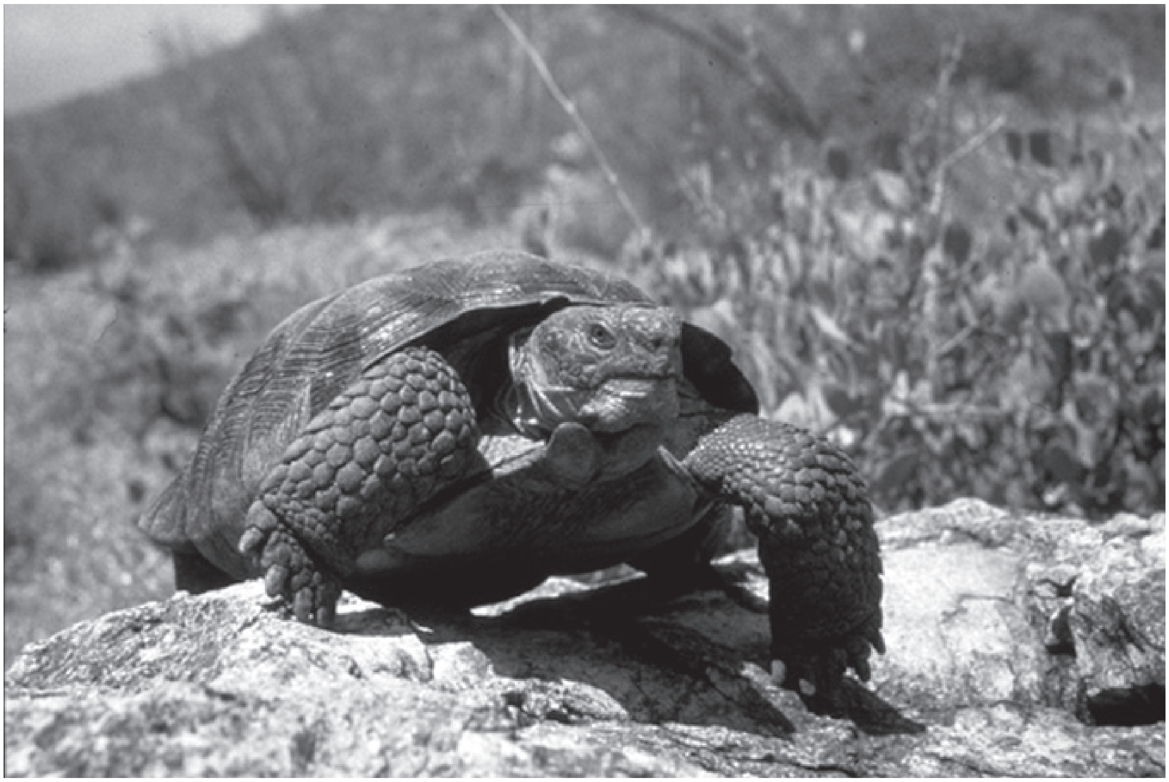

Figure 1
The desert tortoise
(*Gopherus agassizii*)
Photo by Don Swann

Home Is Where You Take It; Tracing The Movements Of Desert Tortoises With Molecular Biology

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I can think of no other reptile that people love more than tortoises. In Tucson, the desert tortoise has captured our hearts as a desert icon. However, despite our admiration, desert tortoises are currently threatened by many human activities. Tortoise populations are declining as a result of habitat loss from human development, habitat alteration by off-road vehicles and grazing, illegal collecting, and road mortality. While many of these dangers are immediate, there are also threats we might not readily perceive, such as building highways and canals that result in habitat fragmentation. Tortoises are even in danger of people who want to help them. By releasing their pet tortoises back into the wild, people's good intentions may spread diseases into wild populations.

