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# Refining genetic boundaries for Agassiz's desert tortoise (*Gopherus agassizii*) in the western Sonoran Desert: the influence of the Coachella Valley on gene flow among populations in southern California

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## Abstract

Understanding the influence of geographic features on the evolutionary history and population structure of a species can assist wildlife managers in delimiting genetic units (GUs) for conservation and management. Landscape features including mountains, low elevation depressions, and even roads can influence connectivity and gene flow among Agassiz's desert tortoise (*Gopherus agassizii*) populations. Substantial changes in the landscape of the American Southwest occurred during the last six million years (including the formation of the Gulf of California and the lower Colorado River), which shaped the distribution and genetic structuring of tortoise populations. The area northwest of the Gulf of California is occupied by the Salton Trough, including the Coachella Valley at its northern end. Much of this area is below sea level and unsuitable as tortoise habitat, thus forming a potential barrier for gene flow. We assessed genetic relationships among three tortoise populations separated by the Coachella Valley. Two adjacent populations were on the east side of the valley in the foothills of the Cottonwood and Orocopia mountains separated by Interstate 10. The third population, Mesa, was located about 87 km away in the foothills of the San Bernardino Mountains at the far northwestern tip of the valley. The Cottonwood and Orocopia localities showed genetic affiliation with the adjacent Colorado Desert GU immediately to the east, and the Mesa population exhibited affiliation with both the Southern Mojave and Colorado Desert GUs, despite having a greater geographic distance (0.5x–1.5x greater) to the Colorado Desert GU. The genetic affiliation with the Colorado Desert GU suggests that the boundary for that GU needs to be substantially extended to the west to include the desert tortoise populations around the Coachella Valley. Their inclusion in the Colorado Desert GU may benefit these often overlooked populations when recovery actions are considered.

## Highlights

- The complex geologic and climatic history of the Desert Southwest region of the United States during the last 6 million years shaped the past and present distribution, diversity, and evolution of plants and animals.
- Gene flow and connectivity among populations of Agassiz's desert tortoises, a conservation-reliant species, are influenced by landscape features including mountains, low-elevation depressions, and even roads.
- Sub-sea level areas northwest of the Gulf of California occupied by the Salton Trough and the Coachella Valley are unsuitable as tortoise habitat, thus forming a potential barrier for gene flow.
- Comparison of tortoise populations separated by the northern Coachella Valley revealed genetic linkages with the Colorado Desert (part of the Sonoran Desert) genetic unit, despite the presence of the low elevation barrier formed by the valley.
- Genetic connectivity is postulated to be through the mountains at the north end of the valley, and the Colorado Desert genetic unit should be extended to the west to reflect this connectivity.

## Keywords:

Agassiz's desert tortoise, Barrier, Conservation, Genetic Boundaries, Genetic Unit, *Gopherus* biogeography, Gulf of California, Salton Trough

## Introduction

The Desert Southwest region of the United States has a complex geologic and climatic history that shaped the past and present distribution, diversity, and evolution of plants and animals. Biological patterns in the region are a reflection of the effects of processes driven by tectonic, volcanic, glacial-interglacial cycling, and shorter-term climatic events across widely varying time scales (Dolby et al. 2015). Situated at the tectonic and zoologically important interface of the North American and Pacific Plates (Atwater 1970, Gottscho 2016), the Desert Southwest has been profoundly shaped and influenced by two major geological processes in the last 6 million years: the formation of the Gulf of California (Dolby et al. 2015) and the evolution of the lower Colorado River (Howard et al. 2019), both of which presented aquatic barriers to some plants and animals. Disentangling the effects of those and other geological processes on the evolution of the biota of the region is an ongoing topic of interest to researchers (Dolby et al. 2015, 2019).

Vicariant events in the region caused by these and other geographic barriers to dispersal (e.g., high mountains and deep valleys) play important roles in the genetic structuring of species. Genetic analyses that assess gene flow, or the lack thereof, can serve to identify barriers to dispersal. Syntheses of geographic and genetic analyses can objectively identify critical management units for conservation, defined as genetic units (GUs; Wayne and Morin 2004). As such, genetic analyses are essential for the effective management of species, especially those that are threatened or endangered.

Agassiz's desert tortoise (*Gopherus agassizii* Cooper) is a threatened species with a wide range involving large portions of both the Mojave and Sonoran deserts of Arizona, California, Nevada, and Utah (Luckenbach 1982, Germano et al. 1994). Desert tortoises are obligate herbivores that avoid some of the harshest conditions of their arid environment by constructing deep burrows and spending the majority of their annual cycle sheltering within. With delayed maturity, a long lifespan, and relatively low egg production, they are very sensitive to additive mortality from human activities and subsidized predators (see reviews in Ernst and Lovich 2009, Berry and Murphy 2019). Despite their wide distribution, declines in the densities of tortoises led to the species being listed as threatened under the U.S. Endangered Species Act in 1990. Declines have continued since then (Allison and Mcluckie 2018), despite significant efforts to recover populations (Averill-Murray et al. 2012).

