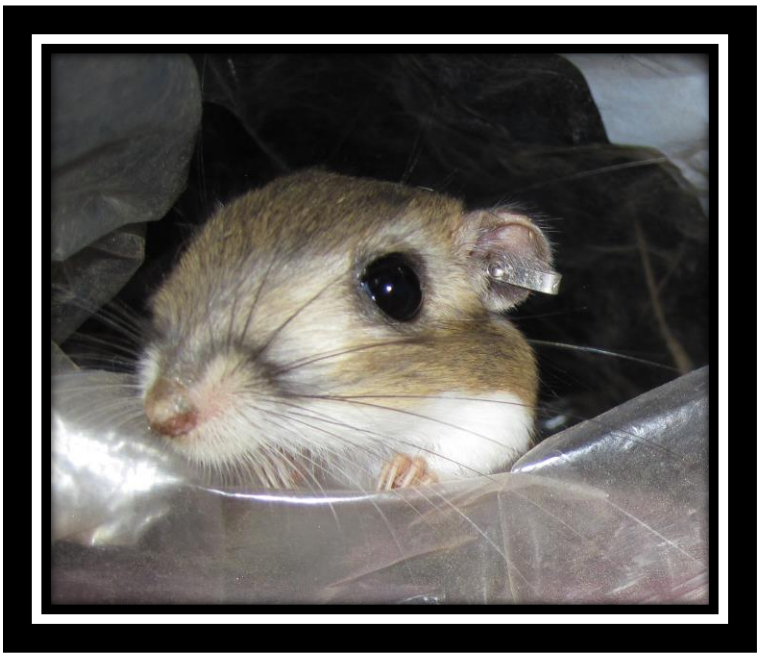
RANGE-WIDE GENETICS OF THE STEPHENS' KANGAROO RAT (*DIPODOMYS STEPHENSI*)



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INTRODUCTION

The Stephens's kangaroo rat (*Dipodomys stephensi; SKR*) currently exists only in fragmented populations separated by urban landscapes. The species is listed as threatened by the state of California and endangered by the USFWS. The draft recovery plan for the species (1997) calls for conservation, funding and management within an established reserve system in Riverside and San Diego Counties. Species management plans are in place within some reserves and a translocation program is being established for the species to develop methods to reduce Take due to development (Shier 2009, 2010, 2011, Shier and Swaisgood 2012). Translocation may mitigate habitat fragmentation and restore historical gene flow by relocating animals between reserves or from areas slated for development projects onto reserves. It is not clear whether translocation is required to manage the species range-wide because, to date, the species landscape genetics and phylogeography are not well understood. To develop a successful range-wide long term management plan for the species that conserves extant genetic variation, it is critical to understand the genetic structure, dispersal characteristics and population histories of the fragmented populations in an evolutionary context.

There were two previous genetic studies conducted on the SKR. The first was a study assessing within and between population genetic variability based on allozyme (protein) variation (McClenaghan and Truesdale 1991, McClenaghan and Truesdale 2002). The results of this study indicated that genetic divergence between and among loci were not significantly correlated with geographic distances between populations, suggesting that habitat fragmentation has influenced population genetic structure in this species (McClenaghan and Truesdale 1991, McClenaghan and Truesdale 2002).

A second study used mitochondrial DNA to assess the patterns of genetic diversity across 16 dispersed collection sites (Metcalf et al. 2001). The results of this study contrast with the results in the study based on allozyme variation and the authors suggested that the mitochondrial genetic variation demonstrated subpopulation structuring, indicating the presence of three geographic subregions (north, mid and south ranges; Metcalf et al. 2001). Yet, small sample sizes and grouping of samples from multiple locations limits the study's ability to accurately describe the species landscape genetics and thus it is considered preliminary (U.S.F.W.S. 2010).

The analysis of highly variable nuclear DNA microsatellite loci provides a powerful tool for quantifying dispersal patterns and reconstructing population histories. As part of a multiyear program to design a translocation model for Stephens' kangaroo rat, we have developed a set of species-specific microsatellite loci for SKR (Shier 2010, 2011). To date, we have genotyped over 300 individual SKR, from multiple locations in the southern, mid and north part of the species range. Our preliminary genetics results indicate that the geographic restriction may not be necessary. Current results suggest that genetic distance and geographic distance are not correlated and there is no observed higher-level population structuring beyond that observed at the individual population level (due to genetic drift) (Shier, unpublished data). These results support the allozyme findings by McClenaghan and Truesdale (1991, 2002) and suggest that the species may have restricted female philopatry and that gene flow may occur between population fragments within a severely fragmented landscape. If our results hold across the range, this would have important management implications for SKR.

This project is a high priority because: 1) current federal regulations limit translocations between reserves until the species range-wide genetics are elucidated (Mark Pavelka, USFWS, pers.comm) and hundreds of animals have been lost to development because development

sites and established release sites were not located within the same subregion (Shier, pers. obs.), 2) the Western Riverside County Multiple Species Habitat Conservation Plan (WRMSHCP) was put into effect only 12years ago, thus resource issues such as fragmentation of populations are just emerging in the plan area and management actions such as Reserve establishment and development are ongoing with a planned 25 year land acquisition phase; and 3*)* small isolated populations may be losing genetic diversity and could become extirpated because they are unable to adapt to new selection pressures such as climate change or a shift in available resources. Understanding SKR range-wide genetics will provide information to help address these issues.

Necessity for Implementation of the Natural Community Conservation Plan (NCCP). The long term Stephens' Kangaroo Rat Habitat Conservation Plan (SKR HCP) was issued to the Riverside County Habitat Conservation Agency (RCHCA) in 1996. The Western Riverside County Multiple Species Habitat Conservation Plan (WRMSHCP), issued in 2004, provides Take Authorization outside the boundaries of the SKR HCP, but within the plan boundaries. The MSHCP serves as an HCP pursuant to Section 10(a)(1)(B) of the Federal Endangered Species Act of 1973 (FESA), as well as a Natural Communities Conservation Plan (NCCP) under the NCCP Act of 2001. The core reserves established by the SKR HCP are being managed as part of the MSHCP Conservation Area. The SKR HCP outlined specific conservation objectives for the species which are to be implemented by the WRMSHCP. The species specific objectives require conservation of a minimum of 15,000 acres of occupied habitat in 6 core areas with at least 30% of the populations maintained at medium or higher densities. These core areas are to be managed for SKR in perpetuity (WRMSHCP species accounts, p M-197-198). In addition, several sections of the WRMSHCP planning agreement call for data collection in order to facilitate adaptive management of core areas. They are as follows: Section 10.4, p21 "Core Reserves and Viable Habitat Linkages; Section 11.4, p25 "Measurable criteria for assessing progress toward core reserves and viable habitat linkages assembly"; Section 11.7 (p 25) of the WRMSHCP agreement covers implementation measures to be accomplished including: 1) 11.7.2 "Refinement of adaptive management principles" and 2) 11.7.4 "Collection of additional data necessary for the MSHCP".

