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RESEARCH ARTICLE

Are captive tortoises a reservoir for conservation? An assessment of genealogical affiliation of captive *Gopherus agassizii* to local, wild populations

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Abstract The conservation of tortoises poses a unique situation because several threatened species are commonly kept as pets within their native ranges. Thus, there is potential for captive populations to be a reservoir for repatriation efforts. We assess the utility of captive populations of the threatened Agassiz's desert tortoise (*Gopherus agassizii*) for recovery efforts based on genetic affinity to local areas. We collected samples from 130 captive desert tortoises from three desert communities: two in California (Ridgecrest and Joshua Tree) and the Desert Tortoise Conservation Center (Las Vegas) in Nevada. We tested all samples for 25 short tandem repeats and sequenced 1,109 bp of the mitochondrial genome. We compared captive genotypes to a database of 1,258 *Gopherus* samples, including 657 wild caught *G. agassizii* spanning the full range of the species. We conducted population assignment tests to determine the genetic origins of the captive individuals. For our total sample set, only 44 % of captive individuals were assigned to local populations based on genetic units derived from the reference database. One individual from Joshua Tree, California, was identified as being a Morafka's desert tortoise, *G. morafkai*, a cryptic species which is not native to the Mojave Desert. Our data suggest that captive desert tortoises kept within the native range of *G. agassizii* cannot be presumed to have a genealogical affiliation to wild tortoises in their geographic proximity. Precautions should be taken before considering the release of captive tortoises into the wild as a management tool for recovery.

Keywords Captivity · Conservation · Genetics · *Gopherus* · Repatriation · Testudinidae · Translocation

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Introduction

Tortoises are endearing animals and people in many cultures commonly keep native tortoises as pets. The phenomenon is global and includes species in Madagascar (*Astrochelys* [*Geochelone*] *yniphora*: Curl et al. 1985), Argentina (*Chelonoidis* [*Geochelone*] *chilensis:* Buskirk 1993), Spain and the Mediterranean (*Testudo* spp.: Perez 2004), South Africa (*Stigmochelys pardalis:* Wimberger et al. 2011), Mexico (*Gopherus morafkai:* Bury et al. 2002) and the United States (*Gopherus* spp.: Edwards et al. 2010; Kampher and Love 1998; Schwartz and Karl 2008). In some cases, the number of captive animals can be significant; Perez (2004) estimates tens of thousands of *Testudo graeca* are held in captivity in Spain and that backyard breeding is common. In Arizona, USA, over 4,600 native *Gopherus morafkai* were legally adopted in the state between 1982 and 2005, but the majority of backyard tortoises kept without permits potentially number in the tens of thousands (Jones 2008). Unfortunately for tortoises, of the 31 species listed by the International Union for the Conservation of Nature's (IUCN) Red List with sufficient information, 25 (81 %) are considered threatened with extinction (IUCN 2011). For threatened tortoise species that are kept as pets there is potential to use captive, native tortoises for conservation through repatriation. (We define

the term *repatriation* as the return of a captive tortoise to within its geographic range.)

One risk of repatriating captive animals of unknown origin is the potential for compromising the genetic integrity of wild populations. This concern is reasonable for tortoises because many species exhibit genetic differentiation throughout their ranges and may hybridize with closely related species (Edwards et al. 2010; Perez 2004; Schwartz and Karl 2008). For example, the anthropogenic movement of genetically differentiated lineages of Galápagos tortoises (*Chelonoidis* spp.) from island to island has complicated efforts to define natural evolutionary units for conservation (summarized in Poulakakis et al. 2012). For some species, large numbers of captives may escape or be released as part of undirected translocations; translocations may also occur during development (Glenn et al. 1990; Murphy et al. 2007). (We define *translocation* as human-mediated movement of a tortoise from one place to another within its geographic range.) Several studies of *Gopherus* have detected evidence of translocated tortoises in their genetic datasets from samples collected in the wild (Clostio et al. 2012; Fujii and Forstner 2010; Murphy et al. 2007; Schwartz and Karl 2005). In addition, genetic studies of captive tortoises show high incidences of individuals with mixed lineages or of unknown origins (Benavides et al. 2012; Edwards et al. 2010; Salinas et al. 2011; Schwartz and Karl 2008).

