

DISTRIBUTION AND GENETIC STATUS OF *XEROSPERMOPHILUS* GROUND
SQUIRRELS IN THE BARSTOW REGION,
SAN BERNARDINO COUNTY, CALIFORNIA

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RH = *XEROSPERMOPHILUS* GROUND SQUIRRELS

ABSTRACT

The Mohave ground squirrel (*Xerospermophilus mohavensis*) is endemic to the western Mojave Desert of California and is state-listed as “Threatened”. It is of conservation concern because of the potential for large-scale renewable energy development within its range. Although recent reports have documented current knowledge of its distribution, there are certain parts of its range where its status is unclear. In particular, there is uncertainty about its range limits in the Hinkley Valley west of the city of Barstow. Although this region has traditionally been considered to be occupied by the Mohave ground squirrel, there are recent records of the closely-related round-tailed ground squirrel (*Xerospermophilus tereticaudus*) here. This report assembles all available evidence from trapping surveys and from genetic analyses in an effort to clarify the distribution of these two ground squirrel species in the Hinkley Valley. An analysis of the distribution of the two ground squirrel species based upon trapping surveys confirms that there are no reliable current reports of Mohave ground squirrels in the Hinkley Valley, while there are a number of round-tailed ground squirrel records here dating back to 1977. A genetic study was carried out using 13 nuclear microsatellite loci from 55 ground squirrel samples collected in this region. The results of this analysis indicate that most of the samples represented genetically pure Mohave or round-tailed ground squirrels. However, 3 individuals collected in Hinkley Valley that were identified as round-tailed ground squirrels on the basis of external characteristics showed evidence of Mohave ground squirrel ancestry. In similar fashion, 3 animals from the desert region west of Hinkley were found to have some round-tailed ground squirrel genetic ancestry in spite of external features typical of Mohave ground squirrels. While round-tailed ground squirrels appear to be the dominant species in Hinkley Valley and Mohave ground squirrels in the desert environment to the west, hybridization does occasionally occur, at least some hybrids are fertile, and backcrossing appears to occur in both parental

directions. It will be important to investigate this phenomenon more closely to evaluate possible threats to the genetic integrity of the threatened Mohave ground squirrel.

INTRODUCTION

The Mohave ground squirrel (*Xerospermophilus mohavensis*) is found only in the western Mojave Desert of California. It was originally listed as “Rare” in 1971 under the California Endangered Species Act (CESA). With the re-authorization of CESA in 1984, the species was categorized as “Threatened”. It is believed to have suffered extensive loss, fragmentation, and degradation of habitat as the result of a number of anthropogenic threats, including urbanization, agricultural development, livestock grazing, military training, and off-road vehicle use. In 2006, the Bureau of Land Management (BLM) adopted the West Mojave Plan, which established development limitations on approximately 1.7 million acres of BLM-managed lands within the range of the Mohave ground squirrel (BLM 2005). Because of the potential for renewable energy development within its range, the Mohave ground squirrel is of great conservation interest. The current distribution and status of the Mohave ground squirrel has been documented in two recent publications (Leitner 2008; Leitner 2015).

The Mohave ground squirrel is closely related to the round-tailed ground squirrel (*Xerospermophilus tereticaudus*), which is distributed from the deserts of eastern California and southern Nevada throughout much of Arizona and south into Mexico. The two species are very similar in external appearance, the most obvious difference being the shape and length of the tail. While the tail of the Mohave ground squirrel is relatively short and flattened, that of the round-tailed ground squirrel is longer and circular in cross-section. The Mohave ground squirrel is found only in the western Mojave Desert where winter rainfall predominates, while the round-tailed ground squirrel occupies a very extensive range to the east and south that is characterized by hotter and drier conditions with rainfall primarily in the summer.

It has been assumed for many years that these two species are allopatric, with their geographic ranges meeting but not overlapping. This contact zone was first described as stretching from Fort Irwin south through the Barstow area, then south along the Mojave River toward Victorville (Wessman 1977). However, recent evidence suggests that round-tailed ground squirrels occur some distance west of the Mojave River in the Hinkley Valley (Leitner 2014). This report analyzes all available data on the current geographic distribution of the two species and on the genetic make-up of *Xerospermophilus* ground squirrels found in the Hinkley Valley and its environs.

METHODS

COMPILATION OF GROUND SQUIRREL LOCALITY RECORDS

Ground squirrel locality records were compiled from a number of different sources. The primary source of occurrence data for the Mohave ground squirrel was the California Natural Diversity Database (CNDDDB), which is maintained by the California Department of Fish and Wildlife as a central repository of information on special status plants, animals, and natural communities. Additional data on Mohave ground squirrel distribution was obtained from museum records, unpublished reports, and consultation

with knowledgeable biologists. The CNDDDB does not provide data on the round-tailed ground squirrel, since it is not a special status taxon. Locational data for this species were collected from the Natural History Museum of Los Angeles County, Museum of Vertebrate Zoology (UC Berkeley), and the VertNet data portal (www.vertnet.org). Additional information was obtained from unpublished reports and consulting biologists. A final important source was the 1998-2012 data base of occurrence records for both species maintained by one of the authors (PL).