The proposed project assists in achieving the NCCP's species specific goals by using landscape level genetics to provide data on genetic structure, dispersal, population history and, thus, information on the viability of core areas and populations and the efficacy of the habitat linkages between them. This information is vital for adaptive SKR management because core areas are isolated and if habitat linkages are ineffective then small populations in some reserves may require translocation to maintain genetic diversity and viability. This approach will also be important to other NCCPs and could be applied to other listed heteromyids such as the San Bernardino kangaroo rat and the Los Angeles Pocket Mouse.

The proposed project is a targeted study for adaptive management of the state and federally endangered Stephens' kangaroo rat (*Dipodomys stephensi*). The primary objective of this project was to use landscape level genetics of Stephens' kangaroo rat to provide critical information for species management across the species range. The funding provided by the California Department of Fish and Wildlife NCCP-Local Assistance Grant (LAG) program was used to: sample the remaining 5-7 sites, genotype the samples, conduct a population structure analysis and prepare the draft and final reports that include genetic management recommendations.

PERSONNEL

The following people conducted research on SKR associated with this research. Dr. Debra Shier conducted and supervised all field research. Brian Shomo, Dr. Thea Wang, Jennifer Hoffman, Emily Gray, Matt Lucero, Melanie LaCava, Susanne Marczak and Steve Montgomery assisted with sample collection. Asako Navarro conducted all genetic analysis.

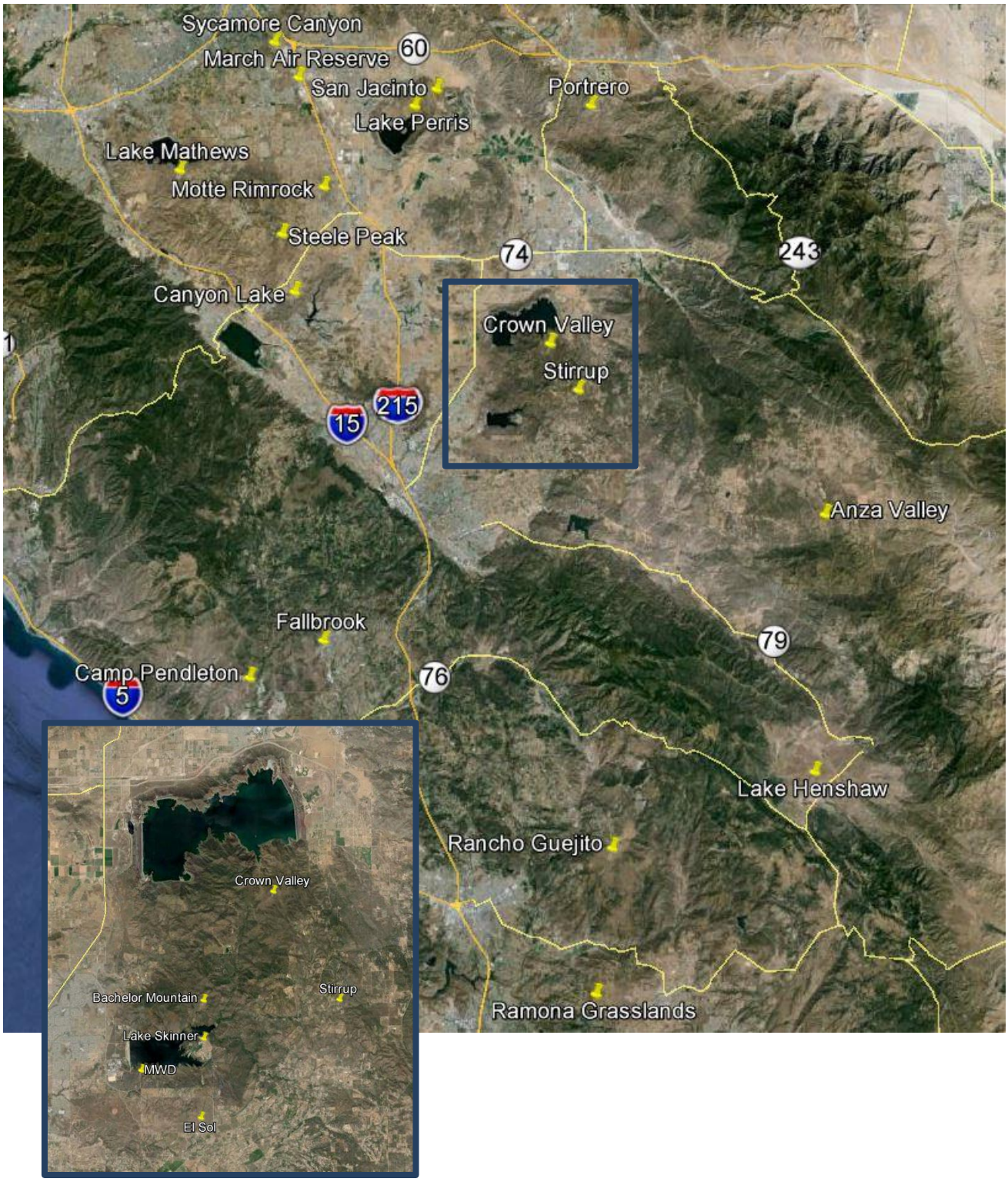
STUDY AREA

We sampled 21 sites within Riverside and San Diego Counties spread throughout the occupied range of SKR (Table 1; Figure 1). Our goal was to collect samples from all SKR occupied sites used by Metcalf et. al. 2001 and additional sites in each subregion in order to have the statistical power necessary to provide a robust determination of genetic structure across subregions. We collected samples from the following sites as part of this grant: Anza Valley/Silverado Ranch, Canyon Lake, Lake Henshaw, Rancho Guejito, San Jacinto Wildlife Area and Ramona Grasslands (Appendix A). All other sites were sampled prior to this grant contract.

Table 1. Sampling sites and site locations.

