

Biogeographic Perspective of Speciation Among Desert Tortoises in the Genus *Gopherus*: A Preliminary Evaluation

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Abstract—The enduring processes of time, climate, and adaptation have sculpted the distribution of organisms we observe in the Sonoran Desert. One such organism is Morafka's desert tortoise, *Gopherus morafkai*. We apply a genomic approach to identify the evolutionary processes driving diversity in this species and present preliminary findings and emerging hypotheses. The Sonoran Desert form of the tortoise exhibits a continuum of genetic similarity spanning 850 km of Sonoran desertscrub extending from Empalme, Sonora, to Kingman, Arizona. However, at the ecotone between desertscrub and foothills thornscrub we identify a distinct, Sinaloan lineage and this occurrence suggests a more complex evolutionary history for *G. morafkai*. By using multiple loci from throughout the tortoise's genome, we aim to determine if divergence between these lineages occurred in allopatry, and further to investigate for signatures of past or current genetic introgression. This international, collaborative project will assist state and federal agencies in developing management strategies that best preserve the evolutionary potential of Morafka's desert tortoise. Ultimately, an understanding of the evolutionary history of desert tortoises will not only clarify the forces that have driven the divergence in this group, but also contribute to our knowledge of the biogeographic history of the Southwestern deserts and how diversity is maintained within them.

Introduction

The diverse assemblages of flora and fauna in the Sonoran Desert have been sculpted by the enduring processes of time, climate, and adaptation. The Sonoran Desert developed gradually over the last 8 million years through multiple glacial and interglacial climatic cycles that resulted in repeatedly expanding and shrinking woodlands, desertscrub, and thornscrub communities (Van Devender 1990). The modern characteristic assemblage that includes columnar cactus and

palo verde trees that define Arizona Upland Sonoran desertscrub likely did not (re)emerge until roughly 9,000 years ago (Van Devender 1990). We are interested in how this fluctuating vegetation community has shaped the evolution of one of the Sonoran Desert's most charismatic reptiles, the Morafka's desert tortoise, *Gopherus morafkai*. In particular, we explore how genetic variation within this species fits the biogeographic history of the landscape.

The genus *Gopherus* contains two species of desert tortoise, one native to the Mojave and Colorado deserts (Agassiz's desert tortoise; *G. agassizii*) and another in the Sonoran Desert region (Morafka's desert tortoise, *G. morafkai*; Murphy and others 2011). *Gopherus agassizii* occurs almost entirely north and west of the Colorado River and *G. morafkai* inhabits regions south and east of the Colorado River, extending southward into tropical deciduous forests of Southern Sonora, Northern Sinaloa, and extreme Southwestern Chihuahua. The Mojave and Sonoran lineages of *Gopherus* differ in morphology, seasonal activity, reproductive ecology, habitat selection, and genetics

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(e.g., Lamb and others 1989; McLuckie and others. 1999; Berry and others 2002).

The U.S. Fish and Wildlife Service federally listed the Mojave population of the desert tortoise (*G. agassizii*) as threatened (USFWS 1990). The Sonoran population (*G. morafkai*) is not federally listed in the United States, but became a candidate for federal listing in 2010 and is considered a Species of Special Concern in Arizona (USFWS 2010). Mexican populations of *Gopherus* receive protection as threatened species (Category A “Amenazada” in NOM-059; D.O.F. 2002). To assess trends in Morafka’s desert tortoise, it is necessary to consider populations in Mexico that occupy about two-thirds of the geographic range. Unfortunately, little data exist on the distribution, ecology, and taxonomic status of the tortoise in Mexico. Driven by this lack of knowledge, in 2005 a team of Mexican, Canadian, and American collaborators began a multifaceted study of desert tortoises throughout their range in Mexico focusing on the tortoises’ health, genetics, general biology, and ecology to investigate their status and assess conservation needs. Here we present preliminary findings and our emerging hypotheses.

Across its large geographic range in Mexico, Morafka’s desert tortoise occupies a variety of habitats including desertscrub, foothills thornscrub, and the northern part of semiarid Sinaloan tropical deciduous forest (TDF) of southern Sonora and northern Sinaloa (Fritts and Jennings 1994). Behavioral patterns and ecological challenges for tortoises in these different environments can vary greatly, but there is currently very little published on the behavior or ecology of tortoises in thornscrub and TDF communities. Genetically, mitochondrial DNA (mtDNA) sampled from tortoises in the southern portion of the range is deeply divergent from haplotypes in the central part of the species’ range, suggesting the potential for a unique lineage (Lamb and others 1989; Berry and others 2002). Thus, one challenge currently facing conservation initiatives for *G. morafkai* is to determine the taxonomic status and distribution in the southern portion of its range. The “Sinaloan” type tortoise may be a cryptic species (Murphy and others 2011).