GENETIC ANALYSIS

Sampling

A total of 55 samples were available for the focal study area, which included the Hinkley Valley and desert habitats out to about 10 km (6 mi) west of Hinkley. These samples included a set of 14 specimens collected in Hinkley Valley in 2012 and 2013 (Vanherweg 2012, 2013). Other samples were derived from field studies conducted within the region from 2006-2014. In order to place the focal study area into the context of species and population diversity of Mohave ground squirrels and round-tailed ground squirrels of the region, we augmented these samples with an additional 72 specimens previously genotyped in Bell and Matocq (2011).

Nuclear microsatellite analyses

For all 127 samples, we amplified thirteen microsatellite loci with the following primer sets: GS14 (Stevens et al. 1997, designed for *Spermophilus columbianus*); IGS 110b and IGS 6 (May et al. 1997, designed for *Spermophilus brunneus*); B126 (Garner et al. 2005, designed for *S. brunneus*); and loci developed for *X. mohavensis*, XmohC109, XmohC114, XmohC9, XmohB3, XmohC10, XmohD116, XmohA114, XmohB108, and XmohB118 (Bell and Matocq, 2010). We carried out DNA amplification in 10 μ l reactions including 1 μ M of each primer (forward primer fluorescently labeled), 5 μ l of Reddy Mix (0.25 units of Taq polymerase, 75mM Tris-HCl (pH 8.8), 20 mM (NH)₄SO₄, 1.5 mM MgCl₂, 0.01% (v/v) Tween 20, and 200 μ M of each dNTP; ABgene) and 3.0 μ l of H₂O, with thermocycler settings of: initial denaturation of 94°C for 2 minutes, then 40 cycles of 93°C for 30 seconds, annealing temperature for 30 seconds, 72°C for 1 minute, and a final extension at 72°C for 30 minutes, on a PTC-200 thermocycler (MJ Research). We combined amplified products with a fluorescent size standard prior to running on an ABI 3730 (Applied Biosystems) at the Nevada Genomics Center. We identified allele sizes using STRand (University of California, Davis, Veterinary Genetics Lab). To confirm allele calls, we generated duplicate genotypes for 30% of the individuals at each locus.

To identify the number of genetic clusters represented in these data without imposing prior spatial information, we used a Bayesian assignment approach as implemented in the program structure ver. 2.2 (Pritchard et al. 2000; Falush et al. 2003). We performed 10 independent Markov chain Monte Carlo (MCMC) sampling runs with 100,000 burn-in steps and 1,000,000 search steps for each K from K = 1 to 20. We used an admixture model, correlated allele frequencies and lambda = 1. We identified the most probable number of clusters based on the rate of change in log probability of the data over successive K (ΔK) as suggested by Evanno et al. (2005). This analysis first identifies the optimal number of genetic clusters that best describe the variation held in the dataset, and then assigns individuals to one or more of these genetic clusters. Each individual is

assigned to a genetic cluster, but individuals can have partial assignment to more than one genetic cluster, suggesting hybrid or admixed ancestry. Despite having a priori knowledge of the genetic identification of the samples included in Bell and Matocq (2011), we ran all samples without locality or putative species identification in order to test consistency of the analyses across the two studies.

To further distinguish individuals of hybrid origin, we used the software NEWHYBRIDS 1.1 Beta 3 (Anderson & Thomson, 2002) to assign individuals to one of six genotypic classes (pure round-tailed ground squirrel, backcross (BC) to round-tailed ground squirrel, F1, F2, BC to Mohave ground squirrel, and pure Mohave ground squirrel). We ran three independent MCMC chains with random starting points and Jeffreys-type priors for allele frequencies and mixing proportion for 2,000,000 sweeps for each chain after an initial burn-in of 1,000,000 sweeps.

