C for 30s, 61°C (GOA17) or 55°C (GOAG4) for 30s (annealing), 72°C for 30s, and 1 cycle of 72°C for 30min. All amplified microsatellite segments underwent a multi-color fluorescence-based DNA fragment size analysis in five separate panels using a fully automated ABI 3730 DNA sequencer. We amplified microsatellites and completed fragment analysis in collaboration with the Nevada Genomics Center (http://www.ag.unr.edu/Genomics/). All alleles were scored with GeneMapper 5.0 (Applied Biosystems).

Descriptive Population Genetic Analyses

Descriptive statistics, including observed heterozygosity and expected heterozygosity and number of alleles per locus, were calculated using GENEPOP (Raymond and Rousset 1995). Tests for linkage disequilibrium for each pair of loci, and deviation from Hardy-Weinberg equilibrium, were performed in FSTAT (version 2.9.3.2, February 2002; Goudet 2001). All loci that were significantly linked to another locus consistently across all sampling locations were removed from subsequent analyses. We also performed a test for null alleles in MICROCHECKER (version 2.2.3; van Oosterhout et al. 2004). If the combined probability of expected heterozygote classes ($P<0.05$) was significant consistently across sampling locations, we removed the locus from analyses. An estimate of F_{IS} was calculated for each locus and across loci for each sampling location to test for significant heterozygote deficits, which would indicate a deviation from Hardy-Weinberg equilibrium. We tested for statistical significance using $\alpha = 0.05$, and we controlled for multiple testing using the Bonferroni correction (Rice 1989).

Identifying genetic clusters

We investigated the genetic population structure of the desert tortoise in the Mojave and Colorado Deserts using two Bayesian clustering models. Program STRUCTURE (version 2.1; Pritchard et al. 2000, Falush et al. 2003, Pritchard et al. 2007) was used to infer the number of genetic clusters without a priori knowledge about putative population clusters. Program GENELAND (Guillot et al. 2005b) has similar assumptions and uses a similar resampling algorithm, but also incorporates spatial data for each individual into the analysis.

STRUCTURE Procedures and Parameters:

STRUCTURE is a modeling algorithm that finds the best probable fit of assigning individuals to a specified number of genetic clusters (K) based upon the individuals' genotypes. The most likely number of clusters is inferred as that which maximizes the probabilities associated with allele frequencies conforming to linkage equilibrium and Hardy-Weinberg equilibrium (i.e., characterizing a randomly mating population). The variables of the model (genetic cluster of origin, and allele frequencies of each cluster) are estimated using a Markov Chain Monte Carlo (MCMC) re-sampling algorithm over a range of possible clusters (K) (Appendix 2). We used an admixture model, which allows for multiple origins of individuals, with correlated gene frequencies (Falush et al. 2003), and we did not provide any prior information on population origin. We performed 10 independent simulations for each K, from $K = 1$ to $K = 10$. We ran initial simulations which suggested that $K > 10$ were unlikely. The burn-in for the final simulations was 750,000 iterations and the number of MCMC iterations was 750,000 for 10 independent simulations per K.

For each value of K, an estimate of the posterior probability of the model fit, $Pr (X \mid K)$, was used and the best fit (Appendix 2) was determined from the "estimated natural log of the probability of data" or $Ln\ P(D)$. We calculated the mean $Ln\ P(D)$ and standard deviation around the estimate from the 10 iterations per K. Inferring the most probable number of genetic clusters, $Pr (X \mid K)$, is not straightforward, and can only be approximated using *ad hoc* procedures (Pritchard et al. 2000, Pritchard et al. 2007). The most probable number of clusters is taken to be the value at which the estimate of $ln\ Pr(X \mid K)$ (or $ln\ P(D)$) is highest, and the value of K that maximizes consistency among the parameter of individual admixture (α) (Pritchard et al. 2007). Thus, the smallest value of K explaining the structure in the data well is taken to be the most parsimonious solution. The second-order rate of change in the posterior probability (ΔK) has been advocated as a more reliable statistic to determine the appropriate K (Evanno et al. 2005). This more formal criterion uses the largest change in the slope of the distribution of $ln\ P(D)$ as an indication of the most likely K (Appendix 2). We compared the results of each of these ad hoc criteria, in conjunction with other basic diagnostics such as the value of the admixture parameter (α) and the pattern of assignment to clusters, to estimate the true number of genetic clusters (Evanno et al. 2005, Pritchard et al. 2007). In all cases, additional information about the biology of the species, and consistency among runs, is necessary to make inferences.

Simulations of populations with more complicated structural organization than an island model indicate that the ΔK statistic often identifies the uppermost level of structuring among putative populations (Evanno et al. 2005). We used additional STRUCTURE simulations with similar parameter values to detect any potential sub-structuring within the clusters identified by the initial model simulations. Individuals assigned to each of the inferred clusters were separated into K data sets, and those models were simulated in STRUCTURE to determine the likelihood of additional hierarchical clustering (Evanno et al. 2005, Rowe and Beebee 2007, Pritchard et al. 2007). We continued to analyze subsequent, putative clusters until the model did not support additional subdivision.

Unequal sample sizes of individuals across the entire distribution of sample effort may lead inaccurately to inferring more clusters than those actually occurring. Spuriously inferred clusters also could result due to the inability of STRUCTURE to delineate populations largely isolated by distance rather than discrete barriers to gene flow (McRae et al. 2005, Pritchard et al. 2007). To account for potential bias in the number of populations inferred from STRUCTURE caused by unequal sampling among locations, we reduced the number of genotypes in locations

that had more than 30 sampled individuals. We randomly selected 30 individuals from each location and used the reduced number of genotypes to infer the number of genetic clusters. Locations with less than 30 individuals remained unchanged in the analyses. Individual genotypes were re-sampled with replacement to produce ten replications of the analysis. Procedures as described above were completed for each replicate and compared for consistency. Hierarchical analyses were also completed with reduced data sets.

GENELAND Procedures and Parameters:

Program GENELAND implements a Bayesian clustering algorithm similar to STRUCTURE, and also uses an MCMC re-sampling method to estimate unknown parameters including the number of genetic clusters. However, GENELAND additionally incorporates spatial data (geo-referenced coordinates) for each individual (Guillot et al. 2005b). GENELAND uses an hierarchical strategy, inferring genetic structure of populations based upon the spatial organization of the populations. Thus, an additional assumption in this model is that populations are spatially organized as a set of non-overlapping polygons with no gaps through the colored Poisson-Voronoi tessellation (Guillot et al. 2005a, 2005b). One key difference between STRUCTURE and GENELAND is that the number of clusters must be inferred using ad hoc approximations in the former, but the number of genetic clusters is treated as a parameter and processed in GENELAND (Guillot et al. 2005a).

Four individuals were removed from GENELAND analyses because we did not have reliable spatial coordinates for them. GENELAND simulations were performed with the GENELAND GUI in the R-PACKAGE. In our simulations, we used spatial (spatial = TRUE) and genetic data (Dirichelet model of allele frequencies) as *a priori* information. We included uncertainty (1 km) into the spatial coordinates for each individual to account for any measurement error, movement of individuals, and the potential for observed locations to reflect the true locations inaccurately (Guillot et al 2005a, Coulon et al. 2006). The first set of MCMC chains was used to determine the modal number of inferred populations (as suggested in Guillot et al. 2005a). The MCMC algorithm was repeated 10 times (allowing K to vary among simulations) using the following parameters: (1) minimum number of populations was 1, (2) initial number of populations was 2, (3) maximum number of populations was 15, (4) 500,000 MCMC iterations, (5) 10 thinning (saving only 1 iteration per 10), (6) maximum number of nuclei in the Poisson-Voronoi tessellation was 300 (default), and (7) maximum rate of Poisson process was 100 (default). After the modal number of populations (K) was estimated from the initial 10 simulations, the previously inferred value of K was used as the initial and maximum number of populations in five additional runs with the same model parameters. Mean assignment probabilities were calculated for each individual from the five runs. During post-processing, we used 200 pixels along the X-axis and Y-axis and a burn-in of 1,000 MCMC cycles. The model identified the modal population of each individual and the probability of assignment of each individual to the modal population. Hierarchical clustering was evaluated using this model with the same method described for STRUCTURE.

Statistics for inferred populations:

First, we determined population differentiation among all sampling locations. Then, we estimated population differentiation among the populations from the two Bayesian techniques. If the two techniques provided different population boundaries, those boundaries were compared. Pair-wise F_{ST} values (Weir and Cockerham 1984) were calculated for all combinations of

inferred populations and sampling locations in program FSTAT 2.9 (Goudet 2001). We tested for pair-wise genetic differences among clusters and sampling locations (not assuming Hardy-Weinberg equilibrium) using a permutation test that randomized genotypes across populations and created new data sets that are evaluated with the log-likelihood statistic *G* (Goudet et al. 1996). Statistical significance ($\alpha = 0.05$) was evaluated after the Bonferonni correction for multiple comparisons (Rice 1989). An analysis of molecular variance (AMOVA) (Excoffier et al. 1992) was also conducted to test the significance of the inferred population structure, which was implemented in Arlequin 2.0 (Schneider et al. 2000). Finally, a frequency-based assignment approach, implemented in DOH, was used to evaluate the hypothesis of genetic clusters provided by the Bayesian approaches (Paetkau et al. 1995).

Mantel tests were used to test for correlation between genetic distance and geographic distance matrices using the web-based package, Isolation by Distance, web service (IBDWS, Jensen et al. 2005). We tested for isolation by distance among sampling locations using pair-wise geographic distances and $F_{ST} /(1- F_{ST})$ (Rousset 1997). The geographic distance matrix was developed for all sampling locations using ArcGIS 9.0 (ESRI). Straight-line distances between the centroids of sampling locations were used as an estimate of geographic distance between locations.

RESULTS AND EVIDENCE OF THE RESULTS

DESCRIPTIVE POPULATION GENETIC ANALYSES

Average heterozygosity (gene diversity) of the Mojave desert tortoise ranged from 0.643 in Gold Butte to 0.799 in Southeast Las Vegas Valley ($\bar{X} = 0.742 \pm 0.040$) (Table 2). These high values of gene diversity and allelic richness ($\bar{X} = 8.352 \pm 3.354$) provided sufficient diversity to investigate genetic structure within the sampling area.

No pair of microsatellites exhibited significant linkage disequilibrium among locations or in any particular group after the Bonferroni correction ($P < 0.000011$ after 95,000 permutations). Although some pairs of loci were statistically linked prior to the Bonferonni correction (i.e. $P < 0.05$), these pairs were not statistically linked in multiple sampling locations. If a pair of loci was in a state of linkage disequilibrium, that pair should be linked in several populations. The pairs of microsatellites were not linked statistically in multiple locations, reducing the chance that the loci violated the assumption of linkage equilibrium. Therefore, we did not remove any microsatellites due to a violation of this critical assumption.

Six of the 25 sampling locations (GB, MD, EL, PI, CM, EP; see Table 1 for abbreviations) had significant F_{IS} values after the Bonferroni correction ($P < 0.0001$) (Table 2), indicating that these sampling locations are not in Hardy-Weinberg equilibrium. However, these significant values were influenced by two loci (GOA 6 and GP 61) (Supplement 1). If these two loci were influencing the F_{IS} values due to problems with amplification, they would cause high F_{IS} values in several, if not all of the sampling locations. It was likely that each sampling location did not represent a discrete randomly mating population, which would create conditions outside of Hardy-Weinberg Equilibrium. The test for the presence of null alleles complemented the F_{IS} values. GOA6, GOA9, GOA12, and GP61 had multiple populations (between 6-9 populations) with a significant combined probability of expected heterozygote classes ($P < 0.05$). However, this evidence for the presence of null alleles did not occur consistently across all tested populations, and we chose to retain those loci for the subsequent analyses.

IDENTIFYING GENETIC CLUSTERS

Bayesian clustering without spatial information (STRUCTURE):

Using Program STRUCTURE with a sample size of 748 tortoises, *ln P(D)* across 10 independent runs reached a plateau after $K = 9$ (Table 3). This plateau indicated that nine clusters were more parsimonious than ten despite a slight increase in the posterior probability. The proportion of admixture (α) also was lowest and reached a plateau at $K = 9$ (Table 3). However, the largest increase in the likelihood that a model was effective occurred between $K = 1$ and $K = 2$. ΔK, which measures the second order rate of change between K and K-*1*, also peaked when K = 2 (Table 3; Fig. 2a). Several independent runs of STRUCTURE for $K = 1$ never converged, thus, it may be inappropriate to compare STRUCTURE results for $K = 1$ with results using other values of K. When we removed $K = 1$ from analysis to find the best fit to the data, $K = 3$ became the most probable configuration because the probability of fit became much higher between $K = 2$ and $K = 3$, and because the amount of admixture jumped to much lower values between $K = 2$ and $K = 3$. The ΔK for $K = 3$ was at least two times higher than ΔK for subsequent values of K (Table 3; Fig. 2a). A large reduction in the admixture parameter (α) also occurred between $K = 2$ and $K = 3$ (Table 3).

Multimodality in the model fit prohibited clear interpretation of our data set. At least two local maxima were reached within different independent MCMC simulations for each K value (not shown). Different local maxima for independent simulations occurred when $K \geq 4$. Multimodality did not occur when K was less than 4 or when $K = 9$, which was also the model with the highest mean *ln P(D)*.