An important part of the recovery plan for the desert tortoise, developed by the U.S. Fish and Wildlife Service (USFWS 2011), is maintaining and managing for genetic variability and linkages among tortoise populations. Linkages are necessary to offset the negative effects of human-caused barriers in the desert, including roads (Latch et al. 2011) and utility-scale wind and solar energy developments (Lovich and Ennen 2011, 2013). Genetic variability of populations of *G. agassizii* is largely a reflection of the

effects of natural geographic barriers on movements (Britten et al. 1997, Murphy et al. 2007, Gaillard et al. 2017). For *G. agassizii*, significant impediments to dispersal and habitation include high mountain ranges and extremely low elevation areas with high summer temperatures (Hagerty et al. 2011), both of which are inhospitable to tortoises. In the Mojave Desert, the latter includes Eureka, Saline, and Death valleys. Similarly, tortoises in the Sonoran Desert of California (including the Colorado Desert subdivision) are all but absent from the low elevation Salton Trough, including the Coachella Valley (Dimmitt 1977, Luckenbach 1982, Berry and Murphy 2019), Cadiz valley, and the lower Colorado River region (Morafka and Berry 2002). Agassiz's desert tortoises tend to prefer valley bottoms and bajadas at moderate elevations (e.g., 300–920 m; Luckenbach 1982) as habitat. Thus, mountain ranges over about 2,000 m, like the New York (2,296 m) and Providence (2,183 m) mountains, are barriers to gene flow (Hagerty and Tracy 2010). At the local scale, variables such as slope and roads may influence tortoise movements and, thus, gene flow (Latch et al. 2011). Not surprisingly, geographic distance also influences gene flow (Murphy et al. 2007, Hagerty and Tracy 2010, Averill-Murray and Hagerty 2014).

Although GUs have been defined for *G. agassizii* (Murphy et al. 2007, Sánchez-Ramírez et al. 2018), these studies did not include sampling from near the southwestern limits of the species range (e.g., populations surrounding the Coachella Valley; Berry and Murphy 2019). The low density or absence of tortoises in much of the area surrounding the Coachella Valley is due to the lack of suitable habitat in such low elevation areas (much of which is below sea level) and environmental conditions that are physiologically challenging for sustaining *G. agassizii* populations (reviewed by Ernst and Lovich 2009, Berry and Murphy 2019). The valley is one of the driest and hottest parts of the Sonoran Desert (Barrows et al. 2010). Its short flowering season for winter annual plants challenges survival of the herbivorous tortoise (Morafka and Berry 2002). Further, large areas of the Coachella Valley are affected by urban development and agriculture (Beatley 1992), rendering it even less suitable for desert tortoises and other wildlife.

Because conditions in the Coachella Valley are not suitable for tortoises, we hypothesize that it acts a barrier to gene flow for disjunct populations of tortoises that occur on the upland slopes of various mountains and bajadas surrounding the valley, including the Chocolate, Orocopa, Cottonwood, Little San Bernardino, San Bernardino, San Jacinto, and Santa Rosa mountains (e.g., Lovich et al. 2015, 2018a; Berry and Murphy 2019). Comparing the genetic composition of three tortoise populations separated by the Coachella Valley allows a preliminary test of our hypothesis. It is important to note that inferring the natural distribution of tortoises today is complicated by intentional introductions by humans (Murphy et al. 2007, Edwards et al. 2010, Edwards and Berry 2013). For example, tortoises are known to have been released in Anza Borrego Desert State Park in the uplands on



the west side of the Salton Trough between 1971 and 1972 (Luckenbach 1982; J. Manning, Washington State University, personal communication), far from our the study sites. In addition, possible releases by Native American tribes that used tortoises for food or other cultural purposes (Schneider and Everson 1989) likely occurred throughout the range of the species. The success of those and other introductions depends on the ability of translocated tortoises to survive under local conditions, something that is not assured (Germano and Bishop 2008) and that varies among individuals (Germano et al. 2017).

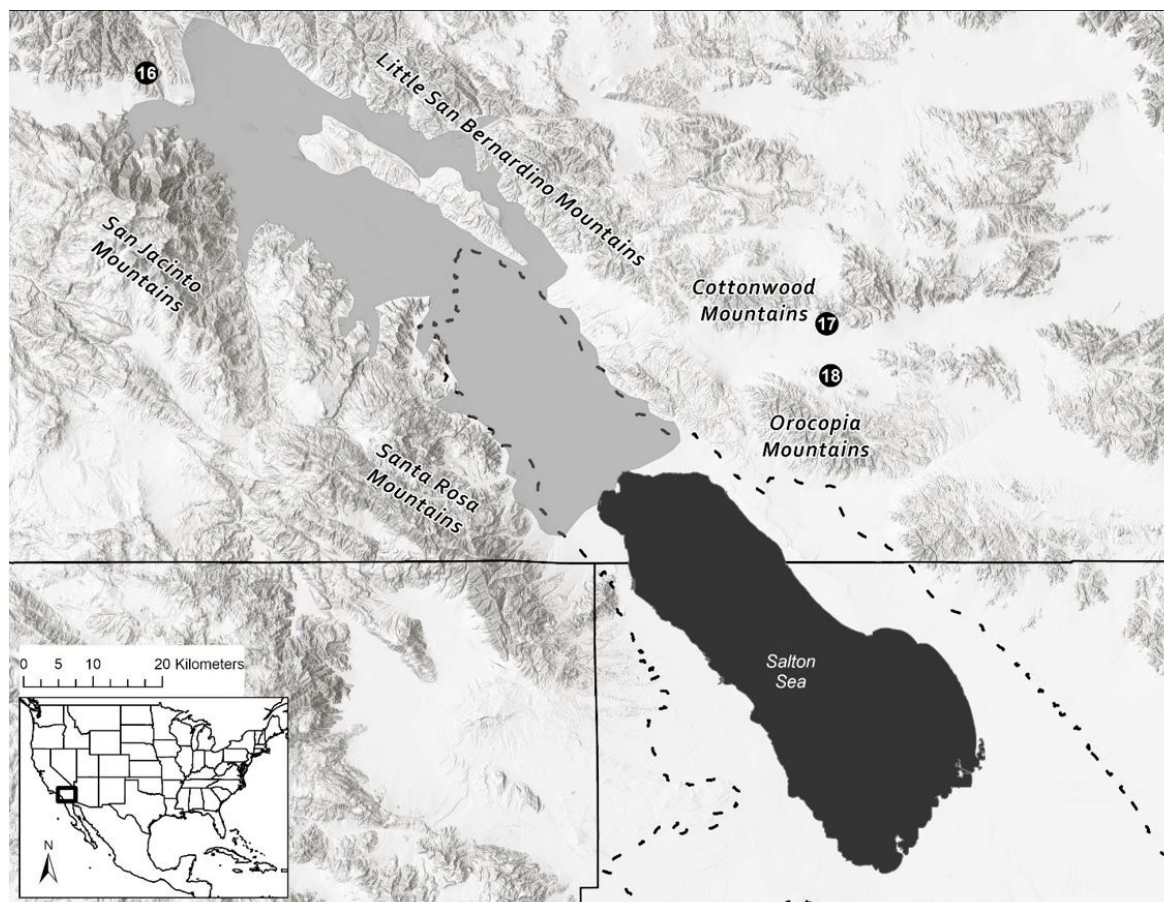
Herein, we provide the first genetic assessment of tortoise populations surrounding the Coachella Valley. The first two sampled populations were east of the southern portion of the valley and a third was located about 85 km away at the northwestern tip of the valley. If the Coachella Valley is a barrier to tortoise dispersal and gene flow, genetic distances between sampled populations should be greater