RESULTS

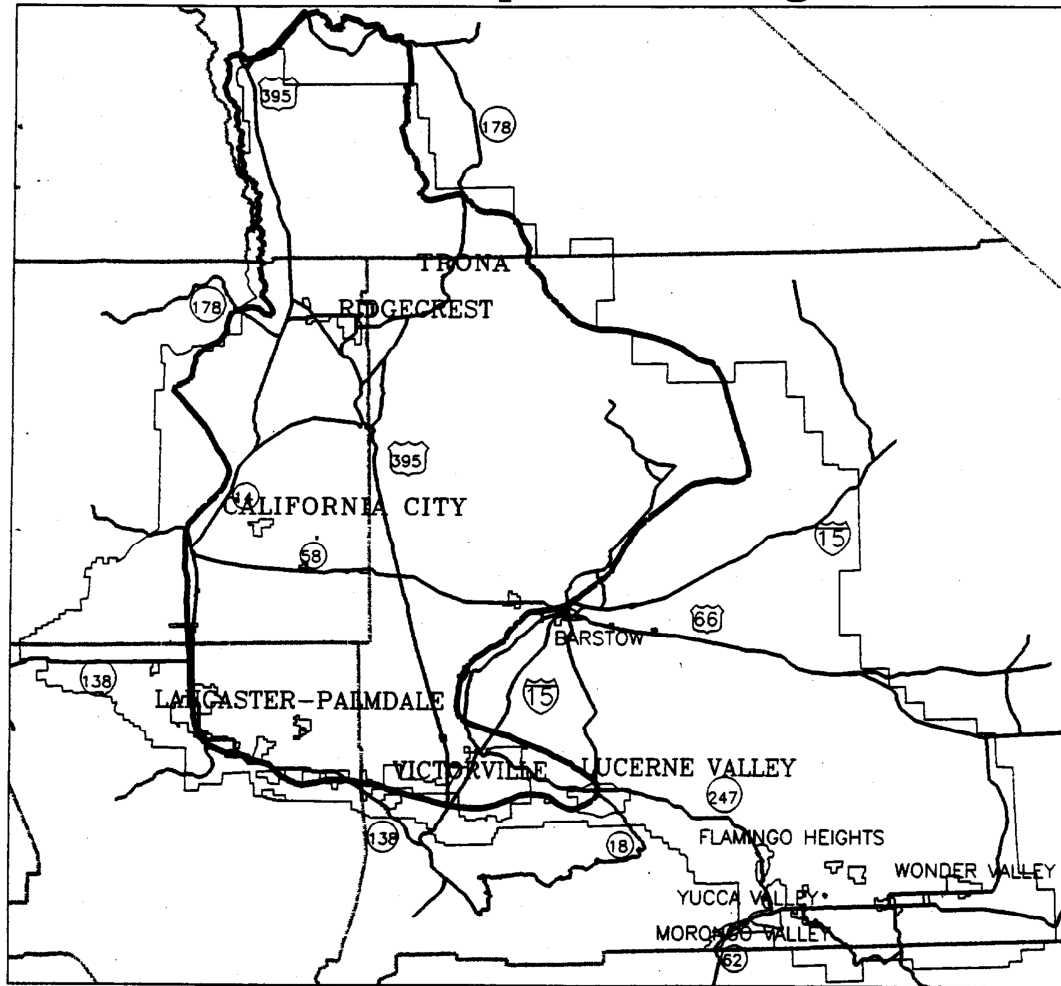
HISTORIC RANGE OF THE MOHAVE GROUND SQUIRREL

The California Department of Fish and Wildlife¹ first provided a range map for the Mohave ground squirrel in its 1980 biennial report on the status of State-listed species (CDFG 1980). This official map was revised in 1992 as a result of advice from the Department's Mohave Ground Squirrel Working Group. The revised map was included in an official status review prepared by Gustafson (1993) and is reproduced in Figure 1. It shows the eastern boundary of the Mohave ground squirrel range as passing through the Barstow area and then southwest and south along the course of the Mojave River.

The 1993 location of the eastern boundary of the Mohave ground squirrel range in the Barstow area was based upon a total of 29 records in the CNDDDB, the earliest of these dating from trapping surveys conducted in 1975 and 1977. Figure 2 shows the locations of these records in relation to Barstow. There were a number of occurrences north of Barstow on Coolgardie Mesa and Superior Valley, with two others along Ft. Irwin Road (Wessman 1977). There were eight CNDDDB records to the west of Barstow during the period from 1975-1988, including 5 near Harper Lake and 3 along State Route 58. Mohave ground squirrels were also reported in the CNDDDB as having been collected in 1981 at a location west of Coyote Dry Lake and at another site east of Daggett (Hafner and Yates 1983). The CNDDDB listed 4 occurrences in Barstow itself dating from 1982 and 1993; these were based upon visual and auditory detections only. Finally, Mohave ground squirrels were reported to have been observed in 1990 at a site about 3 km (2 mi) north of Lenwood in 1990 (CNDDDB Occ. No. 277).

¹ California Department of Fish and Game prior to Jan. 1, 2013

Mohave Ground Squirrel Range



- Mohave Ground Squirrel Range
- West Mojave Conservation Planning Region
- County Boundaries
- Major Roads

Source :

Bureau of Land Management



Figure 1. Map of the Mohave ground squirrel range as presented in Gustafson (1993).