We chose $K = 3$ as the basal, most parsimonious number of genetic clusters because of the high ΔK and because of the occurrence of multimodality for runs when K was greater than three (Fig. 2a). This level of clustering was interpreted to represent the uppermost level of clustering across the landscape. Proportional membership for each sampling location to one of the three clusters was high and ranged from 62 to 97%. Cluster 1 (Northern Mojave or NM) encompassed seven sampling locations in Utah and Nevada (RC, BD, MM, GB, MD, CS, NEL; Fig 2b). The transition between cluster 1 and cluster 2 occurred gradually across several mountain ranges such as the Arrow Canyon Range, which extend North to South and are potential partial barriers among locations north and east of Mormon Mesa to areas south and west of Mormon Mesa. Cluster 2 (Las Vegas or LV) encompassed 9 sampling locations in Nevada and along the Nevada/California border (NWL, AM, PA, SH, IV, SI, SWL, SEL, EL; Fig 2b). A transition zone between cluster 2 and cluster 3 was apparent across Searchlight Pass, a connection point for the Eldorado, Newberry, and Highland ranges. This low pass (1500 m) separates Eldorado and Piute Valleys near the Nevada/California border. Cluster 3 (California or CA) contained individuals from the remaining nine sampling locations in California and in Piute Valley, Nevada (PI, CM, EP, WP, CK, PM, OR, SC, FK; Fig 2b).

We examined the potential for hierarchical sub-structuring within each of the three basal clusters. Hierarchical sub-structuring could explain the ostensible discrepancy between ΔK and the peak mean *ln P(D)*. Each cluster had an additional level of structuring. Cluster 1 (NM) was divided further into two clusters (Table 4). Cluster 2 (LV) was divided further into three clusters (Table 4). Cluster 3 (CA) was divided further into four clusters (Table 4). The additional clusters that were identified in the hierarchical analyses aligned exactly with the clusters identified by the model when K = 9 (Fig. 3), providing evidence that some additional level of structure does exist within the Mojave Desert populations of the desert tortoise. Proportional membership of sampling locations to each of the nine clusters from the complete analysis was variable (Fig. 3).

Although, several locations were clearly assigned to a particular cluster (e.g. proportional membership of Mormon Mesa to cluster 1 was 88%), others were split among clusters (e.g. proportional membership of Ivanpah Valley to each cluster was < 30%; Fig 3). When K=2 was chosen as the most basal number of clusters and used to investigate sub-structuring, the resulting clusters were identical (not shown). Additional sub-structuring was not present in any of the nine genetic clusters when they were analyzed separately (not shown).

Finally, we investigated the potential for unequal sampling effort to influence model choice for the number of genetic clusters. When sampling effort was more evenly distributed among locations (with n ≤ 30), *ln P(D)* peaked when K = 6. The reduction in the number of genetic clusters when K = 6 resulted in no subdivision of the "California" cluster (Fig. 4). Using all of our data, the "California" cluster was split into additional clusters. ΔK with the reduced dataset was also K = 3, and these three basal clusters were identical to those identified with the full data set. With the reduced data set, the resulting number of individuals assigned to each of these three basal clusters was similar ($N_1 = 165$, $N_2 = 212$, $N_3 = 208$). The reduction in sample size also lowered the total number of genetic clusters identified in hierarchical clustering analyses to seven. However, these differences did not align with results of the full model using the reduced dataset where *ln P(D)* was highest at $K = 6$, as they did in the previous analyses (i.e. clusters identified using a hierarchical analysis with the complete data set were identical clustering when $K = 9$, but hierarchical results were not the same as best fit of the full model with the reduced dataset). The sub-structuring for cluster 1 (NM) remained unchanged (two clusters). However, cluster 2 (LV) was only subdivided into two clusters, which removed the Eldorado Valley cluster (Fig 5a). Cluster 3 (CA) was subdivided into three clusters, which removed the Piute Valley cluster (Fig 5b).

Bayesian clustering with spatial information (GENELAND):

Spatial information was included as *a priori* information to infer population boundaries using Program GENELAND. K=4 consistently resulted as the modal number of genetic clusters, though K=3 was chosen twice out of 10 simulations (Table 5). Two of the four genetic clusters were similar to those resulting from analyses using STRUCTURE when K = 3. The Northern Mojave cluster and the Las Vegas Cluster were delineated with similar boundaries to those identified without the spatial information in the STRUCTURE analyses (Fig. 6). The California cluster, identified by STRUCTURE, was split into two clusters in the GENELAND analyses (Fig. 6). The West Mojave was separated from the remainder of the California sampling locations (Eastern Colorado sites, Northern Colorado sites) (Fig. 6). Assignment to these clusters was consistently greater than 90%. When the model was constrained to K = 3, a majority of independent simulations (4 out of 5) identified the same three clusters identified by STRUCTURE. Hierarchical structuring was not detected in the NM or LV cluster; however, the Eastern and Northern Colorado separated as hierarchical clusters within the CA cluster in subsequent analyses.

Statistics for the inferred populations and sampling locations

The Bayesian clustering methods did not provide consistent, definitive delineations for population structure. Therefore, we tested multiple configurations of genetic clusters using an analysis of molecular variance (AMOVA). We compared K=3, K=7, and K=9 from STRUCTURE and K=4 from GENELAND. In all cases, the amount of variation explained by differences among population was low (< 5%), and most genetic variation was explained by

differences within populations (more than 80%) for all configurations (K=3, K=4, K=7, K=9; Table 6). However, all variance components, including the among-population portion, contributed significantly to the genetic variation among clusters ($P < 0.0001$).

Pair-wise F_{ST} values (Weir and Cockerham 1984) among sampling locations ranged from 0.003 (Chemehuevi - East Providence Mountains) to 0.162 (Beaver Dam Slope– Pinto Mountains) (Supplement 2). Almost all pair-wise comparisons for population differentiation were significant after Bonferroni correction ($P < 0.000167$ after 6000 permutations), except for a few locations that were in close proximity (adjacent locations). Southeast Las Vegas had a small sample size (n=12), which likely affected significance values for several pair-wise comparisons that were not directly adjacent (Supplement 2). Similar to the AMOVA results, F_{ST} values suggest that only a very small amount of genetic variation results from population substructure, except for the most distantly paired locations, which had greater yet still only moderate levels of differentiation. When locations were combined to correspond to the 7 or 9 inferred genetic clusters from STRUCTURE, pair-wise F_{ST} values ranged from 0.012 (Amargosa – South Las Vegas) to 0.132 (Virgin River – Eastern Colorado), and they followed a pattern similar to comparisons among all sampling locations (Table 7). Each pair-wise comparison for genetic differentiation was statistically significant after Bonferroni correction ($P < 0.001389$ after 720 permutations).

Self-assignment of individuals to sampling locations was variable (7.14% - 89.1%). However, the percentage of self-assignment improved dramatically when sampling locations were clustered to resemble the inferred populations (K = 9; 64.7% - 92.4%; Table 8). Additionally, no random assignments occurred in any of the 7 or 9 populations after 10,000 re-sampling events.

Isolation by distance was evident across the range of the Mojave desert tortoise (Fig. 7). Genetic and geographic distances were strongly correlated among the sampling locations ($Z = 4392.398$, $r = 0.824$, $P < 0.0001$).

EVALUATION/DISCUSSION OF RESULTS

IDENTIFYING MEANINGFUL GENETIC CLUSTERS FOR THE MOJAVE DESERT TORTOISE

Conservation and management actions for the Mojave desert tortoise are implemented in a spatial context, using previously delineated conservation units (called Recovery Units; USFWS 1994). Multiple forms of evidence, including previously completed population genetic studies, were used to establish these delineations. Our main goal in the research reported here was manifold. First, we used recent advances in molecular techniques, and analysis of population genetic data, to determine to what extent the Mojave desert tortoise exhibits population structure. Second, we evaluated how closely do putative genetic populations reflect current understanding of the population biology. Finally, we wanted to use new insights into the genetics of desert tortoise to translate biology into justifiable management practices.

The interface among natural populations typically is complicated and involves variable rates of gene flow among demes that display structure at multiple scales (Hanski and Gilpin 1997, Hey and Machado 2003, Manel et al. 2003). Many species do not display clear population structure resulting from present geographic or ecological barriers, or as a result of historical interactions (e.g. Pilot et al. 2006, Spinks and Shaffer 2005, Sponer and Roy 2002). Often these species have large distributions, and they have the ability to disperse over large geographic regions (e.g. caribou, Boulet et al. 2007; cougars, McRae et al. 2005; grey wolves, Pilot et al.

2006; lynx, Rueness et al. 2003). This situation can be contrasted to species with an obvious potential for stark genetic differentiation such as amphibians whose close physiological relationship with water can limit dispersal, especially in desert regions where amphibian habitat is separated by formidably desiccating desert environments (Bradford et al. 2003, Simandle 2006). Recent developments in the analysis of genetic data using assignment tests do not require an a priori definition of what constitutes a population (Beaumont and Ranala 2004, Manel et al. 2003, Manel et al. 2005). These analyses offer the potential for improved understanding of population structure for species lacking definitive boundaries (Pritchard et al. 2000, Falush et al. 2003, Guillot et al. 2005b). This approach is ideal to investigate population structure in the Mojave desert tortoise.

Using two Bayesian clustering methods implemented in STRUCTURE (Pritchard et al. 2000) and GENELAND (Guillot et al. 2005b), we successfully identified spatial structure in the Mojave desert tortoise. The gamut of STRUCTURE results consistently identified three basal desert tortoise populations in the Mojave assemblage, using the ΔK metric (Evanno et al. 2005). Previous analyses of simulated data have demonstrated that STRUCTURE is able to identify the uppermost level of structuring for migration models that are more complex than the traditional island model, which is presumably common for most species (Evanno et al. 2005). Identical cluster boundaries were identified in a majority of the trials when the spatial Bayesian model (GENELAND) was constrained to $K = 3$. However, the addition of spatial context also highlighted the Western Mojave as a separate cluster in the modal number of genetic clusters (four clusters). The differences between these two clustering methods, and how this affects interpretation of inferred genetic clusters, will be discussed below.

Across the range of the Mojave desert tortoise, the three basal clusters follow a north to south gradient (Fig. 1). The Northern Mojave cluster is comprised of sampling locations in the northern part of the range (as far north as St. George, UT) with localized transitional zones between Mormon Mesa and Coyote Springs (through Moapa Valley) and across the Muddy and Virgin Rivers. This area is topographically complex and most likely provides a mosaic of available habitat for tortoises, interspersed among mountain peaks taller than 1000m. Prior to extensive urban development, Las Vegas Valley provided a continuous tract of tortoise habitat with open corridors to the northwest and south. Previously, researchers hypothesized that this area was a transitional corridor between locations to the north and south (Britten et al. 1997). As a result of this historic potential for connectivity, the Las Vegas Cluster is extensive, including locations as far northwest as Oasis Valley in Nevada and southeast to Eldorado Valley, NV. The transitional area between the Las Vegas cluster and the California cluster occurs in two main areas: across Searchlight Pass between the Eldorado and Piute Valleys and across the major montane barriers of the New York and Providence Mountains, which bisects the Mojave National Preserve. Additionally, these montane barriers were clearly identified with GENELAND. The California cluster contains most of the Mojave and Colorado Deserts in California, except for transitional zones occurring near the California and Nevada border. The habitat in this cluster is relatively continuous, despite the occurrence of several different vegetation communities.

The three basal genetic clusters inferred with microsatellite markers (Northern Mojave, Las Vegas, and California) closely resemble the distribution of the three mtDNA haplotypes found previously (Lamb et al. 1989). These haplotypes had very few restriction length differences in comparison to the Sonoran and Sinaoloan haplotypes; less than 0.5% nucleotide differences were found among Mojave haplotypes (Lamb et al. 1989). Recent mtDNA

sequencing also corroborated this low divergence rate among Mojave haplotypes (Edwards 2003, Murphy et al. 2007). Two major mtDNA lineages were identified (Murphy et al. 2007) and these lineages correspond to genetic clustering using microsatellite data in this study. The combination of microsatellite and mtDNA evidence provides support for the existence of population structure at the landscape scale, despite a weak mtDNA signal. Inferences from our study were made without any presuming underlying population structure, and they complement mtDNA results from previous research. Differences in the mutation rate of mtDNA offer a perspective from a deeper evolutionary time scale, suggesting only recent divergence of any Mojave groups (Lamb et al. 1989, Lamb and McLuckie 2002). In contrast, Mojave and Sonoran populations may have diverged approximately 5 million years ago (Edwards et al. 2004, Lamb et al. 1989, Lamb and McLuckie 2002,) and may constitute separate species (Berry et al. 2002, Murphy et al. 2007).

Previous studies using simulated and real data sets successfully identified fine scale structure in complex systems using hierarchical clustering methods (Evanno et al. 2005, Rowe and Beebee 2007). When we separated and reanalyzed the basal clusters (2 or 3) to detect any hierarchical sub-structuring, we identified structure at a finer geographic scale within each cluster. Fine-scale delineations highlighted by hierarchical analyses were also apparent in the number of genetic populations when the complete data set was analyzed. A majority of these delineations were robust to the random removal of individuals used to create an equal sampling effort across the range. However, locations with intense sampling (e.g. Piute Valley) did appear to increase the chance that the models identified additional, potentially spurious clusters. When the sample size was experimentally reduced to assess the importance of sampling design, locations that were deemed to be distinct using the full data set no longer separated as distinct genetic clusters (this was particularly the case in the hierarchical analyses). For example, both Eldorado Valley and Piute Valley were not identified as distinct clusters when the sample size was reduced for those locations. Therefore, we are skeptical that some of the finer delineations of clusters are anything more than artifact of sampling design, and not likely biologically meaningful. Our results highlighted the reported effect of sampling intensity on the STRUCTURE model that was found in a previous study (McRae et al. 2005). We emphasize the importance of sampling design in studies intended to identify population boundaries, as well as careful interpretation of results from Bayesian clustering methods.

Fine scale clustering using these data are relevant to previous hypotheses of the structure of desert tortoise populations in the listed portion of the range. Prior to the original Recovery Plan for the desert tortoise (USFWS 1994), few genetic data were available to distinguish among regions in Nevada and Utah. Despite morphological, ecological, and behavioral differences among tortoises in the current Upper Virgin River recovery unit located entirely in north of the Beaver Dam mountains (USFWS 1994), we found no genetic evidence that this area is distinct from adjacent location along the Beaver Dam Slope (the southern face of the Beaver Dam Mountains), further south in Utah and into Nevada (Mormon Mesa). Previous mtDNA, allozyme, and morphometric data from the original Northeastern Mojave recovery unit (Britten et al. 1997) lead to the hypothesis that additional variation existed in this region and that original conservation units did not reflect this diversity.