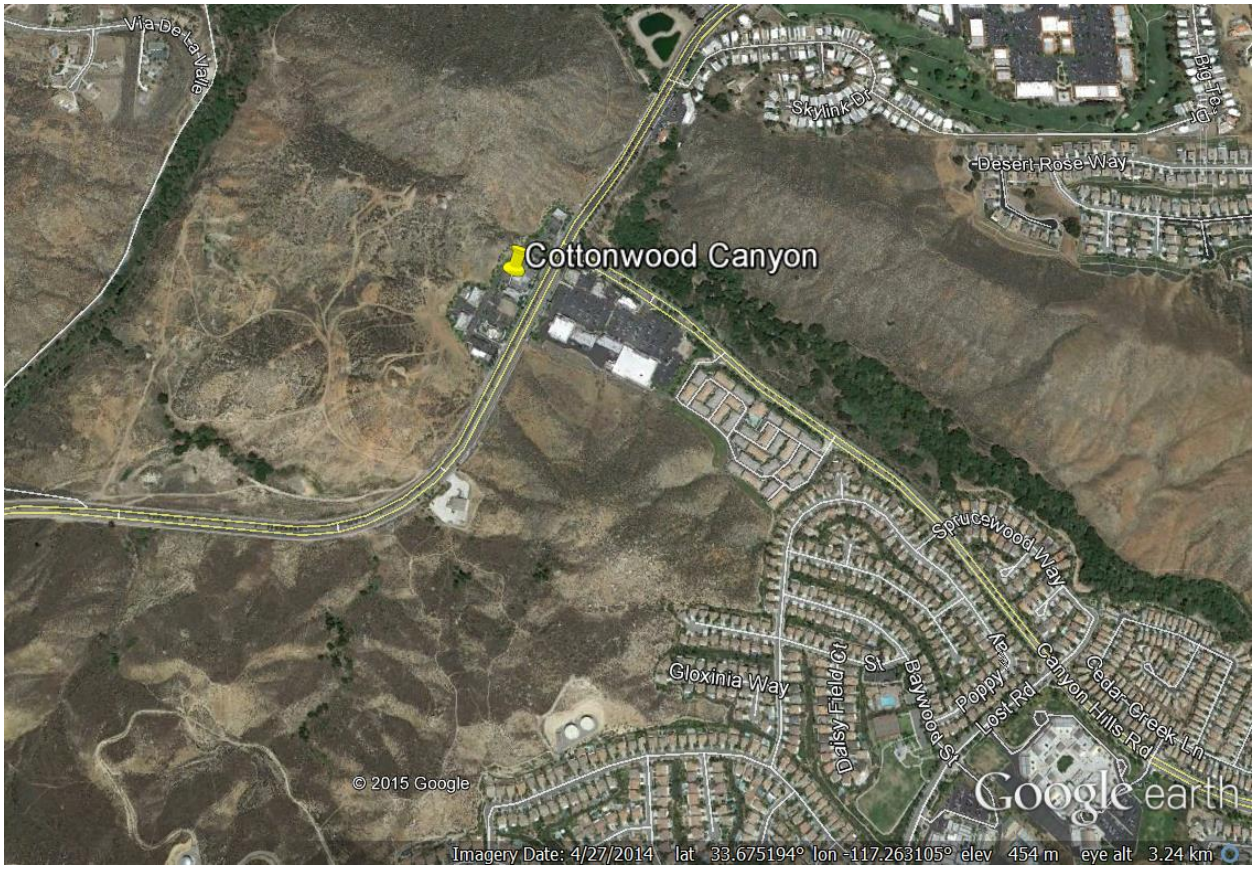
No.	List of Sites in Metcalf	Used in Metcalf	Region**	GPS location		Distance to closest site (km)	Closest Site	Sample Size
1	Anza Valley/Silverado Ranch	No	South	33.480727	-116.691474	21.09	Lake Henshaw	20
2	Bachelor Mountain*	No	South	33.606379°	-117.036477°	1.71	Lake Skinner	9
3	Camp Pendleton	Yes	South	33.333889°	-117.344444°	8.78	Fallbrook	21
4	Canyon Lake	Yes	Mid	33.699900°	-117.293596°	6.26	Steele Peak	23
5	Crown Valley (Shippley)*	Yes	Mid	33.650147°	-117.005333°	5.61	Stirrup	11
6	El Sol	No	South	33.562433°	-117.037965°	3.46	MWD/Monofil	14
7	Fallbrook Naval Weapons Station	Yes	South	33.344883	-117.272752	8.78	Camp Pendleton	20
8	Lake Henshaw/Warner Springs	Yes	South	33.245186°	-116.703392°	22.82	Rancho Guejito	21
9	Lake Mathews	Yes	North	33.821842°	-117.468047	12.71	Steele Peak	21
10	Lake Perris	Yes	?	33.876658	-117.161846	2.91	San Jacinto Wildlife Area	23
11	Lake Skinner*	Yes	South	33.590317°	-117.033583°	3.26	MWD	14
12	March Air Reserve	No	North	33.903695°	-117.309176°	4.57	Sycamore Canyon	21
13	Motte Rimrock Reserve	Yes	Mid	33.799502°	-117.260295°	6.61	Steele Peak	20
14	MWD - Monofil	No	South	33.578100°	-117.067692°	3.26	El Sol	18
15	Portrero Creek	Yes	North	33.876167°	-116.955833°	16.42	San Jacinto Wildlife Area	20
16	Ramona Grasslands	No	South	33.034539°	-116.950889°	15.44	Rancho Guejito	23
17	Rancho Guejito	Yes	South	33.173270°	-116.932299°	15.44	Ramona Grasslands	20
18	San Jacinto Wildlife Area	No	?	33.891963°	-117.131888°	2.91	Lake Perris	27
19	Steele Peak	Yes	North	33.750458	-117.3028596	6.26	Canyon Lake	18
20	Stirrup	No	South	33.608778	-116.962469	6.78	SWCMSR - Lake Skinner	6
21	Sycamore Canyon	Yes	North	33.92075	-117.3096944	4.57	March	21
* Sites within Southwestern Riverside County Multispecies Reserve (SWRCMSR)								
** Region as defined by Metcalf et al.								

Figure 1. Map of Sampling Sites. Locations of sites adjacent to and within Southwestern Riverside County Multispecies Reserve (SWRCMSR) are shown in the zoomed in section below.



Sites that have been developed since Metcalf's study include: Allesandro Heights, Norco and Cottonwood Canyon. Though a small population persists at Norco (Montgomery, pers. comm.), it was decided through consultation with USFWS (Mark Pavelka, pers. comm.) that acquiring 20 samples from this site would be difficult to achieve and the site was not used in this study. Cottonwood Canyon was included in our LAG proposal, however, since that time, we acquired the GPS location of Metcalf et. al's sampling site and learned that the original site used in their study is developed with no remaining SKR habitat in the vicinity (See Figure 2). Thus, this site could not be used for the range-wide genetics study. In addition, our trapping efforts revealed that the kangaroo rats located at the Safari Park are Dulzura kangaroo rats (DKR; *Dipodomys simulans*) and thus no samples were collected from this site. Sites added to this study included San Jacinto Wildlife Area (Figure 1; San Jacinto), Bachelor Mountain and Stirrup. The samples from San Jacinto Wildlife Area were collected opportunistically as part of a separate research effort. Bachelor Mountain is within the SWRCMSR and Stirrup is a private property on Stirrup Road, Temecula, Riverside County, CA. These samples were collected as part of our translocation efforts in the vicinity.