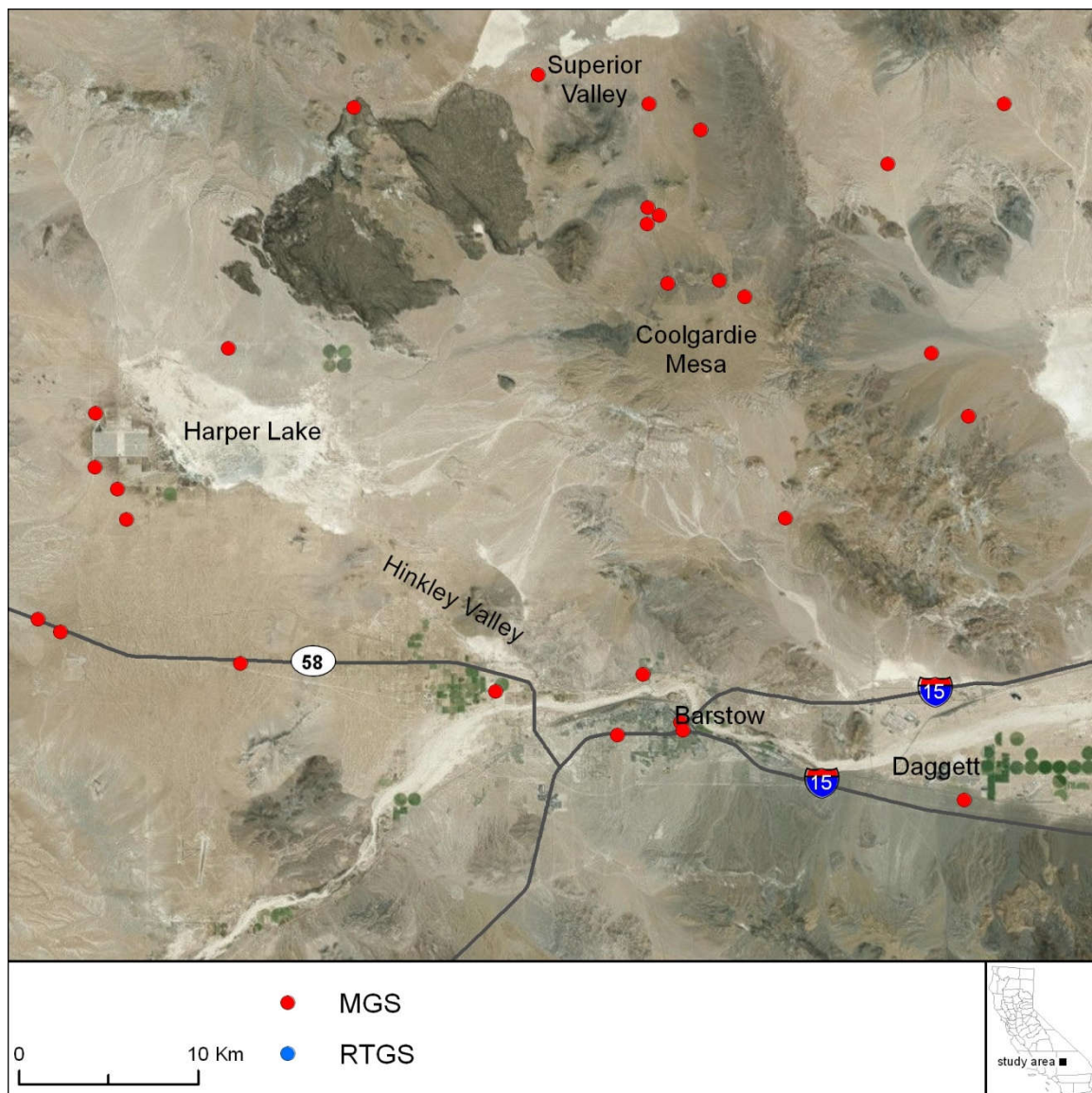


Figure 2. Geographic distribution of CNDDDB Mohave ground squirrel records in the Barstow area from the period 1975-1993.

NEW EVIDENCE CONCERNING THE RANGE BOUNDARY

The placement of the Mohave ground squirrel range boundary in the Barstow area has been generally accepted in recent literature (Leitner 2008). However, more recent evidence suggests that this may not accurately reflect the current distributional limits of the species in this area.

First, five of the CNDDDB occurrences shown in Figure 2 have recently been removed from the Mohave ground squirrel database. These include 3 visual and auditory detections made within the city of Barstow in 1993. The original observer concluded that it was not possible to determine the species identity with certainty because of the similarity in physical appearance and vocalizations between Mohave and round-tailed ground squirrels (D. LaBerteaux, pers. comm.). The 1981 Mohave ground squirrel CNDDDB occurrences near Coyote Lake and Daggett were pointed out to be in error (D. Hafner, pers. comm.), as the original report had clearly identified these as round-tailed

ground squirrel records (Hafner and Yates 1983). There were only two other Mohave ground squirrel CNDDDB records in the Barstow area prior to the 1993 designation of the range boundary. In 1982 a Mohave ground squirrel was detected in Barstow just north of the Mojave River, while in 1990 there were reported observations of the species north of Lenwood.