than the genetic distances these populations have to adjacent tortoise populations outside of the valley to the east or northwest. Analyses integrate data from 24 short tandem repeats (STRs; microsatellites) from these samples with homologous information from Murphy et al. (2007).

## Methods and Materials

### Study area

The Salton Trough is the northern extension of the basin occupied by the Gulf of California. It is a pull-apart basin caused by subsidence from oblique extension across strike-slip faults (Brothers et al. 2009). Extending for about 225 km, the Salton Trough in California includes (from north to south) the Coachella Valley, Salton Sea, Imperial Valley, and in Mexico the Colorado River Delta (Fig. 1). The terrestrial portion of the trough extends northwest from the Gulf of California to the San Gorgonio Pass, near Palm Springs,



**Figure 1.** Map showing Agassiz's desert tortoise sampling sites around the Coachella Valley (shaded in gray) in California, USA, the northern extension of the Salton Trough. Study sites are numbered for consistency with other figures and tables: 16=Mesa, 17=Cottonwood, 18=Orocopia. The northwestern part of the Coachella Valley between the label for study site 16 and the San Jacinto Mountains is the San Gorgonio Pass. The light-colored area around and south of the Salton Sea is a continuation of the terrestrial portion of the Salton Trough before it ends at the Gulf of California in Mexico. County lines are shown for reference. The horizontal line is the southern boundary of Riverside County and the vertical line separates San Diego (left) from Imperial counties (right). Dashed line demarcates the approximate high stand of Lake Cahuilla at approximately 12 m ASL during the Pleistocene obtained from <https://www.arcgis.com/home/item.html?id=ac2b6de1149047b9af934acd4d01fdca>

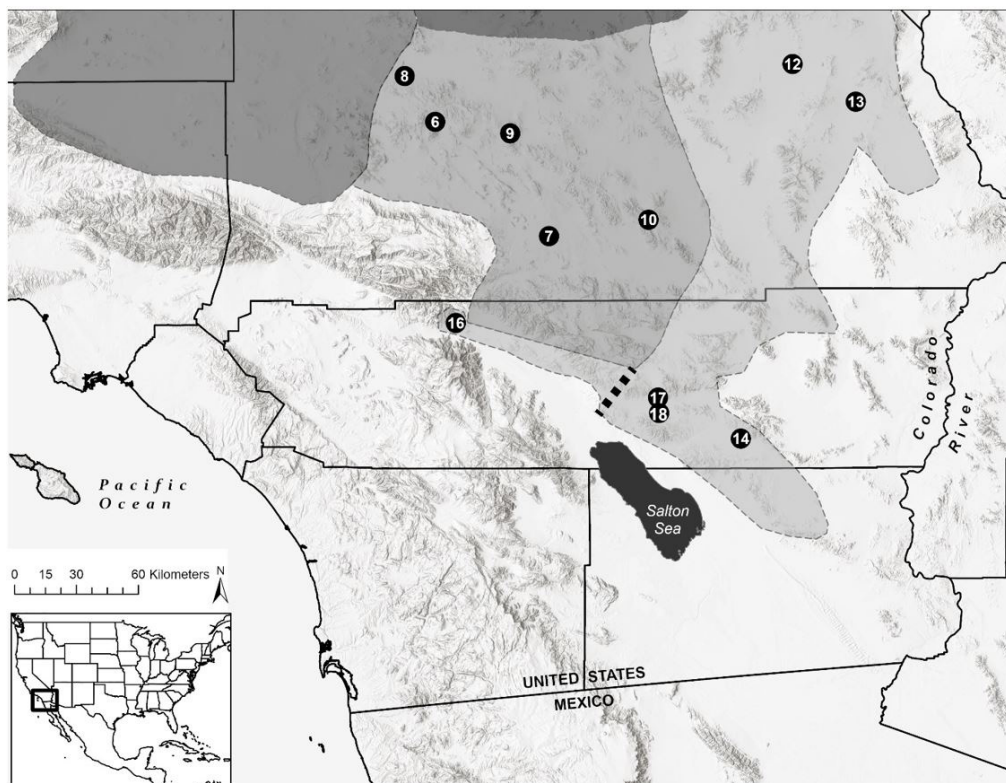
California. At its lowest point in the Salton Sink, the depression is about 85 m below sea level, although some of that area is now inundated by the Salton Sea with a surface elevation of about 71 meters below sea level. Approximately 5,400 km<sup>2</sup> of the trough (including the Eastern Coachella Valley) is below sea level, and it is surrounded by mountains on all sides except in the south near the Gulf of California (Waters 1983). The Coachella Valley covers about 777 km<sup>2</sup> in the northernmost portion of the Salton Trough (Beatley 1992). As mentioned earlier, the low elevation habitats of the Salton Trough, including the Coachella Valley, are unsuitable as tortoise habitat (Nussear et al. 2009) and expected to be barriers to dispersal and gene flow.