Indeed, we detected genetic clusters within this overall region supporting the Britten et al. hypothesis. Four genetic clusters were apparent in the current Northeastern Mojave recovery unit. The Virgin River cluster split and transitioned into a Muddy Mountains cluster. Additionally, the northern portion of Las Vegas Valley (including Coyote Springs Valley) was separated from the

southern portion of Las Vegas Valley (including Eldorado Valley). The Amargosa Desert, Oasis Valley, Pahrump Valley, Greenwater Valley (in Death Valley National Park) and Shadow Valley also formed the distinct Amargosa cluster. These locations were outliers in a previous analysis (Britten et al. 1997); however, sampling locations in California (some of which clustered with this group) were not included in the Britten et al. study. We were able to detect cluster boundaries by sampling randomly and extensively across the range of the Mojave assemblage.

Habitat differences driven by variation in climate (predominantly rainfall) as well as correlated behavioral and life history differences were used previously to distinguish among regions within the California cluster (Henen et al. 1998, Lovich et al. 1999, Peterson 1994, Peterson 1996, Tracy et al. 2004, USFWS 1994, Wallis et al. 1999). We identified three groups within the basal California cluster. The Northern Colorado cluster, which also contains Piute Valley, borders the South Las Vegas Cluster and transitions to that cluster at the Searchlight Pass. The Eastern Colorado cluster is the most southern cluster in the assemblage, and the Baker Sink separates it from the Northern Colorado cluster. The Baker Sink is part of a potential low-elevation barrier extending from Saline Valley in California in the north, then south through Death Valley, Silurian Valley, Baker Sink, and Cadiz Valley. This barrier reflects the formidable effects of the lower elevations and extremely hot climates along this line, which divides the ecological western Mojave Desert, with its quite variable winter-spring precipitation regime, lower elevations, and Mojave River hydrology, from the more eastern Mojave Desert, subject to more predictable winter and summer monsoon precipitation, more variable elevations, and closed basin and Colorado River hydrology (Tracy et al. 2004). The Western Mojave cluster is separated from the Eastern Colorado cluster in the Pinto Mountains, and from the Amargosa cluster in the low elevation area near Death Valley. The Western Mojave cluster was also highlighted as a distinct cluster using spatial data in GENELAND. We found no additional hierarchical clustering within the Western Mojave cluster, which contradicts another recent study using microsatellites to delineate desert tortoise populations (Murphy et al. 2007). We address inconsistencies between these two studies below. In summary, we identified three basal genetic clusters that were further delineated into seven groups at a finer scale (Fig. 1).

Limitations to identifying desert tortoise populations

Despite the inferred presence of broad and fine scale population structure identified by the Bayesian analyses, only low genetic differentiation was detected among most sites using F-statistics. Moderate differentiation occurred only among the most geographically distant sites, and great differentiation occurred nowhere. F_{ST} values provide a summary statistic that describes the result of cumulative gene flow across multiple generations, and these statistics do not allow us to differentiate among different hypotheses for population dynamics (i.e. reflecting historically moderate to high levels of gene flow that no longer occur, or reflecting current gene flow; Neigel 2002, Pearse and Crandall 2004).

To explore past demography, coalescent-based methods are necessary to provide estimates of population parameters (Beerli 1998, Beerli and Felsenstein 1999, 2001, Nordborg 2001, Pearse and Crandall 2004). Estimates of long-term gene flow can be complemented by assignment methods, which can detect recent gene flow and potentially first generation migrants (Manel et al. 2005, Paetkau et al. 2004). The current level of habitat fragmentation, and the isolation of critical habitat, supports an hypothesis of historically high levels of gene flow. In addition, the majority of genetic variation can be explained by differences among individuals within populations (as determined by the AMOVA). Although the amount of genetic variation explained by population

structure is significant, the percentage of variation explained was small relative to individual variation.

The low to moderate levels of genetic differentiation seen among desert tortoise populations also follows a gradient that is consistent with "isolation-by-distance" (Wright 1943). Isolation by distance is the simplest and most parsimonious model explaining differentiation among populations in the absence of barriers to gene flow (Wright 1943, Kimura and Weiss 1964, Slatkin 1993). Typically, genetic distance increases with geographic distance where the dispersal ability of the species limits interactions among individuals beyond the local scale in comparison to the whole range (Kimura and Weiss 1964, Manel et al. 2003, Slatkin 1993). The Mojave desert tortoise exhibits a strong correlation between geographic distance and genetic distance; geographic distance explained 67% of the variation in genetic distance among sampled locations. These results are consistent with the lack of major barriers to movement at the landscape scale, and consistent with the recognized ability of tortoises to move long distances.

Unfortunately, the dispersal ecology of this species is not well understood (Morafka 1994). However, individual tortoises have the potential to move long distances to forage or reproduce. Although few long forays (greater than 30km) have been recorded (Edwards et a. 2004), long-distance dispersal events are difficult to detect using direct methods (Koenig et al. 1996, Nathan 2001). The long life span of tortoises, coupled with annual opportunities for reproduction during non-drought periods, allows individuals potentially to move longer distances over their reproductive lifetime (Edwards et al. 2004, Esque et al. unpub). This expanded period of influence and long generation time increases the potential for gene flow to homogenize populations over relatively short distances, causing isolation by distance to be a primary mechanism for any population differentiation.

Although the basal and hierarchical clusters identified by the Bayesian analysis were robust and informative, there are reasons to be cautious interpreting the results. Using STRUCTURE to determine the most likely number of populations is arbitrary and based on ad hoc criteria (Pritchard et al. 2000, Evanno et al. 2005, Pritchard et al. 2007). Although the ad hoc criteria coupled with diagnostics and biological context are thought to be relatively reliable, there is still the potential for misinterpretation (Pritchard et al. 2007). In particular, populations that are separated by distance with localized dispersal are not delineated well using STRUCTURE (Pritchard et al. 2007). When allele frequencies differ only slightly between adjacent populations due to localized gene flow, the underlying model may produce results that are difficult to interpret because the algorithm is forced to search for distinct components whether distinct components exist or not.

In a previous study with simulated data, the model was able to detect the basal clusters in a contact zone migration model (Evanno et al. 2005). A hierarchical clustering analysis was effective in this scenario, which suggests that it is possible to make inferences from Bayesian models when demes are connected by localized gene flow (Evanno et al. 2005). However, the level of differentiation in these simulations was higher (F_{ST} = 0.16-0.43) (Evanno et al. 2005) than the levels detected in the Mojave desert tortoise (F_{ST} = 0.01 – 0.16). The number of genetic markers used in our study, and their variability, gave us very high power to detect small differences in allele frequencies (Ryman et al. 2006, Hedrick 1999, Hedrick 2001, Waples 1998) potentially magnifying differences of marginal biological significance. Thus, decisions concerning conservation actions should be fortified with ecological and behavioral information as well as genetic information.

Multimodality of the fitted models in STRUCTURE, and the varied effects of sampling design, also caused us to scrutinize the interpretation of our data. Low differentiation among populations, isolation by distance, or a combination may have caused the re-sampling algorithm to find more than one local maximum for simulations fixing the number of clusters to be more than four. However, multimodality did not occur when K was fixed to 2, 3, and 9, and also multimodality did not occur in the hierarchical analyses. Lack of multimodality in these instances provided support for scenarios of 2, 3, and 9 population clusters. Differences in sampling intensity also resulted in different numbers of genetic clusters and the boundaries to those clusters. Six clusters had the highest likelihood with reduced (and even) sample sizes, and this simulation indicated that the California cluster remained the same for each replicated analysis. However, hierarchical analyses with reduced and even samples resulted in a partitioning of the California cluster in a biologically meaningful way by merging locations (e.g. Piute Valley) that was sampled more intensely in the full data set. It is difficult to reconcile the discrepancy between the analysis of the entire range using the reduced and even data set and the hierarchical analysis of basal clusters using the reduced and even datasets, except to acknowledge that sampling effort was not equal among all locations. Therefore, we support the use of caution with interpreting these analyses and stress the importance of sampling design for future assessments.

Finally, minor differences between the two types of Bayesian analysis require discussion. Both methods clearly identified broad scale population structure, including barriers to gene flow such as the New York and Providence Mountains. However, GENELAND identified different population boundaries for a fourth basal cluster in the Western Mojave Desert. Although this cluster was identified in subsequent hierarchical analyses with STRUCTURE, we assume that the addition of geographic information increased the likelihood that this cluster really exists. Minor irregularity in our sampling scheme (i.e. lack of sampling outside of Desert Wildlife Management Areas in the Western Mojave region) may have contributed to the detection of this area as a separate cluster. However, the model implemented in GENELAND appears to be robust to this type of irregularity in sampling design, though the detection of boundaries can be affected (Guillot et al. unpublished). Clearly, using multiple types of analyses to make informed inferences from population genetic data is a valuable approach (Manel et al. 2004, Rowe and Beebee 2007).

Comparison to other North America tortoise microsatellite studies

We detected low genetic differentiation among sampling locations in the range of the Mojave desert tortoise, which supported the major conclusions of other recent studies of *Gopherus agassizii* populations (Edwards et al. 2004; Murphy et al. 2007). A majority of genetic variation was captured within the populations, and isolation-by-distance was characterized as a major determinant of the pattern of differentiation. F_{ST} values for the desert tortoise (0.01 – 0.16) appear to be particularly low when compared to the gopher tortoise (*Gopherus polyphemus*), which inhabits sand hill, longleaf pine, and scrub ecosystems of the southeastern United States (Schwartz et al. 2005). Levels of genetic differentiation were notably higher in this species ($\bar{F}_{ST}$ = 0.24 ± 0.12) (Schwartz et al. 2005). Further, geographic distance accounted for approximately 15% of the observed genetic variation for gopher tortoises (Schwartz unpublished). In striking contrast, 65% of observed genetic variation is explained by geographic distance for the Mojave desert tortoise (this study and Murphy et al. 2007). Gopher tortoises are known to have limited migratory ability and very small home ranges (McRae et al. 1981; Diemer 1992; Eubanks et al.

2003; Schwartz et al. 2005), and existing gopher tortoise populations are restricted mainly to protected park land due to extensive habitat destruction and fragmentation (Kautz 1993; Schwartz et al. 2005). Behavioral differences, and naturally limited migration, could elucidate the different patterns of genetic differentiation. Although Sonoran desert tortoises also exhibit a pattern of isolation by distance, only 30% of the observed variation is explained by distance (Edwards et al. 2004). Differential use of available habitat may account for the disparity in the amount of genetic variance explained between Mojave and Sonoran desert tortoise populations (Van Devender 2002). Sonoran desert tortoises tend to inhabit rocky foothills, which are more fragmented than are the bajadas typically occupied by Mojave desert tortoises (Van Devender 2002).

Despite a similar global F_{ST} value (0.06) and similar patterns of differentiation (isolation-by-distance and majority of variation occurring within populations), conclusions from our study differ very much from another recent assessment of the Mojave desert tortoise using microsatellites (Murphy et al. 2007). The genetic clusters identified by Murphy et al. (2007) align closely with the current six recovery units described in the original Recovery Plan (USFWS 1994); however, the authors also detected additional substructuring within the Western Mojave Recovery Unit (Western, Southern, Central Mojave units). Therefore, they suggested that the original Western Mojave recovery unit should be further bisected into three units, increasing the total number of recovery units to eight. The authors did not recommend any other changes to recovery unit boundaries.

The boundaries of genetic units detected in our study differed from Murphy et al. 2007 and from the original recovery units (USFWS 1994). We identified seven genetic clusters for the Mojave desert tortoise that reflect isolation-by-distance coupled with geographic barriers preventing localized gene flow. The main boundary differences between the two studies exist in the northern portion of the range, where we detected additional genetic variation requiring further delineation of the Northeastern Mojave recovery unit. The population in the Upper Virgin River recovery unit has been cited as ecologically and behaviorally dissimilar from other populations, which was supported by extreme allele frequency differences (Murphy et al. 2007). However, we determined that the tortoises in the region surrounding St. George, UT consistently, and strongly, cluster with adjacent locations in the Beaver Dam Slope, Mormon Mesa, and Gold Butte. Additionally, we detected a boundary along the New York, Providence, and McCullough Mountains, which separates a portion of the Eastern Mojave recovery unit and the Northern Colorado recovery unit. The locations west of these mountain ranges grouped with a genetic cluster not previously recognized (Amargosa cluster). Finally, we did not detect any further sub-structuring in the Western Mojave recovery units.

Differences between these two studies in the delineating population boundaries can be attributed to sampling design. Careful investigation of population structure requires comprehensive, and thorough, sampling of all potential populations (Evanno et al. 2005). Furthermore, population structure should be inferred from random sampling across the landscape (Manel et al. 2003; Guillot et al. 2005). We favored spreading our sampling effort across more populations, even if we could not get the desired sample size in each area, over sampling to get large sample sizes in fewer locations (Pons and Chaouche 1995). Additionally, we selected microsatellite markers with several alleles improved our ability to estimate genetic parameters (Lowe, Harris, and Ashton 2004; Ryman et al 2006).

Previously, genetic research for the Mojave desert tortoise was conducted in conjunction with other studies, or was limited in geographic scope, which constrained sampling design and the ability to detect population boundaries (Lamb et al. 1989; Britten et al. 1997; Berry et al.

2002; Tracy et al. 2004). As a result, additional research with the expressed intent of identifying genetic units was recommended (Berry et al. 2002; Tracy et al. 2004). Although Murphy et al. (2007) sampled representative individuals from each of the six original recovery units (USFWS 1994), a majority of the sampling (73%) was confined to the Western Mojave Recovery Unit, with 30% of the total samples collected within a single 60km area (Marine Corps Air Ground Combat Center, Twentynine Palms, CA). Murphy et al. used samples collected over a ten-year period from previous studies mainly related to disease detection within Desert Wildlife Management Areas. As a result, sampling was opportunistic, and many samples were collected on plots of varying size, but usually only a few square kilometers in area.