Taxonomic assessments of tortoises in Mexico can be bolstered by identifying geographic barriers to dispersal that contribute to genetic isolation, analogous to the Colorado River, which separates *G. agassizii* and *G. morafkai*. In contrast, the identification of genetic clines infers that taxonomic recognition of multiple species is unwarranted because such designations would serve to recognize the extremes only. It is equally critical to understand the rates and patterns of genetic differentiation expected between two populations. Previous work has established that in both Mojave and Sonoran environments, desert tortoises exhibit a population genetic structure of isolation by distance (IBD; Edwards and others 2004; Murphy and others 2007; Hagerty and others 2011). IBD occurs when the geographic distance between populations is the major limitation to gene flow. Panmixia is expected at all sampling localities yet allele frequencies at sites separated by great geographic distances may differ significantly. In contrast, when alleles that distinguish species boundaries are under strong selection, the rate of genetic introgression should be near zero in one or both directions (Payseur 2010).

Methods

Between 2005 and 2011 we collected blood samples from over 130 wild-caught *G. morafkai* throughout the species’ range in Mexico and in three different vegetation communities: TDF, foothills thornscrub, and Sonoran desertscrub. Targeted sampling involved the ecotone

between foothills thornscrub and Sonoran desertscrub (fig. 1). We collected whole blood via subcarapacial or brachial venipuncture. We also used salvaged red blood cells stored at the University of Arizona Genetics Core. Blood collection followed Animal Use Protocols approved by the Royal Ontario Museum Animal Care Committee, and by the University of Arizona (IACUC 09-138). All genetic procedures and analyses were conducted in the University of Arizona Genetics Core following procedures described in Edwards and others (2004) and Murphy and others (2007). We genotyped individuals for 16 microsatellite loci (Edwards and others 2004; Murphy and others. 2007) and sequenced approximately 1,200 base pair portions of mtDNA (Murphy and others 2007). In addition, we utilized additional samples from throughout the range of desert tortoises in the United States (Edwards and others 2004; Murphy and others 2007; Edwards and others 2010).

We used PAUP* 4.0b10 (Swofford 2002) to reconstruct the maternal (mtDNA) phylogeny using a 50% majority rule consensus tree based on unweighted maximum parsimony analysis. In addition to the samples collected in this study, we also included data from *G. morafkai* collected in Arizona, *G. agassizii* collected in California and Utah (Murphy *et al.* 2007), and we utilized other *Gopherus spp.* (*G. berlandieri*, *G. flavomarginatus* and *G. polyphemus*) as outgroups.

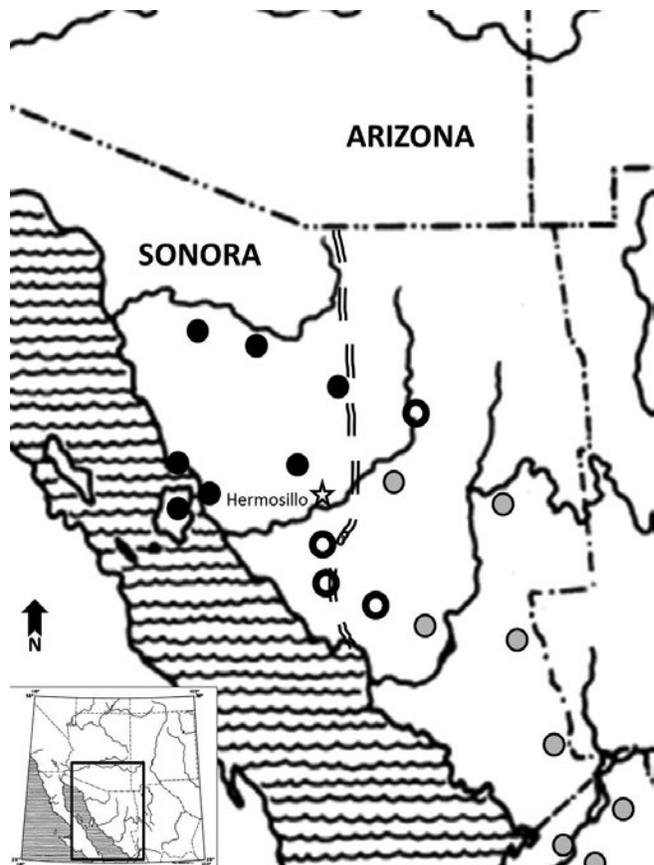


Figure 1—Sample locations in Mexico for this study are represented by circles; solid circles represent sites where the sampled mitochondrial haplogroup is strictly “Sonoran,” gray-shaded circles represent sites where the sampled mitochondrial haplogroup is strictly “Sinaloan” and open circles represent sites where a mix of Sonoran and Sinaloan haplotypes are observed. The double-dashed vertical line running down the middle of the image roughly defines the boundary of Sonoran desertscrub.

