Mojave National Preserve

National Park Service
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Science Newsletter

A History of Mojave Desert Tortoise Movement: A View through the Window of Population Genetics

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This edition of the Mojave National Preserve Science Newsletter highlights the desert tortoise, with important new findings on genetics and disease. The ongoing problem of traffic-related mortality on highways is the topic of our resource management concern section.

The desert tortoise is a charismatic flagship species throughout the deserts of the southwestern United States. We used tools from the field of population genetics to identify where populations occur in the listed portion of the species' range and to recommend revisions to the existing recovery units that are critical to managing the desert tortoise.

Decisions regarding species of conservation concern are often informed by analyses of genetic data that are used in conjunction with other population data such as habitat requirements and estimates of population size, survival, and reproduction (*1*). Previously, general recommendations such as the maintenance of genetic variation and the prevention of inbreeding were the main contributions of population genetics to conservation biology (*1*). Beyond these general principles, neutral genetic markers can be used to answer many questions related to individuals and populations. For example, we can infer if individuals within a population are closely related, how many individuals disperse from a population and reproduce in

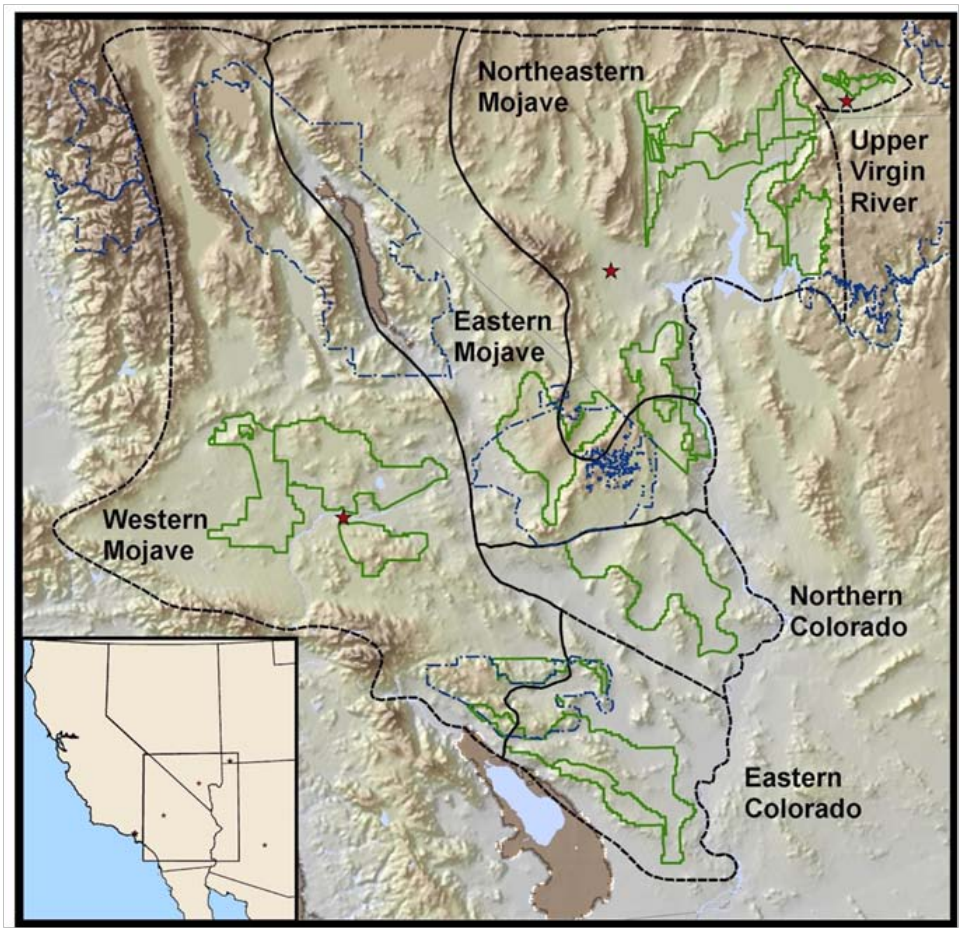

Figure 1. Map of the listed portion of desert tortoise habitat and original recovery units from the 1994 Recovery Plan. Black dotted lines indicate the six recovery units, green solid lines indicate Desert Wildlife Management Areas (DWMAs), and blue dotted lines indicate National Parks, including Mojave National Preserve. The stars indicate some major cities in the southwestern United States including, St. George, UT, Las Vegas, NV, Barstow, CA, Los Angeles, CA (inset only), and Phoenix, AZ (inset only). The Baker Sink follows the black line that separates the Western Mojave Recovery Unit (RU) from the Eastern Mojave RU, and then the Northern Colorado RU.

In this Issue:

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This Science Newsletter:

The Mojave Desert is internationally known as a place to conduct scientific research on desert ecosystems. In fact Mojave National Preserve was designated in part to "retain and enhance opportunities for scientific research in undisturbed ecosystems" as stated in the California Desert Protection Act of 1994. Significant research is conducted through the Sweeney Granite Mountains Desert Research Center, part of the University of California Natural Reserve System, and the Desert Studies Center, operated by the California Desert Studies Consortium of California State Universities. Both are located in the Preserve.

The purpose of this newsletter is threefold. First, we would like to highlight some of the research being done by scientists in the Preserve and to distribute this information to park staff and management. Second, this periodical will allow us to inform the public and research community about science being done by Preserve staff or funded through the National Park Service. And most importantly, we would like to build collaboration between scientists and resource managers so that scientists are made aware of the needs of managers and top quality science is brought to bear on the problems facing resource managers.

This newsletter is published twice per year, in the spring and fall. Copies are available in print at our Kelso Depot Visitor Center, Barstow Headquarters, Desert Studies Center, Sweeney Granite Mountains Desert Research Center, and electronically as pdf documents on the web[1]. Articles range from non-technical news stories to highly technical research reports. All material in this newsletter has been peer-reviewed by subject-matter experts.

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[1]http://www.nps.gov/moja/naturescience/sciencenews.htm

another, and how many populations exist in a given area.

Historical and present demographic processes (including dispersal) cause the structure of populations within a species. The processes are intimately tied to geographic, landscape, and habitat features (*2*). The result is a cumulative genetic signature that biologists can detect with variable, neutral genetic markers such as microsatellites. The underlying genetic population structure can provide an indication of how individuals move across the landscape. When individuals disperse (along with their genes), and they reproduce in the new population, gene flow occurs. Gene flow is important because it helps maintain connectivity among populations. This connectivity can be valuable because it can help prevent local extinctions, helps to prevent inbreeding, and fosters adequate responses to environmental change (*3*).

Investigating population structure can be valuable to managers of species of concern for several reasons. We care about genetic diversity because it can indicate the ability of a species to adapt under changing environmental conditions. Understanding population structures also can help us to prioritize habitat for restoration and inform designations of conservation units for proper management of threatened populations (*1, 4*). Here, we discuss how the inferences made from highly-variable, neutral genetic markers (e.g., microsatellites) can complement other ecological information to inform conservation decisions for a threatened species in the Mojave Desert.

A flagship species of the Mojave desert, the desert tortoise (*Gopherus agassizii*), is listed as threatened under the U.S. Endangered Species Act of 1973 (55 FR 12178, April 2, 1990) in the region located north and west of the Colorado River (Figures 1 and 2). Declines in population numbers are a result of habitat destruction, invasive species, disease, and many other threats related to increased human land use (*5, 6*). Desert tortoises are long-lived, have low growth rates and delayed sexual maturity (age 13-20), and have low annual reproductive rates over a long adult life span (*7*). Reproduction and growth rates vary with heterogeneity in the harsh, environmental conditions of the Mojave Desert; individuals capitalize on rare years with high rainfall and productivity (*8*). These traits cause tortoise populations to respond slowly to management actions, making evaluation of those actions difficult. Extreme and variable climatic conditions also reduce daily and seasonal activity of tortoises. Individuals spend a majority of each day and each season underground in burrows, making these animals elusive to human observers and researchers (*9*). Additionally, the Mojave desert tortoise has a large geographic distribution, which extends across four states in the southwestern United States. Thus, management requires coordination among multiple federal, state, and local agencies, communities, and various non-governmental organizations to implement actions that are important to recovering the population.