We address this issue for Agassiz's desert tortoise (*Gopherus agassizii*), native to the Mojave and Colorado desert regions of the USA and distributed in southeastern California, southern Nevada, southwestern Utah, and northwestern Arizona. Individuals that occur north and west of the Colorado River (the vast majority of the species range) were federally listed as threatened by the U.S. Fish and Wildlife Service (USFWS) (1990). Management of captive Agassiz's desert tortoise is a serious issue and is regulated by state and federal agencies. However, Agassiz's desert tortoises are commonly kept both legally and illegally as backyard pets and number in the tens of thousands across the geographic range. Although backyard breeding is discouraged, it frequently occurs and since desert tortoises are a long lived species, they often outlive their caretakers. (We use the word *caretaker* to define a person or family who possesses and cares for an Agassiz's desert tortoise. Caretakers are not owners because captive Agassiz's desert tortoises are owned by the government.) In addition, wild tortoises displaced during construction projects may be entered into adoption programs. Currently more tortoises in need of caretakers exist than there are people to adopt them. As a result, government-approved adoption programs have been put into place in many desert communities to help facilitate the growing population of captive tortoises.

The largest rehabilitation/adoption facility is the Desert Tortoise Conservation Center (DTCC) in Clark County, Nevada. The DTCC was established in 1990 and is currently managed by the San Diego Zoo's Conservation Centers for Species Survival in partnership with the USFWS, Bureau of Land Management and the Nevada Department of Wildlife. For two decades, the DTCC has served as a repository for wild tortoises displaced from their habitats due to development, injury, and unwanted pets. In the recent past the DTCC has received up to as many as 1,000 tortoises annually, and as of August 2012, the center maintained a population of approximately 2,200 tortoises (DTCC; A. Walsh *personal communication*). The majority of tortoises received by the DTCC are unwanted pets (USFWS; C. Mullen *personal communication*), however the DTCC is transitioning to receive fewer pet tortoises and its current holdings are being reduced through planned translocations.

At the same time wild populations of Agassiz's desert tortoises and available habitat are declining (USFWS 2010) the numbers in captivity are rising through unregulated breeding. Determining the ethicality of managed repatriation involves consideration of multiple parameters (McCoy and Berry 2008; Snyder et al. 1996) such as potential for the transfer of infectious diseases (Jacobson 1993; Jacobson et al. 2012; Johnson et al. 2006), inappropriate behaviors (Berry 1986), anticipated mortality (Ashton and Burke 2007; Wimberger et al. 2009), and the genetic consequences for wild populations (Snyder et al. 1996). In this paper, our objectives are to (1) address whether the growing captive population of Agassiz's desert tortoise is a viable reservoir for repatriation into depauperate wild populations by assessing genetic affinity of captive tortoises to local wild populations, (2) compare the results to the potential geographic location of origin of the captive as described by the current caretaker and as predicted by genetic assignment, and (3) discuss the ethical and management conundrums of release of captives to the wild.

Materials and methods

Samples and localities

We sampled 105 captive desert tortoises from two localities in California: 30 samples from health clinics held at Crestwood Animal Hospital (Dr. Jeff Novak, DVM) in Ridgecrest, Kern County, and 75 samples from Critter Care Animal Hospital (Dr. Thad Thorson, DVM) in Joshua Tree, San Bernardino County (Fig. 1). In addition, we collected 40 samples from the Desert Tortoise Conservation Center (DTCC) in Las Vegas, Nevada (Fig. 1). At the California clinics, each caregiver of a tortoise provided name, address, the length of time the tortoise had been cared for, the

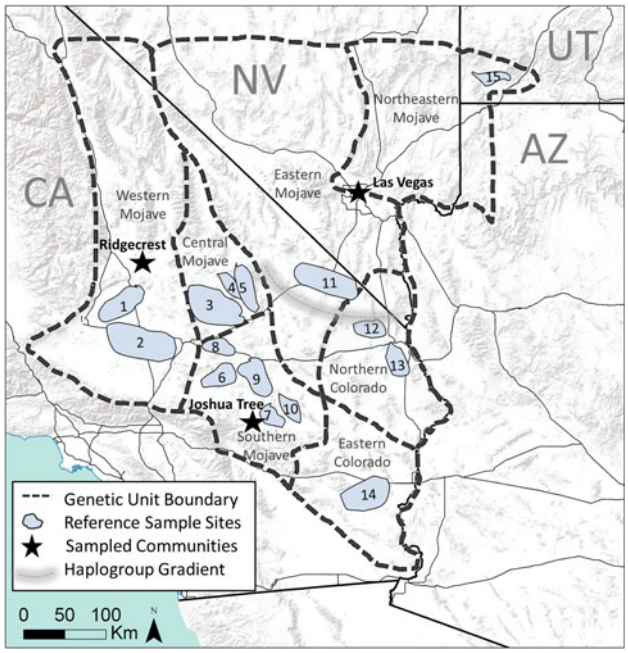

Fig. 1 Sampled communities, genetic units, and reference sample collection localities: Ridgecrest, Kern County and Joshua Tree, San Bernardino County, California and the Desert Tortoise Conservation Center (DTCC) in Las Vegas, Nevada. Genetic units (GUs) defined as: Western Mojave (sites *1* and *2*; $n = 143$); Central Mojave GU (sites *3–5*; $n = 129$); Southern Mojave (sites *6–10*; $n = 213$); Eastern Mojave (site *11*; $n = 59$); Northern Colorado (sites *12* and *13*; $n = 48$); Eastern Colorado (site *14*; $n = 37$); and Northeastern Mojave (site *15*; $n = 28$). "Haplogroup Gradient" approximates the cline between "Mojave" (MOJ_A) and "Northern Mojave" (MOJ_B) haplogroups (Murphy et al. 2007)