We performed a bootstrap analysis with 1,000 replicates of 10 random addition sequences per replicate to determine the support for the inferred relationships. For our microsatellite analyses we incorporated samples previously collected in Arizona and estimated the degree of population differentiation within and among populations using AMOVA (analysis of molecular variance) in ARLEQUIN 3.5.1.2 (Excoffier and others 2005). We calculated genetic distances based on pairwise Φ_{ST} (F_{ST} sensu Weir and Cockerham 1984) and defined populations by their proximity to major cities; Kingman, Phoenix, and Tucson, Arizona, USA, and Hermosillo, Ciudad Obregón, and Álamos, Sonora, Mexico.

Results

Analyses of mtDNA sequences resolved three distinct maternal lineages corresponding to the Mojave, Sonoran, and Sinaloan populations. Sonoran and Sinaloan tortoises differentiated along the ecotone between foothills thornscrub and Sonoran desertscrub. Distinct Sinaloan haplotypes occurred only in foothills thornscrub and TDF environments while Sonoran haplotypes were mostly restricted to desertscrub environments (fig. 1). Several sample sites along the ecotone of foothills thornscrub and desertscrub exhibited individuals representing both Sonoran and Sinaloan maternal lineages (fig. 1). Modal Sonoran haplotype SON01 (Murphy *et al.* 2007) was observed at all sites exhibiting Sonoran lineages.

In the analyses of bi-parentally inherited microsatellite loci we observed a continuum of genetic similarity spanning 850 km of Sonoran desertscrub, ranging from Hermosillo, Sonora, northwards to Kingman, Arizona (table 1). This corresponded with the distribution of Sonoran mtDNA haplotype SON01. Tortoises within this continuum had a population structure consistent with IBD (Mantel test: $r^2 = 0.75$, $p < 0.01$). However, strength of the correlation began to break down when assessed across the ecotonal boundary between desertscrub and thornscrub-TDF (Mantel test: $r^2 = 0.38$, $p < 0.01$). Fixed differences in microsatellite alleles occurred in foothills thornscrub and TDF in southern Sonora and clearly distinguish a unique Sinaloan genotype.

Discussion

These analyses suggest that tortoises living in foothills thornscrub and TDF in southern Sonora and Sinaloa have a level of differentiation equal to that between *G. agassizii* and *G. morafkai* from Arizona

(Murphy and others 2011). All three lineages—Mojave, Sonoran, and Sinaloan—likely diverged from a common ancestor 5–6 million years ago (fig. 2; Lamb and others 1989; Avise and others 1992; McLuckie and others 1999). A cursory analysis of the three mtDNA lineages suggests they are on independent evolutionary trajectories and potentially constitute separate species. However, spatial overlap of several haplotypes occurs at the eastern and southern boundaries

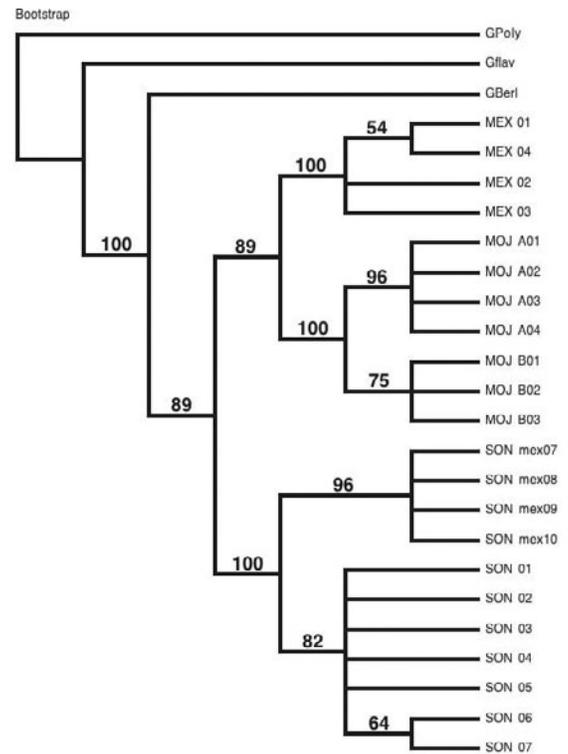


Figure 2—A 50% majority rule consensus tree based on maximum parsimony for 1,200 base pairs of sequence from the ND4 region of the mitochondrial genome of desert tortoises (*Gopherus* spp.); SON = *G. morafkai*, SON mex = mtDNA haplotypes observed only in Sonora, Mexico, MOJ = *G. agassizii*, MEX = Sinaloan populations of *Gopherus morafkai* and outgroups *G. berl* (*G. berlandieri*), *G. flav* (*G. flavomarginatus*), and *G. poly* (*G. polyphemus*). Numbers above branches are bootstrap values.