Second, surveys using live-trapping and trail cameras have yielded many new MGS detections since 1993. Figure 3 shows these newer well-documented occurrences along with other records dating back to 1975. There is a clear geographic pattern with the great majority of occurrences well to the north and west of Barstow. The 3 remaining records from the Barstow area are all based upon visual observations. These include the 1982 sighting in Barstow, the 1990 observations north of Lenwood, and a visual record in 2005 about 6 km (4 mi) south of Barstow near the municipal landfill. In none of these cases were specimens actually trapped and examined, so it is difficult to be certain of the accuracy of the species identification.

When the Mohave ground squirrel range boundary was originally proposed to include the Barstow area, there was no consideration of distributional records of the round-tailed ground squirrel. Examination of museum records indicates that 4 round-tailed ground squirrel specimens are known from Barstow and vicinity, dating from 1914 to 1966. Wessman (1977) reported trapping round-tailed ground squirrels in the vicinity of Coyote Lake and at a site near Hinkley. Round-tailed ground squirrels were recorded in 1981 by Hafner and Yates (1983) at 3 locations to the east of Barstow. During the period 2006-2009, there were a number of trapping surveys carried out in the Barstow area. These studies resulted in many captures of round-tailed ground squirrels throughout this region, from Hinkley and Hodge east to Daggett. In attempting to accurately define the current range boundary, it is important to focus on data from trapping studies in which external diagnostic features could be closely examined. Figure 4 presents the geographic distribution of Mohave and round-tailed ground squirrel records through 2009 based upon trapping studies in which more reliable identification could be obtained. It can be seen that round-tailed ground squirrel records extend west from Barstow and the Mojave River into the Hinkley Valley.

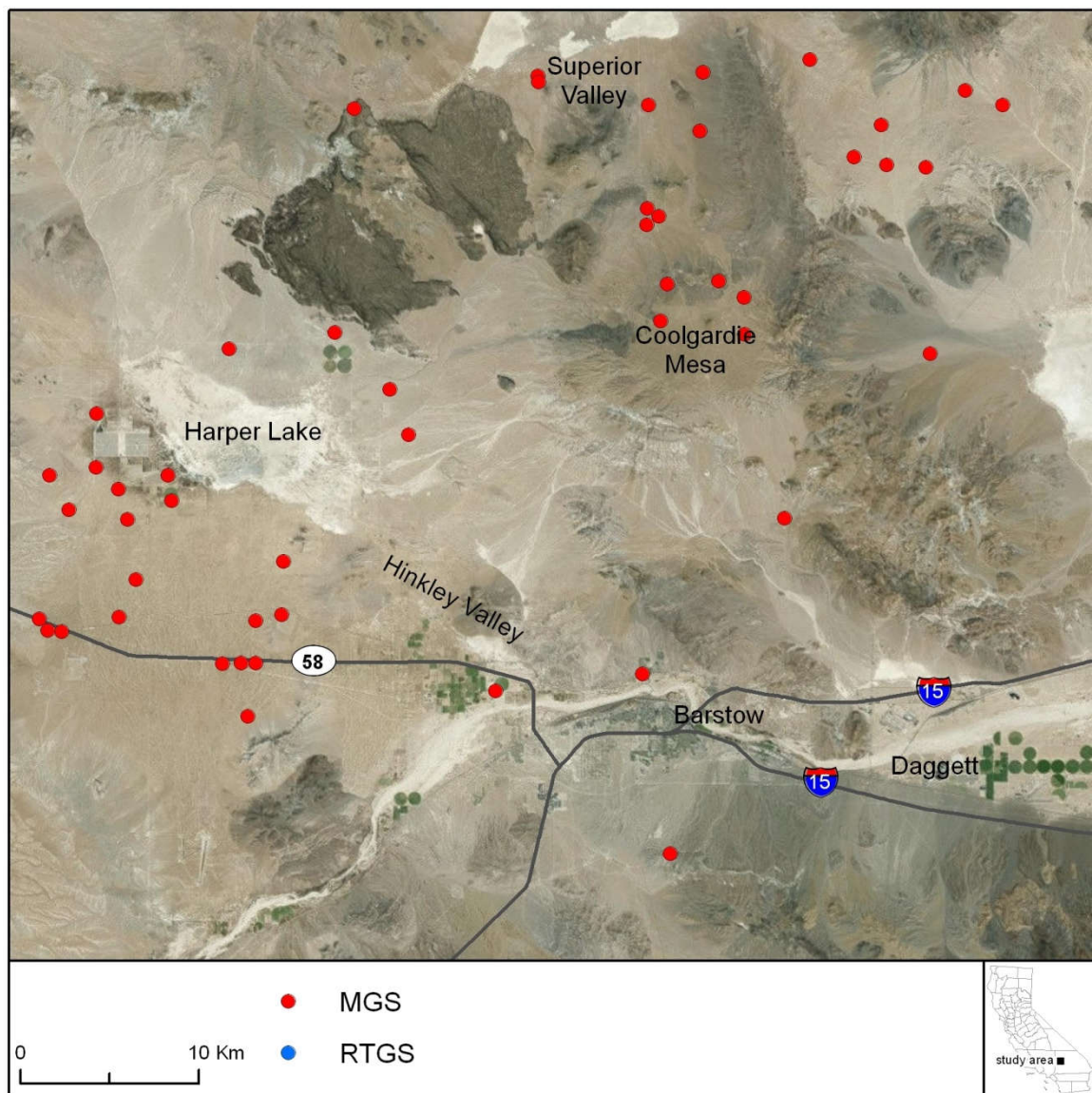


Figure 3. Geographic distribution of Mohave ground squirrel records in the Barstow area from the period 1975-2014.