The Mesa study site, near Palm Springs, California, is located in the foothills (600–900 m) of the San Bernardino Mountains just above the northwestern tip of the Coachella Valley, on the extreme western edge of the Sonoran Desert ecosystem. The site has been extensively developed for wind energy production (Lovich et al. 2011). Vegetation at the site is a combination of coastal, montane, and desert plant species in a fire-prone landscape (Lovich et al. 2018b). Additional details of the study site and a history of research conducted there are presented by Lovich and Daniels (2000), Agha et al. (2015), and Lovich and Ennen (2017).

The other two study sites (both near Chiriaco Summit, California) are located in the uplands about 28 km due east of the edge of the Coachella Valley floor and about 85 km southeast of Mesa. The Cottonwood study site is located in the southern part of Joshua Tree National Park along the base of the Cottonwood Mountains. The site is characterized by sloping bajadas and desert washes originating in the steep foothills (520–780 m) of the Cottonwood Mountains to the north. Further details of the vegetation in the area are provided by Lovich et al. (2018a). The Orocopia study site is relatively flat, with elevations (480–620m) increasing to the south and east, and a gentle uphill grade to the Orocopia Mountains to the south. This site is characterized by creosote scrub vegetation (*Larrea tridentata* Coville) with widely-scattered ocotillos (*Fouquieria splendens* Engelm.) and blue palo verde trees (*Parkinsonia florida* Watson). The surface is characterized by areas of desert pavement (Wood et al. 2005) interspersed with sandy to gravelly soil and numerous tank and jeep track scars from World War II training activities (Prose 1985) associated with nearby Camp Young in the early 1940's.

**Field techniques**

From 2000 to 2018, we collected DNA samples from Agassiz's desert tortoises at sites that are outside but hydrologically part of the Coachella Valley (Fig. 2).



**Figure 2.** Map showing centroids of locations of groups listed in Table 1. Group 16 is the Mesa study site and Groups 17 and 18 are Cottonwood and Orocopia study sites, respectively. The other sites are listed in Table 1. Shaded areas with fine dashed line borders show the adjacent genetic units modified from Sánchez-Ramírez et al. (2018). From left to right they are: Western Mojave GU, Central Mojave GU (upper sliver), Southern Mojave GU (lower), and Colorado Desert GU. The southern boundary of the Colorado Desert GU is further modified from Sánchez-Ramírez based on our data supporting an extension (left of the bold dashed line) of the GU going northwest from Groups 17 and 18 to Group 16.



We used the subcarapacial venipuncture technique (Hernandez-Divers et al. 2002, Drake et al. 2012) to collect blood and lymph from tortoises, obtaining up to 0.5 ml samples from each adult tortoise using a 23-gauge needle irrigated with sodium heparin. For samples from hatchling tortoises at the Mesa site collected in 2000, we clipped small amounts of keratinous scute tissue from the marginal scutes as part of our individual marking system (Cagle 1939).

### Laboratory Processing

A total of 55 samples from our three study sites around the Coachella Valley were processed at the University of Arizona Genetics Core (UAGC), Tucson, AZ, USA. Of these, 31 samples based on scute tissue were received from the University of Southern Mississippi as extracted DNA, and 24 blood samples were extracted and processed at UAGC. Usable DNA was obtained from 53 of the 55 samples.

We mixed whole blood with lysis buffer and incubated overnight with proteinase K at 55°C, followed by robotic extraction using a QIAGEN BioSprint 96 robotic magnetic-particle purification system (Qiagen; Valencia, California, USA) and Aline Biosciences Buccal Swab gDNA Kit (Aline Biosciences; Woburn, Massachusetts, USA). Scute samples were extracted using a Qiagen DNeasy tissue kit. We quantified recovered DNA using a BioTEK Synergy HT (BioTEK; Vermont, USA). We analyzed an approximately 1,100 base pair portion of mitochondrial *ND3/ND4* for samples from Orocochia and Mesa to establish a baseline for the populations and to help identify native vs. translocated individuals (Edwards and Berry 2013). We genotyped all samples for 24 previously described short tandem repeats (STRs) following the methods of Edwards and Berry (2013). Fragment analysis and DNA sequencing were performed following standard procedures by UAGC.

We scored the STR fragment data using Genemarker v.1.85 (SoftGenetics; State College, Pennsylvania, USA). We aligned mtDNA sequences using CLC DNA Workbench v.5.7.1 (CLC Bio; Denmark) to established reference haplotypes in Genbank (Murphy et al. 2007).

### Descriptive statistics

We calculated diversity indices for each population based on polymorphic STR loci. We used Arlequin (v. 3.5.1.2; Excoffier and Lischer 2010) to detect significant departures from Hardy-Weinberg expectations and FSTAT v.2.9.3.2 (Goudet 1995) to generate estimates of gene diversity and allelic richness per locus. We used GENEPOP v.4.2 (Raymond 1995, Rousset 2008) to estimate inbreeding coefficients ( $F_{IS}$ ; Weir and Cockerham 1984). We used default parameters in FSTAT, ARLEQUIN, and GENEPOP for all Markov-chain tests and permutations.