Table 1—Comparison of pairwise genetic distance, Φ_{ST} (F_{ST} sensu Weir and Cockerham 1984), among sites spanning the geographic range of desert tortoises in the Sonoran Desert. Data generated from 16 microsatellite loci. Sites are categorized by their proximity to major cities; Kingman, Phoenix, and Tucson, Arizona, USA, and Hermosillo, Ciudad Obregón, and Álamos, Sonora, Mexico. Dotted line indicates transition from localities representing desertscrub (above) and thornscrub and tropical deciduous forest (below). This analysis was performed excluding samples from the 4 sites with mixed mitochondrial lineages.

	Kingman	Phoenix	Picacho	Tucson	Hermosillo	Obregón
Kingman						
Phoenix	0.007					
Picacho	0.043	0.024				
Tucson	0.067	0.042	0.008			
Hermosillo	0.090	0.055	0.051	0.041		
Obregón	0.251	0.201	0.185	0.181	0.145	
Álamos	0.231	0.191	0.173	0.172	0.153	0.072

of Sonoran desertscrub and this suggests a complex history in this vegetative transition zone (fig. 1). The transitions from desertscrub to thornscrub to TDF are generally considered gradual, clinal transitions between somewhat artificial categories. Even though about 85% of foothills thornscrub is in Sonora, phytogeographers have characterized it as “Sinaloan” as a result of floristic affinities to TDF (Brown 1982). This ecotone is likely to have expanded and retreated many times during the relatively recent transformation into the Sonoran Desert (Van Devender 2000) and this dynamic system has undoubtedly influenced genetic differentiation among desert tortoise lineages.

Whereas the divergence between *G. agassizii* and *G. morafkai* is a classic example of allopatric speciation resulting from geographic isolation by the Colorado River (Lamb and others 1989; Avise and others 1992; McLuckie and others 1999; Murphy and others 2011), divergence of Sonoran and Sinaloan *G. morafkai* suggests that a different mechanism is in play. Sonoran and Sinaloan tortoises differentiate along an ecotone of foothills thornscrub and Sonoran desertscrub communities; ecotones can play critical roles in evolution because hybridization can occur in these settings (Barton and Hewitt 1985). Interestingly, our analyses do not detect clear signals of hybridization between Sinaloan and Sonoran type tortoises despite the spatial proximity of lineages in this extensive vegetative transition zone (fig. 1). This is in contrast to *G. agassizii* and *G. morafkai* where hybridization has been observed at locations of secondary contact (Edwards and others 2010). Unlike the clear allopatric pattern of divergence between *G. agassizii* and *G. morafkai*, Sonoran-Sinaloan lineages of *G. morafkai* may have diverged under a parapatric model where the current contact zone in Mexico may be a result of recent secondary contact after isolation prior to the formation of the Sonoran Desert. Chance interbreeding (during times of sympatry) may have driven or be driving the development of reproductive isolating mechanisms. Alternatively, the sharp break in the cline may be a result of insufficient time for genes to flow far beyond the boundary. Our future efforts will attempt to discern whether genetic differentiation is a result of past periods of isolation, or if differentiation has evolved in the absence of physical barriers to gene flow.

Taxonomic clarification is of utmost importance for management of *G. morafkai*. Our continuing effort to resolve the taxonomy of the species in the context of its geographic distribution will provide a basis for prioritizing resources and conservation measures where they are most needed and will ensure that data are available to maintain genetic diversity across the species' range. Future efforts will resolve the taxonomic uncertainty of *G. morafkai* in Mexico by testing the null hypothesis of conspecificity and determining the mechanism(s) of divergence between the Sonoran and Sinaloan lineages. Clarification of these evolutionary processes will directly inform efforts to preserve the evolutionary potential of the species. Ultimately, an understanding of the evolutionary history of desert tortoises will not only clarify the forces that have driven differentiation in this group, but also contribute to our knowledge of the biogeographic history of the Southwest deserts and how diversity is maintained within them.

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