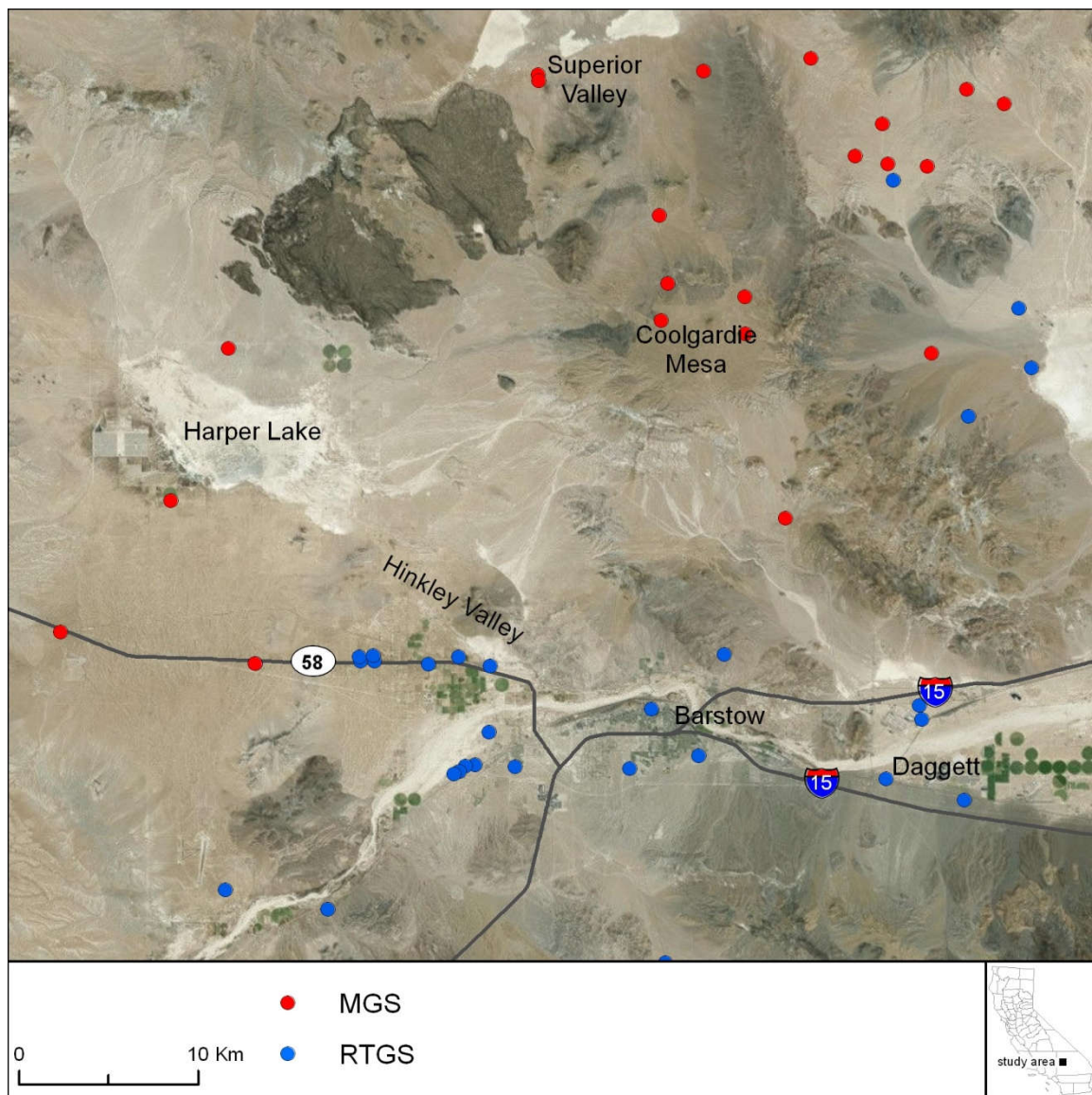


Figure 4. Geographic distribution of Mohave ground squirrel and round-tailed ground squirrel trapping records from the Barstow area from the period 1914-2009.