Figure 2. Location of Cottonwood Canyon site used in Metcalf et al. The site is developed and could not be used for this study as visual surveys indicate that no SKR remain in the vicinity.



GENERAL METHODS

Sample Collection -- Trapping

We set up trapping grids or targeted trap lines using active burrows at each sampling site and trapped each site for 5 consecutive nights or until all 20 samples were collected. Multiple trapping efforts at a site were occasionally necessary to achieve our sample size goal if SKR densities were low (see Appendix A for trapping results by site). Trapping grids/lines contained no more than 100 traps (grids: 10m spacing between traps in each direction). We placed a flag next to each trap and a small piece of colored reflective tape on traps to improve our ability to locate them at night. We captured SKR using Sherman live traps (8 x 9 x 23 cm) with a long bridge and 0.5 cm space in the door to prevent tail severance. We baited traps with sterilized raw oats and/or millet to prevent germination in the wild. We set traps just before dusk and checked them at least 2 times per night. Traps were emptied of seed and closed before dawn. We took a GPS location at all traps in which we captured an SKR. Each SKR captured was sexed, aged, weighed, and either individually marked for identification or marked with a Sharpey non-toxic pen to ensure that a single sample was collected from each individual.

Ear snip protocol

We collected genetic samples from individuals by taking a tiny ear snip sample from each individual SKR captured. Ear snipping is a common method for collection of genetic data in the rodent family Heteromyidae (Metcalf et al. 2001, Alexander and Riddle 2005, Loew et al. 2005, Waser et al. 2006) and has been used with endangered species within the family (*D. stephensi*, Metcalf et al. 2001; *D. ingens*, (Loew et al. 2005). Ear snips can be as small as a pencil point and provide ample genetic data for analysis of parentage, genetic relationships and dispersal (Waser et al. 2006). Obtaining an ear snip involves sterilizing scissors with 70% ethanol, holding the scissors on a tangent from the edge of the pinna, and snipping a sliver (~0.5mm) off the edge. Tissue samples were transferred to and stored in a vial with 95% ethanol. Scissors were sterilized between animals. Samples were transferred to our Genetics Division at the San Diego Zoo Institute for Conservation Research (Escondido, CA) for genetic analysis.

Genetic Processing and Analysis

We processed a total of 424 earsnip samples from SKR collected between 2008 to 2015. Though a minimum of 20 samples we taken at each of the sites trapped specifically for this purpose, some samples (n=5: Canyon Lake) failed to amplify and were unable to be used for either genotyping or sequencing. In addition, in a small number of cases (n=5 Ramona Grasslands and n=1 Anza Valley), kangaroo rats were misidentified as SKR but genetic analyses revealed that they were DKR.

Mitochondrial control region sequencing

A 660-bp fragment of the mitochondrial D-Loop control region was sequenced from a total of 377 samples using a modified version of primers L16007 and H00651 (Kocher et al. 1989, unpublished). Amplification was verified on a 1.5% TBE agarose gel, and products were purified using ExoSAP-IT® (Affymetrix). Cycle sequencing was performed using BigDye™ 3.1 and sequencing products were sequenced bi-directionally on an ABI 3130 Genetic Analyzer (Applied

Biosystems). Contigs were assembled and edited using SEQUENCHER 5.1 (Gene Codes Corp.), and sequences were aligned using the built-in version of CLUSTAL W.

Microsatellite marker validation

A microsatellite library was developed specifically for SKR in 2010. The Savannah River Ecology Laboratory at the University of Georgia was contracted to develop the library using next generation 454 sequencing technology. This method turned out to be extremely productive, resulting in ~725 loci with greater than 10 tetra-nucleotide repeats. Using an algorithm implemented in the programs MSATCOMMANDER and PRIMER3 (Rozen and Skaletsky 2000, Faircloth 2008) that searches for repeat regions and suitable primers, the Georgia lab was also able to provide 150 primer pairs which had no primer warnings (e.g. primer dimer). A set of 24 highly polymorphic microsatellite markers were used to genotype 424 samples for the range-wide study. PCRs were performed using the Qiagen Multiplex PCR Kit (Qiagen Inc.) and organized into seven multiplex and one singleplex schemes. PCR reactions were performed in a total volume of 12.5 µl containing 1.0 µl DNA template, 0.2 µM each primer, 1X Multiplex PCR Master Mix, and 0.5X Q-Solution. PCR cycling conditions were 95°C, 15 min; 35X (94°C, 30s; 60°C, 90s; 72°C, 60s); 60°C, 30 min. Amplification products were verified on a 1.5% TBE agarose gel. Fragment analysis was performed using capillary electrophoresis on an ABI 3130xl genetic analyzer (Applied Biosystems) and alleles were scored relative to an internal size standard (500 ROX) using GENEMAPPER 3.0 (Applied Biosystems).

Microsatellite markers were evaluated for deviations from Hardy-Weinberg equilibrium and signs of linkage disequilibrium at the population level using the software GENEPOP 4.1 (Rousset 2008). Markers were further assessed for evidence of null alleles and genotyping errors using the software MICRO-CHECKER (van Oosterhout et al. 2004) and FREENA (CHAPUIS AND ESTOUP 2007). Significance at an initial p-value of 0.05 was corrected for multiple tests using the B-Y False Discovery Rate method (Benjamimi and Yekutieli 2001). Exclusionary power of loci were calculated using GENALEX 6.5 (Peakall and Smouse 2012) for each population separately as well as for all populations combined.

Sequencing analysis

The software TCS 1.21 (Clement et al. 2000) was used to create an unrooted, maximum parsimony haplotype network depicting the genealogical relationships between various D-loop haplotypes. A 95% connection limit was applied. Phylogenetic analyses were performed using Bayesian inference (BI) and maximum-likelihood (ML) methods. MRBAYES (Ronquist and Huelsenbeck 2003) was used to generate a BI consensus tree under the best-fit model chosen using JMODELTEST 2.0 (Posada 2008). The General Time Reversible model gamma distributed with invariant sites (GTR+I+G) was selected using the Akaike Information Criterion (AIC) and the program was run for 2 million generations and the first 8000 trees were discarded as burn-in. The maximum likelihood tree was constructed using MEGA (Tamura et al. 2011) with 1000 bootstrap replicates. Species *D. simulans* and *D. merriami* were used as outgroups for the BI and ML analyses.

Microsatellite analysis

Multiple genetic diversity estimates were calculated for each range-wide population. The software HP-RARE (Kalinowski 2005) was used to calculate allelic richness (AR) and private allelic richness (PAR) averaged over all loci, incorporating sample size variation by method of rarefaction. The computer program GENALEX 6.5 (Peakall and Smouse 2012) was used to calculate observed (H_O) and expected (H_E) heterozygosity. Inbreeding coefficients (F_{IS}) per

population were estimated using FSTAT 2.9.3 (Goudet 1995). We also employed FSTAT 2.9.3.2 to calculate pairwise genetic differentation between populations expressed as F_{ST} values (Weir and Cockerham 1984). Significance was corrected from an initial significance level of $p<0.05$ to account for multiple comparisons using the B-Y False Discovery Rate method.