The nature of being a tortoise leaves this species particularly vulnerable to changes in their environment. Tortoises are long-lived animals with long generation times and therefore they do not recover quickly from population declines. To ultimately approach conservation of tortoises, we must think on the time scale of tortoises - not just how they will persist in our lifetime, but hundreds of years from now. There is growing interest in understanding the natural history of desert tortoises so that we can apply long-term approaches to management and conservation. In my research, I used molecular biology techniques to address the implications of anthropogenic landscape change on the inter-population movements of desert tortoises.

The desert tortoise (*Gopherus agassizii*) is recognized as having distinct Mojave and Sonoran populations. The Mojave population is defined as all tortoises north and west of the Colorado River and the Sonoran population contains all tortoises south and east of the Colorado River. The Mojave and Sonoran populations differ in morphology, seasonal activity, reproductive ecology, habitat selection, and mitochondrial DNA divergence. My study focused on tortoises from the extreme eastern and western parts of their range. Tortoises in the western Mojave primarily occur in valleys, alluvial fans, and bajadas in Mojave desertscrub (Joshua tree, creosote bush, and white bursage). In this environment, tortoises excavate deep burrows in washes and surrounding desert flats. In contrast, tortoises in southern Arizona generally inhabit areas of rocky foothills associated with leguminous trees and mixed cactus (foothill paloverde, saguaro, prickly pear, and cholla) characteristic of Arizona Upland Sonoran desertscrub. Tortoises in southern Arizona generally den in rocky outcrops and caliche caves in habitat patches separated by low desert valleys.

Adult tortoises have few natural predators and very low adult mortality. A mountain lion is perhaps the only native animal that can effectively eat an adult tortoise. Unfortunately, tortoises now face a wealth of human caused threats. Tortoises are very long lived (>40 years in the wild) and have long generation times (estimated at 25 years – the same as humans). Demographic studies indicate that for long-lived species that have high adult survivorship, a minor increase in the mortality of adult females in a population can have serious repercussions. This has implications for management because if we recognize that a population is declining, then we shouldn't expect that it would easily rebound. Tortoises also exhibit strong site tenacity, and return to the same dens year after year. Such "hard-wiring" may also make them less able to cope with lasting disturbances to their environment.

The Mojave population of the desert tortoise was federally listed as threatened by the U.S. Fish and Wildlife Service (USFWS) in 1990. In 1994, the USFWS established six recovery units within the Mojave population. Recovery units were identified based on evolutionarily significant units (ESUs) using published and unpublished data on genetic variability, morphology, and behavior patterns of populations as well as ecosystem types. ESUs are groups of desert tortoises that appear to be on independent evolutionary trajectories, but how they move and relate to each other within ESUs is not well understood. Investigation into the movement patterns (gene flow) that occur within ESUs and whether those patterns differ among ESUs relative to geographic features can help facilitate conservation efforts for this species. The Sonoran population is not federally listed but is currently considered a Species of Special Concern by the Arizona Game and Fish Department. Arizona is faced with a privileged situation in that we are making efforts to protect tortoises before they are threatened or endangered. Unfortunately, many species do not receive this kind of valuable research attention until after their populations have declined.

The biggest current threat to tortoises in the Sonoran Desert is direct loss of habitat. The upland hillsides surrounding Tucson and Phoenix where tortoises live are also prime real estate for people. Because Sonoran desert tortoises are associated with rocky foothills, populations are seemingly isolated from each other by low desert valleys. Tortoises sometimes make long-distance movements across valley bottoms, but the importance of these movements to tortoise population viability is unknown. Concern has arisen because recent human landscape changes such as roads, agricultural lands, the CAP, and urban development may act as barriers to tortoise movement. It is important to determine what effect these barriers may have on the long-term survival of tortoise populations. In addition, the frequency of tortoise long distance movements may be influenced by the heterogeneity of the landscape, such that the widespread and continuous tortoise habitat in the western Mojave suggests a greater potential there for gene flow than in the Tucson area. Therefore, delineation and management of ESUs might need to reflect this.