source of the tortoise (the desert, a friend or acquaintance, a desert tortoise adoption group, federal agency, state agency), and the town or city where the source was located. Caretakers further provided information on the number of desert and other tortoises and turtles in their possession. No such information was available for the tortoises from the DTCC.

DNA isolation

We isolated total DNA from salvaged red-blood cells by overnight lysis 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 Invitrogen Dynal bead extraction chemistry (Life Technologies, Carlsbad, California USA). We quantified recovered DNA using a BioTEK Synergy HT (BioTEK, Vermont, USA) and diluted working stocks to 5 ng/µl for polymerase chain reaction (PCR) amplifications.

Mitochondrial DNA

We amplified an approximately 1,500 base pair (bp) portion of the ND3, arginine tRNA, ND4L, and part of the ND4 genes using primers Nap2 and New Gly (Arévalo et al. 1994; Britten et al. 1997). We followed the methods in Edwards (2003) and Murphy et al. (2007) for PCR conditions. We submitted PCR products to the University of Arizona Genetics Core for DNA sequencing on a 3730XL DNA Analyzer (Applied Biosystems, Carlsbad, California USA). We aligned an 1,109 bp sequence using CLC DNA Workbench ver. 5.7.1 (CLC bio, Denmark) and compared individuals to a reference database of mtDNA haplotypes representing all *Gopherus* species: *G. agassizii* ($n = 125$), *G. morafkai* ($n = 192$), *G. berlandieri* ($n = 58$), *G. flavomarginatus* ($n = 78$), and *G. polyphemus* ($n = 1$). A portion of these same reference individuals were used previously in *Gopherus* population genetic studies (Edwards 2003; Edwards et al. 2010; Fujii and Forstner 2010; Murphy et al. 2007). The different species of *Gopherus* exhibit fixed differences in mtDNA (Edwards et al. 2010; Lamb et al. 1989; McLuckie et al. 1999; Murphy et al. 2007, 2011).

STRs

We tested all samples for 25 previously described short tandem repeats (STRs): Cm58 (FitzSimmons et al. 1995); Goag03, Goag04, Goag05, Goag06, Goag07, Goag32 (Edwards et al. 2003); Test56 (Hauswaldt and Glenn 2003); GP15, GP19, GP30, GP55, GP61, GP81, GP96, GP102 (Schwartz et al. 2003); ROM01, ROM02, ROM03, ROM04, ROM05, ROM07, ROM10 (Edwards et al. 2011); and ROM08, ROM09 (Davy et al. 2011). We combined loci into nine different multiplex PCRs following methods described in Edwards (2003), Murphy et al. (2007), and Edwards et al. (2011). For loci that failed to amplify, we reran samples in uniplex reactions. We combined post-PCR products prior to fragment analysis and submitted them to the University of Arizona Genetics Core for fragment analysis on a 3730 DNA Analyzer (Applied Biosystems, Carlsbad, California, USA). We analyzed electropherograms using Genemarker 1.85 (SoftGenetics, State College, Pennsylvania USA).

Analysis

For STR data, we generated F-statistics for population differentiation among the three captive localities using AMOVA in ARLEQUIN (Ver. 3.11, Excoffier et al. 2005). We assessed population association of the captive samples using the assignment test in program WHICHRUN (Ver. 4.1; Banks and Eichert 2000). The program calculates the likelihood of a given captive individual originating from ≥2 candidate populations, on the basis of its multilocus STR genotype. We made the assumption of Hardy–Weinberg and linkage equilibrium in reference populations based on previous publications (Edwards et al. 2004, 2010;

Murphy et al. 2007). We assessed stringency for population allocation with the selection criterion of the log of the odds ratio (LOD) for the 2 most likely source populations. Assignments with a LOD ratio of ≥2 have a ≤0.01 chance of type I error. In addition, we evaluated the affinity of our reference samples to their populations of origin using the jackknife method in WHICHRUN. The jackknife method samples individuals from the reference dataset one at a time, recalculates allele frequencies in the absence of the individual, and then determines the most likely population origin for that individual (Table 1).