Unfortunately, this sampling design is not ideal to evaluate spatial population structure, as it may not capture spatial variation (Storfer et al. 2007). Sampling many individuals in close proximity may increase the probability of sampling very closely related individuals within demes, which violates assumptions of the Bayesian analyses, and can lead to an overestimation of the number of distinct genetic clusters (Pritchard et al. 2007). Our work also shows that unequal sampling intensity increases the potential to overestimate the number of genetic clusters even if the samples are not dominated by sampling demes. This result has been discussed previously (McRae et al. 2005). Therefore, the intensive sampling in the Western Mojave recovery unit (Murphy et al. 2007) likely created spurious, additional clusters in the Bayesian analyses. In our study, pair-wise F_{ST} values among locations in these regions were < 0.02 and were not statistically significant. Although the sampling design for our study was not completely randomized or inclusive, we accounted for unequal sampling intensity and adjusted the interpretation of any putative genetic clusters.

Incomplete sampling across portions of the range (e.g. Nevada) also potentially caused spurious results in the study by Murphy et al (2007). They noted that the Upper Virgin River recovery unit and the Northeastern Mojave recovery unit were the most differentiated groups and were more isolated than the other sampled groups. However, they sampled no locations in Nevada, and that likely artificially created led to the conclusion of great differentiation in the northern-most sample site. Including locations in Nevada in our study revealed a gradient of small genetic differentiation. Thus, our analyses do not support great differentiation in the northern-most locations of the desert tortoise.

Recent simulations have addressed the potential problem of not sampling “ghost populations”, and the effect of this inadequate sampling on estimating migration rates (Beerli 2004; Slatkin 2005). The interdependence of populations is complicated, and although it may not be completely necessary to sample all populations, high levels of migration from unsampled populations impact estimates of migration rates (Beerli 2004). In a similar way, the allele frequencies used to infer population structure likely will be different with the absence of known locations of the species. The comparison of the Murphy et al. study to ours underscores the potential for markedly different interpretations of population genetic data and analyses when study design and sampling differ. Although the microsatellite markers used in each of these two studies were not identical, multiple metrics, including F-statistics, analysis of molecular variance, and Mantel tests, were strikingly similar. Despite these similarities, the inferences in each study were markedly different. Therefore, we contend that highly skewed sampling intensity coupled with lack of sampling in a portion of the core distribution of a species prohibited Murphy et al. from making accurate inferences from population genetic assessments at the landscape scale. The shift from population-based analyses to individual-based analyses in population genetics research requires a change in the design of studies and how samples are collected. If sampling is not

modified to reflect these new approaches to data analysis, inferences and conclusions drawn from data may be misleading.

Recommendations for conservation practices

The life history traits of the desert tortoise (i.e. very long life span and generation time) framed the temporal scale of our investigation. Therefore, we described population structure that was shaped by many generations (and therefore many hundreds of years) prior to the recent surge of anthropogenic impacts on the southwestern United States. As such, our analyses cannot detect potential isolation due to recent urbanization of the Mojave and Colorado Deserts. Indeed, the long generation time of desert tortoise makes it unlikely that our analyses could assess the impacts of anthropogenic relocations of tortoises that have occurred within the last several decades. Fortunately, this perspective allows us to infer the spatial genetic structure of tortoise populations prior to severe human influence, which can offer direction for the maintenance of natural (or at least semi-natural) population dynamics. We are able to make several recommendations to revise conservation strategies for the Mojave desert tortoise (see below).

Delineation of conservation units

Recovery planning was initiated subsequent to the federal listing of the Mojave Desert tortoise in 1990 under the Endangered Species Act of 1973 (55 FR 12178, April 2, 1990). This planning included crafting a recovery plan and delineating biologically distinct populations within the range of the Mojave desert tortoise as a means to facilitate management and preserve intraspecific diversity. Original authors of the Recovery Plan used the concept of the evolutionarily significant unit (ESU; Ryder 1986, Waples 1991, Mortiz 1994) to describe geographic units (termed Recovery Units), which were deemed important to conserve adequately the diversity of the listed entity (USFWS 1994). The concept of the ESU has remained widely used and debated in the academic literature (Crandall et al. 2000, Green 2005, Pennock and Dimmick 1997, Paetkau 1999, Taylor and Dizen 1999, Waples 1998). However, the management unit (Moritz 1994, Palsboll et al. 2006, Paetkau 1999) is more applicable conservation unit to diagnose entities within the Mojave desert tortoise. Management units (MU), which can be defined as populations with independent dynamics, are typically considered as less isolated than ESUs and are useful for identifying local conservation and monitoring (Palsboll et a. 2006). Although these terms and the discussion surrounding them have merit and are necessary to establish a biological basis for delineations, the MU and the ESU are not policy terms or legally binding.

Currently, the Mojave Desert tortoise is listed as a distinct population segment (DPS) (55 FR 12178, April 2, 1990), which is a legal entity under the ESA (ESA Section, 4). As such, its status can be changed independently of other DPS or species/subspecies. The policy that defined a DPS originally pertained to salmonids and was based on the ESU (NMFS 1991, Waples 1991, Waples 1995). Revisions to this policy further clarifies the definition and provides guidelines for how a DPS can be listed, delisted, or reclassified using the criteria of discreteness, significance, and conservation status for all vertebrate taxa (USFWS and NOAA 1996, Rosen 2007). Genetic data are relevant to satisfying the criteria for both discreteness and significance; however other morphological, ecological, and behavioral evidence are also applicable (USFWS and NOAA 1996).

Within the scope of recovery planning, the recovery unit remains the workhorse of conservation unit delineations for many listed species. Delineated recovery units contain features

that ensure the recovery and long-term viability of the listed entity (NMFS 2006). In contrast to the DPS, the recovery unit is described only within a recovery plan and is not formally listed through the ESA. Therefore, recovery units are not protected individually by the Act, nor can their status be changed separately from other units. Although these units may be treated as individual management units, the population(s) contained in each recovery unit within the listed entity must exhibit signs of recovery before it can be removed from the endangered species list (NMFS 2006). Therefore, the subsequent discussion of conservation units for the desert tortoise refers generally to diagnosing management units (generally) and recovery units (specifically). If further delineations of distinct population segments for the desert tortoise were considered by the USFWS in the future, these data would be applicable to that decision as well.

We recommend that the boundaries of conservation units for the Mojave desert tortoise be revised to reflect fine-scale genetic structure identified in this investigation. Although we detected only low-to-moderate levels of genetic differentiation range wide, these delineations reflect differentiation derived from natural levels of gene flow in a system driven by isolation-by-distance. Additionally, localized dispersal and natural barriers have prevented homogenization of these populations over many generations. Across the range, we recommend delineating seven conservation units. However, simply rejecting panmixia may not be sufficient for the delineation of conservation units, and some types of population structure (e.g. isolation-by-distance) do not lend themselves to definitive boundaries (Palsboll et al. 2006). The recommended changes to delineations should be treated as a new hypothesis that is tested with additional genetic, demographic, ecological, and behavioral data, including estimates of dispersal rates among proposed genetic clusters, and biotic interactions within the ecologically different areas of the range of the Mojave desert tortoise (Palsboll et al. 2006).

Delineating conservation units (or recovery units) should not be based solely on population genetics (Green 2005, Paetkau 1999, Taylor and Dizon 1999). Genetic clusters described with neutral markers provide an excellent starting point for delineating conservation units (Palsboll et al. 2006); however, these data and analyses do not exclusively translate into populations with unique evolutionary potential, nor do they reflect conservation status (Green 2005). In the case of the desert tortoise, the temporal scale of analyses prevents us from detecting any population genetic signatures from recent fragmentation of habitat, anthropogenic influences on habitat or populations, or population declines. However, known threats to population persistence differ dramatically across the range and population declines are spatially heterogeneous (Tracy et al. 2004). Conservation units should not only reflect genetic considerations and conservation status, but also ecological considerations broadly speaking to include landscape differences as well as local differences in geography, vegetation, and physiognomy. Diversity of food and shelter resources must be captured in conservation units to ensure temporal and spatial abilities to meet the needs of individual tortoises as a way to bolster the viability of populations and avoid periodic natural threats to persistence including climate change. Therefore, these units must capture unique habitats and unique ecological interactions, as well as variability in behavior and life history traits.

Our data provide no direct link between genetic variation and traits that could provide a selective advantage across the habitat types that exist throughout the range of the Mojave desert tortoise. However, the genetic clusters that we have identified encompass variation in life history characteristics, activity patterns, behavioral traits, and habitat types. Each of the recommended conservation units contains a portion of the regional variation in survival rates, causes of mortality, and reproductive output (Germano 1994b, Henen et al. 1998, Lovich et al. 1999,

Mueller et al. 1998, Nagy and Medica 1986, Peterson 1994, Peterson 1996, Tracy et al. 2004). For example, tortoise reproduction varies across a longitudinal gradient; tortoises in the western Mojave Desert (which typically receives mostly summer rains) produce relatively larger eggs, produce fewer eggs overall, and lay their second clutches later than do tortoises in the adjacent eastern Mojave Desert (Wallis et al. 1999). Behaviorally, western Mojave tortoises are much less active during summer than are tortoises in other regions (Marlow 1979, Nagy and Medica 1986). Extremely winter-dominant rainfall and resultant effects on the vegetation community, as well as its position on the western end of the distribution, contribute to the significance of this conservation unit (USFWS 1994).

The tortoises located near St. George, Utah represent the northern-most extent of the distribution of this species. The genetic data presented here do not support the delineations of the current Upper Virgin River recovery unit to be entirely in Utah; however, other unique features of these tortoises warrant additional protection. Desert tortoises in this regions experience long, cold winters (about 100 freezing days) and mild summers, during which the tortoises are continually active (Woodbury and Hardy 1948). Here tortoises live in a complex topography consisting of canyons, mesas, sand dunes, and sandstone outcrops where the vegetation is a transitional mixture of Sagebrush Scrub, Creosote Bush Scrub, Blackbrush Scrub, and a psammophytic community. Desert tortoises use sandstone and lava caves instead of tortoise-constructed burrows, travel to sand dunes for oviposition, and use still other habitats for foraging. Often, two or more desert tortoises use the same burrow or cave (Woodbury and Hardy 1948, Esque 1994), which is less common in the southern and western portions of the range. Clearly, these tortoises have conservation potential despite the lack of supporting genetic differentiation. Thus, it seems prudent not to manage this tortoise population in complete isolation due to the evidence for historic gene flow with adjacent locations.

Maintenance of population structure

Severe anthropogenic impacts to desert tortoise habitat, including fragmentation due to highways, has only occurred in the past five to six decades (Hunter et al. 2003, Lovich and Bainbridge 1999). Desert tortoises have a relatively long generation time (estimated as more than 25 years; USFWS 1994). The age of first reproduction is determined by body size in females (sexual maturity occurs approximately at minimum size of 180 mm; Germano 1994a, Turner et al. 1986) and individual growth rate varies in relation to available forage and drought (Germano 1994b, Mueller et al. 1998). Assuming a 25-year generation time, a conservative estimate of four generations may have occurred in the past century. Any potential genetic signature of habitat fragmentation and subsequent reduction in gene flow should not be observable yet. Further, fencing major roadways and public lands has made movement among critical habitat effectively impossible (Edwards et al. 2004).

We speculate that urban development has severely disrupted the natural migration and dispersal patterns of the desert tortoise, and that it is not possible to detect these disruptions due to the long temporal scale over which population dynamics occur in this species. The low levels of genetic differentiation that we have detected suggest that, until recently, tortoise populations were well connected. Recent habitat suitability models supported our hypothesis of past population connectivity (Nussear et al. in prep). In a future population genetic assessment, researchers may have the power to detect the isolation of tortoise populations.

Desert tortoises have been translocated among locations in recent history (a) for management purposes (Murphy et al. 2007), (b) for research (Field et al. 2007, Nussear 2004),

and prior to the listing of the species, (c) tortoises were removed from the wild as part of the pet trade (Murphy et al. 2007). Captive releases of individuals have been recorded periodically. These translocations have the potential to interfere with the ability to detect a population genetic signature (Murphy et al. 2007). However, many of these translocations were poorly documented, and there is scant information beyond anecdotes to suggest that these translocations resulted in successful reproduction in the new locations. Early translocations were often executed when the seasonal temperatures were inhospitable for tortoises, and shelter was not provided for the translocated tortoises, which generally results in the translocated tortoises dying (Cook et al. 1978, Cook 1983, Field et al. 2007, Nussear 2004). Indeed, many early translocations were not successful because tortoises were exposed to lethal thermal environments or novel predators (Cook 1983, Field et al. 2007, Nussear 2004). However, translocations have been successful (i.e. high survivorship and typical egg-laying behavior) when they occurred during the spring when seasonal temperatures were below lethal limits and forage was available (Nussear 2004). The only evidence of potentially successful translocations threatening to taint our data set is in the Red Cliffs Desert Reserve near St. George, UT. Reportedly, individuals had been moved from California to the St. George area, however, we were able to discern likely translocated individuals, and they all had a genetic signature from south Las Vegas Valley, not to California. Further, all individuals in our analyses were assigned to their original cluster, or to an adjacent cluster. This is consistent with isolation-by-distance and historically high levels of gene flow. The possibility that translocations have augmented the signal of gene flow among clusters does exist; however, there is limited evidence that this factor warrants scrutiny. Similar to other anthropogenic impacts, translocations have only occurred in recent history. Unless the actual translocated animals were sampled in a population genetic assessment, the long generation time of these animals would prevent any potential progeny showing up in a sample of adult tortoises, and thus would not be detected in our study or other recent studies.