Our goals were to identify population structure for this threatened population segment and to use population genetic data and analyses in combination with other relevant biological data to make recommendations for revisions to the existing recovery units. The species' traits described above and the complications of coordination across many political boundaries present unique challenges for management. Additionally, a tortoise may take 25 or more years to replace itself in the population (*5*). This long generation time affects how long it takes for changes in allele frequencies to occur and causes population structure to change more slowly (*10*). Therefore, the genetic data that we collected likely reflect ecological processes that were occurring prior to

Figure 2. (Top Panel) Mojave desert tortoise foraging in the Newberry Mountains, Nevada. (Bottom Panel) Desert tortoise in typical creosote scrub (*Larrea tridentata*) habitat along a genetics sampling transect in Mojave National Preserve.

anthropogenic changes in the Mojave Desert. This provides opportunities to make recommendations based upon population dynamics prior to severe human influences.

An army of dedicated individuals who collected data to estimate population density across the range also collected close to 750 blood samples that we used for genetic material. Samples were collected from 25 geographic locations that could be divided into valleys of tortoise habitat (*11*). Transects were distributed across the states where the tortoise is listed, and they were laid out so as to provide complete coverage of the geographic range of the species. DNA was amplified with primers specifically designed for microsatellies that were developed for the Mojave desert tortoise. Microsatellites are nuclear genetic markers that mutate quickly and are neutral with respect to natural selection. Therefore, they are one type of genetic marker that can be used to evaluate population structure on a more recent time scale (10-100 generations). We amplified tortoise DNA with 20 microsatellite markers that had 3-40 alleles or variants (*11, 12*). Alleles are determined to be different when the number of the tandem repeats in the DNA sequence changes. The frequencies of the alleles are the main currency for population genetics and can be used to identify populations that could be considered demographically independent. We investigated the population structure using Bayesian statistical tests to assign tortoises to different populations based on the frequency of their alleles (*13*). Bayesian assignment approaches offer an objective way to identify populations based on the similarities among allele frequencies without previous knowledge of where the populations exist. Once we had a clearer understanding of population structure, we evaluated the populations that we identified using population genetic metrics such as *F*-statistics that provide an indication of the extent of genetic differences among populations (11).

Mojave desert tortoises are structured hierarchically, and the structure is visible at two scales (*11*). We detected a broad scale of structure consisting of three main clusters: Northern Mojave, Las Vegas, and California (*11*) (Figure 3). Following a north to south gradient of allele frequencies, each cluster has a core area that is clearly identified and transition zones where assignment of individuals to a particular cluster is not 100% (*11*). The distribution of mitochondrial DNA haplotypes, or variants of the same DNA

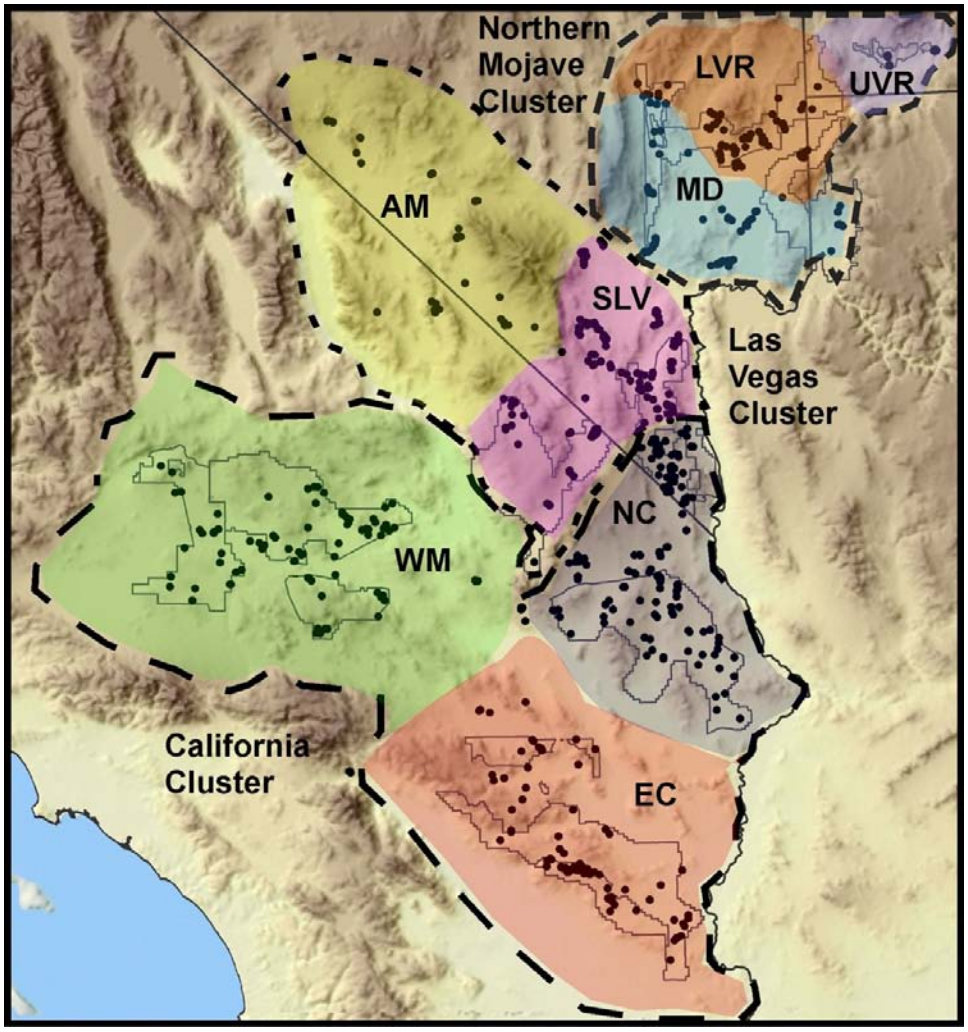

Figure 3. Map showing the recommended boundary revisions for recovery units based on the population structure of the Mojave desert tortoise. Each black dot indicates a location where a blood sample was collected. The three basal genetic groups are marked as dotted lines. The smallest hatching is the Las Vegas Cluster, medium hatching is the Northern Mojave cluster, and the largest hatching is the California cluster. The colors within each basal cluster indicate the recommended recovery units based on the finer-scale structure and complementary ecological data: Upper Virgin River (UVR) = purple, Lower Virgin River (LVR) = orange, Muddy Mountains (MD) = light blue, Amargosa Desert (AM) = yellow, South Las Vegas (SLV) = pink, Northern Colorado (NC) = dark blue, Eastern Colorado (EC) = red, and Western Mojave (WM) = green (adapted from *11*).

sequence, identified previously, closely resembles the microsatellite clusters (*14, 15*). Within these main clusters, the Mojave desert tortoise can be further divided into seven additional clusters that span the diversity of habitat, reproductive traits, and behavior (*11*) (Figure 3). We used these analyses to make recommendations to the U.S. Fish and Wildlife Service to revise the recovery units that were designated in the 1994 Recovery Plan (*5*) (Figure 1). Our genetic analyses are the backbone for the proposed changes; the boundaries for each unit are designated based on the significantly different allele frequencies among genotype clusters. Genetic analyses alone are not sufficient to designate recovery units or any other type of conservation unit (*16*). Therefore, where possible, we complement our data with other information regarding ecology, behavior, and habitat for the Mojave desert tortoise. These proposed changes should be treated as a hypothesis that can be revised with new data and analyses as they become available. According to the most recent National Marine Fisheries Service policy, as adopted by the U.S. Fish and Wildlife Service, recovery units should be geographically identifiable and essential to the recovery of the Mojave population of the desert tortoise (*17*). Each unit should contain elements necessary to conserve characteristics of the population that are required to sustain it in the long term, including genetic diversity (*17*). The original six recovery units were based on the best available data at the time, but the boundaries had poor justification in some cases, and they do not follow the current policy (*6*). The critical habitat identified within each original recovery unit (Desert Wildlife Management Areas or DWMAs) remains vital for the recovery of the desert tortoise (Figure 1).