We first compared a 16 locus STR genotype (Murphy et al. 2007) in our captive samples to a database of 1,258 *Gopherus* samples, including *G. agassizii* from throughout its range ($n = 656$). Other *Gopherus* samples in the database included: *G. morafkai* divided into samples collected in Arizona ($n = 348$), central Sonora, Mexico ($n = 35$), and the "Sinaloan" type (Murphy et al. 2011) collected in southern Sonora and Sinaloa, Mexico ($n = 41$); *G. berlandieri* collected in Texas ($n = 118$; Fujii and Forstner 2010); and *G. flavomarginatus* collected from Chihuahua, Mexico (Morafka et al. 1994) and from captivity ($n = 60$). Using a jackknife method for reallocation of individual samples from the baseline data (Banks and Eichert 2000), 100 % of samples reassigned to the correct population of origin at the species level. For the *G. agassizii* samples, we divided this initial database into "Mojave" and "Northern Mojave" populations (Fig. 1). This division complements the geographic distribution of the two primary mtDNA lineages (haplogroups MOJ_A and MOJ_B; 0.6 % sequence divergence) observed by Murphy et al. (2007). Using the 16 locus STR genotype, 98.77 % of samples reassigned correctly to the "Mojave" population and 95.40 % of samples reassigned correctly to the "Northern Mojave" population. Hagerty and Tracy (2010) also observed a division in *G. agassizii* between the northern and southern portions of the species range.

For captive individuals assigned to *G. agassizii*, we then compared their 25 locus STR genotypes to a reference database of 657 *G. agassizii* sampled throughout their range. These reference samples were previously used by Murphy et al. (2007) to describe population structure in *G. agassizii*. However, we increased the number of loci from 16 to 25 and removed several individuals from the dataset that were identified as likely to have been translocated (Murphy et al. 2007).

Genetic units

We defined population structure of the 657 wild caught *G. agassizii* samples according to Murphy et al. (2007) and designated 7 genetic units (GUs: Fig. 1). Murphy et al. (2007) determined the geographic boundaries for these genetic units using data available on differences in vegetative communities, physical attributes of the habitat, climate, choice and availability of forage plants, use of cover sites (burrows, dens), and denning behavior. We used additional information from Hagerty and Tracy (2010) and the Revised Desert Tortoise Recovery Plan (USFWS 2011) to refine the geographic boundaries of GUs. The genetic structure among GUs is characteristic of isolation by distance (Table 2; Hagerty et al. 2011; Murphy et al. 2007).

For the three communities where we sampled captive tortoises, the GU for Ridgecrest occurs within the Western Mojave, Joshua Tree is located in the Southern Mojave GU, and the DTCC is located on the boundary between the Eastern Mojave and Northeastern Mojave GUs (Fig. 1). It is important to note that our reference database does not include wild desert tortoise samples collected in Nevada; however, it does contain samples from surrounding areas including Red Cliffs Recreation Area in Utah and the Ivanpah Valley in northeastern San Bernardino County, California (Fig. 1). This northern portion of the species range has been identified as having additional population

Table 1 Evaluation of comparative databases of *G. agassizii* samples genotyped for 25 STR loci using a jackknife method for reallocation of individual samples from the baseline data (Banks and Eichert 2000)

Genetic unit	*n*	Western Mojave	Central Mojave	Southern Mojave	Eastern Colorado	Northern Colorado	Eastern Mojave	Northeastern Mojave	% correctly assigned to population of origin
Western Mojave	143	126 (122)	6 (4)	6 (4)		4 (3)	1 (1)		88.11
Central Mojave	129	10 (10)	101 (98)	6 (6)		11 (8)	1 (1)		78.29
Southern Mojave	213	9 (9)	3 (3)	158 (148)					74.18
Eastern Colorado	36			1 (1)	31 (30)	4 (3)			86.11
Northern Colorado	48				1 (1)	47 (42)			97.92
Eastern Mojave	59					2 (1)	55 (53)	2 (2)	93.22
Northeastern Mojave	28							28 (28)	100.00

Genetic units modified from Murphy et al. (2007). Population assignment results after parenthetical values indicate number of population assignments of LOD ≥ 2.00

genetic structure that our dataset does not capture (Britten et al. 1997; Hagerty and Tracy 2010).