### Population analyses

We compared data from our three study sites (n=53 samples) around the Coachella Valley (described above) to a reference database of 709 samples of *G. agassizii* collected from throughout the species' range (Murphy et al. 2007) as updated by Edwards and Berry (2013) for additional loci that match our 24 STR loci. The samples in these previous analyses clustered into 15 groups (our sites 17 and 18 combined into one new group for this analysis) according to Murphy et al. (2007), which we assigned to 5 genetic units (GUs) based on the results of Sánchez-Ramírez et al. (2018) (Table 1). We generated an  $F_{ST}$  matrix among all groups using ARLEQUIN. Relationships between genetic and geographic distances were considered qualitatively only because geographic features affected linear travel by tortoises (Dutcher et al. 2020). Thus, relatedness among populations relied on assignment tests using

**Table 1.** Desert tortoise study sites and groups modified from Murphy et al. 2007, including our three study sites around the Coachella Valley (sites 16, 17, 18). Sites 17 and 18 were combined into one group due to their proximity. All are grouped into 5 genetic units for *G. agassizii* as described in Sánchez-Ramírez et al. (2018). Modified sample set for Southern Clade California samples excludes Northern Clade groups 11, 12 and 15. Abbreviations are as follows: NTC = National Training Center, MCAGCC = Marine Corp Air Ground Combat Center, CMAGR = Chocolate Mountain Aerial Gunnery Range.

Genetic Unit	Study Site	# of Samples	Group	# of Samples in Group
Western Mojave	Desert Tortoise Natural Area	56	1	60
	Fremont-Valley	4	1	
	Hinkley	12	2	83
	Kramer	3	2	
	Edwards Air Force Base	57	2	
	Fremont-Kramer	11	2	
Central Mojave	Superior-Cronese	10	3	52
	Fort Irwin (Goldstone)	9	3	
	Fort Irwin (NTC)	33	3	
	Fort Irwin (Tiefort)	31	4	31
	Fort Irwin (Control Site)	33	5	46
	Fort Irwin (Eastgate 2)	13	5	

Table 1. Continued...

Genetic Unit	Study Site	# of Samples	Group	# of Samples in Group
Southern Mojave	Lucerne Valley	11	6	25
	Ord-Rodman	14	6	
	MCAGCC (Emerson)	9	7	70
	MCAGCC (Sand Hill)	61	7	
	Daggett	72	8	72
	MCAGCC (Lavic Lake)	8	9	27
	MCAGCC (Maumee Mine)	7	9	
	MCAGCC (Sunshine Park)	12	9	
	MCAGCC (Bullion)	16	10	19
	MCAGCC (Lava)	3	10	
Colorado Desert	Fenner	4	12	31
	Goffs	27	12	
	Chemhuevi	7	13	17
	Upper Ward Valley	10	13	
	Chuckwalla	17	14	36
	CMAGR	19	14	
Northern Mojave	Ivanpah	33	11	59
	Ivanpah (site 14)	23	11	
	Shadow Valley	3	11	
	Upper Virgin River. UT	28	15	28
<i>unassigned</i> (Coachella Valley)	Mesa	30	16	30
	Cottonwood	10	17	23
	Orocopia	13	18	

WHICHRUN (Ver. 4.1; Banks and Eichert 2000), which calculates the likelihood of a given individual originating from  $\geq 2$  candidate populations on the basis of its multilocus STR genotype. We assessed stringency of population allocation based on the log of the odds ratio (LOD) for the two most likely source populations. Assignments with a LOD ratio of  $\geq 2$  had a  $\leq 0.01$  chance of type I error.

We used STRUCTURE v.2.3.4 (Pritchard et al. 2000) to assess associations of the Coachella Valley samples with the database without prior population assignments. Analyses were run for  $K = 1-12$  with 10 trials per  $K$ , and each run for 500,000 iterations following a burn-in period of 50,000 MCMC iterations under the admixture model, assuming allele frequencies were correlated between populations. We used STRUCTURE HARVESTER Online (Earl and von Holdt 2012) to evaluate the results. Independent STRUCTURE runs were compiled and visualized using the Greedy  $K$  algorithm in CLUMPAK (Kopelman et al. 2015). We performed analyses two ways: 1) using all samples shown in Table 1, and 2) just the “Southern Clade”. The latter included all locations with mitochondrial “haplogroup” MOJ\_A, found throughout most of

California by Murphy et al. (2007), but excluded haplogroup MOJ\_B (groups 11, 12, 15 in Table 1) from the northeastern Mojave Desert.

## Results

For the three study sites around the Coachella Valley, 34 individuals were sequenced for mtDNA, including 9 from Orocopia and 25 from Mesa; all individuals had haplotype MOJ\_A01 (identified by Murphy et al. 2007 as a widely distributed haplotype of haplogroup MOJ\_A in California). STRs genotypes were obtained for 53 individuals, including 30 from Mesa, 10 from Cottonwood, and 13 from Orocopia. All populations exhibited heterozygosity consistent with each other (Table 2) and other reported populations of *G. agassizii* (Edwards and Harrison 2014). Allelic richness and gene diversity were relatively low, but this was not unexpected for small populations that are relatively isolated from a geographic perspective (Table 2).

Data for populations at the nearby study sites Cottonwood and Orocopia were combined, and they were most similar (Table 3) to their closest Colorado Desert GU (Group 14; Chuckwalla and CMAGR) in our comparison with the reference database. The Mesa

population was equidistant in relatedness between locations in the Southern Mojave and Colorado Desert GUs, despite having a greater geographic distance (0.5x–1.5x greater) to the former locations. Assignment tests associated most (21/23) Cottonwood and Orocopia individuals to the Colorado Desert GU, whereas individuals from Mesa were split between the Colorado Desert GU and the Southern Mojave GU, and two individuals were assigned further west to the Western Mojave GU (Table 4).

When the Coachella Valley samples were analyzed against the Southern Clade using STRUCTURE

(Figs. 3–4), the best fit was  $K = 5$ , when evaluated using the DeltaK method of Evanno et al. (2005). In all iterations, Cottonwood, Orocopia, and Mesa samples primarily clustered with the “Colorado Desert” (group 14), with a few exceptions (Table 5). Analysis using all samples in the reference database did not change the clustering. As  $K$  was increased, Daggett (group 8, Table 1) fell out as a cluster before any of the unassigned Coachella Valley samples broke away from the Colorado Desert GU (group 14). Mesa became distinct only when evaluated for  $K = 7$  (Fig. 3).