The revised boundaries for the eight recovery units that we describe differ from those described in the 1994 Recovery Plan, with most differences occurring in Nevada (*11, 18*) (Figure 3). Differences from the original recovery units occur within the major range limitations for the species including the Colorado River, which forms a substantial physical barrier to the east. Based on Bayesian clustering analyses, we identified four populations within the original Northeastern Mojave Recovery Unit: Lower Virgin River, Muddy Mountains, South Las Vegas, and Amargosa Desert (*11*) (Figure 3). These modifications support a previous hypothesis stating that the original Northeastern Mojave Recovery Unit contained additional genetic diversity (*19*). The proposed Amargosa Desert Recovery Unit contains a previously undescribed genetic subpopulation, and therefore, lacks any critical habitat. Inadequate sampling caused tortoises in this area to be overlooked in the past. Importantly, this recovery unit is genetically distinct from neighboring units, bordered by Death Valley on the west and the Spring Mountains in the east (*11*). Both of these ecological boundaries are formidable for tortoises,

particularly the extreme thermal environment in Death Valley. We recommend that these tortoises be included in future research on ecology and behavior to characterize putative differences in this region.

Boundaries for the three recovery units in California remain almost identical to the descriptions in the 1994 Recovery Plan (*18*) (Figures 1 and 3). The Northern Colorado recovery unit, which also contains Piute Valley, borders the South Las Vegas recovery unit. The boundary occurs at Searchlight Pass, which is a low elevation pass (1500 m). The Eastern Colorado recovery unit represents the southern-most extent of the listed population. A low elevation barrier, known as the Baker Sink, extends from Saline Valley in California in the north, then south through Death Valley, Silurian Valley, Baker, Amboy, and Cadiz Valley. This barrier separates the Northern and Eastern Colorado and reflects the formidable effects of the lower elevations and extremely hot and dry climates along this line. The Western Mojave cluster is separated from the Eastern Colorado cluster in the Pinto Mountains, and from the Amargosa cluster in the low elevation area near Death Valley.

Population genetics information alone does not clearly indicate that a recovery unit has unique evolutionary potential or adaptive differences (*16*). Additional information should be used to support delineations. For example, differences in vegetation and climate tend to shape differences in how tortoises forage, use habitat, reproduce, and survive in each recovery unit (*e.g. 5, 6, 20, 21*). Many ecological differences in the Mojave and Colorado Deserts, including climate and vegetation, also occur along gradients. For example, west of the Baker Sink, variable rainfall typically occurs in the winter and spring, and it almost never occurs as summer monsoonal rains. Less variable winter rains as well as summer monsoon precipitation characterize the

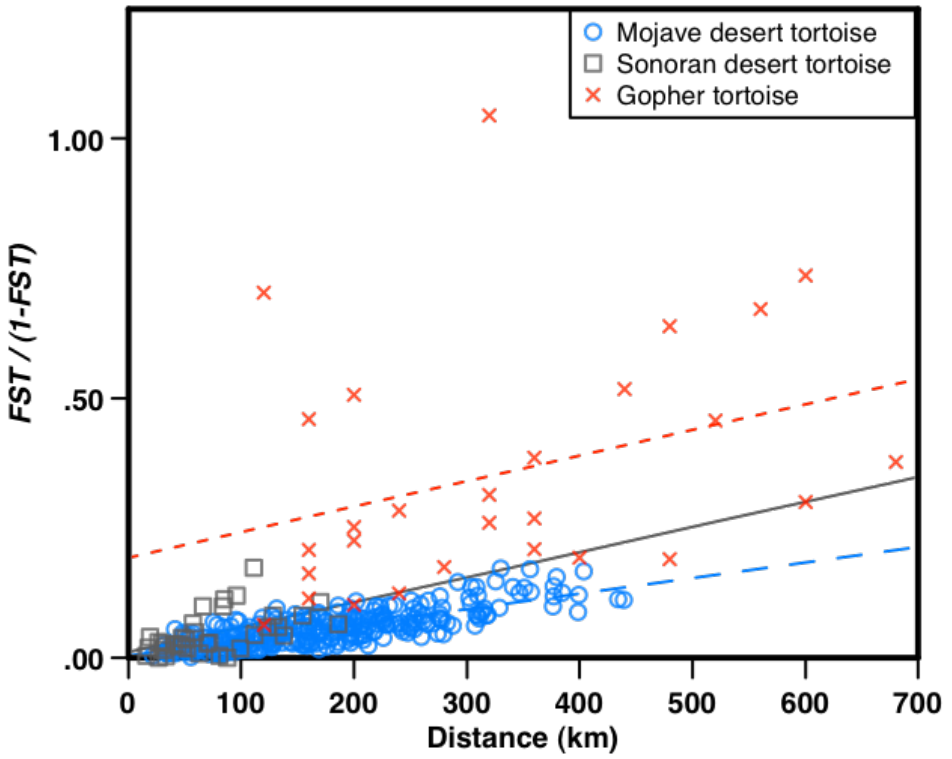

Figure 4. Isolation by distance across sampling locations of the Mojave desert tortoise (blue circles; R^2 = 0.678, P < 0.0001 (*18*)), the Sonoran Desert tortoise (grey squares; R^2 = 0.304, P = 0.005 (*28*)), and the gopher tortoise (red crosses; R^2 = 0.158, P = 0.036 (*29*)). Points represent comparisons of genetic distance (F_{ST} /1-F_{ST}) as a function of geographic distance between central points for sampling locations. Lines correspond to the linear regressions for each data set.

Mojave Desert east of the Baker Sink (*6, 22*). The most noticeable differences in tortoise ecology result from this climatic gradient and the variation in plant communities between the western and eastern Mojave Desert (*6*). Tortoises in the Western Mojave Recovery Unit produce relatively larger eggs, produce fewer eggs overall, and lay their second clutches later than do tortoises in the adjacent eastern Mojave Desert (*21*). Behaviorally, Western Mojave tortoises are less active during summer than are tortoises in other proposed recovery units (*23*). Therefore, we used evident geographic barriers, such as mountain ranges and hot and dry low elevation playas to delineate boundaries for the recovery units that reflect the core genotype groups (*11, 18*).

Although tortoises in the northern-most extent of the range near St. George, Utah were genetically similar to adjacent locations in Nevada, morphological, ecological, and behavioral data distinguish those individuals (*11*). Therefore, we characterized the Upper Virgin River as the eighth recovery unit (*11, 18*) (Figure 3), which reflects the original recovery unit designation (*5*). The tortoises in the Upper Virgin River Recovery Unit represent the northern-most extent of the current distribution of this species. Desert tortoises in this region experience long, cold winters and mild summers, live in a complex topography where the vegetation is a transitional mixture of Great Basin and Mojave communities, and tortoises use natural caves in sandstone and lava instead of burrows excavated by the tortoises (*5, 24, 25*). Tortoises also travel to sand dunes to lay eggs and use other habitats for foraging (*24*). The unique habitat and resulting behavioral differences in tortoises in this region warrant explicit protection (*11, 18*).

Population structure of the Mojave desert tortoises exists at broad and fine spatial scales; however, genetic differentiation among most sites is low (*11*). We used F_{ST} values as an index that describes genetic difference between pairs of sampling locations and genotype clusters. F_{ST} provides a summary of the genetic changes that have occurred over time, with higher values closer to 1 indicating that populations are genetically very different and do not exchange many migrating individuals. Pair-wise values among the seven clusters were statistically significant and ranged from 0.012 between the Amargosa and South Las Vegas clusters to 0.132 between the Virgin River and Eastern Colorado clusters (*11*). Therefore, we can infer from the low to moderate F_{ST} values that tortoises and their genes moved among populations often within each generation (approximately 25 years) (*5*). We cannot distinguish effectively between current exchange of genes and exchanges that occurred hundreds to thousands of years ago with this metric. However, we can assume from the extensive habitat fragmentation that has occurred within the Mojave Desert over the past century that it is very difficult for tortoises to move among these clusters (*11, 18*). Gene flow is likely no longer occurring at the same spatial scale due to human impacts. Genetic differentiation for the Mojave desert tortoise also follows a pattern that is consistent with a phenomenon known as isolation-by-distance (*26*), where the average genetic distance increases as the distance between two populations increases (Figure 4). This pattern suggests that how far tortoises disperse plays a major role in determining population structure (*11, 27*).