Results

Captive tortoise data

Most tortoises sampled were adults but had not been in the custody of a single caretaker for the life of the animal. Caretakers from the Ridgecrest clinic had their tortoises an average of 3.6 years ($N = 23$, range: 1 week to 15 years), and those attending the Joshua Tree clinic had their tortoises an average of 6.5 years ($N = 61$; range: 1 week to 47 years). Many caretakers of captive tortoises sampled in Ridgecrest and Joshua Tree had few details about the sources of their tortoises, and many tortoises apparently had been transferred from one adoption group, person, or family to another over time. Many rescued or adopted tortoises had been injured by vehicles or attacked by dogs in desert urban or ex-urban areas. Of the 24 captives from Ridgecrest for which data were available, only one tortoise was reported to have come from a source outside the local area, Ft. Irwin in the Central Mojave GU. Caretakers from the Joshua Tree clinic reported more varied origins. We collected information for 66 tortoises from Joshua Tree and 39 of these reportedly came from tortoise rescue groups. Although most of these rescue groups were centered in the desert (e.g., Joshua Tree Tortoise Rescue, $N = 25$; Yucca Valley, $N = 2$; Palm Desert/Palm Springs, $N = 2$; Victorville, $N = 2$), eight of the 39 were from adoption groups potentially affiliated with the California Turtle and Tortoise Club located in the Los Angeles basin and the cities of Chino and Santa Barbara. One tortoise sampled in Joshua Tree was taken from the wild near the Salton Sea in the Colorado Desert 35 years prior to sampling. Caretakers of 18 tortoises sampled at the Joshua Tree clinic resided in the Joshua Tree community; an additional 22 tortoises were brought to the clinic by caretakers who resided outside the local area. We excluded these samples from our analysis of the Joshua Tree community and instead reported them as from "other locations". Three of these were from the desert community of Barstow (within the Western Mojave GU) and 19 were from outside the Mojave Desert region including the cities of Los Angeles, Downey, Temecula, Brea, Carson, Culver City, Costa Mesa, Ontario, and Nuevo, California.

Most caretakers reported keeping more than one desert tortoise (presumably *G. agassizii*). Where data were available, 100 % of 23 tortoises at Ridgecrest and 97 % of 64 tortoises sampled at the Joshua Tree clinic lived in households with multiple tortoises. Two of the 24 Ridgecrest tortoises and nine of the 66 Joshua Tree tortoises lived at households where other chelonian species were present, including: ornate box turtle (*Terrapene ornata*), Horsfield's tortoise (*Testudo horsfieldii*), African spurred tortoise (*Geochelone sulcata*), Galapagos giant tortoises (*Chelonoidis nigra* species complex), and leopard tortoise (*S. pardalis*).

Genetic analyses

Of our 145 samples, we recovered viable DNA from 130 individuals. We generated STR profiles for 128 samples and successfully generated mtDNA sequences for 111 samples (Tables 3, 4). For the two California captive populations, 72 of 75 samples genotyped within one-step of mtDNA haplogroup MOJ_A01, which is ubiquitous in the Mojave and Colorado deserts in California (Western, Central, and Southern Mojave and Northern and Eastern Colorado GUs; Murphy et al. 2007). Also for the California samples, we observed 2 haplotypes (MOJ_B01 and MOJ_B02) strongly associated with the "Northern Mojave" (Eastern Mojave and Northeastern Mojave GUs; Murphy et al. 2007). The caretaker of the Ridgecrest MOJ_B01 individual reported that the tortoise had "walked into the yard" and the caretaker of the MOJ_B02 individual reported that they obtained the tortoise "from the street in Barstow" where the caretaker resides. In contrast,

Table 2 Pairwise F_{ST} (Weir and Cockerham 1984) among 7 genetic units generated for the comparative databases of *G. agassizii* samples genotyped for 25 STR loci

Genetic unit	#	Western Mojave	Central Mojave	Southern Mojave	Eastern Colorado	Northern Colorado	Eastern Mojave
Western Mojave	143	0.000					
Central Mojave	129	0.037	0.000				
Southern Mojave	213	0.038	0.032	0.000			
Eastern Colorado	36	0.097	0.069	0.056	0.000		
Northern Colorado	48	0.080	0.052	0.046	0.031	0.000	
Eastern Mojave	59	0.084	0.067	0.070	0.107	0.079	0.000
Northeastern Mojave	28	0.131	0.117	0.121	0.147	0.132	0.055

Genetic units modified from Murphy et al. (2007)

Table 3 MtDNA haplotype results of captive tortoises sampled from three locations: Joshua Tree (JT) and Ridgecrest (RC), California, and from the Desert Tortoise Conservation Center (DTCC) in Las Vegas, Nevada

Site	*n*	mtDNA haplotype				
		MOJ_A01	MOJ_B01	MOJ_B02	MOJ_B03	SON_01
JT	32	31				1
RC	24	23 (3)	1			
DTCC	36	15	17		4	
Other	19	18 (1)		1		

"Other" represents tortoises sampled at the Joshua tree clinic that were brought to the clinic by caretakers who resided with their tortoises outside the local area, including cities in Los Angeles and Orange counties, California. We compared a 1,109 bp sequence for each individual to a database of 35 identified haplotypes identified in 454 tortoises (*Gopherus* spp.). Haplotypes were defined per Murphy et al. (2007): The "MOJ_A" haplogroup is ubiquitous in the Mojave Desert in California while the "MOJ_B" haplogroup is strongly associated with the Northern part of the range of *G. agassizii*. SON_01 is the most abundant haplotype observed across the range of *G. morafkai*. Parenthetical values indicate novel mtDNA haplotypes that had single-step differences from the modal haplotype MOJ_A01

21 samples from the DTCC genotyped as the regionally observed "MOJ_B" haplogroup (haplotypes MOJ_B01 and MOJ_B03) while 15 genotyped as MOJ_A01 from the southern portion of the range (Table 3).