Studying the importance of dispersal in tortoises is challenging because of the long life span of tortoises and the rarity of movements. Therefore, I turned to molecular biology to define population structure, estimate the amount of gene flow, and assess the importance of tortoise long distance movement for population viability. I examined tortoise movements (gene flow) on 3 different spatial scales:

1. Between Mojave and Sonoran Populations-
I compared patterns of gene flow within each desert region to assess whether landscape heterogeneity influences population structure.

2. Among disjunct habitat patches in the Sonoran Desert-
I assessed the amount of gene flow that occurred historically among habitat patches (mountain ranges) in the Sonoran Desert to determine if movement between disjunct patches is currently hindered by landscape changes and what affect this might have on population viability. Although anthropogenic barriers are currently in place, because of the long-generation time of tortoises, I expected to see a historic pattern of gene flow NOT influenced by current landscape changes.

3. Within continuous habitat at a single mountain range-
I examined the genetic relatedness of individual tortoises within continuous habitat at Saguaro National Park to determine if tortoise behavior (home range) or geographic features (such as washes or ridges) have an influence on population structure.

From the Mojave population, I sampled 36 desert tortoises from 4 study sites (valleys) northeast and southeast of Barstow in the Western Mojave Recovery Unit in San Bernardino County,

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California. The maximum distance between any 2 sites was 157 km and the minimum distance 52 km. In Arizona, I sampled desert tortoises from 8 study sites (mountain ranges) in Pima and Pinal counties in the Tucson vicinity and from 1 site northeast of Phoenix in Maricopa County for a total of 170 tortoises from the Sonoran population. The maximum distance between the 8 Tucson area sites was 128 km and the minimum distance 16 km. The single site in Maricopa County (Sugarloaf) is 59 km north of the closest study site in the Tucson area.

The author uses a syringe to draw a small amount of blood from the front leg of a tortoise.
Photo by Cecil Schwalbe

Since tortoises can be difficult to find, this project would not have been possible without a tremendous volunteer effort. I owe tremendous gratitude to the members of the Tucson Herpetological Society and the other volunteers for all their support. Once located, I processed tortoises on site and collected general information on health, size, etc. Only a small blood sample was needed to conduct the genetic research since reptiles have nucleated red blood cells.

I targeted sites where tortoise research had previously occurred so that I could also contribute to long-term data. For example, in the Picacho Mountains, a volunteer search party found an individual tortoise that had been previously marked by Sherry Barrett (USFWS). We located the tortoise in essentially the same area that it was originally captured over a decade earlier, emphasizing this species' longevity and strong site tenacity. Another interesting case was a recaptured tortoise we found in a remote part of the West Silverbell Mountains. When Peter Woodman originally located the tortoise 1995 its carapace was completely painted green, white, and red in a Christmas scene. It was unknown whether this individual was a captive that had escaped or was released, or if someone had come to this remote site and painted it. (The DNA evidence ultimately indicated that this individual did not differ significantly from others tortoises at the site).

To examine population structure within continuous habitat, I simultaneously conducted radio-telemetry on 35 tortoises at Saguaro National Park. These tortoises are also part of other, ongoing studies and these data were collected with the assistance of several other tortoise researchers. I sampled from two sites at the park that were ~4 km apart and separated geographically by Box Canyon/Tanque Verde Ridge. During the study, no telemetered tortoises moved between the two sites. With several years of data on each tortoise, I was able to estimate the home range (the area used by an animal that supplies the resources necessary for its survival) for each individual.

I conducted all lab work at the Genomic Analysis and Technology Core at the University of Arizona. To compare differences between the Mojave and Sonoran populations, I examined mitochondrial DNA (mtDNA) in a sub-set of my samples (36 Mojave, 40 Sonoran). MtDNA is an excellent marker for population studies and has been the main focus of previous desert tortoise genetic research. I used the technique of DNA sequencing which allowed me to examine genetic variation based on differences between individual nucleic acids. The genetic code in DNA is represented by the pattern of 4 nucleic acids: adenine, thiamine, cytosine, and guanine, represented as A, T, C, and G. Divergence is characterized by differences in the DNA sequence between individuals, such that we might see the pattern ATGC**C**TCTAC**T**TCA… in one population or individual and a different pattern ATGC***T***TCTAC***A***TCA… in another population or individual. With some understanding of how frequently these changes occur over evolutionary time and how likely they are to become characteristic in a population, I could estimate how frequently tortoises moved between and within populations.