For the Mojave desert tortoise, we detected low genetic differentiation among sampling locations, which supported the major conclusions of other recent studies of *G. agassizii* (*15, 28*). The distances that tortoises disperse appear to be a major determinant of the pattern of differentiation (*11, 27*). F_{ST} values between sampling locations for the Mojave desert tortoise (0.01 – 0.16) appear to be particularly low when compared to the gopher tortoise (*Gopherus polyphemus*), which inhabits sand hill, longleaf pine, and scrub ecosystems of the southeastern United States (*29*). Levels of genetic differentiation were notably higher in this species (F_{ST} = 0.24 ± 0.12) (*29*). Even more striking is how little variation in the genetic data is explained by geographic distance between locations. Geographic distance accounted for approximately 15% of the observed genetic variation for gopher tortoises (*18*) (Figure. 4). In striking contrast, 68% of observed genetic variation is explained by geographic distance for the Mojave desert tortoise (*11*). Gopher tortoises are known to have limited migratory ability and very small home ranges (*29*), and existing gopher tortoise populations are restricted mainly to protected parkland due to extensive habitat destruction and fragmentation (*29*). Behavioral differences, and naturally limited migration, could cause the different patterns of genetic differentiation (*18*).

Although dispersal distances are also important for the Sonoran desert tortoises, only 30% of the observed variation is explained by geographic distance (*28*) (Figure 4). Differential use of available habitat may account for the disparity in the amount of genetic variance explained between Mojave and Sonoran desert tortoise populations (*30*). Sonoran desert tortoises tend to inhabit rocky foothills, which are more naturally fragmented than are the bajadas typically occupied by Mojave desert tortoises (*30*). The Mojave desert tortoise is quite unique in this regard, not only when compared to other North American tortoises (*18, 27*). Many vertebrate species that have been studied have low correlations between genetic and geographic distances. Mojave National Preserve (MNP) is topographically diverse, containing some of the highest peaks within the range of the Mojave desert tortoise, which are likely effective dispersal barriers. We collected genetic samples from tortoises within MNP and from adjacent habitat, covering locations both east and west of the New York and Providence Mountains including Kelso Wash, Ivanpah Valley, Clipper Valley, and Shadow Valley. Several distinctive tortoise populations converge within MNP. A majority of tortoises located northwest of the mountains near Ivanpah and Shadow Valley assign to the main Las Vegas Cluster and to the fine-scale South Las Vegas cluster (Figure 3). However, tortoises south of the Kelso Mountains cluster with the California tortoises and assign to the Western Mojave cluster (Figure 3). On the eastern side of the New York and Providence Mountains, most tortoises can be grouped within the main California cluster and in the Northern Colorado fine-scale cluster along with tortoises from Piute Valley (Figure 3). Tortoise diversity within the preserve is likely higher than essentially any other areas of critical habitat and should be protected and managed with that in mind.

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Upper Respiratory Tract Disease (URTD), Mycoplasmosis, and Antibody-Responses in the Mojave Desert Tortoise

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Upper respiratory tract disease (URTD) in the Mojave desert tortoise (*Gopherus agassizii*) was recognized as a potential threat to the persistence of wild populations shortly before its listing as a threatened species under the U.S. Endangered Species Act (*1, 2*). The term URTD has been used to refer to visible signs of respiratory disease (i.e., mucous in and around the nares, swelling of the eyes, lesions in the tissue of the respiratory tract, and in severe cases, lethargy and death) (*3, 4*) (Figure 1). Therefore, URTD describes the symptoms that accompany an illness, regardless of the pathogen(s) responsible for them (*5*), and we treat it as such throughout this paper. *Mycoplamsa agassizii* is thought to be the predominant causal agent of URTD in the desert tortoise (*3, 5, 6*). URTD and *M. agassizii* have been the focus of both research and management over the course of the past 20 years (*5*). Here we review our most recent research, describing induced and natural antibodies that bind *M. agassizii*, in the desert tortoise. Our research has been aimed at increasing the general understanding of the tortoise immune system, in particular their production of antibodies, in order to increase the efficacy of conservation strategies for the management of wild tortoise populations in the Mojave Desert.

Diagnoses of current mycoplasmal infections would ideally be based on the direct quantification of *M. agassizii* in the

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Figure 1. Mojave desert tortoises.
(Top Panel) This tortoise is symptomatic for URTD. Specifically, this tortoise shows clear evidence of dried mucous and chronic mucal discharge from the nares, with damage to the scales around the nares.
(Bottom Panel) This tortoise is asymptomatic for URTD, and is being examined by University of NV, Reno field-technicians.

respiratory tract, but has been difficult to apply accurately to field studies of mycoplasmosis in live animals (*5*). In practice, desert tortoises are commonly diagnosed with mycoplasmosis by the quantification of antibodies produced as a response to infection with *M. agassizii* (or, possibly, a very similar species of *Mycoplasma*) via an ELISA (enzyme-linked immunosorbant assay) (*3, 7-9*). Two different ELISAs exist to quantify antibodies that bind *M. agassizii* in the bloodstream of tortoises. The first ELISA, developed by Schumacher and others (*7*), was designed to measure induced antibodies (*3, 7, 9*) —or the type of antibody produced by the adaptive immune system in response to infection (*10*). We have developed a similar ELISA, with the difference that it quantifies both natural (innate) and induced (adaptive) antibodies that recognize and bind to *M. agassizii* (*11*). Technically, this ELISA uses a polyclonal instead of a monoclonal reagent (*11*). It quantifies all types of antibodies produced by desert tortoises, including IgM (presumably the isotype of all natural antibodies and some induced antibodies) as well as IgY and IgYΔFc (presumably the isotypes of antibodies formed in the later stages of an induced immune response) (*11*). A Western blot is then used to distinguish between natural and induced antibodies that recognize *M. agassizii* (*11*). This gives us the capacity to detect definite, past exposure to *M. agassizii*, and also allows us to quantify levels of natural antibodies in individuals (*11*). Natural antibodies are one aspect of the innate immune system and have been shown to provide defense against certain pathogens in a variety of vertebrate species (e.g. humans (*12*), mice (1*3-17*), and fish (*18, 19*)). However, the ability of either induced or natural antibodies to reduce or prevent URTD after exposure to *M. agassizii* is currently unknown and would be a fruitful direction for future research. Interestingly, immune responses to mycoplasmal infections in a variety of vertebrate host species may both limit the spread of mycoplasmal infections throughout the body and exacerbate symptomatic disease (20).

Here, we review three aspects of our recent research, focused on the roles of induced and natural antibodies that recognize and bind *M. agassizii* in the Mojave desert tortoise. We review research pertaining to: *1)* the apparent prevalence (distribution and abundance) of *M. agassizii* across the range of the Mojave desert tortoise, *2)* a tortoise's ability to make a detectable antibody-response to pathogens, and *3)* possible differences in tortoise populations' immunocompetence (relative ability of the immune system to minimize infections) across their range. These studies support the hypothesis that URTD, and the interaction between the desert tortoise and *M. agassizii*, may be more complex than previously thought (5). In particular, both individual tortoises and