In this dataset, we observed 3 novel mtDNA haplotypes with single-step differences from MOJ_A01. One occurred in a single individual from the Joshua Tree clinic (but the caretaker resides in Carson, California) and two novel haplotypes were genotyped in three individuals from Ridgecrest. The shared, novel haplotype observed in the two tortoises from Ridgecrest were kept by the same caretaker and these tortoises may have been related (mother and offspring or siblings). In addition, we identified a single individual from Joshua Tree that was a different (but cryptic) species; Morafka's desert tortoise, *G. morafkai*. This individual genotyped with the most abundant haplotype observed across the range of *G. morafkai*, SON_01 (Murphy et al. 2007) and the STR assignment was characterized as being from the Arizona population of *G. morafkai*. This individual had been held in captivity in California for at least 8 years at the time of sampling; the source was a friend or acquaintance from the town of Joshua Tree.

The number of correctly assigned individuals to their GU of origin differed significantly between the captive and wild populations in the locality's GU (Fisher's exact test; Ridgecrest $p < 0.012$, Joshua Tree $p < 0.001$ and DTCC Tree $p < 0.001$). STR population assignments indicated that captive individuals from Ridgecrest have a higher affinity to their GU of geographic origin (66.67 %) than those from Joshua Tree (34.88 %) or the DTCC (41.03 %) (Table 4). Most individuals that were not assigned to the GU associated with their sampled communities were assigned to a neighboring GU; however several individuals appeared to have originated from more distant areas. Two individuals from the DTCC were assigned to GUs in the southern portion of the range (Central Mojave and Eastern Colorado GUs in California); each individual also exhibited a corresponding MOJ_A01 mtDNA haplotype (Table 3). Two individuals sampled at the Joshua Tree clinic (and held by the same caretaker who resides in Barstow, CA) were assigned to GUs in the Northern part of the range. While we could not successfully amplify the mtDNA for one of these samples, the other had a corresponding "northern" MOJ_B02 mtDNA haplotype. This corroboration between mtDNA and STR assignment for both the Joshua Tree and

Table 4 Population assignment results for captive tortoises sampled from three locations: Joshua Tree (JT) and Ridgecrest (RC), California and the Desert Tortoise Conservation Center (DTCC) in Las Vegas, Nevada

Site	*n*	Western Mojave GU	Central Mojave GU	Southern Mojave GU	Eastern Colorado GU	Northern Colorado GU	Eastern Mojave GU	Northeastern Mojave GU	% assigned to local GU
JT	43	3 (3)		15 (13)	12 (11)	13 (10)			34.88 %
RC	24	16 (16)	5 (3)	2 (2)			1 (1)		66.67 %
DTCC	39		1 (1)		1 (1)	10 (9)	16 (12)	11 (10)	41.03 %
Other	22	8 (8)	1 (1)	5 (3)		6 (6)	2 (1)		n/a
								Overall	44.34 %

"Other" represents tortoises sampled at the Joshua tree clinic and brought to the clinic by caretakers who reside with their tortoises outside the local area, including cities in Los Angeles and Orange counties, California. We compared a 25 locus genotype for captive individuals to a database of 657 tortoises sampled throughout the species range. Genetic Units modified from Murphy et al. (2007). Parenthetical values indicate number of population assignments of LOD $\geq$ 2.00

DTCC individuals suggests that these tortoises have genetic origins distant from the local community. In contrast, the Ridgecrest sample with northern Mojave mtDNA haplotype was assigned to the Central Mojave GU with its STR genotype, but with an LOD of 1.39.

The two California captive populations exhibited genetic differentiation between them consistent with expectations for tortoises separated by the geographic distance between the sampled communities; $F_{ST} = 0.052$ (Weir and Cockerham 1984). The genetic differentiation between the DTCC samples and the two California locations was much greater: F_{ST} between the DTCC and Joshua Tree and Ridgecrest were 0.147 and 0.133, respectively. While these F_{ST} estimates are greater than the average F_{ST} observed across the range of the *G. agassizii*, they are consistent with genetic distances for larger geographic distances (Table 2).