**Table 2.** Mean diversity indices based on 24 microsatellite (STR) loci:  $n$  = number of individuals genotyped; richness and diversity estimated using FSTAT. Randomization tests for Hardy–Weinberg equilibrium calculated with ARLQUIN, where Obs Het = observed heterozygosity and Exp Het = expected heterozygosity.  $F_{IS}$ , inbreeding coefficient (Weir and Cockerham 1984) estimated using GENEPOP. Italicized values indicate standard deviation of the mean.

Pop	n	# alleles	Allelic range	Allelic richness	Gene diversity	Obs Het	Exp Het	$F_{IS}$
Mesa	30	6.60 <i>(3.59)</i>	13.85 <i>(9.82)</i>	2.52 <i>(0.61)</i>	0.65 <i>(0.21)</i>	0.655 <i>(0.259)</i>	0.647 <i>(0.221)</i>	0.000
Cottonwood	10	6.28 <i>(2.82)</i>	13.17 <i>(10.59)</i>	2.81 <i>(0.54)</i>	0.74 <i>(0.15)</i>	0.698 <i>(0.216)</i>	0.739 <i>(0.158)</i>	0.059
Orocopia	13	5.80 <i>(3.24)</i>	12.05 <i>(10.14)</i>	2.56 <i>(0.73)</i>	0.65 <i>(0.24)</i>	0.561 <i>(0.274)</i>	0.649 <i>(0.244)</i>	0.147

**Table 3.** Population pairwise genetic distance ( $F_{ST}$ ; below diagonal) and geographic distance (Km; above diagonal) between unassigned populations around the Coachella Valley and adjacent genetic units to the east and north-northwest. Group 16 = Mesa, Group 17 = Cottonwood, and Group 18 = Orocopia. Orocopia and Cottonwood locations combined for analysis due to proximity.

Genetic Unit	Group	Southern Mojave					Colorado Desert	Unassigned (Coachella Valley)	
		6	7	8	9	10	14	16	17&18
Southern Mojave	6	0	49	32	24	77	173	81	144
	7	0.014	0	79	38	35	120	51	81
	8	0.020	0.041	0	52	104	202	100	166
	9	0.014	0.012	0.023	0	55	156	79	124
	10	0.026	0.017	0.047	0.020	0	107	88	74
Colorado Desert	14	0.067	0.047	0.085	0.059	0.045	0	124	36
Unassigned	16	0.056	0.060	0.069	0.060	0.065	0.068	0	87
	17&18	0.068	0.058	0.079	0.060	0.050	0.011	0.054	0

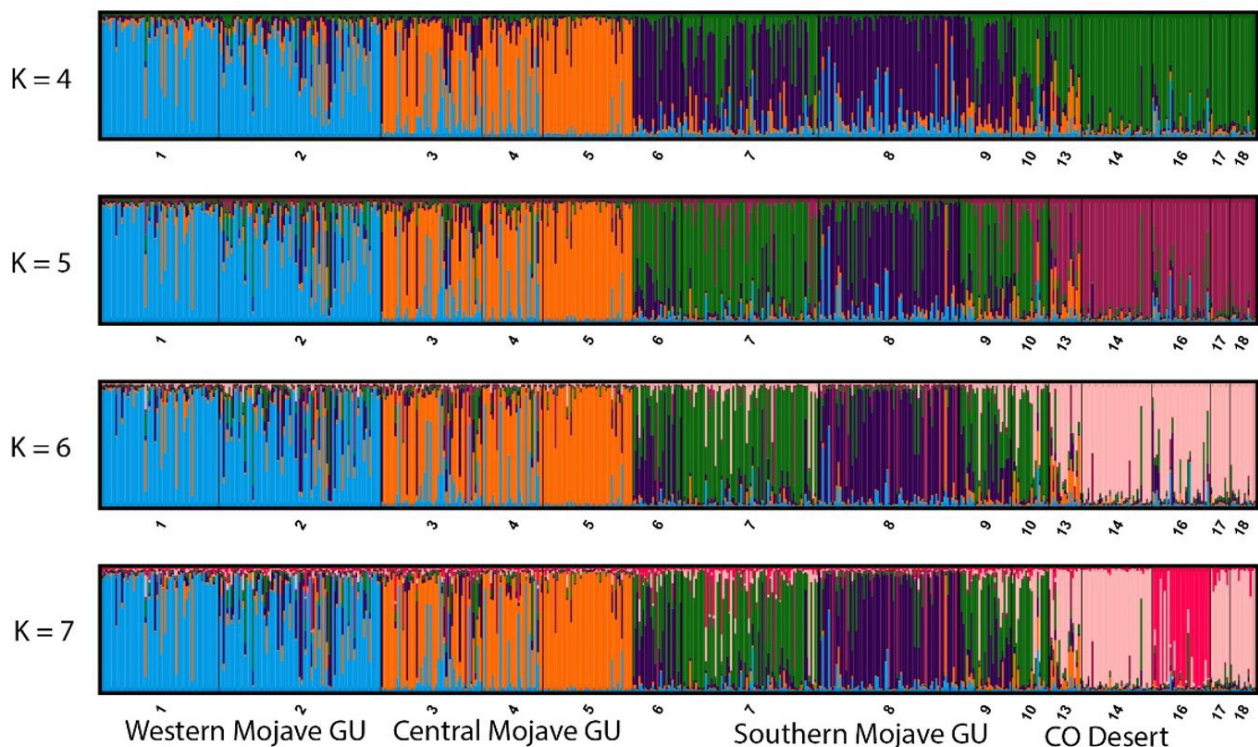
**Table 4.** Population assignment of three tortoise populations around the Coachella Valley to a reference database of 709 *G. agassizii* samples designated into five genetic units based on the genetic structure reported by Sánchez-Ramírez et al. (2018). Parenthetical values are assignments with  $LOD > 2$ .

	n	Genetic unit assignment		
		Western Mojave	Southern Mojave	Colorado Desert
Mesa	30	2 (2)	12 (11)	16 (15)
Cottonwood	10	0	1 (1)	9 (9)
Orocopia	13	0	1 (1)	12 (11)



**Table 5.** STRUCTURE analysis Q-values for K = 5 clusters averaged across 10 iterations. Standard deviations in italics.