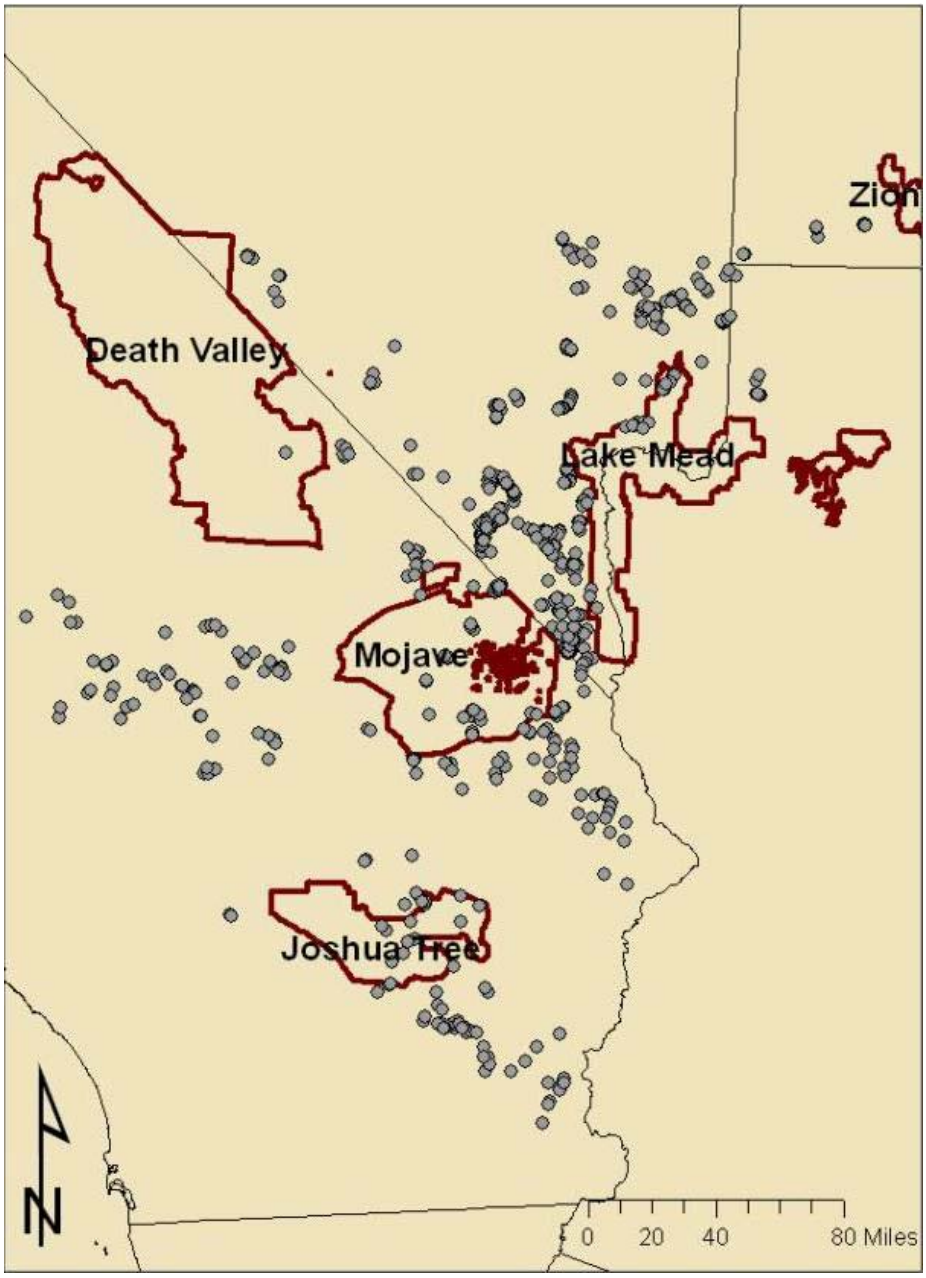

Figure 2. Mojave desert, including southern California, southern Nevada, southeastern Utah, and northwestern Arizona (modified from *21, 26*) showing tortoise point locations in relation to national parks within the region.

geographically segregated populations of tortoises present different patterns of significant exposure to *M. agassizii* (i.e. significant enough to induce an immune response) and of innate levels of natural antibodies to *M. agassizii*. This suggests that wildlife managers, in the future, may tailor disease-management strategies to the particular physiological and genetic characteristics of tortoises and populations under their jurisdiction.

Similar to past research of URTD in desert tortoises, we measured the presence of *M. agassizii* indirectly, via positive, induced-antibody responses (*5, 11*). Therefore, an important caveat of our conclusions is that we detected only the past or present exposure to *M. agassizii*, and only exposures severe enough to induce an adaptive immune response (*21*). As used in the following section, tortoises that are *M. agassizii*-positive have been infected for 2-4 weeks and may include tortoises that have been infected in the past, regardless of whether they subsequently minimized or cleared the infection (*3, 6, 21-23*).

Tortoise blood samples were collected both within Desert Wildlife Management Areas (2004, 2005) and outside of these protected areas (2004 – 2006) in order to cover the occupied range of the Mojave desert tortoise (*24, sensu 25*) (Figure 2). We divided the Mojave and Colorado Deserts into 24 relatively discrete valleys or interconnected valleys, or geographic populations (*21, 26*) (Figure 3). Approximately 20 tortoises were sampled in each geographic population, and these populations were also used as the sampling-unit in our statistical analyses (*21, 26*). Blood samples, spatial data, body measurements, and clinical signs of URTD (*sensu 3, 4*) were taken and recorded (*21, 26*). To account for the large number of independent observers, we defined URTD conservatively as clear evidence of fresh or dried mucous on the head or forelimbs (*21, 26*). This description also included evidence of chronic mucal discharge (e.g., eroded nares, de-pigmentation of scales around the nares and beak, etc.) (*21, 26*) (Figure 1 Top).

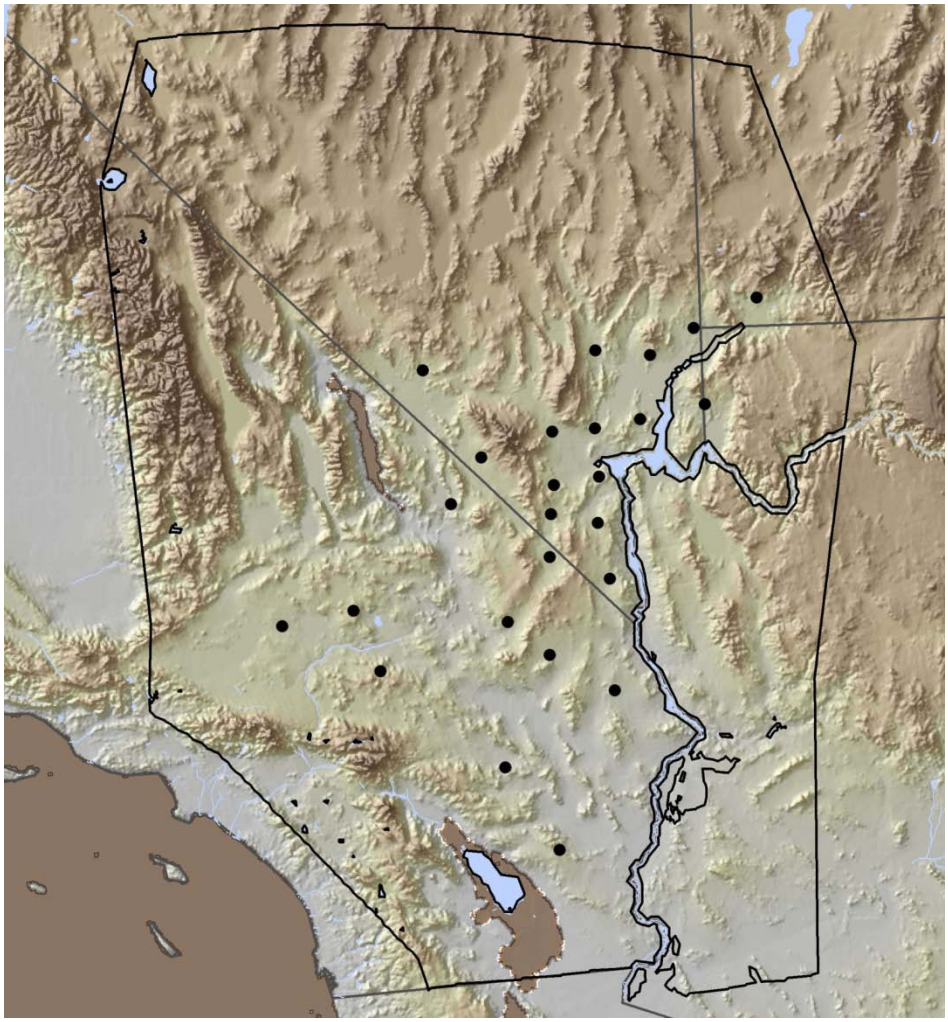

Figure 3. Mojave desert, including southern California, southern Nevada, southeastern Utah, and northwestern Arizona (modified from *21, 26*). Each point is the centroid of one of the 25 geographic locations used in our sampling design. Due to sample numbers and location, two locations were combined to form the 24 geographic populations used in our analyses. Each geographic population is either one discrete valley, or two or more inter-connected valleys. Dark gray lines represent state boundaries, and the black line is a rough approximation of the boundary of the Mojave Desert ecoregion.