Future management should take into account our analyses using genetic markers. If adult individuals are translocated to supplement declining populations, or they are cleared from habitat that is slated for urban development, care should be taken to transport individuals to within their genetic cluster. This consideration should complement other recommendations from previous studies (Field et al. 2007, Nussear 2004). Additionally, managers should avoid transporting tortoises across the potential boundaries to gene flow identified here. Head-starting populations with young recruits is a potential management action that may be implemented to augment poor recruitment in some locations (Iverson 1991, Congdon et al. 1993, but see Heppell et al. 1996). However, in any head starting program, mated adults should originate from the same genetic cluster, and offspring should be released in that cluster to maintain current levels of genetic diversity and avoid excessive inbreeding or outbreeding (Frankham et al. 2002).

Although we can infer from summary F-statistics that high levels of gene flow occurred among adjacent tortoise populations, estimates of migration rates from F-statistics are not reliable (Whitlock and McCauley 1999). Therefore, additional analyses are necessary to infer the number migrants per generation among clusters. If possible, coalescent methods (Beerli 1998, Beerli and Felsenstein 1999) should be used to quantify historic levels of migration among clusters and estimate the effective population size. These data can provide valuable information about the ecology of the species, but the analyses also have management implications. In the past, long-distance migration may have been critical to the persistence of desert tortoise populations. Catastrophic die-offs have been documented periodically (Peterson 1994), and recolonization from adjacent valleys may be necessary to ensure population viability. This rescue

effect (Brown and Kodric-Brown 1977, Hanski and Gilpin 1997) could have profound implications across the temporal scale in which tortoise populations operate.

Translocations or other management actions, such as the addition of culverts under highways to allow natural movement, may be necessary to improve connectivity and maintain historic levels of gene flow across cluster boundaries that have been ablated by human actions. Although the effectiveness of culverts as habitat linkages has been demonstrated for other species (Clevenger et al. 2001), limited research has been conducted on how well culverts facilitate tortoise movement (Boarman et al. 1996, Fusari 1985, Ruby et al. 1994). Results from this research are promising, suggesting that culverts (if large enough) have the potential to be an effective method for maintaining connectivity (Ruby et al. 1994). Fencing of major roadways has certainly decreased mortality of adult tortoises (Boarman et al. 1996), but this management action has fragmented habitat (von Seckendorff Hoff and Marlow 2002), and halted putative gene flow within and among tortoise populations (Edwards et al. 2004, Ruby et al. 1994). Facilitating movement among populations may be a critical component to management strategies for this threatened distinct population segment.

CONCLUSIONS

Isolation-by-distance and low levels of genetic differentiation characterize population structure in the Mojave desert tortoise. Using individual-based Bayesian assignment tests, we identified hierarchical structuring in this threatened distinct population segment. The three basal clusters corresponded to mtDNA haplotypes, and we detected additional spatial structure within the basal clusters. Uneven sample sizes in some areas appear to have created spurious clusters; however, seven of the finer scale clusters were robust to our sampling scheme.

RECOMMENDATIONS

We recommend that the boundaries of conservation units for the Mojave desert tortoise be changed to account for these new analyses. Our recommended boundaries do not align with recommendations from other genetic studies of the Mojave desert tortoise using microsatellites; however, the noticeable differences in sampling design between the studies account for these differences.

LITERATURE CITED

Andersen, M. C., J. M. Watts, J. E. Freilich, S. R. Yool, G. I. Wakefield, J. F. McCauley, and P. B. Fahnestock. 2000. Regression-tree modeling of desert tortoise habitat in the central Mojave Desert. Ecological Applications 10:890-900.

Beerli, P. 1998. Estimation of migration rates and population sizes in geographically structured populations. In G.R. Carvalho (ed.) Advances in Molecular Ecology. IOS Press, pp 39-53.

Beerli, P. 2004. Effect of unsampled populations on the estimation of population sizes and migration rates between sampled populations. Molecular Ecology 13:827-836.

Beerli, P., and J. Felsenstein. 1999. Maximum-likelihood estimation of migration rates and effective population numbers in two populations using a coalescent approach. Genetics 152:763-773.

Beerli, P., and J. Felsenstein. 2001. Maximum likelihood estimation of a migration matrix and effective population sizes in n subpopulations by using a coalescent approach. Proceedings of the National Academy of Sciences of the United States of America 98:4563-4568.

Berry, K.H., J. Mack, R.W. Murphy, and W. Quillman. 2006. Introduction to the special issue on the changing Mojave Desert. Journal of Arid Lands 67: 5-10.

Berry, K. H., D. J. Morafka, and R. W. Murphy. 2002. Defining the desert tortoise(s): our first priority for a coherent conservation strategy. Chelonian Conservation and Biology 4:249-262.

Boarman, W. I., M. Sazaki, G. C. Goodlett, and T. Goodlett. 1996. Reduction in mortalities of desert tortoises and other vertebrates along a fenced highway. Page(s) 108-108 in The Proceedings of the Desert Tortoise Council, V. Dickinson, editor. 20th Annual Symposium of the Desert Tortoise Council; March 31 - April 2, 1995, Las Vegas, Nevada. San Bernardino, California: Desert Tortoise Council, Inc. 116 pp.

Boulet, M., S. Couturier, S. D. Cote, R. D. Otto, and L. Bernatchez. 2007. Integrative use of spatial, genetic, and demographic analyses for investigating genetic connectivity between migratory, montane, and sedentary caribou herds. Molecular Ecology 16:4223-4240.

Bradford, D. F., A. C. Neale, M. S. Nash, D. W. Sada, and J. R. Jaeger. 2003. Habitat patch occupancy by toads (*Bufo punctatus*) in a naturally fragmented desert landscape. Ecology 84:1012-1023.

Britten, H. B., B. R. Riddle, P. F. Brussard, R. Marlow, and T. E. Lee. 1997. Genetic delineation of management units for the desert tortoise, Gopherus agassizii, in Northeastern Mojave Desert. Copeia:523-530.

Burk, J.H. 1977. Sonoran Desert. p. 869-889. In: MG Barbour and J. Major (eds.). Terrestrial vegetation of California. New York, New York.

Bury, R.B., T.C. Esque, L.A. DeFalco, and P.A. Medica.1994. Distribution, habitat use, and protection of the desert tortoise in the Eastern Mojave Desert. In R.B. Bury and D.J. Germano (eds), Biology of the North American tortoises. United States Department of the Interior National Biological Survey.

Clevenger, A.P., B. Chruszcz, and K. Gunson. 2001. Drainage culverts as habitat linkages and factors affecting passage by mammals. Journal of Applied Ecology 38:1340-1349.

Congdon, J. D., A. E. Dunham, and R. C. Von Loben Sels. 1993. Delayed sexual maturity and demographics of Blanding's turtles (*Emydoidea blandingii*): implications for conservation and managment of long-lived organisms. Conservation Biology 7:826-833.

Cook, J. C., Wever, A. E., & Stewart, G. R. 1978. Survival of captive tortoises released in California. Proceedings of the Desert Tortoise Council, Las Vegas, NV.

Cook, J. C. 1983. Rehabilitation of the desert tortoise *Gopherus agassizii*. California State Polytechnic University, Pomona.

Coulon, A., G. Guillot, J. F. Cosson, J. M. A. Angibault, S. Aulagnier, B. Cargnelutti, M. Galan, and A. J. M. Hewison. 2006. Genetic structure is influenced by landscape features: empirical evidence from a roe deer population. Molecular Ecology 15:1669-1679.

Crandall, K. A., O. R. P. Bininda-Emonds, G. M. Mace, and R. K. Wayne. 2000. Considering evolutionary processes in conservation biology. Trends in Ecology & Evolution 15:290-295.

Diemer, J. E. 1992. Home Range and Movements of the Tortoise (*Gopherus polyphemus*) in Northern Florida. Journal of Herpetology 26:158-165.

Edwards, T. C. 2003. Desert tortoise conservation genetics. Masters Thesis, University of Arizona, Tucson, AZ.

Edwards, T., C. S. Goldberg, M. E. Kaplan, C. R. Schwalbe, and D. E. Swann. 2003. PCR primers for microsatellite loci in the desert tortoise (*Gopherus agassizii*, Testudinidae). Molecular Ecology Notes 3:589-591.

Edwards, T., C. R. Schwalbe, D. E. Swann, and C. S. Goldberg. 2004. Implications of anthropogenic landscape change on inter-population movements of the desert tortoise (*Gopherus agassizii*). Conservation Genetics 5:485-499.

Esque, T. 1994. Diet and diet selection of the desert tortoise (*Gopherus agassizii*) in the northeast Mojave Desert. Masters Thesis, Colorado State University, Fort Collins, CO.

Eubanks, J. O., W. K. Michener, and C. Guyer. 2003. Patterns of movement and burrow use in a population of gopher tortoises (*Gopherus polyphemus*). Herpetologica 59:311-321.

Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology 14:2611-2620.

Excoffier, L., P. E. Smouse, and J. M. Quattro. 1992. Analysis of Molecular Variance Inferred from Metric Distances among DNA Haplotypes - Application to Human Mitochondrial-DNA Restriction Data. Genetics 131:479-491.

Falush, D., Stephens, M., and Pritchard, J. K. 2003. Inference of population structure: Extensions to linked loci and correlated allele frequencies. Genetics, 164:1567-1587.

Field, K. J., C. R. Tracy, P. A. Medica, R. W. Marlow, and P. S. Corn. 2007. Return to the wild: Translocation as a tool in conservation of the Desert Tortoise (*Gopherus agassizii*). Biological Conservation 136:232-245.

Frankham, R., J.D. Ballou, and D.A. Briscoe. 2002. Introduction to Conservation Genetics. Cambridge University Press, New York, New York.

Fusari, M. 1985. A study of the reactions of desert tortoises to different types of fencing. Page(s) 125-132 in The Proceedings of the Desert Tortoise Council, K. Hashagen, editor. The Seventh Annual Symposium of the Desert Tortoise Council; March 27-29, 1982, Las Vegas, Nevada and St. George, Utah. Long Beach, California: Desert Tortoise Council, Inc. 173 pp.

Germano, D.J. 1994a. Comparative life histories of North American tortoises. In Bury, R.B and Germano, D.J. (Eds.) Biology of North American Tortoises. Washington, D.C.: U.S.D.I. National Biological Survey.

Germano, D.J. 1994b. Growth and age of maturity of North American tortoises in relation to regional climates. Canadian Journal of Zoology 72: 918-931.

Germano, D.J., R.B. Bury, T.C. Esque, T.H. Fritts, and P.A. Medica. 1994. Range and habitats of the desert tortoise (*Gopherus agassizii*). In Bury, R.B and Germano, D.J. (Eds.) Biology of North American Tortoises. Washington, D.C.: U.S.D.I. National Biological Survey.

Goudet, J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3)

Goudet, J. 1995. FSTAT (Version 1.2): A computer program to calculate F-statistics. Journal of Heredity 86:485-486.

Green, D. M. 2005. Designatable units for status assessment of endangered species. Conservation Biology 19:1813-1820.

Guillot, G., A. Estoup, F. Mortier, and J. F. Cosson. 2005. A spatial statistical model for landscape genetics. Genetics 170: 1261-1280.

Guillot, G., F. Mortier, and A. Estoup. 2005. GENELAND: a computer package for landscape genetics. Molecular Ecology Notes 5: 712-715.

Hedrick, P. W. 1999. Perspective: Highly variable loci and their interpretation in evolution and conservation. Evolution 53: 313-318.

Henen, B. T., C. C. Peterson, I. R. Wallis, K. H. Berry, and K. A. Nagy. 1998. Effects of climatic variation on field metabolism and water relations of desert tortoises. Oecologia 117: 365-373.

Heppell, S. S., L. B. Crowder, and D. T. Crouse. 1996. Models to evaluate headstarting as a management tool for long-lived turtles. Ecological Applications 6: 556-565.

Hoff, K.V.S., and R. W. Marlow. 2002. Impacts of vehicle road traffic on desert tortoise populations with consideration of conservation of tortoise habitat in southern Nevada. Chelonian Conservation and Biology 4: 449-456.

Hunt, C.B. 1974. Natural Regions of the United States and Canada, W.H. Freeman and Co, San Francisco.

Iverson, J.B. 1991. Patterns of survivorship in turtles (order: Testudines). Canadian Journal of Zoology 69: 385-391.

Jensen, J. L., A. J. Bohonak, and S. T. Kelley. 2005. Isolation by distance, web service. Bmc Genetics 6.

Kautz, R.S. 1993. Trends in Florida wildlife habitat 1936-1987. Florida Scientist 56: 7-24.

Kimura, M. and G.H. Weiss. 1964. The stepping stone model of population structure and the decrease of genetic correlation with distance. Genetics 49: 561-576.

Koenig, W.D., D. Van Vuren, and P.N. Hooge. 1996. Detectability, philopatry, and the distribution of dispersal distances in vertebrates. Trends in Ecology and Evolution 11: 514-517.

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

Lovich, J. E., and D. Bainbridge. 1999. Anthropogenic degradation of the southern California desert ecosystem and prospects for natural recovery and restoration. Environmental Management 24:309-326.

Lovich, J., P.A. Medica, H. Avery, K. Meyer, G. Bowser, and A. Brown. 1999. Studies of reproductive output of the desert tortoise at Joshua Tree National Park, the Mojave National Preserve, and comparative sites. Park Science 19: 22-24.

Lowe, A., P. Ashton, and S.E. Harris. 2004. Ecological Genetics: Design, Analysis, and Application. Oxford: Blackwell Publishing. Malden, MA.

Luckenbach, R.A. 1982. Ecology and management of the desert tortoise (*Gopherus agassizii*) in California. In North American Tortoise Conservation and Ecology. Washington, D.C.

Manel, S., E. Bellemain, J. E. Swenson, and O. Francois. 2004. Assumed and inferred spatial structure of populations: the Scandinavian brown bears revisited. Molecular Ecology 13:2107-2107.