To evaluate hierarchical population genetic structure, a Bayesian clustering analysis was performed using the computer program STRUCTURE 2.3.2 (Pritchard et al. 2000). We ran the analysis with typical default settings, including an admixture model with correlated allele frequencies. This analysis is used to objectively determine the number of genetically distinct clusters that exist among a set of individuals using their multilocus genotype data, without any preconceived notions regarding possible population structure. Bayesian simulations were run for each population number (*K*) ranging from 1–30. For each value of *K*, we ran 10 independent replicates with a burn-in period of 250,000 steps followed by 750,000 iterations, thereby assuring convergence on the inferred likelihood values. We follow the widely-accepted protocol of (Evanno et al. 2005), wherein the true value of *K* is inferred using an ad hoc statistic, ΔK, based on the rate of change in the log probability of data between successive *K* values ($\Delta K = m(|L''K|) / s[L(K)]$). In addition to the Bayesian approach using STRUCTURE, population structure was also assessed using a multivariate approach. The Discriminant Analysis of Principal Components (DAPC) feature in the R package, ADEGENET, was used to determine the possible number of genetic clusters (Jombart et al. 2010) using a retention of 100 PC axes. The number of clusters (K) was varied from 1 to 30. The optimum K value was chosen based on the lowest value of K that reflected the least changes in BIC (Jombart 2008).

Isolation by distance

An isolation by distance analysis was performed to identify any possible genetic variation that might be distributed clinally, with populations closest to each other tending to be the most genetically similar—a so-called pattern of "Isolation By Distance" (IBD). Mantel tests (50000 permutations) were performed using Genepop 4.1 (Rousset 2008) to test for significant patterns of IBD.

Results

Sequencing analysis

A total of 377 SKR samples were sequenced at a 660-bp region of the mitochondrial D-loop control region (Table 2). A total of 42 haplotypes were observed, representing 41 variable sites (6.2%) and 24 (57%) private haplotypes. San Jacinto Wildlife Area and Lake Perris had the greatest haplotype diversity and Ramona Grasslands had the least, represented by only one haplotype. Anza Valley had the greatest number of unique haplotypes (n=4) followed by Stirrup (n=3).

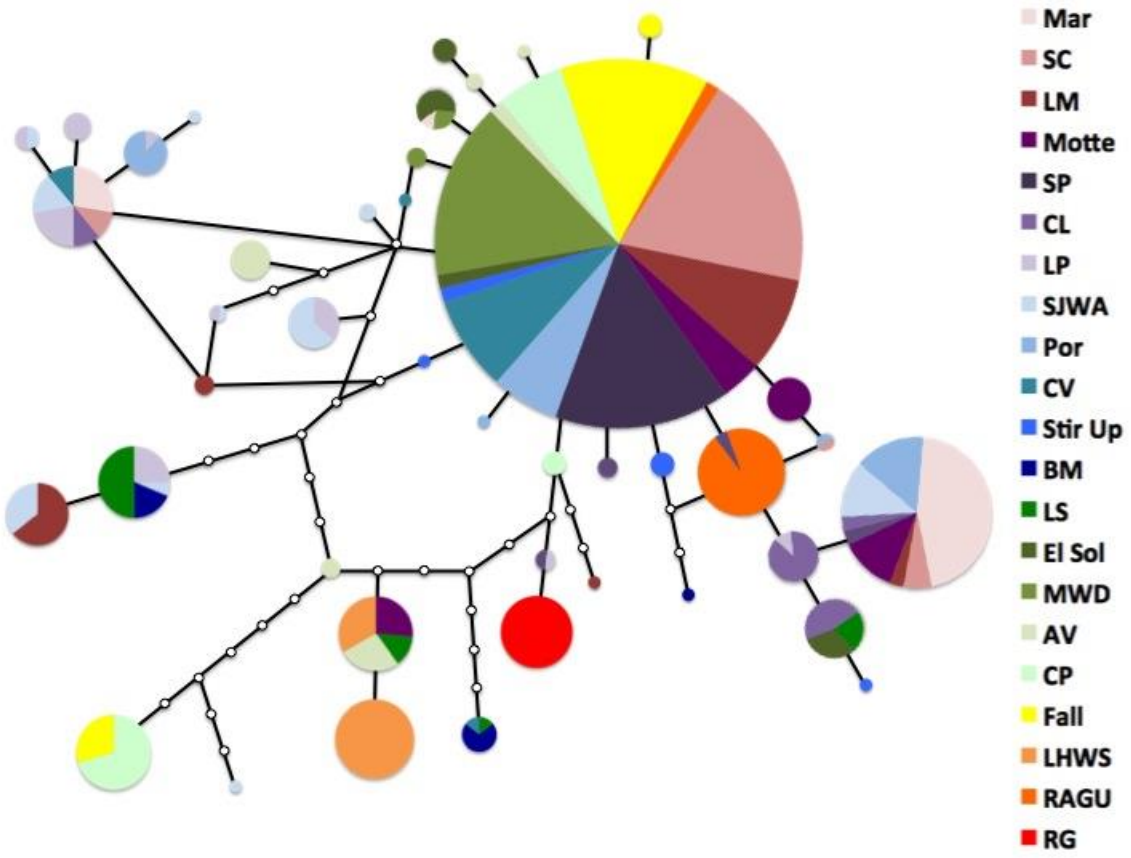
The statistical parsimony network (Figure 3) does not reveal any clear geographic structure in SKR haplotypes based on locality. There was a frequently observed haplotype (Contig2) found in 84 of 377 sequences (22%), and it was widely distributed throughout 13 range-wide sites from as far north as Sycamore Canyon and as far south as Rancho Guejito, with no obvious geographical trend. Slightly different regions of the D-loop were sequenced by Metcalf et al. (2001); however, they also found a very common and widespread haplotype they refer to as the "CC" haplotype. Unlike Metcalf et al. (2001) which observed most CC haplotypes in the South, we observed Contig2 mostly in the central and northern parts of the range. All haplotypes were

fairly closely related, with the most distant haplotype (Contig7) being only seven mutational differences from the closest haplotype.

Table 2. Range-wide sites with associated abbreviation (Abbr.), samples sequenced per site (n), number of haplotypes from the site, and number of private haplotypes unique to the site.