Blood samples were tested via ELISA and Western blot (*sensu 11*), and scored against a standard sample that was run on each plate and blot (described in detail in *21, 26*). This standard sample was taken from captive animals that were known to be unexposed to *Mycoplasma*-species (*21, 26*). Briefly, the ELISA and Western blot used the same polyclonal reagent that detects the full range of natural (IgM) and induced (IgM, IgY, and IgΔFc) antibodies (*11*). ELISA titers were determined via end-point titration curves (*11*). One researcher (FS) scored Western blots according to the following criteria. “Negative” Western blots had the same number of visible protein bands, or one to three additional bands, as the standard sample (i.e., bands due to natural antibodies, including the slight natural variation observed due to individual differences and assay conditions). “Suspect” Western blots had five to seven additional bands, or more than seven, faint, additional bands, in comparison to the negative standard. “Positive” Western blots had more than eight additional, strong bands or so many additional bands that they formed a solid

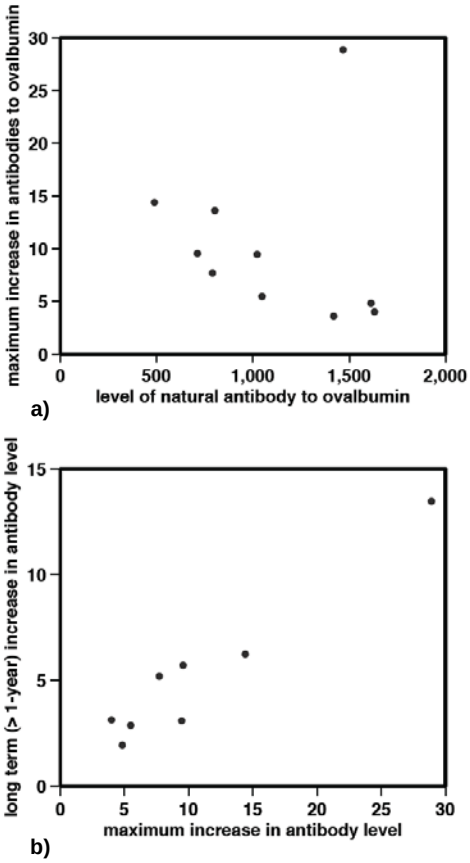

Figure 4. Tortoises' antibody levels to ovalbumin were quantified by ELISA, as endpoint titers, before immunization, after immunization (at their maximum levels), and one year after immunization (modified from *21, 23*). a) We detected a negative relationship between the tortoises' inherent natural antibody levels and their maximum induced-antibody response. Natural antibody levels are reported as end-point antibody titers. The maximum increase in antibodies is reported as the ratio of antibody titers after immunization to natural antibody titers (i.e., an x-fold increase above baseline, natural antibody titers). Only tortoises that made significant induced-antibody responses to ovalbumin are shown. b) We detected a positive relationship between the tortoises' maximum induced-antibody response (x-fold increase in antibody levels due to immunization) and the magnitude of long-term elevation of antibody levels (persistent, x-fold increase in antibody levels more than one year post-immunization treatments). Increase in antibody levels is the ratio of antibody titers (measured post-treatment and more than one year post-treatment) to baseline, natural antibody titers.

smear. In statistical analyses, we counted both "suspect" and "positive" samples as tortoises positive to exposure to *M. agassizii* (*sensu 27*). Due to known strain-variation and variable binding-patterns, this method was calibrated in such a way as to diagnose all infected animals in our own experiments (*28*) and in the experiments presented by Wendland and others (*27*) as "suspect" or "positive" (*21, 26*).

Using generalized linear regression models, we detected a number of patterns among attributes of these 24 geographic populations (*21, 26*). Namely, we found that positive exposure to *M. agassizii*—as detected via induced - antibody responses—varied among populations and ranged from 0 – 73 % (*21, 26*). The highest levels of positive exposure were detected in the northeastern portions of the Mojave Desert (*21, 26*). Furthermore, these levels of exposure to *M. agassizii* were independently and positively associated with the population's mean levels of natural antibodies and with the local mean number of days below freezing (averaged over 15 years) (*21, 26*) (Table 1).

Therefore, by conducting a range-wide survey of *M. agassizii*, we were able to determine that one measure of the innate immune system (natural antibody levels) and one climatic variable (colder and longer winters) were correlated with levels of *M. agassizii* in wild desert tortoise populations over the time-frame of our study (*21, 26*) (Table 1). This conclusion led us to hypothesize that mean immunocompetence of tortoise populations and local levels of *M. agassizii* may be functionally related, and that host and/or pathogen populations may be influenced by the local thermal regime (colder and longer winters) (*21, 26*).

We conducted a controlled experiment, on a group of captive, adult tortoises at the University of Nevada, Reno, to measure the magnitude and time-course of a general induced-antibody response (*21, 23*). Instead of using *M. agassizii* as the pathogen we used ovalbumin, or chicken-egg-white protein, as the antigen (*21, 23*). An antigen is the molecule that triggers the immune response (*10*), and ovalbumin is a common antigen, specifically known to stimulate strong antibody-responses in vertebrates (*29*). An advantage of using this type of antigen is that ovalbumin does not replicate and we can therefore control the tortoises' level of exposure to it.

We used ovalbumin to artificially immunize 16 tortoises (described in more detail in *21, 23*). Briefly, each tortoise was exposed to the ovalbumin three times (a primary immunization and two subsequent boosts). Antibody levels were measured via a polyclonal ELISA, which essentially was a modification of the *M. agassizii*-ELISA used in the range-wide survey (described above). Unexpectedly, we found that tortoises had natural antibodies to ovalbumin, and that these levels were relatively constant over time (within each individual) but variable among individuals. However, we were able to use each tortoise's antibody level prior to exposure as a negative control, against which to measure the increase in antibodies over time (*21, 23*).

We found a significant, negative correlation between an animal's natural antibody levels to ovalbumin and the magnitude of its induced-antibody response to ovalbumin (*21, 23*) (Figure 4a). In other words, tortoises that had the highest, innate levels of natural antibodies produced the lowest levels of induced-antibody to ovalbumin (Figure 4a). The ability of natural antibodies to limit the increase in induced antibodies in

Table 1. Regression models of population-level measures of disease and natural antibody levels in tortoise populations (n = 24). All significant models were univariate.

Response variable	Predictor Variable	R^2	p
Seroprevalence	mean annual days below freezing	0.434	< 0.0005
	mean natural antibody levels	0.232	< 0.0173
Mean natural antibody levels	genotype group	0.594	< 0.0001

response to pathogen exposure or artificial immunization is termed “epitope-masking”, and may lead to increased inaccuracy when diagnosing individuals solely on the magnitude of their antibody responses (*21, 23*). In addition, we detected significantly elevated levels of induced antibody to ovalbumin more than a full year after the immunizations and boosts were completed (*21, 23*) (Figure 4b). Therefore, tortoises showed positive antibody responses to a non-replicating, foreign molecule, more than a year after exposure (*21, 23*).

Together, these two results suggest that caution is needed in interpreting induced -antibody responses of tortoises as evidence of current infection with a pathogen (*21, 23*). For example, a tortoise with high natural antibody levels to a specific pathogen possibly could fail to make an induced-antibody response, even after repeated exposures. Conversely, if a tortoise did make a strong induced-antibody response, it would tend to keep a positive, induced-immune response for more than a year. Therefore, antibody responses may not be the most accurate way in which to diagnose tortoises for many types of pathogenic diseases.

Consequently, we are directing our future research towards measuring *Mycoplasma* spp. directly via quantitative PCR of preserved nasal lavages [saline rinses of the nares, preserved with RNA-later (Qiagen; Valencia, CA)]. Although this technique is not expected to provide absolute quantification of *Mycoplasma* within the respiratory tract of individual tortoises, it should allow us to quantify the relative exposure to *Mycoplasma* experienced by the various tortoise populations of the Mojave Desert (*sensu 28*).

In our range-wide survey of disease, we not only detected significant differences among populations’ levels of natural antibody, but we also detected a correlation between natural antibody levels and a population’s genetic identity (*sensu 24*). Specifically, populations belonging to the California genotype group had significantly lower mean levels of natural antibodies than did the populations belonging to the Las Vegas and North Mojave genotype groups (*21, 26*) (Table 1). One, possible explanation is that genetic history may constrain the production of natural antibody levels (*21, 26*). In other words, not just types of natural antibodies (i.e., an individual’s natural antibody repertoire) but also their levels of expression are often genetically-determined in vertebrates (*17*), and this may be the case in tortoises as well.