Manel, S., O. E. Gaggiotti, and R. S. Waples. 2005. Assignment methods: matching biological questions techniques with appropriate. Trends in Ecology & Evolution 20:136-142.

Manel, S., M. K. Schwartz, G. Luikart, and P. Taberlet. 2003. Landscape genetics: combining landscape ecology and population genetics. Trends in Ecology & Evolution 18:189-197.

McRae, W. A., J. L. Landers, and J. A. Garner. 1981. Movement Patterns and Home Range of the Gopher Tortoise. American Midland Naturalist 106:165-179.

McRae, B. H., P. Beier, L. E. Dewald, L. Y. Huynh, and P. Keim. 2005. Habitat barriers limit gene flow and illuminate historical events in a wide-ranging carnivore, the American puma. Molecular Ecology 14:1965-1977.

Morafka, D. 1994. Neonates: missing links in the life histories of North American tortoises. Pages 161-173 *in* R. B. Bury and D. J. Germano, editors. Biology of North American Tortoises. National Biological Survey, Fish and Wildlife Research, Washington D.C.

Moritz, C. 1994. Defining Evolutionarily-Significant-Units for Conservation. Trends in Ecology & Evolution 9:373-375.

Mueller, J. M., K. R. Sharp, K. K. Zander, D. L. Rakestraw, K. R. Rautenstrauch, and P. E. Lederle. 1998. Size-specific fecundity of the desert tortoise (*Gopherus agassizii*). Journal of Herpetology 32:313-319.

Murphy, R. W., K. H. Berry, T. Edwards, and A. M. McLuckie. 2007. A genetic assessment of the recovery units for the Mojave population of the desert tortoise, *Gopherus agassizii*. Chelonian Conservation and Biology 6:229-251.

Nathan, R. 2001. The challenges of studying dispersal. Trends in Ecology and Evolution 16: 481- 483.

National Marine Fisheries Service. 2006. Iterim Endangered and Threatened Species Recovery Planning Guidance. July 2006, Washington D.C.

Neigel, J. E. 2002. Is F-ST obsolete? Conservation Genetics 3:167-173.

Nordborg, M. 2001. Coalescent theory. In “Handbook of Statistical Genetics” D.J. Balding, M.J. Bishop, and C. Cannings (eds). Wiley, Chichester, England.

O'Connor, M. P., L. C. Zimmerman, D. E. Ruby, S. J. Bulova, and F. R. Spotila. 1994. Home range size and movements by desert tortoises, *Gopherus agassizii*, in the eastern Mojave desert. Herpetological Monographs 8:60-71.

Paetkau, D., W. Calvert, I. Stirling, and C. Strobeck. 1995. Microsatellite Analysis of Population-Structure in Canadian Polar Bears. Molecular Ecology 4:347-354.

Paetkau, D. 1999. Using genetics to identify intraspecific conservation units: a critique of current methods. 13:1507-1509.

Palsboll, P. J., M. Berube, and F. W. Allendorf. 2007. Identification of management units using population genetic data. Trends in Ecology & Evolution 22:11-16.

Pearse, D. E., and K. A. Crandall. 2004. Beyond F-ST: Analysis of population genetic data for conservation. Conservation Genetics 5:585-602.

Pennock, D. S., and W. W. Dimmick. 1997. Critique of the evolutionarily significant unit as a definition for "distinct population segments" under the US Endangered Species Act. Conservation Biology 11:611-619.

Peterson, C. C. 1994. Different rates and causes of high mortality in two populations of the threatened desert tortoise, *Gopherus agassizii*. Biological Conservation 70:101-108.

Peterson, C. C. 1996. Ecological energetics of the desert tortoise (*Gopherus agassizii*): Effects of rainfall and drought. Ecology 77:1831-1844.

Pilot, M., W. Jedrzejewski, W. Branicki, V. E. Sidorovich, B. Jedrzejewska, K. Stachura, and S. M. Funk. 2006. Ecological factors influence population genetic structure of European grey wolves. Molecular Ecology 15:4533-4553.

Pons, O., and K. Chaouche. 1995. Estimation, Variance and Optimal Sampling of Gene Diversity .2. Diploid Locus. Theoretical and Applied Genetics 91:122-130.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945-959.

Pritchard, J.K., X. Wen, and D. Falush. 2007. Documentation for STRUCTURE software: Version 2.2., http://pritch.bsd.uchicago.edu/software.

Qiagen. 2001. Isolation of genomic DNA from dried blood spots (QA22.doc October 2001). www1.qiagen.com/literature/protocols/pdf/QA22.pdf

Raymond M, and F. Rousset. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity, 86, 248-249.

Rice, W.R. 1989. Analyzing tables of statistical tests. Evolution 43: 223-225.

Rousset, F. 1997. Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. Genetics 145:1219-1228.

Rowe, G., and T. J. C. Beebee. 2007. Defining population boundaries: use of three Bayesian approaches with microsatellite data from British natterjack toads (Bufo calamita). Molecular

Ecology 16:785-796.

Rowlands, P., H. Johnson, E. Ritter, and A. Endow. 1982. The Mojave Desert. Pages 103-162 In. Bender, G.L. (editor) Reference Handbook on the Deserts of North America. Greenwood Press, Westport, CT.

Ruby, D.E., J.R. Spotila, S.K. Martin, S.J. Kemp. 1994. Behavioral responses to barriers by desert tortoises: Implications for wildlife management. Herpetological Monographs 8: 144-160.

Rueness, E. K., P. E. Jorde, L. Hellborg, N. C. Stenseth, H. Ellegren, and K. S. Jakobsen. 2003. Cryptic population structure in a large, mobile mammalian predator: the Scandinavian lynx. Molecular Ecology 12:2623-2633.

Ryder, O. A. 1986. Species Conservation and Systematics - the Dilemma of Subspecies. Trends in Ecology & Evolution 1:9-10.

Ryman, N., S. Palm, C. Andre, G. R. Carvalho, T. G. Dahlgren, P. E. Jorde, L. Laikre, L. C. Larsson, A. Palme, and D. E. Ruzzante. 2006. Power for detecting genetic divergence: differences between statistical methods and marker loci. Molecular Ecology 15:2031-2045.

Schneider, S., D. Roessli, and L. Excoffier. 2000. Arlequin ver. 2.00: A software for population genetic analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Schwartz, T. S., and S. A. Karl. 2005. Population and conservation genetics of the gopher tortoise (*Gopherus polyphemus*). Conservation Genetics 6:917-928.

Schwartz, T. S., M. Osentoski, T. Lamb, and S. A. Karl. 2003. Microsatellite loci for the North American tortoises (genus Gopherus) and their applicability to other turtle species. Molecular Ecology Notes 3:283-286.

Simandle, E.T. 2006. Population structure and conservation of two rare toad species (*Bufo exsul* and *Bufo nelsoni*) in the Great Basin, USA. Doctoral Dissertation , University of Nevada, Reno.

Slatkin, M. 1993. Isolation by Distance in Equilibrium and Nonequilibrium Populations. Evolution 47:264-279.

Slatkin, M. 2005. Seeing ghosts: the effect of unsampled populations on migration rates estimated for sampled populations. Molecular Ecology 14:67-73.

Sneath, P.H.A., and R.R. Sokal. 1973. Numerical Taxonomy: The principles and practice of numerical classification. Freeman. San Francisco, CA, USA. 573p.

Spinks, P. Q., and H. B. Shaffer. 2005. Range-wide molecular analysis of the western pond turtle (*Emys marmorata*): cryptic variation, isolation by distance, and their conservation implications. Molecular Ecology 14:2047-2064.

Sponer, R., and M. S. Roy. 2002. Phylogeographic analysis of the brooding brittle star *Amphipholis squamata* (Echinodermata) along the coast of New Zealand reveals high cryptic genetic variation and cryptic dispersal potential. Evolution 56:1954-1967.

Storfer, A., M. A. Murphy, J. S. Evans, C. S. Goldberg, S. Robinson, S. F. Spear, R. Dezzani, E. Delmelle, L. Vierling, and L. P. Waits. 2007. Putting the 'landscape' in landscape genetics. Heredity 98:128-142.

Taylor, B. L., and A. E. Dizon. 1999. First policy then science: why a management unit based solely on genetic criteria cannot work. Molecular Ecology 8:S11-S16.

Tracy, C.R., R.C. Averill-Murray, W. I. Boarman, D. Delehanty, J. S. Heaton, E.D. McCoy, D. J.Morafka, K.E. Nussear, B.E. Hagerty, P.A. Medica. 2004. Desert Tortoise Recovery Plan Assessment. Report to the U.S. Fish and Wildlife Service, Reno, NV.

Trimble, S. 1989. The Sagebrush Ocean: A Natural History Of The Great Basin. University of Nevada, Reno. Reno, NV.

Turner, F. B., P. Hayden, B. L. Burge, and J. B. Roberson. 1986. Egg production by the desert tortoise (*Gopherus agassizii*) in California. Herpetologica 42:93-104.

U.S. Department of the Interior and U. S. Department of Commerce. 1996. Policy regarding the recognition of distinct vertebrate population segments under the Endangered Species Act. Federal Register 61: 4722-4725.

U.S. Fish and Wildlife Service. 1994. Desert tortoise (Mojave population) Recovery Plan. U.S. Fish and Wildlife Service, Portland, Oregon. 73 pp.

Van Devender, T.R., 2002. Natural history of the Sonoran tortoise in Arizona. Pages 3-28 In T.R. Van Devender (editor). The Sonoran Desert Tortoise: Natural History, Biology, and Conservation. University of Arizona Press.

van Oosterhout. C, W.F. Hutchinson, D.P.M. Wills, and P. Shipley. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data, 4, 535-538.

van Oosterhout, C., D. Weetman, and W. F. Hutchinson. 2006. Estimation and adjustment of microsatellite null alleles in nonequilibrium populations. Molecular Ecology Notes 6:255-256.

Wallis, I. R., B. T. Henen, and K. A. Nagy. 1999. Egg size and annual egg production by female desert tortoises (*Gopherus agassizii*): The importance of food abundance, body size, and date of egg shelling. Journal of Herpetology 33:394-408.

Waples, R.S. 1991. Pacific salmon, *Oncorhynchus* spp. and the definition of "species" under the Endangered Species Act. Marine Fisheries Review 53: 11-22.

Waples, R. S. 1998a. Evolutionarily significant units, distinct population segments, and the endangered species act: Reply to Pennock and Dimmick. 12:718-721.

Waples, R. S. 1998b. Separating the wheat from the chaff: Patterns of genetic differentiation in high gene flow species. Journal of Heredity 89:438-450.

Weir, B. S., and C. C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. Evolution 38: 1358-1370.

Whitlock, M. C., and D. E. McCauley. 1999. Indirect measures of gene flow and migration: F-ST not equal 1/(4Nm+1). Heredity 82:117-125.

Woodbury, A.M. and R. Hardy. 1948. Studies of the desert tortoise, Gopherus agassizii. Ecological Monographs 18: 145-200.

Wright. S. 1943. Isolation by distance. Genetics 28: 114-138.

TABLES

Table 1. Sampling locations based on geography (including the state and abbreviation for the site), the number of individuals from each location, and how samples were collected (STS = systematic transect sampling; LDS = Line distance sampling (random)). Each site is associated with a desert tortoise Recovery Unit, however, these delineations are only approximate due to sampling sites crossing Recovery Unit boundaries.

Recovery Unit	Sampling location	Abr.	State	Sample collection	Sample size
Upper Virgin River	Red Cliffs Desert Reserve	RC	UT	STS	33
Northeastern Mojave	Beaver Dam Slope	BD	UT, NV	LDS, STS	12
	Mormon Mesa	MM	NV	LDS, STS	43
	Gold Butte-Pakoon Basin	GB	NV, AZ	LDS, STS	17
	Coyote Springs	CS	NV	LDS, STS	26
	Muddy Mountains	MD	NV	STS	30
	Northeast Las Vegas Valley	NEL	NV	STS	20
	Northwest Las Vegas Valley	NWL	NV	STS	21
NA	Pahrump Valley	PA	NV	STS	27
NA	Amargosa Desert, Oasis Valley, Greenwater Valley	AM	NV, CA	STS	18
	Southwest Las Vegas Valley	SWL	NV	STS	28
	South I-15 Corridor (Goodsprings, Jean Dry Lake, Sloan)	SI	NV	STS	29
	Southeast Las Vegas Valley (River Mountains)	SEL	NV	STS	12
	Eldorado Valley	EL	NV	LDS, STS	49
Eastern Mojave	Piute Valley	PI	NV	LDS, STS	80
	Ivanpah Valley	IV	CA	LDS, STS	16
	Shadow Valley	SV	CA	STS	17
	East Providence Mountains	EP	CA	LDS, STS	38
	West Providence Mountains	WP	CA	LDS, STS	14
Northern Colorado	Chemehuevi DWMA	CM	CA	LDS	59
Eastern Colorado	Chuckwalla DWMA	CK	CA	LDS	56
Eastern Colorado/West Mojave	Pinto Mountains DWMA/Joshua Tree NP	PM	CA	LDS	25
Western Mojave	Ord-Rodman DWMA	OR	CA	LDS	14
	Superior-Cronese DWMA	SC	CA	LDS, STS	45
	Fremont-Kramer DWMA	FK	CA	LDS	19
	TOTAL				748

Table 2. Mean gene diversity (± 1 standard deviation), mean allelic richness (± 1 standard deviation), and F_{IS} (significant values after Bonferroni correction of P < 0.0001 are in bold) for each sampling location.