GENETIC ANALYSIS

Genotypes generated during this study from the subset of individuals included in Bell and Matocq (2011) were consistent with the original genotypes. This suggests high repeatability of our lab methodology and consistency between the studies. All individuals expected to be round-tailed ground squirrels or Mohave ground squirrels from the previous study were confirmed based on the new analysis. Table 1 shows the number of samples genotyped from each region and the number of individuals of each type that were recorded.

Table 1. Mohave ground squirrel (MGS) and round-tailed ground squirrel (RTGS) genetic samples analyzed in this study by general locality. The total number (N) of individuals examined is shown for each locality. Each individual is listed in the genetic class to which it was assigned in a NEWHYBRIDS analysis (pure RTGS or MGS, first or second generation hybrid, backcross (BC) to either parental class, or uncertain assignment).

General Location	N	RTGS	MGS	F1/F2	BC-RTGS	BC-MGS	uncertain
Boron	15		15				
Coolgardie Mesa	7		7				
EAFB	16		16				
Fremont Peak	5		5				
Ft. Irwin WEA	8		8				
Hinkley	55	31	18	2		3	1
Kramer Junction	21		21				

In the analysis of unknown samples from recent collections in the Hinkley area, we uncovered genetically pure individuals of Mohave ground squirrels and round-tailed ground squirrels as well as hybrid specimens. In the vicinity of Hinkley, we found evidence of Mohave ground squirrels and round-tailed ground squirrels occurring in proximity to one another, as well as evidence of hybridization. Individuals with genomic compositions (q values) greater than 90% one taxon or the other were considered genetically “pure”. The vast majority of individuals had q values > 0.97 for either round-tailed ground squirrel or Mohave ground squirrel, which we took as unambiguous assignment to either pure parental type. However, our structure analysis identified 2 individuals as hybrids ($q < 0.90$) with a high degree of confidence, that is, with 95% credibility intervals not encompassing zero. The same analysis identified another 3 individuals as probable hybrids based on their mean genomic composition ($q < 0.90$), but with a lower level of confidence given that their 95% credibility intervals encompassed zero and one, suggesting that at least some runs found them to be pure individuals. Our NEWHYBRIDS analysis confirmed the hybrid status of the same 5 individuals as found in the structure analysis, but was able to classify these individuals into their genotypic class (i.e. first generation hybrid, versus second generation and further backcrosses to either parental type). NEWHYBRIDS identified MGS765 and MGS777 as second generation hybrids, suggesting these individuals were the offspring of first generation animals that would have been the outcome of a direct intercross between pure round-tailed ground squirrel and Mohave ground squirrel. MGS784, MGS785, and MGS854 were identified as backcross Mohave ground squirrel. Finally, a single sample, GS601, could not be classified by NEWHYBRIDS because of partial probability in multiple classes, namely pure round-tailed ground squirrel and back-cross round-tailed ground squirrel. The structure run showed this individual to have a mean genomic composition of 0.916 round-tailed ground squirrel, suggesting that this animal may have had admixed ancestry, although it was slightly above our 90% threshold cutoff value.

The spatial distribution of the samples from the Hinkley area plotted according their genotype is shown in Figure 5. The majority of individuals from the agricultural areas of Hinkley Valley were classified as round-tailed ground squirrels. Two samples (MGS765 and MGS777) collected in the northern portion of the Hinkley area, while another sample (GS601) that may have had mixed ancestry was found farther south near SR58. A number of individuals from the desert habitat west of Hinkley were genotyped as pure Mohave ground squirrel. Nevertheless, there were 3 animals (MGS784, MGS785,

MGS854) from that region that were classified as backcross Mohave ground squirrel, indicating some round-tailed ground squirrel genetic contribution in their ancestry.