However, it is important not to extrapolate this pattern to mean that populations in the California group have lower, overall immunocompetence than populations in the Las Vegas and North Mojave genotype groups. Natural antibody levels are just one component of the innate immune system. To quantify relative differences in immuncompetence, it is important to measure multiple, functionally-distinct components of the immune system (*30, 31*). Evidence of regional variation in this one, quantified component (natural antibodies), has led us to include multiple assays of different immune-functions in plans of future research to quantify disease and physiological health within Mojave populations of the desert tortoise.

Through a range-wide survey of disease and a controlled experiment to measure aspects of antibody-responses in the desert tortoise, we have shown that both mycoplasmal URTD in the Mojave Desert and the immune system of the tortoise are more complex than has been assumed in the past. For example, the California genotype group (*sensu 24*) showed lower levels of natural antibodies to and lower apparent prevalence of *M. agassizii* (*21, 26*). Interestingly, this is also the region in which large outbreaks of seemingly, locally devastating URTD was first observed (*5, 32*). However, *M. agassizii* was not absent from this region, is not expected to have the same levels of prevalence through time, and was present in all three, genotype groups of the desert tortoise (*21, 26*).

These observations have led us to suggest that disease dynamics may be surprisingly different in different regions of the Mojave Desert (*21, 26*). Specifically, some regions may experience epizootic disease, characterized by a disease that occurs at an unexpected level or in an unexpected time or place (*33, 34*). In other regions, URTD may occur as an enzootic disease, or a disease that is characterized by predictable regularity and prevalence in its host population (*33, 34*). Knowledge of the epizootic and/or enzootic characteristics of wildlife diseases has, in other systems, enabled managers and conservation biologists to better manage threatened host populations (*e.g. 33-36*). We are gearing future research towards this goal of characterizing disease dynamics across the range of the Mojave desert tortoise.

In particular, we hope to provide managers with a variety of tools to assess disease, immunocompetence, and potential changes in disease dynamics in desert tortoise populations. With a combination of new and old tools, management decisions—in regard to mycoplasmosis and URTD—should become increasingly tractable and more amenable to adaptive management. With a deeper understanding of individual diseases and tortoise -immunocompetence, managers and conservation biologists ultimately should be able to gather the data necessary for predicting disease-dynamics (e.g., cyclic or chaotic epizootics and stable or fluctuating enzootics) under variable environmental conditions and in diverse desert tortoise populations.

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Desert Tortoise and Highway Traffic:

a Resource Management Concern

D. Hughson and N. Darby[1]

Mojave National Preserve was created in 1994 by the California Desert Protection Act, in part, to “perpetuate in their natural state significant and diverse ecosystems of the California desert.” This area of the Mojave Desert is important habitat for the desert tortoise, California’s state reptile. Approximately 48% of the Preserve is designated critical habitat for the species, including portions of the Ivanpah Valley and Fenner Valley critical habitat units.

There are 168 miles of two-lane paved highways in the Preserve, 145 miles of which cross designated habitat for the tortoise (Figure 1). A major north-south thoroughfare connects inland cities of southern California to Las Vegas, Nevada and often carries up to 2 vehicles per minute on busy weekends traveling at an average speed of nearly 70 miles per hour (mph). Observations by park staff indicate that as many as 10 tortoises are killed annually on highways in the Preserve.

We began a project in 2008 to improve our understanding of the rate that tortoises were being killed on roads in the Preserve, where these mortalities were occurring, and to increase law enforcement protection of tortoises. Specifically we wanted to see if the recommendations in the Preserve’s General Management Plan for heightening awareness through seasonal, temporary signage, and slowing traffic, would reduce tortoise mortalities on roads.

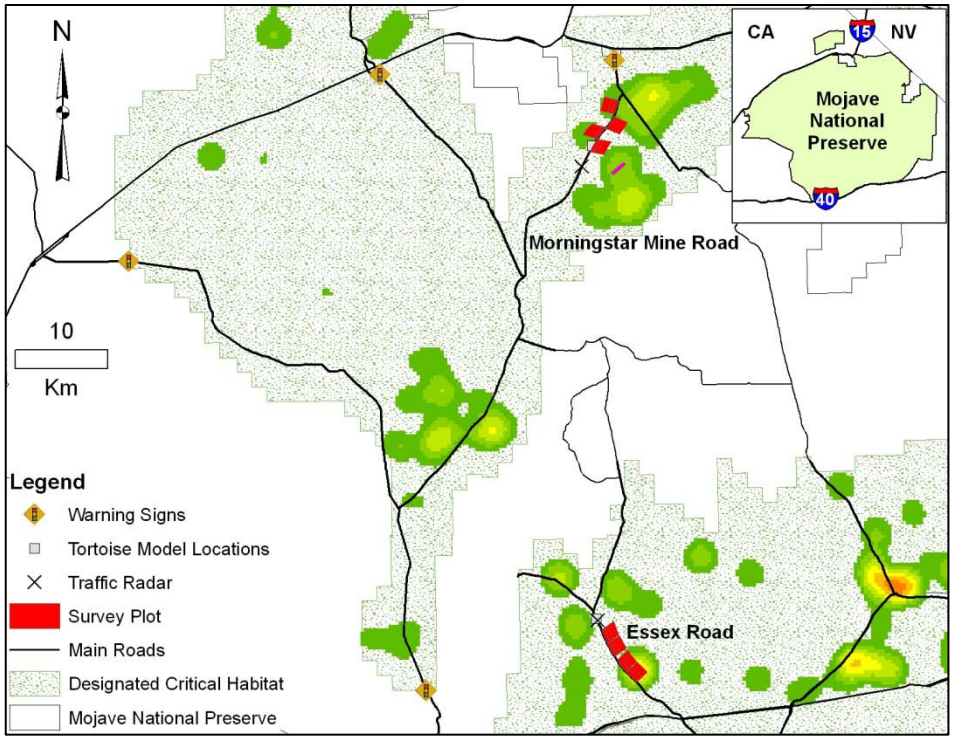

Figure 1. Main roads, designated critical habitat for the desert tortoise, and elements of this study are indicated. The green, yellow, to orange (highest) colors represent relative tortoise concentrations based on live observations from line distance sampling data (*1*).

Figure 2. “Watch for tortoise” warning signs were erected along paved roads at the entrances to the Ivanpah Valley critical habitat unit in Mojave National Preserve in the spring of 2009, and equipped with flashing yellow lights in the spring of 2010. We observed motorist responses to a medium-sized tortoise model on the edge of the pavement before and after sign placement.

In the spring of 2009 we placed warning signs (Figure 2) at the 4 points where paved roads enter the Ivanpah Valley critical habitat unit, leaving roads in the Fenner Valley unit without signage as a control, and equipped the signs with flashing yellow lights in the spring of 2010. We tested the effect of these signs on driver behavior by placing a model tortoise along the edge of the highway and observing motorists’ behavior from concealment. In addition, we collected data on traffic speed and density using a traffic radar device (StealthStat, Kustom Signals, Inc.); conducted transects parallel to roads for tortoise sign (*2*) (Figure 3); drove all highways in tortoise habitat weekly looking for evidence of mortality, and conducted intensive walking surveys of randomly selected one-mile road segments.

Highways in the Fenner Valley unit lead to rough gravel roads, whereas those in

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the Ivanpah unit cross through the Preserve, connecting Interstates 40 and 15. We focused our efforts on Morningstar Mine Road, in the Ivanpah Valley unit, and Essex Road, in the Fenner Valley critical habitat unit. From the spring of 2008 through the spring of 2010 we collected 2285 hours of traffic data on Essex Road and 2497 hours of data on Morningstar Mine Road during the active tortoise season. During this time we recorded 20128 vehicles on Essex Road traveling at an average speed of 32.2 mph (standard deviation = 24.1 mph) and 41324 vehicles on Morningstar Mine Road traveling at an average speed of 66.5 mph (standard deviation = 17.5 mph), which is an average traffic density of 0.15 vehicles per minute on Essex Road and 0.28 vehicles per minute on Morningstar Mine Road. A two-sample t-test assuming unequal variances indicated that traffic patterns on these two roads were significantly different (t = -359, df = 30869, p = 0). The radar device was located 1.8 km from the intersection of Essex Road and Black Canyon Road, which may have resulted in slower speeds, with some drivers anticipating the turn. Our 77.8 hours of observations, of motorist response to a model tortoise, on Essex Road, and 20 hours on Morningstar Mine Road, however, convinced us that the traffic patterns on these two roads are not comparable.