Location	Gene diversity (±) SD		Allelic Richness (±) SD		F_{IS}
RC	0.712	0.207	6.413	2.668	0.072
BD	0.656	0.263	5.568	2.644	0.079
MM	0.687	0.238	6.114	2.737	0.011
GB	0.643	0.279	5.593	2.624	**0.142**
MD	0.750	0.241	7.357	3.380	**0.075**
CS	0.723	0.235	7.078	3.445	0.061
NEL	0.744	0.267	7.416	3.423	-0.003
NWL	0.756	0.215	7.589	3.197	0.061
AM	0.742	0.215	6.999	3.156	0.036
PA	0.765	0.215	7.499	3.199	0.059
SH	0.768	0.188	7.253	3.149	0.051
IV	0.788	0.206	7.655	3.182	0.039
WP	0.780	0.195	7.970	3.515	0.027
SI	0.786	0.169	7.442	3.022	0.035
SWL	0.780	0.209	7.993	3.816	0.038
SEL	0.799	0.173	7.606	3.105	0.047
EL	0.780	0.198	7.406	3.041	**0.069**
PI	0.779	0.209	7.920	3.172	**0.061**
CM	0.739	0.232	7.517	3.345	**0.058**
EP	0.746	0.222	7.556	3.204	**0.06**
CK	0.721	0.253	7.078	3.359	0.044
PM	0.724	0.257	7.288	3.574	0.056
OR	0.737	0.239	7.048	3.392	0.072
SC	0.725	0.234	7.024	3.423	0.026
FK	0.721	0.237	6.916	3.047	0.098
Overall	0.742	0.040	8.352	3.354	0.053

Table 3. Mean *Ln P(D)* (± 1 standard deviation) and the second order rate of change calculations for ΔK when K was fixed to K = 1 through K = 10 in STRUCTURE.

K	Mean *Ln P(D)*	± SD *Ln P(D)*	Mean *L'(K)*	Mean *L''(K)*	*ΔK*	α
1	-64113.	8.37				
2	-60625.	1.39	3487.1	2572.35	**1845.73**	0.187
3	-59918.	2.73	707.06	557.65	**204.28**	0.078
4	-59769.	578.13	149.41	523.2	0.91	0.054
5	-59242.	4.33	527.45	309.63	71.46	0.049
6	-59011	72.55	230.66	144.23	1.99	0.046
7	-58776	77.10	234.65	158.84	2.06	0.045
8	-58595	20.94	180.75	83.62	3.99	0.043
9	-58482	6.79	113.59	98.23	14.46	0.041
10	-58461	20.44	21.94			0.041

Table 4. Mean *Ln P(D)* and ΔK for each of the three basal clusters in STRUCTURE. These additional analyses were used to detect hierarchical clustering within the Mojave population of the desert tortoise. * indicates the K with the highest mean *Ln P(D)* and Δ*K*

Basal cluster	K	Mean *Ln P(D)*	Δ*K*	Description of hierarchical clusters
Northern Mojave	1	-13456.4		The Northern Mojave was divided into two clusters. Cluster 1 (Virgin River) contained RC, BD, MM. Cluster 2 (Muddy Mountains) contained GB, MD, CS, and NEL.
	2*	**-13191.9**	**16.9**	
	3	-13219.1	9.0	
	4	-13359.0	7.3	
	5	-14842.2	4.2	
	6	-14295.2	2.6	
	7	-14497.5	1.7	
Las Vegas	1	-17997.5		The Las Vegas cluster was divided into three clusters. Cluster 1 (Amargosa Desert) contained AM, PA, and SH. Cluster 2 (South Las Vegas) contained NWL, IV, SI, SWL. Cluster 3 (Eldorado) contained El and SEL.
	2	-17925.7	1.8	
	3*	**-17807.7**	**43.1**	
	4	-18187.4	3.2	
	5	-18006.1	2.9	
	6	-18235.4	2.5	
	7	-18905.2	2.4	
	8	-19387.3	1.6	
	9	-19454.3	2.1	
	10	-19686.9	1.4	
California	1	-28618.9		The California cluster was divided into four additional clusters. Cluster 1 Piute Valley) contained PI and WP. Cluster 2 (Northern Colorado) contained CM and EP. Cluster 3 (Eastern Colorado) contained CK and PM. Cluster 4 (Western Mojave contained OR, SC, and FK.
	2	-28184.7	44.7	
	3	-27885.3	38.1	
	4*	**-27683.7**	**55.7**	
	5	-27753.1	19.1	
	6	-28166.4	2.0	
	7	-28822.3	2.2	
	8	-28965.1	1.7	

Table 5. Log of the posterior density of the model for 10 independent runs of GENELAND. The modal K is the optimal number of genetic clusters for desert tortoises across 500,000 iterations of the model.

Run number	Log Posterior Density	Modal K
1	-57516	3
2	-57954	4
3	-58033	4
4	-57934	4
5	-57643	3
6	-57960	4
7	-57963	4
8	-57958	4
9	-57950	4
10	-57954	4

Table 6. Analysis of molecular variance for 3 genetic clusters as determined via STRUCTURE. The percentage of variation explained by each source was similar for K=3, K=4, K=7, and K=9. The signficance test included 1023 permutations of the data.

Source of Variation	df	Sum of Squares	Variance components	Percentage variation	Significance test
Among groups	2	417.22	0.389	4.94	0.00
Among populations within groups	22	445.61	0.218	2.78	0.0
Among individuals within populations	723	5468.89	0.300	3.82	0.0
Within populations	748	5208.5	6.96	88.46	0.0
Total	1495	11540.22	7.87		

Table 7. Pair-wise F_{ST} values for the 9 inferred genetic clusters. All values were significant using an adjusted P value (P < 0.00139) after 720 permutations. Cluster IDs are: VR = Virgin River; MD = Muddy Mountains; AM = Amargosa Desert; SLV = South Las Vegas Valley; EL = Eldorado Valley; PI = Piute Valley; NCO = Northern Colorado Desert; ECO = Eastern Colorado Desert; WM = Western Mojave Desert.

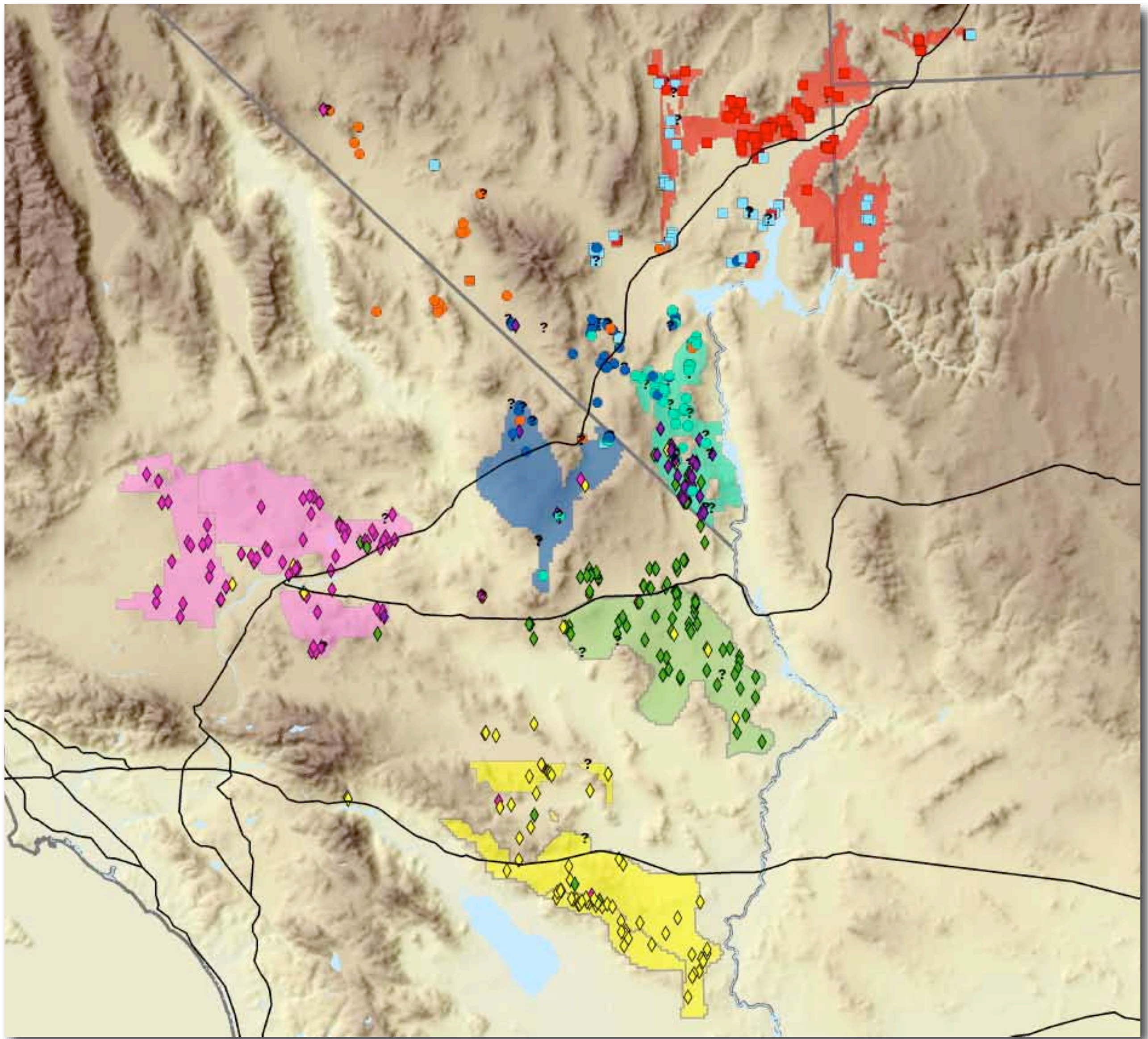
	VR	**MD**	**AM**	**SLV**	**EL**	**PI**	**NCO**	**ECO**	**WM**
VR	-								
MD	0.025	-							
AM	0.044	0.016	-						
SLV	0.048	0.019	0.012	-					
EL	0.067	0.038	0.023	0.014	-				
PI	0.087	0.057	0.041	0.029	0.020	-			
NCO	0.114	0.082	0.062	0.051	0.040	0.011	-		
ECO	0.132	0.097	0.086	0.066	0.057	0.028	0.026	-	
WM	0.125	0.082	0.071	0.057	0.052	0.032	0.032	0.031	-

Table 8. Number of assignments to one of the inferred genetic clusters when K = 9. Cluster IDs are: VR = Virgin River; MD = Muddy Mountains; AM = Amargosa Desert; SLV = South Las Vegas Valley; EL = Eldorado Valley; PI = Piute Valley; NCO = Northern Colorado Desert; ECO = Eastern Colorado Desert; WM = Western Mojave Desert.

	VR	**MD**	**AM**	**SLV**	**EL**	**PI**	**NCO**	**ECO**	**WM**	**Percent Assignment**
VR	92	9	0	2	2	0	0	0	0	87.6
MD	10	58	3	4	1	0	0	0	0	76.3
AM	0	7	57	12	3	4	0	0	0	68.7
SLV	1	3	14	55	11	1	0	0	0	64.7
EL	0	0	2	4	40	3	0	0	0	81.6
PI	0	0	1	1	7	59	8	3	1	73.8
NCO	0	0	1	3	5	7	78	9	8	70.3
ECO	0	0	0	0	0	2	5	68	5	85.0
WM	0	0	0	0	0	0	2	4	73	92.4

FIGURES

Figure 1. Map of subpopulations for the Mojave population of the desert tortoise. Each point indicates each location where a blood sample was collected. The marker type indicates the three basal clusters (square = Northern Mojave, circle = Las Vegas, diamond = California). The color of the marker further indicates substructuring (Virgin River = red, Muddy Mountains = light blue, Amargosa Desert = orange, South Las Vegas = dark blue, Eldorado Valley = teal, Piute Valley = purple, Northern Colorado = green, Eastern Colorado = yellow, Western Mojave = pink). The shaded polygons lines designate Desert Wildlife Management Areas, which indicate where active management and conservation is occurring, and each color corresponds to the genetic subpopulation.



Figures 2. Results from Program STRUCTURE using 20 microsatellites and 748 individuals from 25 sampling locations. (a) Number of genetic clusters based the mean *ln P(D)* (red circles) and ΔK (blue squares and dotted line) using K=1 and K=2 as a starting point for 10 separate MCMC chains with fixed K from K=1 to K=10; (b) Representative bar plot for K=3. Bar plots indicate proportional membership of each individual to one (or more than one) genetic cluster.

Fig 2a

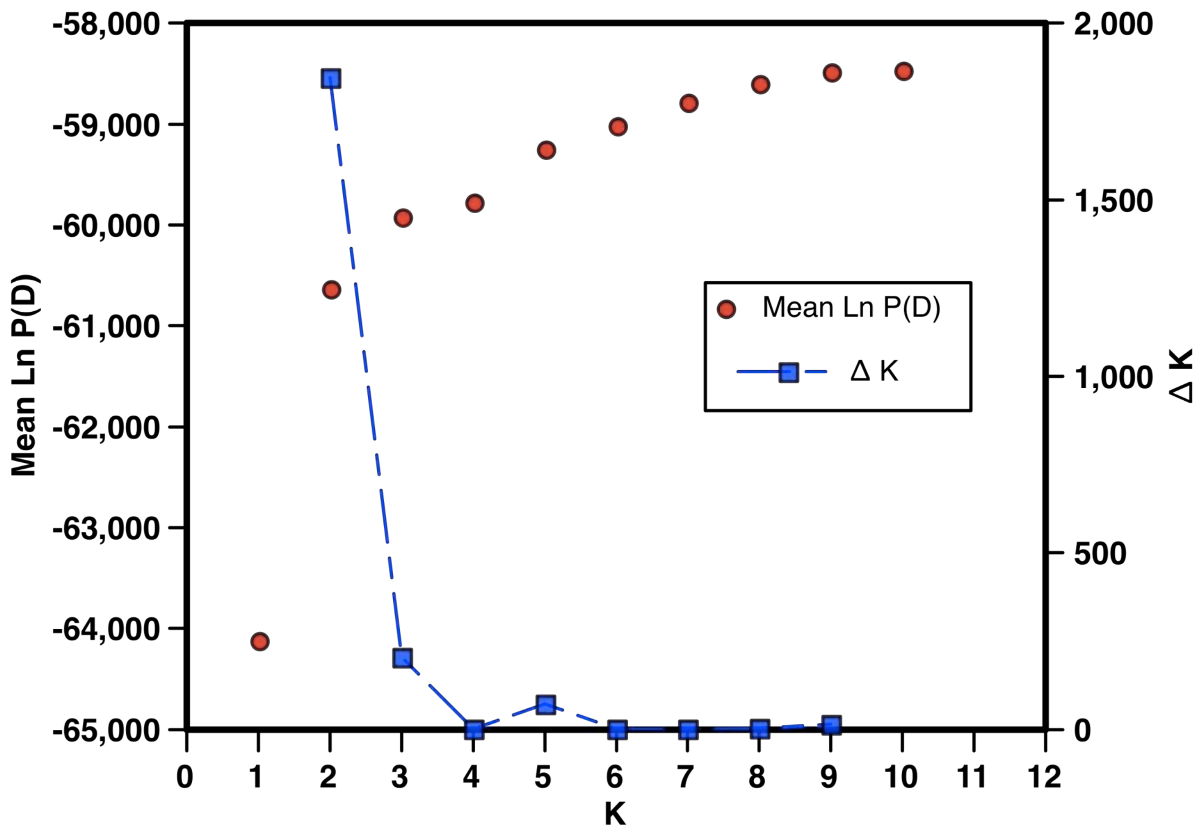


Figure 2b.