When I compared the sequences between tortoises from the Mojave and Sonoran deserts, I found that there were 4.5% fixed differences: these same differences occurred consistently in comparisons between all individuals from each population. This pattern of fixed differences is characteristic in species that have had long-term extrinsic barriers to gene flow. These data support previous calculations that divergence between Mojave and Sonoran desert populations of desert tortoises coincides with the formation of the Bouse

Embayment in the Lower Colorado River Valley between California and Arizona approximately 5 million years ago.

In addition, my data suggests that there was a prehistoric (10,000-45,000 yrs ago) population expansion event that occurred simultaneously in both the Mojave and Sonoran populations. Because fossil evidence documents the occurrence of the desert tortoise in both of these regions during the late Wisconsin glacial, I do not believe this pattern reflects a new range expansion into these areas, but possibly recovery from a population bottleneck associated with the last interglacial period. This pattern could also reflect a more recent bottleneck caused by anthropogenic extirpation, coinciding with the North American megafauna extinctions of the glaciopluvial transition 12,500-10,000 years ago. Several North American turtle species went extinct during this period (*Geochelone* sp., *Terrrapene c. putnami*, *Gopherus laticauda*) and pre-Columbian human-induced extirpation has been well documented for the extirpation of the Bolson tortoise, *Gopherus flavomarginatus.*

Within each desert population, I found relatively few nucleotide differences among individuals. From a genetic standpoint, this low variability demonstrates that tortoises have a history of maintaining small effective population sizes over time. I estimated that within each area sampled, the effective population size is approximately 60,000. This could be considered the number of tortoises that constitute an ESU. While there is high amount of variance associated with this estimate, it is interesting that both the Mojave and Sonoran areas sampled had similar estimates and these correspond with the estimate of effective population size generated from population viability analysis. (PVA is a computer simulation technique that uses demographic information to create a model of the population). Three PVAs conducted on the Mojave population of the desert tortoise recommend that a minimum of 50,000 individuals is necessary for a 50% chance of persistence for 500 years.

I estimated gene flow within each desert area by assessing the level of genetic differentiation among study sties. The Tucson area and the western Mojave both showed the same phylogeographic pattern. Phylogeography is the intraspecific relationship between genealogy (genetic differentiation) and geography (spatial separation). The pattern observed in the Tucson area and the western Mojave is characteristic of intermediate gene flow in a species not subdivided by long-term barriers. However, the Sugarloaf site, in the Sonoran Population outside of Phoenix, did show greater differentiation from the Tucson area sites. While natural geographic barriers certainly play a role in limiting the ability of tortoises to move long-distances, our understanding of desert tortoise dispersal is incomplete. Some barriers may be of long-term consequence, such as the Colorado River maintaining the genetic separation of Mojave and Sonoran populations, or, similarly, the Gila River that more recently may have separated the Sugarloaf site from sites in the Tucson area. However, the general similarity in phylogeographic patterns in both the Mojave and Sonoran populations suggests that heterogeneity of the landscape is not necessarily a good predictor of what constitutes an ESU for the desert tortoise.

To examine patterns of gene flow more thoroughly within just the Sonoran population, I used a molecular technique with greater resolution: microsatellite markers. Microsatellites are bi-parentally inherited (unlike mtDNA), are considered selectively neutral, and evolve rapidly. A microsatellite is simply a tandomly repeated segment of DNA, such as… ATC ATC ATC ATC ATC ATC ATC... Variability in a microsatellite is exhibited by differing lengths of this repeat (number of repeats) in an individual, such that one individual may have this pattern repeated 10 times, another 11 times, or another 9 times. Using a combination of multiple microsatellites is the method of DNA fingerprinting: the same method is used in human genealogy, paternity testing, and forensic science. This technique had never been applied to desert tortoises before, so this posed to be a major challenge of the project. In total, I identified 6 novel microsatellites in the desert tortoise genome useful for population genetic studies. I also tested 11 microsatellite markers already published for other turtle species (including sea turtles) and found one from the green sea turtle that was variable in the desert tortoise.