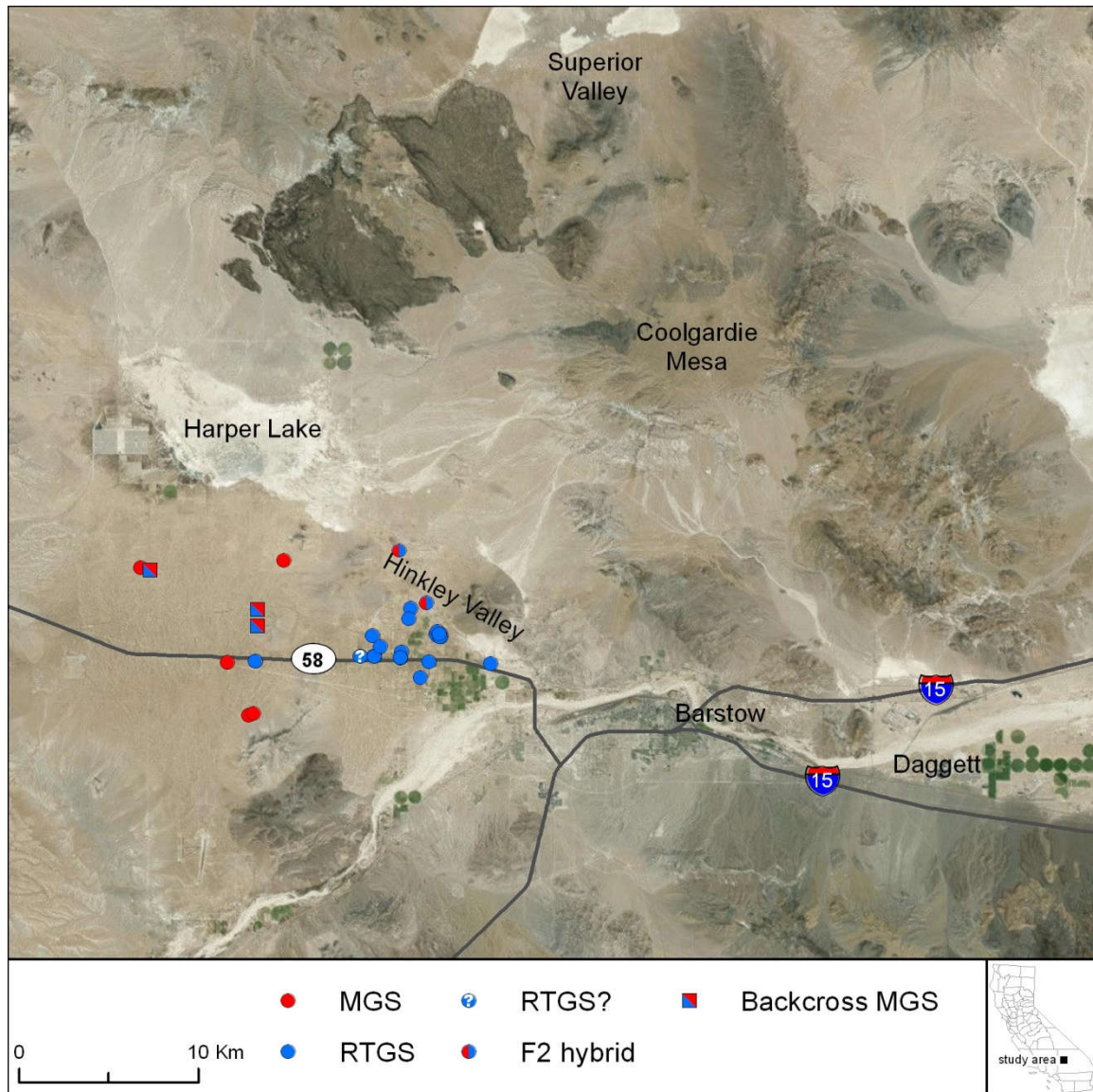


Figure 5. Geographic Distribution of Mohave ground squirrels, round-tailed ground squirrels, and hybrids in the Hinkley area based upon genetic analysis.

DISCUSSION

Recent data strongly indicates that the eastern boundary of the Mohave ground squirrel range lies well to the west of the Mojave River in the vicinity of Barstow. The only recent Mohave ground squirrel record east of the Mojave River is based upon a visual observation in 2005 near the Barstow landfill that has not been confirmed by trapping. Two older Mohave ground squirrel records near Lenwood and Barstow were also based on visual observations and there has been no confirmation of these sightings.

It seems clear from both trapping data and genetic analysis that the dominant *Xerospermophilus* species in the Hinkley Valley is the round-tailed ground squirrel. The earliest trapping record of this species in the area dates to 1977, long after the native

desert habitat there was converted to irrigated agriculture. Since round-tailed ground squirrels appear more tolerant of disturbance, it is possible that they largely replaced an original Mohave ground squirrel population after land conversion. However, although we found no evidence of genetically pure Mohave ground squirrels in Hinkley Valley, there is reason to believe that they are still present. We found 2 specimens that were classified as F2 hybrids, indicating that they had significant Mohave ground squirrel ancestry. These individuals were captured in desert scrub habitat to the north of areas converted to agricultural production. It is note-worthy that when these animals were captured, they were identified as typical round-tailed ground squirrels based upon their external characteristics. Another individual from the western edge of Hinkley Valley showed evidence of possible Mohave ground squirrel genetic ancestry.

Well to the west of Hinkley Valley, in relatively undisturbed desert habitat, Mohave ground squirrels appear to be the dominant species. Nevertheless, we found evidence that at least some round-tailed ground squirrels may be present here. Three individuals that had all the expected external features of typical Mohave ground squirrels showed evidence of genetic input from round-tailed ground squirrels. Camera studies and live-trapping in this region have revealed 2 cases at which both species were present simultaneously (Leitner 2008, 2014).

While the exact ancestry or detailed pedigree of the individuals sampled for this study cannot be inferred from these analyses, our results confirm the spatial proximity of pure parental types, that hybridization does occasionally occur, that at least some hybrids are fertile, and that backcrossing appears to occur in both parental directions. The frequency of hybridization, its spatial distribution and the ecological or demographic factors that contribute to hybridization require further investigation.

ACKNOWLEDGMENTS

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