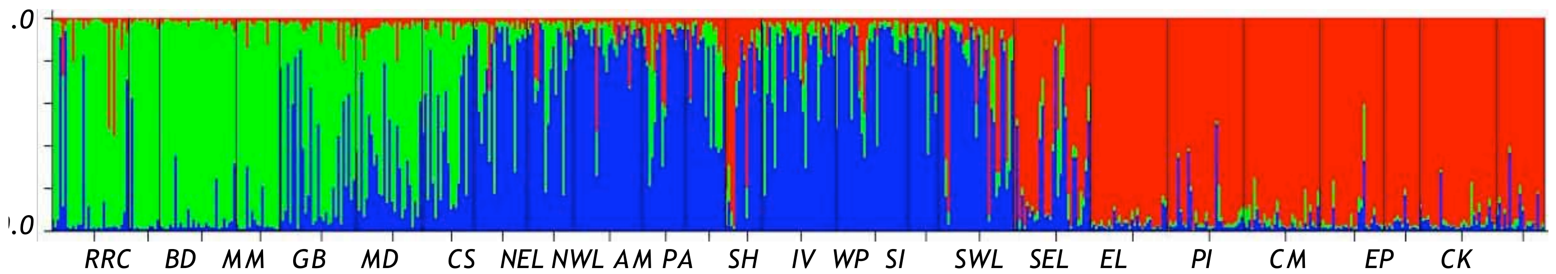


Figure 3. Nine genetic clusters identified with Program STRUCTURE. Mean proportional membership (± 1 standard deviation) to 9 genetic clusters (K=9)

Figure 4. Mean proportional membership (± 1 standard deviation) to 6 genetic clusters identified using STRUCTURE when each sampling location has n ≤ 30.

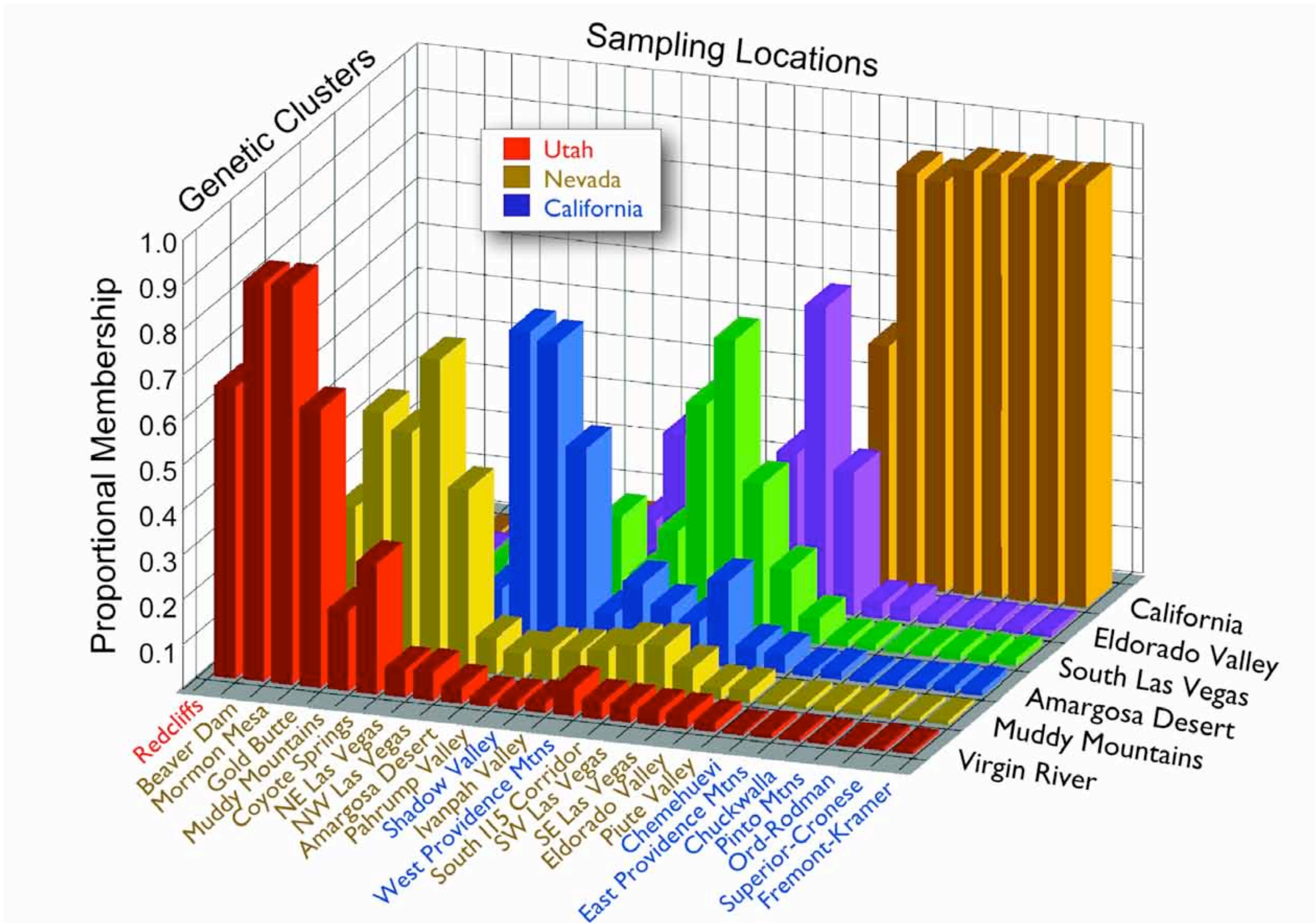


Figure 5. Representative bar plots for hierarchical structuring with a reduced data set. (a) Las Vegas Cluster, (b) California cluster.

Fig. 5a

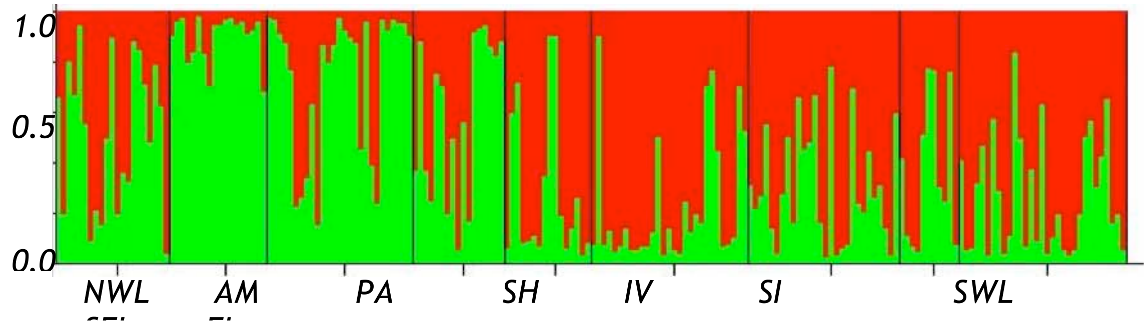


Fig 5b.

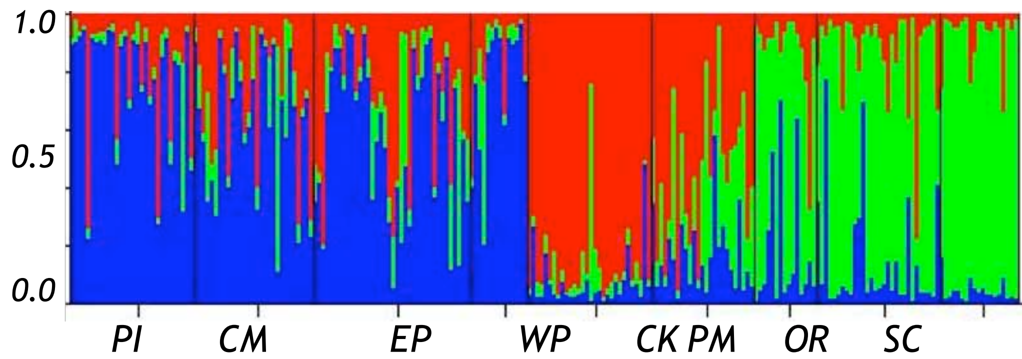


Figure 6. Map of posterior probability of membership to four genetic clusters identified using GENELAND. Each point corresponds to a desert tortoise location. Lighter shading represents higher probability of membership. (a) Western Mojave Desert cluster (WM); (b) Virgin River cluster (VR); (c) Las Vegas cluster (LV); and (d) Colorado Desert cluster (CO).

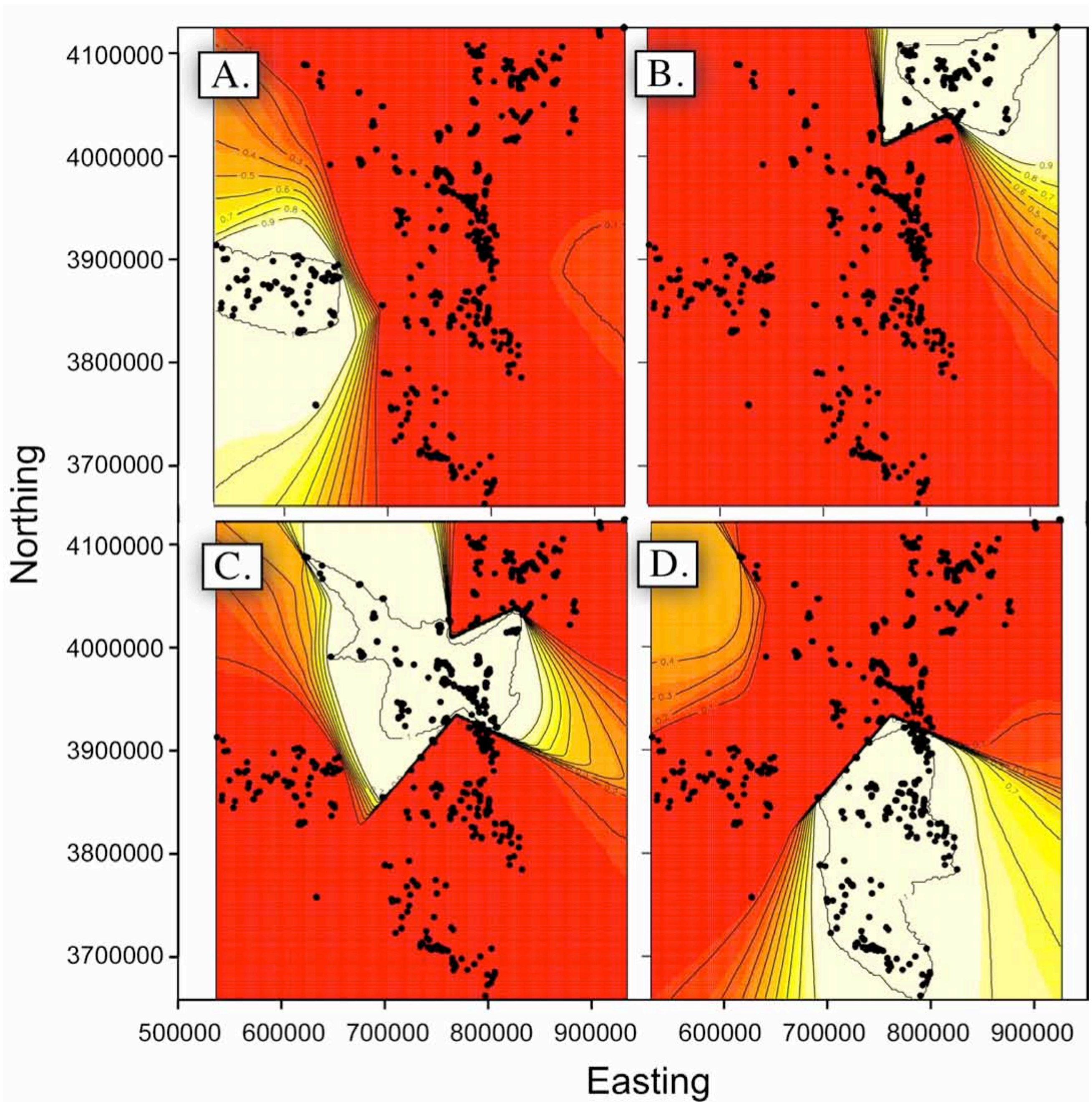


Figure 7. Isolation by distance in populations of the desert tortoise across the Mojave Desert. Points represent comparisons of genetic distance ($F_{ST}/1-F_{ST}$) as a function of geographic distance between centroids for sampling locations ($R^2 = 0.6783$, $P < 0.0001$).