Population		Colorado Desert GU	Southern Mojave GU	Southern Mojave GU (Daggett cluster)	Western Mojave GU	Central Mojave GU
Mesa	Ave	0.752	0.100	0.064	0.063	0.021
	SD	<i>0.230</i>	<i>0.137</i>	<i>0.096</i>	<i>0.103</i>	<i>0.026</i>
Orocopia	Ave	0.893	0.042	0.025	0.019	0.019
	SD	<i>0.086</i>	<i>0.057</i>	<i>0.019</i>	<i>0.014</i>	<i>0.019</i>
Cottonwood	Ave	0.899	0.039	0.023	0.015	0.025
	SD	<i>0.049</i>	<i>0.036</i>	<i>0.011</i>	<i>0.007</i>	<i>0.025</i>



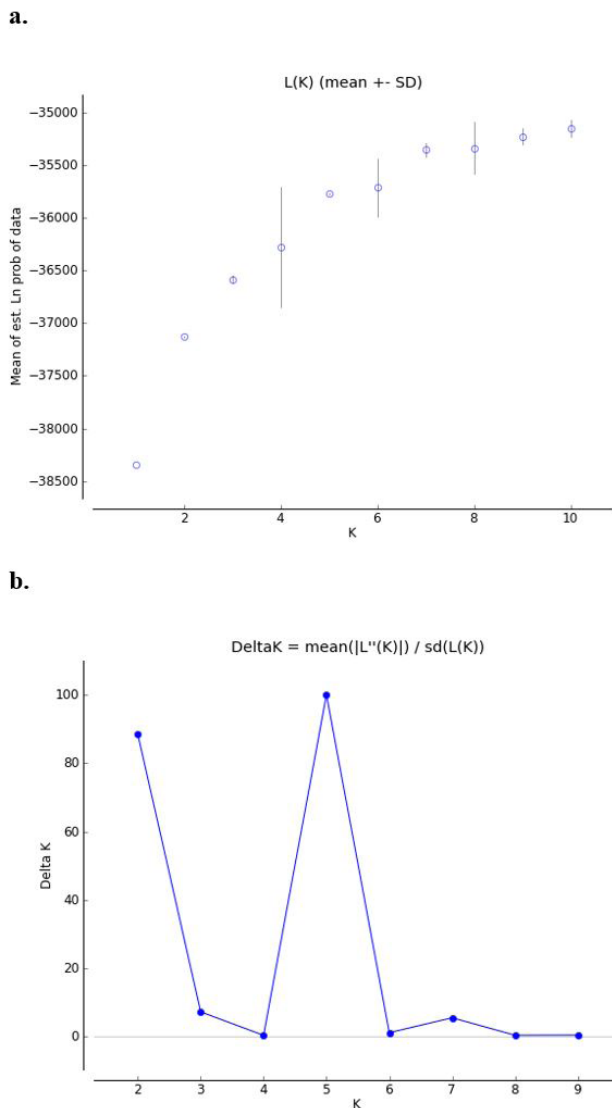
**Figure 3.** STRUCTURE analyses for Agassiz’s desert tortoise without prior population assignments consolidated and visualized using CLUMPAK for genetic clusters (K) 4–7 for three Coachella Valley populations (16–18) and Southern Clade genetic units; Western Mojave GU (groups 1–2), Central Mojave GU (groups 3–5), Southern Mojave GU (groups 6–10) and Colorado Desert GU (groups 13–14). STRUCTURE analyses histograms consolidated and visualized using CLUMPAK for genetic clusters K = 4–7.

### Discussion

Our analyses did not detect deep, lineage divergence among tortoise populations separated by the Coachella Valley, as would be expected if it was a significant barrier to gene flow now or in the past. Instead, the three tortoise populations surrounding the valley show evidence of continuity with the Colorado Desert GU and gene flow consistent with geographic distance, including some evidence of gene flow between Mesa at the northwestern tip of the valley and the adjacent Southern Mojave GU.

The observed lack of differentiation is somewhat unexpected given the long history of geological

change in the region. For example, prehistoric aquatic barriers in the lower Colorado River basin drove divergence between Mojave and Sonoran desert biota (Bell et al. 2010, Dolby et al. 2015). The first barrier, a marine incursion of the Gulf of California, extended northwestward into what is now the Salton Trough as early as 6.3 Ma (Dolby et al. 2019). Fossil-rich marine sediments show that the Gulf extended as far north as San Geronio Pass near Palm Springs, California, and possibly east to Parker, Arizona along what is now the Colorado River (Dolby et al. 2015, 2019). The Colorado River extensional corridor of the Miocene left a series of basins that were flooded in the Pliocene by the precursor to the lower river. Water from the



**Figure 4.** a) The mean log-likelihood of the number of populations of Agassiz’s desert tortoise for each K (ranging from 1–10), with error bars representing the standard deviation around the mean. b) DeltaK, derived through the Evanno et al. (2005) method, is shown for each K 1–10.

Colorado River arrived in the lower basin after 5.24 Ma, forming a second barrier (Howard et al. 2019) or filter (Dolby et al. 2019) to dispersal and gene flow in what is now the Salton Trough.