Range-wide Site	Abbr.	N	Number of haplotypes	Private haplotypes
Anza Valley	AV	19	6	4
Bachelor Mountain	BM	9	3	1
Camp Pendleton	CP	21	3	1
Canyon Lake	CL	17	4	0
Crown Valley	CV	11	4	1
El Sol	El Sol	14	4	1
Fallbrook	Fall	20	3	1
Lake Henshaw/Warner Springs	LHWS	21	2	1
Lake Mathews	LM	21	5	2
Lake Perris	LP	23	9	1
Lake Skinner	LS	14	4	0
March	Mar	21	3	0
Motte/Rim Rock	Motte	20	4	1
MWD	MWD	18	3	1
Potrero	Por	20	5	1
Ramona Grassland	RG	16	1	1
Rancho Guejito	RAGU	20	2	0
San Jacinto Wildlife Area	SJWA	27	10	3
Steele Peak	SP	18	5	1
Stirrup	Stir Up	6	4	3
Sycamore Canyon	SC	21	4	0

Figure 3. Statistical parsimony network depicting mutational relationships between SKR D-loop haplotypes. Each haplotype contains the percent contribution from each population, which is designated by a unique color. The areas of the circles representing each haplotype correspond to its frequency. Each line represents a single mutational event, and each dot represents a putative nucleotide change. A 95% connection limit was applied.



Results from the phylogenetic analyses also revealed low levels of mitochondrial divergence and phylogenetic structure within SKR across its range (Figure 4). Species *D. stephensi, D. simulans* and *D. merriami* are reciprocally monophyletic at this marker. However, within SKR, the phylogram shows overall low resolution with weakly supported nodes except for five cases in which two closely related haplotypes have strong support. These closely related haplotypes do not strictly fall within the three geographical ranges designated by Metcalf et al. 2001 (North, Central, South) and the most basal haplotypes within SKR also show no obvious geographical origin. The lack in pattern is further supported by shared haplotypes that were found in more than one site, labeled as "Contigs", which shows no clear association between locality and haplotype diversity (Figure 4).

Figure 4. Bayesian inference consensus tree constructed using SKR D-loop haplotypes and outgroups *D. simulans* (SBKR) and *D. merriami* (DKR). Only posterior probabilities showing strong support (≥0.95) are labeled at the nodes, followed by bootstrap supports (≥75) from the maximum likelihood analysis. Branches are labeled with haplotype names. Haplotypes identified from one sample are labeled by the site abbreviation and individual ID, while haplotypes obtained from more than one sample are labeled by the contig ID followed by sample sizes from each locality.

Microsatellite marker validation

Results from Hardy-Weinberg equilibrium and genotypic linkage equilibrium tests indicated no significant deviations across all populations; therefore, all loci were retained for genetic analyses. There was some evidence for null alleles, however, uncorrected pairwise Fst values fell within the 95% confidence interval for Fst values corrected for the presence of null alleles. This indicates that null alleles do not have a significant effect on genetic differentiation estimates, therefore; all data were retained for further analyses. The probability of identity (P_{ID}) of the 24 loci for each population was low, ranging from 1.4×10^{-13} to 7.8×10^{-27} and probability of identity for full siblings (PI_{sibs}) ranging from 1.5×10^{-6} to 9.9×10^{-11}. Probabilities were even lower when all populations were combined, with a P_{ID} of 1.4×10^{-33} and PI_{sibs} of 9.0×10^{-12}.

Genetic Diversity

As shown in Table 3, genetic diversity estimates as measured by observed heterozygosity were generally high for all populations, ranging from 0.484 in Ramona Grassland to 0.808 in Canyon Lake. The highest allelic richness was observed in Lake Skinner, followed closely by Lake Mathews, Canyon Lake, Lake Perris, and San Jacinto Wildlife Area. The lowest allelic richness was observed in Rancho Guejito, Ramona Grassland, and Camp Pendleton. Eleven of the 21 range-wide sites had private alleles, with the highest frequency found in Lake Mathews, Anza Valley, Canyon Lake, and Fallbrook. Inbreeding coefficients were relatively low with the exception of Fallbrook, which shows consistency with lower observed heterozygosity values compared to the expected.

Table 3. Summary statistics from microsatellite data among the range-wide sites. Sample size (n), allelic richness (AR), private allelic richness, observed heterozygosity (H_O), expected heterozygosity (H_E), and inbreeding coefficient (F_{IS}). Allelic richness and private allelic richness estimates are rarefied*

Range-wide Site	n	AR	PAR	H_O	H_E	F_{IS}
Anza Valley	19	5.42	0.17	0.66	0.666	0.035
Bachelor Mountain	9	5.17	0	0.764	0.691	-0.047
Camp Pendleton	21	3.92	0	0.583	0.56	-0.017
Canyon Lake	21	7.25	0.13	0.808	0.772	-0.022
Crown Valley	12	6.29	0.08	0.733	0.738	0.05
El Sol	31	6.29	0.08	0.737	0.721	-0.005
Fallbrook	20	5.17	0.13	0.573	0.669	0.169
Lake Henshaw/Warner Springs	21	4.38	0	0.597	0.609	0.044
Lake Mathews	23	7.25	0.21	0.774	0.773	0.022
Lake Perris	24	7.25	0.08	0.745	0.758	0.039
Lake Skinner	31	7.42	0.04	0.751	0.752	0.017
March	21	6.38	0	0.746	0.724	-0.007
Motte/Rim Rock	20	6.83	0.04	0.742	0.76	0.049
MWD	21	4.75	0	0.7	0.607	-0.129
Potrero	20	6	0	0.696	0.717	0.056
Ramona Grassland	16	3.79	0.04	0.484	0.497	0.057
Rancho Guejito	20	3.46	0	0.577	0.572	0.017
San Jacinto Wildlife Area	27	7.25	0	0.772	0.775	0.023
Steele Peak	19	7.04	0	0.794	0.773	0
Stirrup	6	5.67	0.04	0.764	0.751	0.073
Sycamore Canyon	21	6.25	0	0.74	0.719	-0.004

Population structure

Two different methods of clustering analyses were performed in order to evaluate population structure. The most likely number of clusters as measured by Delta K using the Bayesian clustering analysis was clear, at K=15 (Figure 5). The multivariate method using DAPC was less clear-cut, but the most likely number of clusters ranged from K=13 to K=15 clusters (Figure 6). STRUCTURE barplots were obtained for K=13 to K=15 as displayed in Figure 7. The barplot displaying the optimal number of clusters (K=15) as identified by the Bayesian STRUCTURE analysis was in strong accordance with the range-wide populations. Not surprisingly, the closely located sites San Jacinto Wildlife Area and Lake Perris fell within one cluster. Rancho Guejito and Ramona Grassland were also combined into one cluster. Sycamore Canyon, Canyon Lake, Crown Valley, Stirrup, and Bachelor Mountain showed some admixture from clusters represented by March, Steele Peak, and Canyon Lake. At K=14, the two clusters representing Steele Peak and Canyon Lake at K=15 merged to form one cluster. Additionally, at K=13 the distinct cluster represented by MWD was lost.