Using these microsatellite markers, I detected little genetic differentiation among study sites in the Sonoran Desert. I determined that the genetic similarity among sites was in fact a result of gene flow and not a result of recent ancestry, such as a recent range expansion event into the region or the result of a bottleneck event. I found a correlation between the genetic distance (genetic similarity between sites) and the geographic distance between sites. This pattern is called “isolation by distance”, or IBD. The desert tortoise is perhaps the ideal organism for the IBD model, such that its greatest difficulty of dispersal is a function of geography. I estimated that a minimum of 2.9 migrants per generation is exchanged between adjacent mountain ranges. However, this estimate should not be taken as an absolute number because gene flow can be variable and unpredictable among populations due to a wide array of demographic and environmental factors. The most likely scenario for the desert tortoise is that gene flow occurs not at a regular rate,

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but with varying frequencies over time related to environmental fluctuations. What is important is that gene flow occurred historically at a rate greater than one migrant per generation (OMPG), which is the amount necessary to eliminate differentiation caused by drift or mutation.

Among individual, radiotelemetered tortoises at Saguaro National Park, my results also showed lack of differentiation between the two study sites. I conclude that within continuous habitat, geographic features or behavior do not contribute significantly to within-population genetic structure. Coincidentally, one of the radiotelemetered tortoises from Saguaro made a long distance movement during the course of the study. We named her "Thelma", from the movie *Thelma and Louise*, because she was an adult female that took off for no apparent reason. Over the course of two field seasons, Thelma moved over 32 km straight-line distance from the Rincon Mountains to the Santa Rita Mountains. In the process, researchers had to facilitate her movement across several anthropogenic barriers, such as fence lines, railroad tracks, and an interstate highway. She was also captured several times and temporarily adopted by private citizens. While Thelma's movement is complimentary to this study, it does not by itself tell us if these movements contribute to gene flow. However, it does exemplify that tortoises are capable and sometimes motivated to disperse long distances but the urban topography of the modern landscape makes such movements virtually impossible.

Anthropogenic barriers, like the CAP shown here in the Picacho Mountains, hinder the ability of tortoises to make long-distance movements.
Photo by Taylor Edwards

My research demonstrates that tortoises in the Tucson area historically dispersed between mountain ranges and that movements between these disjunct locations may be critical to the persistence of small tortoise populations at each location. Unfortunately, many tortoise populations are becoming islands surrounded by human development and are more vulnerable to stochastic events. Encroachment of human development also increases the threat of road mortality, illegal take, and exposure to disease from escaped or released domestic tortoises. The life history traits of the desert tortoise suggest that there are severe constraints on the ability of populations to respond to chronic disturbances. It is unlikely that a closed population of desert tortoises experiencing a dramatic reduction in adult survivorship would be able to offset that loss through compensatory increase in reproductive output. The high level of gene flow I observed among populations suggests that if a population were to experience a catastrophic decline as a result of drought or other stochastic event, its recovery may rely heavily on the immigration of new individuals from adjacent mountain ranges.

An excellent example of a desert tortoise population imperiled by landscape change is Tumamoc Hill within the city limits of Tucson. Tumamoc Hill hosts a small (<30) population of tortoises. This population is essentially an island completely surrounded by urban development. The effects are apparent in the tortoise population; the population is small and many of the individuals exhibit shell trauma from domestic or feral dog attacks. The proximity of this site to people's homes also makes the tortoise population vulnerable to escaped or released domestic tortoises that are a potential source of disease to wild populations. It is inevitable that this population will (or is) experiencing a population decline from human-related activities. Since there is no dependable way for new tortoises to naturally immigrate into the population due to the proliferation of heavily traveled roads surrounding it, the population will likely be extirpated. Because of the long lifespan of desert tortoises, a local extinction could take decades to be realized.