Discussion

Captive tortoise affiliation

Agassiz's desert tortoise exhibits genetic structure characteristic of isolation by distance (Hagerty et al. 2011; Murphy et al. 2007) and thus gene flow among populations can inhibit discrete genetic assignment to GUs (Table 1). However, all three captive localities exhibit reassignment proportions significantly less than expected if sampling wild populations (Tables 1, 4). In California, local assignment proportions also varied from community to community, with Ridgecrest showing the highest local affinity and Joshua Tree the lowest (Table 4). The differences in affinities could partly be an artifact of differences in reassignment probabilities among GUs (Table 1) or might reflect the community's location relative to geographic boundaries of the GU. In addition, the community's demographic make-up and location may contribute to the captive tortoises resident there. For example, Ridgecrest is not on a major highway while Joshua Tree is a destination point for visitors to the Joshua Tree National Park and also is located near the Marine Corps base at Twentynine Palms where human resident turnover is high. In Nevada, tortoises from the DTCC originated from the largest geographic area and presumably the DTCC population has the greatest admixture. The genetic differentiation observed among the three captive tortoise populations emphasizes that tortoises in captivity differ among communities and thus should not be considered a single genetic pool.

Although we observed a greater proportion of captive individuals that did not originate from their current geographic location than would be expected in samples collected from the wild, only a small proportion appear to have originated from extremely disparate regions. Our data are in contrast to the high numbers of hybrid and mixed lineage individuals observed in captive tortoises in Arizona. More than 30 % of captive tortoises in Phoenix were not of the expected Sonoran Desert origin (*G. morafkai*) but instead were *G. agassizii* or hybrids (Edwards et al. 2010). These differences may reflect the movement of people (and their pet tortoises) between states. Between 2005 and 2009, more than twice as many people moved from California to Arizona than vice versa (U.S. Census Bureau 2012). Likewise, the greater admixture observed in the DTCC samples may reflect the expanding city of Las Vegas where significantly more people moved into Nevada from neighboring California than emigrated to California (U.S. Census Bureau 2012).

A possible explanation for the 17 individuals in our data set assigned to a population with a low LOD (<2) is that they represent first or second generation crosses between captive parents from different localities. However, the three novel mtDNA haplotypes observed in the data emphasize that our reference database does not capture the entirety of the genetic variation of *G. agassizii* and thus may limit our ability to correctly assign all individuals to regions of origin. In addition, our reference dataset lacks resolution in the northern portion of the range of *G. agassizii*. Hagerty and Tracy (2010) present a genetic assessment of the recovery units for *G. agassizii* with increased sampling of the northern portion of the range where they observed additional structuring that our reference samples do not capture.

Management implications

Our dataset suggests that the release of captive tortoises into wild populations whether haphazardly or as part of a management strategy, without first obtaining genotypic information, would likely result in the genetic mixing of disparate populations. Theoretically, this could result in outbreeding depression (disruption of epistasis or dilution of locally adapted alleles: Edmands 2007; Edmands and Timmerman 2003). Arguably the potential for outbreeding depression in admixed desert tortoise populations may be low because they exhibit a genetic structure with gene flow. However that risk increases the greater the time populations have been separated (Frankham et al. 2011). Desert tortoises exhibit a genetic cline across their range and morphological, behavioral, and ecological differences among tortoises at disparate ends of their distribution suggest that sufficient time has passed for populations to develop local adaptations (Berry et al. 2002). Murphy et al. (2007) note an absence of genetic integration in their analysis of over 600 samples collected in the wild despite documenting thousands of released captives and

translocations of wild caught *G. agassizii*. This suggests that tortoises translocated from disparate areas do not successfully integrate into new populations. Although mortality may be higher for translocated animals in general (Ashton and Burke 2007; Wimberger et al. 2009), this could also suggest that the more differentiated individuals lack adaptations necessary to survive on a local level. Without additional analyses, it would be best to assume that there has been adequate time for tortoise populations to acquire local adaptations and translocations of wild animals should be restricted to individuals located within an appropriate genetic distance. Thus, the conservative approach would be to match closest populations (Weeks et al. 2011) or as recommended in Edwards et al. (2004) "nearest-neighbor populations".

Theoretical arguments also exist for purposefully mixing disparate populations to increase the fitness of some declining populations, but this is primarily as augmentation for highly fragmented or inbred populations that previously had potential for genetic exchange (Hedrick 1995; Moritz 1999; Weeks et al. 2011). Desert tortoises do not meet this requirement since they maintain relatively large populations and wild populations still exhibit high genetic diversity (Hagerty and Tracy 2010; Murphy et al. 2007). Unfortunately, it is not possible to reliably predict the outcome of introducing genetically differentiated individuals into new populations (Edmands 2007; Tallman et al. 2004), and the number of genetically distinct individuals introduced into a novel population does not necessarily correlate with the effects (Edmands and Timmerman 2003). The most conservative approach is to maximize the genetic and adaptive similarity of populations (Edmands 2007; Frankham et al. 2011). With the resources available to resolve the genetic identity for *G. agassizii*, it would be prudent to genotype and assign any captive individuals of unknown origin to the most appropriate GU prior to any organized repatriation effort.