Figure 5. Graph from the Bayesian STRUCTURE analysis plotting the most likely number of genetic clusters (K) that best fit the data.

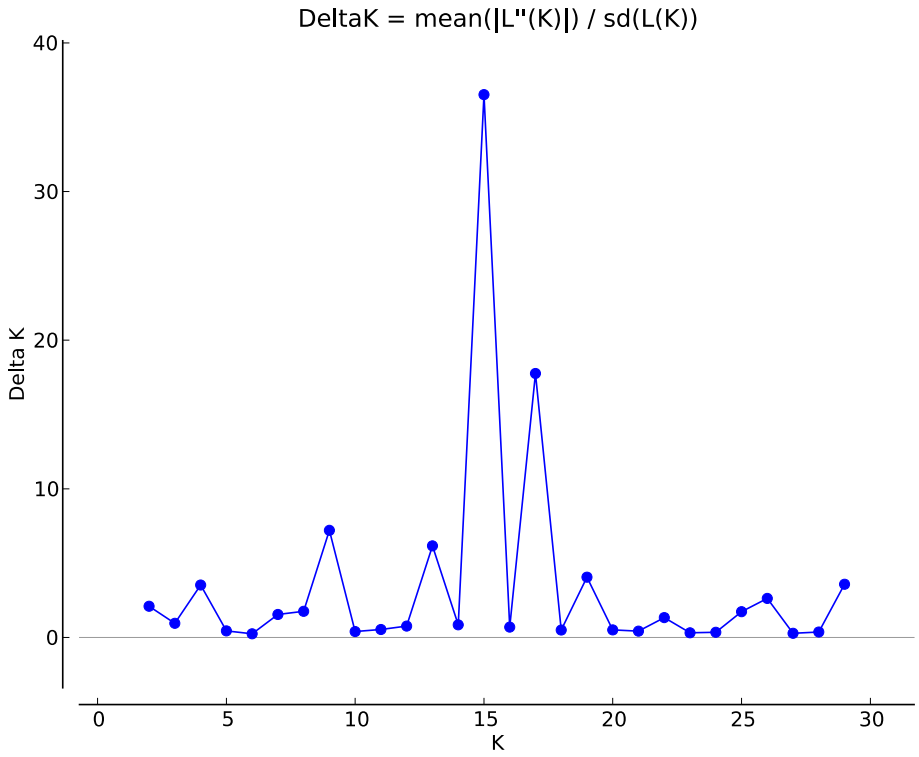


Figure 6. BIC plot obtained from the DAPC analysis. The optimal number of clusters correspond to the lowest BIC values, around K=13-15.

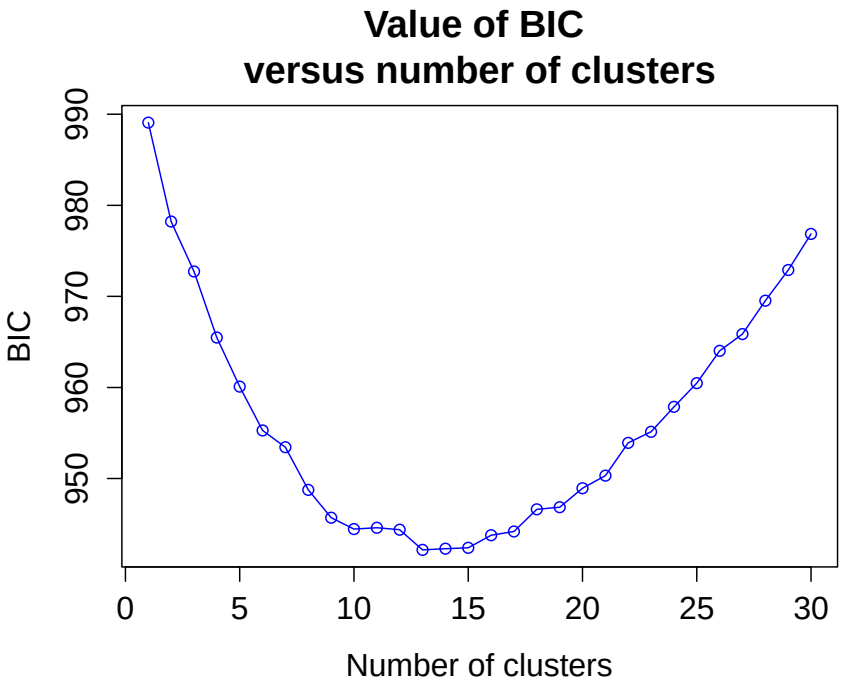
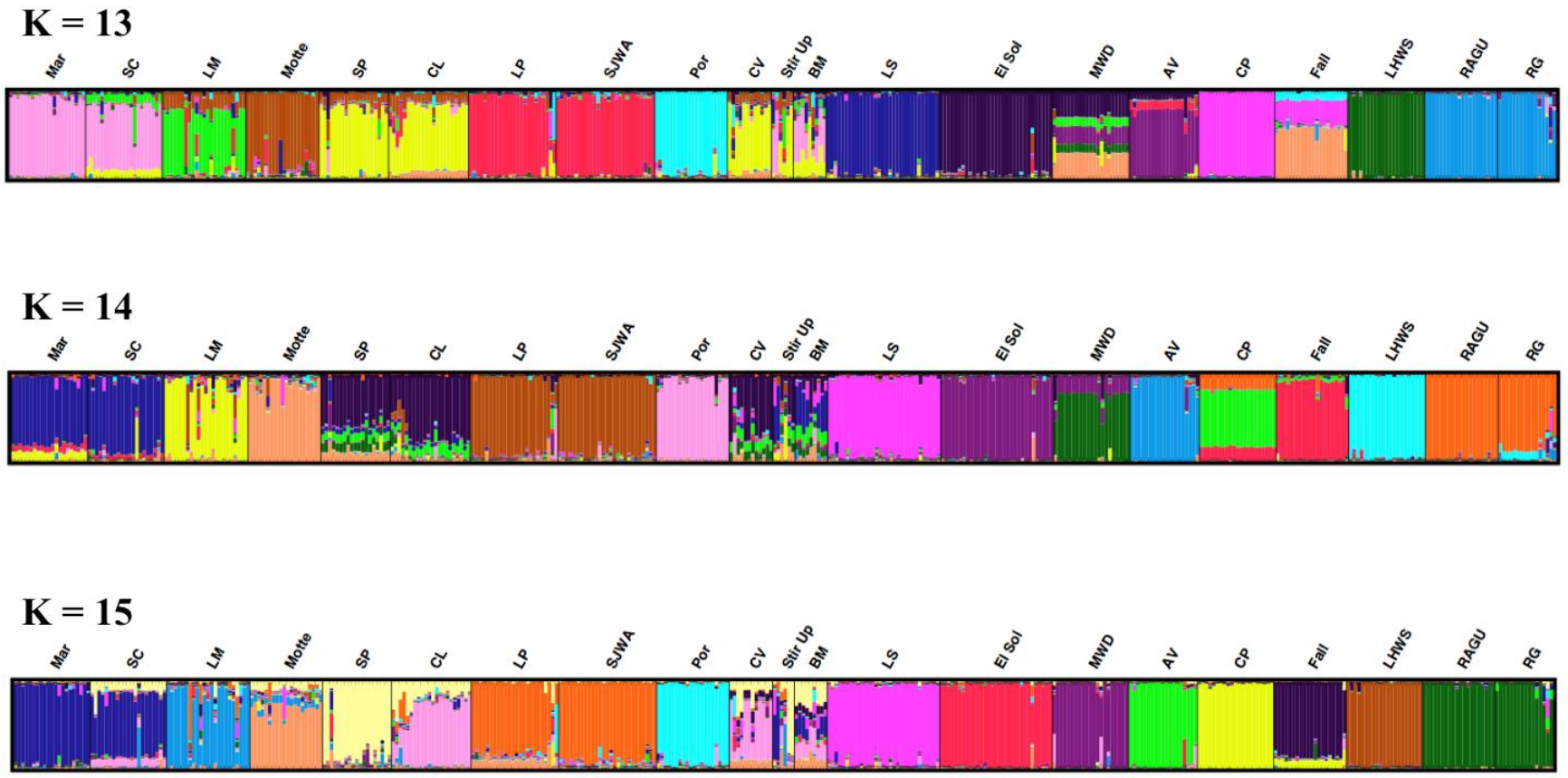