Over the last several thousand years, the Salton Trough has also been inundated periodically with freshwater from overflow of the Colorado River, forming a large waterbody known as Lake Cahuilla (Waters 1983), and it is now partially filled by the hypersaline Salton Sea. Lake Cahuilla (Fig. 1) and its earlier marine precursor would have been dispersal barriers to tortoise movements in the Salton Trough, for while they are capable of floating (Patterson 1973), sometimes for great distances (Gerlach et al. 2006), tortoises are poor swimmers (see review in Strong

and Walde 2006), especially *G. agassizii* (Woodbury and Hardy 1948) since their feet lack webbing present in many aquatic turtles (Pace et al. 2001, Ernst and Lovich 2009).

Aquatic barriers in the Salton Trough and lower Colorado River Valley affected gene flow and evolution not only of tortoises (Lamb et al. 1989), but their impact has also been demonstrated to varying degrees in phylogeographic studies of some other reptile species (e.g., Devitt 2006, Mulcahy et al. 2006), but not others (Lamb et al. 1992, Gottscho et al. 2017).

Past and present conditions in the Salton Trough, including the Coachella Valley, present tortoises with inhospitable habitat that inhibits them from moving from one side to the other in a straight line, but this dispersal barrier does not isolate them. Tortoises may have circumvented the Coachella Valley using its mountainous northern perimeter as a corridor for gene flow. The most likely route of connectivity involves the northern edge of the valley through the foothills, canyons, and bajadas of the Little San Bernardino Mountains. Our analyses cluster together Mesa, Orocopia, and Cottonwood from across the Coachella Valley. Nowadays, Interstate 10 separates Orocopia and Cottonwood, which are only about 6 km apart, and their genetic distance to one another is substantially less than the distance to Mesa. Tortoises at Mesa exhibit evidence of historic gene flow with populations to the north-northwest, yet it still clusters with the Colorado Desert GU.

Our analyses suggest that the range of the Colorado Desert GU should be extended to include populations surrounding the Coachella Valley. Populations around the Coachella Valley appear to show stronger genetic affinities to the Colorado Desert GU to the east (e.g., populations in Chuckwalla and Chocolate Mountain Aerial Gunnery Range, Table 1) than they do to the Southern/Central Mojave Desert GUs to the north. The Little San Bernardino and Cottonwood mountains at the southern boundary of Joshua Tree National Park form a natural barrier for tortoises between the Coachella Valley and the Southern Mojave GU (e.g., Lucerne Valley, Ord-Rodman, MCAGCC, Table 1). The same barrier forms the boundary between the climatically different Mojave and Sonoran desert ecosystems (see Lovich and Bainbridge 1999, Barrows 2011, and references therein), so our results correspond with the zoogeography of the region.

Mesa (Table 1, group 16) appears to have some introgression with the Southern Mojave GU, with decreasing influence moving eastward across the Colorado Desert GU (groups 17, 18 & 14: Fig. 2, Table 5). Thus, historic gene flow at Mesa likely occurred through the Morongo Valley corridor between the San Bernardino and Little San Bernardino mountains, and this is consistent with the model of isolation-by-distance (Murphy et al. 2007, Hagerty et al. 2011) exhibited by *G. agassizii*. No population has been isolated completely over time; gene flow occurs/occurred among neighboring populations.

The shared mtDNA ancestry among the Coachella Valley tortoises suggests that the current distribution

may be attributed either to a recent origin or to unabated dispersal. Edwards (2015) suggested that demographic events or selection reduced diversity within California, leaving tortoises with mtDNA Haplogroup MOJ\_A only. This could have involved a population bottleneck followed by a population expansion (Edwards 2003), possibly coinciding with climate change associated with glacial-interglacial periods during the Pleistocene. The estimated time to the most recent common ancestor of this haplogroup—98,268 years (SD  $\pm$ 48,000 years; Edwards 2003)—is consistent with this hypothesis. Desert tortoises likely experienced multiple population contractions and expansions correlating with Pleistocene glacial and interglacial events. The lower Colorado River Valley appears to have maintained more desert-like conditions during the Wisconsin glacial period (Betancourt et al. 1990), and this area may have acted as a refugium for desert-evolved species. It is unclear how *G. agassizii* may have benefitted from such a refugium since they are not considered to be a desert-evolved species (Morafka and Berry 2002).

The Salton Trough does not appear to be a major driver of population structure in the current distribution of desert tortoises. Instead the Little San Bernardino and Cottonwood mountains appear to limit gene flow to neighboring populations to the north, separating the Southern Mojave GU and the Colorado Desert GU. Although all *G. agassizii* exhibit a fairly recent shared ancestry (within maternal clade MOJ\_A), enough time has passed for local adaptation to occur across the species' large distribution (Sánchez-Ramírez et al. 2018). Tortoises around the Coachella Valley likely retain adaptations unique to the lower Colorado River Valley that allow them to persist at the edge of the species' range.

Low-lying desert areas, currently occupied by desert tortoises, are expected to become unsuitable in a warming and drying climate scenario (Barrows 2011, Barrows et al. 2016), and there is some preliminary evidence that the transition is already underway (Lovich et al. 2014). In the context of species conservation, we cannot predict which adaptive traits will be most critical in the face of environmental change and which individuals will contribute most to the evolutionary potential of the species. Individuals living on the edges of their distribution or in marginal habitat have the potential for being better adapted to changing environmental conditions (like climate change) that are different or more challenging from conditions in the core of their distribution (Eckert et al. 2008, Palstra and Ruzzante 2008, Hardie and Hutchings 2010). Desert tortoise populations around the Coachella Valley, especially west of the Salton Trough, represent an underappreciated extension of the species' range. Their genetic affiliation with the Colorado Desert GU and the lower Colorado River Valley, which potentially acted as a refugium during previous climate change events, suggest that it is essential to maintain the connectivity of these populations for the long-term sustainability of the species in that GU.

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