It is important that management strategies be designed to facilitate gene flow among disjunct tortoise locations. Currently, it is unlikely that successful long-distance dispersal events could occur over much of the range of the desert tortoise due to recent proliferation of major human development. Assessing what constitutes a barrier to movement for tortoises is necessary for maintaining connectivity between sites. While a roadway may not be a barrier to a large ungulate, it may be impenetrable to a tortoise. Tortoises are able to cross some barriers and have been shown to use culverts. Fencing or concrete barriers along highways may also help guide tortoises toward appropriate crossing areas and prevent road mortality.

Placement of culverts and corridors needs to specifically accommodate tortoises, as corridors designed for general wildlife use may not be effective.

Translocation of tortoises from nearest-neighbor populations should be evaluated as a potential management strategy to recover or maintain small populations isolated by anthropogenic barriers. Tortoises generally exhibit strong site tenacity, and translocation studies of reptiles indicate that they generally fare poorly in unfamiliar areas. However, preliminary studies in the Mojave Desert indicate that translocation may be an effective strategy for supplementing depauperate populations of desert tortoises. Currently in Arizona, tortoises are sometimes relocated short distances during construction projects. Before inter-population translocation of tortoises is implemented as a conservation strategy in the Sonoran Desert, effects of translocation on survivorship of relocated individuals and the populations into which they are introduced need to be evaluated and the potential for disease transmission from one population to another needs to be assessed. In addition, translocation will not likely be a sustainable strategy unless threats are also identified and alleviated. While it may be tempting to apply the OMPG rule to isolated tortoise populations not declining, different schedules of supplementation may be appropriate depending on environmental and demographic conditions specific to each population. Management strategies compatible with the evolutionary history of gene flow among disjunct populations will help ensure the long-term persistence of Sonoran desert tortoise populations.

Tortoises are just one species that that faces threats caused by human landscape change. They are a good example of a species that requires long-term management and foresight, such as an ecosystem itself. They are a charismatic species and public interest in the persistence this species will hopefully benefit other species as well.

Acknowledgements

Although I have used the word “I” in this summary of my thesis work, it is actually a “we” and would not have been possible without the assistance of many individuals:

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Selected References:

Avise, J.C., B.W. Bowen, T. Lamb, A.B. Meylan and E. Bermingham. 1992. Mitochondrial DNA evolution at a turtle's pace: Evidence for low genetic variability and reduced microevolutionary rate in the testudines. Molecular Biology and Evolution 9(3):457-473.

Berry, K.H., D.J. Morafka, and R.W. Murphy. 2002. Defining the desert tortoise(s): our first priority for a coherent conservation strategy. Chelonian Conservation and Biology, 4(2):249-262.

Congdon, C.D., A.E. Dunham and R.C. Van Loben Sels. 1993. Delayed sexual maturity and demographics of Blanding's turtles (*Emydoidea blandingii*): implications for conservation and management of long-lived organisms. Conservation Biology 7(4):826-833.

Dodd, C.K., and R.A. Seigel. 1991. Relocation, repatriation and translocation of amphibians and reptiles: are they conservation strategies that work? Herpetologica 47: 336-350.

Lamb, T., and C. Lydeard. 1994. A molecular phylogeny of the gopher tortoises, with comments on familial relationships within the Testudinoidea. Molecular Phylogenetics and Evolution 3(4):283-291.

Mills, L.S., and F.W. Allendorf. 1996. The one-migrant-per-generation rule in conservation and management. Conservation Biology 10(6):1509-1518.

U.S. Fish and Wildlife Service. 1994. Desert Tortoise (Mojave Population) Recovery Plan. U.S. Fish and Wildlife Service, Portland, Oregon, USA. 73pp. and appendices.

Van Devender, T.R., editor. 2002 The Sonoran desert tortoise: natural history, biology, and conservation. University of Arizona Press, Tucson, Arizona, USA. 388pp.