Motorist response to the tortoise model placed on the road edge was significantly different between Morningstar Mine and Essex roads (Table 1); a response being some indication, such as brake lights, slowing, swerving, or stopping, that the driver saw the model. For all years combined $\chi^2 = 29.4$, df = 1, and p=0.0000001. On Morningstar Mine Road approximately 1 of every 25 drivers exhibited an observable response the tortoise model compared to 1 out of 7 drivers on Essex Road. This response rate did not change with the installation of warning signs or flashing yellow lights.

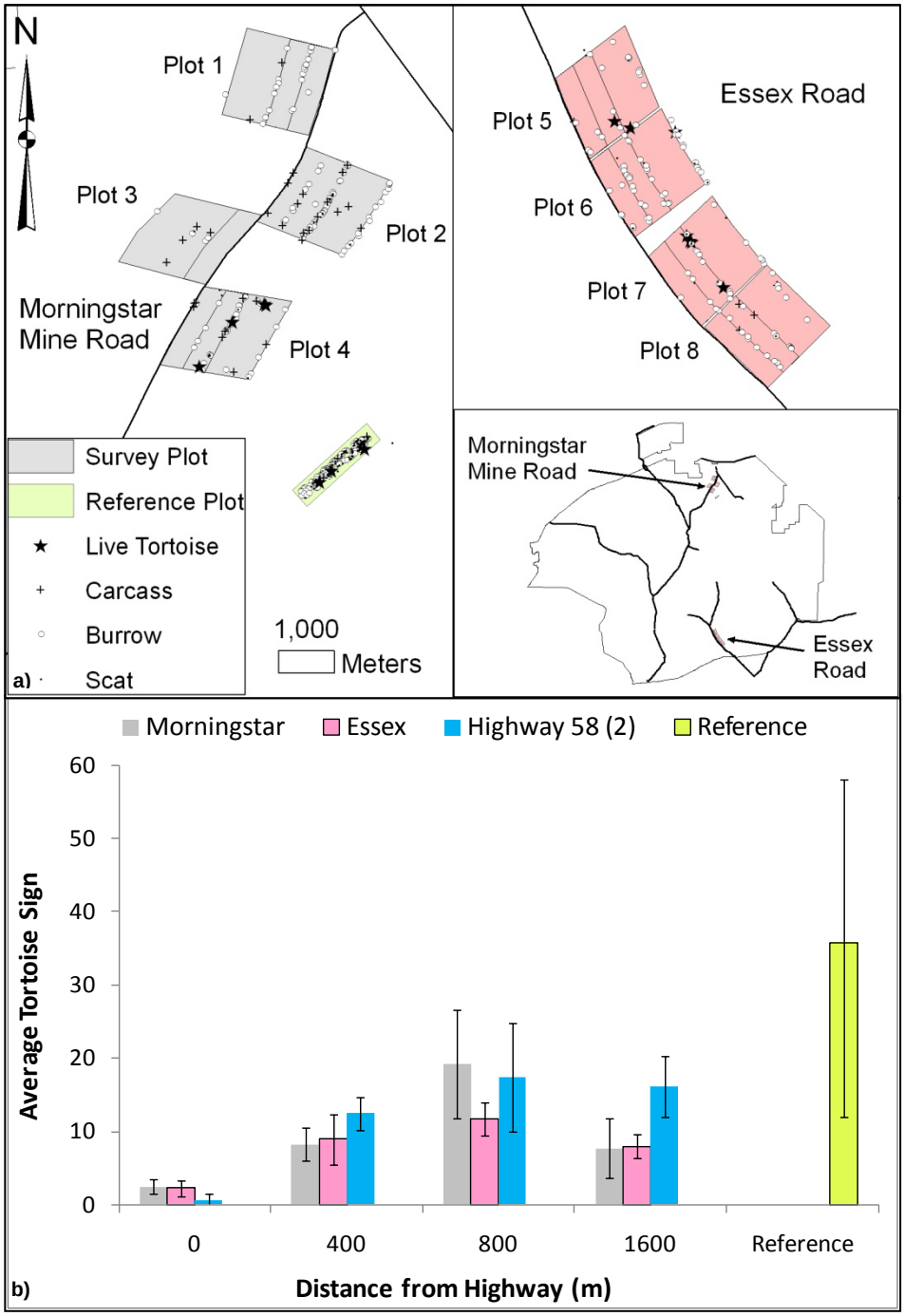

Figure 3. a) Transects parallel to Morningstar Mine road and Essex road were surveyed March 30 – April 2, 2009 for tortoise sign. Transects 1.5 km in length were surveyed at distances of 0, 400, 800, and 1600 m from road edge. Reference transects were surveyed in Ivanpah Valley 3.2 km southeast of Morningstar Mine road. b) Average tortoise sign as a function of distance from the road shows a pattern similar to that found along Highway 58 (*2*). Error bars are standard error except for the reference which is minimum and maximum.

Table 1. Motorist behavior in response to a tortoise model on pavement edge. Data are counts of motor vehicles. Plus signs indicate an observed reaction and negative signs indicate no observed reaction. Signs were placed on Morningstar Mine road starting in 2009.

Road	2008	2009	2010
Essex	+34	+13	+24
	-172	-88	-181
	No Signs	**Warning Signs**	**Flashing Lights**
Morningstar	+7	+6	+7
	-188	-98	-199

Thus we concluded that the warning signs had no effect on motorist behavior.

Despite the differences in traffic density, speed, and driver behavior, both highways had patterns of depressed tortoise sign extending outwards from the road edge similar to Highway 58 west of Barstow (*2*) (Figure 3b). Comparison with the reference transects conducted 3.2 km from Morningstar Mine Road suggests that the road effect may extend farther than 1.6 km (*3, 4*), although the reference transects also may have been located by chance in a relatively high concentration of tortoises. Two-factor analysis of variance with replication indicates that the patterns of tortoise population depression are insignificant between the two roads (*Road F* = 0.46*, df* = 1*, p* = 0.5, *Distance F* = 4.7*, df* = 3*, p* = 0.01, *Interaction F* = 0.62*, df* = 3*, p* = 0.61).

Roads are conduits for many threats to the desert tortoise. Illegal collection is likely greatest along well-traveled roads, potentially removing some individuals from a population. Release of captive tortoises also likely occurs along roads, potentially introducing disease into wild populations. Predators, such as ravens, tend to be attracted to roads by fresh road kill and trash, which could contribute to depression of tortoise populations. Numerous observations year after year of road-killed tortoises on, and adjacent to, heavily traveled highways, however, provide the strongest evidence that direct impact by motor vehicles is a leading cause of tortoise population depression adjacent to roads.

We observed lower speeds, lower traffic density, and greater driver awareness on Essex Road, compared to Morningstar Mine Road, yet the depression of tortoise sign adjacent to the roads was similar. This suggests that, within the Preserve, the impacts of lightly traveled roads, relatively slower traffic, and motorists likely to see and respond to tortoises can be as severe as the more heavily traveled roads carrying higher speed traffic and less-observant motorists. This also suggests that decreasing traffic speed and increasing driver awareness may not result in a corresponding improvement in tortoise populations adjacent to roads.

Tortoise barrier fencing is recommended along paved roads within Mojave National Preserve (*5*). Even if funding could be obtained for a multimillion dollar construction project, a question remains as to whether or the fence could be adequately maintained across many cross-cutting arroyos and gullies long enough for tortoises to reoccupy the habitat (6, *7*). Other concerns are related to fence construction. Will barrier fencing increase habitat fragmentation? Should barrier fencing be placed far from roads to minimize illegal collection opportunities and visual impacts or close to roads to maximize available habitat?

In the absence of tortoise barrier fencing, however, it is almost certain that traffic-related tortoise mortalities and population depression adjacent to paved roads will continue to occur. In the presence of barrier fencing, habitat reoccupation by tortoises could require decades (*6, 7)*.

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[1] http://www.sciencemag.org/about/authors/prep/res/refs.dtl