Despite the ability to assign captive animals to a genetic and geographic location of origin, other genetic considerations should be considered. For example, polyandry and sperm storage have been documented in desert tortoises (Davy et al. 2011; Murphy et al. 2007) thus allowing a single mature female to represent genetic material from multiple males. A captive female tortoise, even if genotypically suitable for repatriation, could still introduce admixed or hybrid progeny into the population after release. In addition, utilizing animals that have been bred or maintained in captivity for extended periods may result in selection for attributes that might reduce fitness in the wild (Christie et al. 2012; Frankham 2008; McDougall et al. 2006). After all, captive propagation is selective breeding. There may even be repercussions of 'successful' repatriation if a few surviving individuals dominate the breeding pool and increase the potential for inbreeding depression (Milinkovitch et al. 2004).

Aside from the genetic risks of repatriating captive animals, other risks need to be considered prior to initiating a managed repatriation effort (Dodd and Siegel 1991). Initial mortality of reintroduced individuals may be high (Ashton and Burke 2007; Wimberger et al. 2009) or cause displacement of resident individuals (Berry 1986) and thus ethical dilemmas and public perception of management actions need to be considered (McCoy and Berry 2008). The greatest risk in releasing captive animals to the wild is the potential for captive animals to spread infectious diseases (Cunningham 1996; Kock et al. 2010; Sainsbury and Vaughan-Higgins 2012; Viggers et al. 1993). Disease transmission is a particularly large risk for tortoises because captive animals may be exposed to a variety of zoonotic diseases through the large international reptile trade market and hobbyists (Jacobson 1993; Martel et al. 2009; Soares et al. 2004; Wimberger et al. 2011). Not all diseases have been identified and tests are not available for screening those that are known. Several new pathogens have been identified in the last few years (e.g., Jacobson et al. 2012) and tests have not been developed to identify them. Even clinically healthy tortoises have the potential to be carriers (Christopher et al. 2003; Jacobson and Berry 2012; Jacobson et al. 1995; Martel et al. 2009). As reported by the caretakers in this study, most have multiple individuals of *G. agassizii* and many also keep other species.

Translocation of some tortoise species can be successful and lead to long-term retention (Ashton and Burke 2007; Bertolero et al. 2007), but this requires intensive management and should be weighed against other options (Ashton and Burke 2007). Repatriation efforts are best suited to habitat already devoid or depauperate of tortoises or into small, inbred populations that are at risk and in need of genetic rescue (Tallman et al. 2004; Weeks et al. 2011). With appropriate resources and information, exemplified by our knowledge of *G. agassizii*, appropriate captive breeding measures could take place (following IUCN guidelines, e.g., for captive breeding, maintenance of studbooks, and re-introductions; IUCN 2002), creating breeding units based on the natural population structure of the species, and allowing precise determination of the geographic origin for reintroduction (Williams and Osentoski 2007). However, captive breeding is currently not a high priority and may not be the most appropriate use of limited conservation resources (USFWS 2011). In many areas, habitats have deteriorated, been infested with alien plant species, or been burned, and these habitats are unlikely to support additional tortoises. In addition, problems with human-subsidized predators have not been solved. These issues should be addressed first, before captive tortoises are released.

Conclusions

For Agassiz's desert tortoise, captive populations should not be considered a reservoir for recovery of wild populations. Repatriation of captive animals and reintroduction of captive bred animals have high risks associated with them and should therefore only be employed where other conservation measures are unavailable (Snyder et al. 1996). While wild populations still persist, from an evolutionary perspective the best approach is to maintain them in situ without artificial influence from new individuals. In fact, vulnerable wild populations may directly benefit from programs that shelter or adopt unwanted and displaced tortoises by ensuring that those animals are not haphazardly released to the wild. The State of California has such a program with the California Turtle and Tortoise Club. Hundreds of captive tortoises find new homes through their government-approved adoption programs. However, elsewhere within the geographic range, unregulated collection and breeding of desert tortoises have resulted in an unfortunate predicament requiring appropriation of limited government and non-government resources and difficult choices by management agencies (i.e., sterilization, euthanasia, and legal enforcement). To create long-term change, we encourage continued educational outreach that addresses the negative consequences of taking tortoises from the wild, breeding them in captivity, or releasing them to the wild. The issues addressed in our study with *G. agassizii* may be useful to scientists and managers considering repatriation efforts with other species of turtles and tortoises because of the similarities in life history traits and conservation challenges.

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