Figure 7. STRUCTURE barplots for K=13 to K=15. Range-wide sites are labeled on top of the barplot with their respective abbreviations (Refer to Table 1). Each bar represents a unique individual and the y-axis represents the proportional membership of each individual to a given cluster. Unique clusters are represented by a unique color.



Genetic Differentiation and Isolation by distance

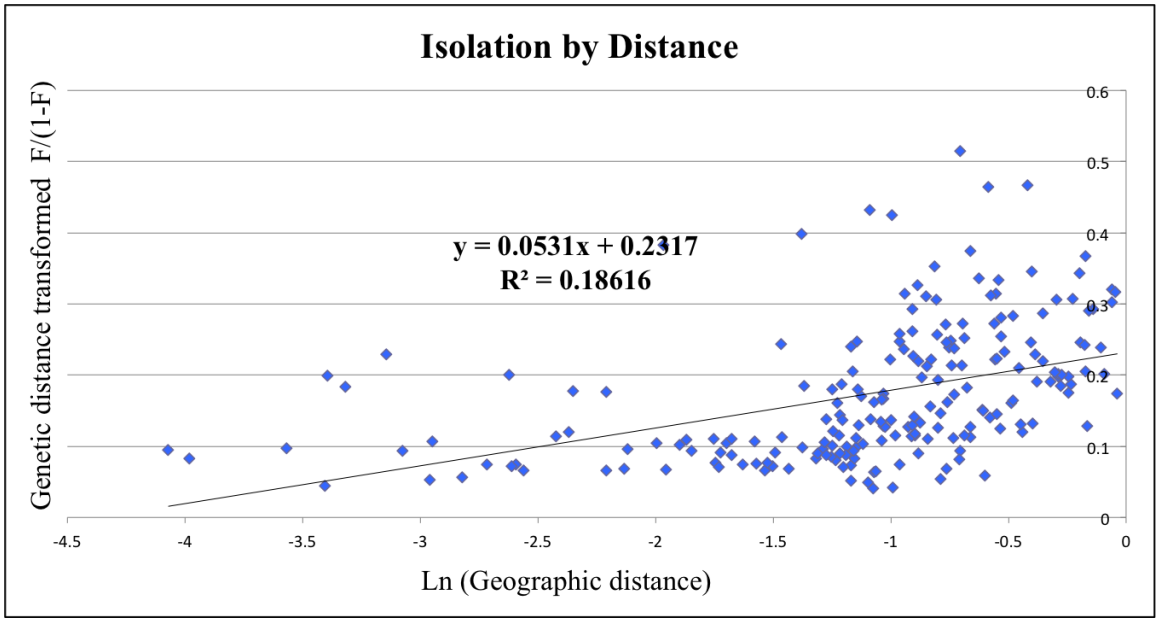
Fst estimates as a measure of genetic differentiation were calculated for each pair of range-wide populations. It is important to note that Fst values reflect the variance among subpopulations relative to the total variance. General guidelines set by (Wright 1978) and (Hartl and Clark 1997) were used to categorize Fst values in Table 3: 0-0.05 little genetic differentiation, 0.05-0.15 moderate differentiation, 0.15-0.25 great differentiation, and >0.25 very great genetic differentiation. There seems to be a slight trend towards range-wide sites north of Lake Henshaw/Warner Springs and Rancho Guejito having less genetic differentiation to each other than the populations south of Lake Henshaw/Warner Springs and Rancho Guejito, with the exception of Fallbrook. The range-wide sites Camp Pendleton, Rancho Guejito, and Ramona Grassland show the highest levels of genetic differentiation to each other and to other range-wide populations. High mutation rates and differences in genetic diversity within sub-populations can influence Fst values, which may explain the discrepancy in the STRUCTURE and Fst results between Rancho Guejito and Ramona Grassland.

Table 3. Fst values between pairs of range-wide populations, categorized into different colors based on guidelines set by Wright (1978) and Hartl & Clark (1997). 0-0.05 little genetic differentation (white), 0.05-0.15 moderate differentiation (yellow), 0.15-0.25 great differentiation (orange), and >0.25 very great genetic differentiation (red). Significance was obtained after 4200 permutations and adjusted for multiple comparisons to P<0.00735 from an initial P<0.05.

	Mar	SC	LM	Motte	SP	CL	LP	SJWA	Por	CV	Stir Up	BM	LS	El Sol	MWD	AV	CP	Fall	LHWS	RAGU	RG
Mar		0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024
SC	0.0864		0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024
LM	0.0841	0.0804		0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024
Motte	0.088	0.0942	0.0666		0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024
SP	0.086	0.0709	0.0656	0.0617		0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024
CL	0.0699	0.0841	0.0616	0.0616	0.0503		0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024
LP	0.0924	0.0985	0.0684	0.0644	0.0688	0.0668		0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024
SJWA	0.095	0.0995	0.0598	0.0628	0.0715	0.0637	0.0429		0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024
Por	0.0975	0.1141	0.1012	0.0908	0.1035	0.0695	0.0961	0.0993		0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024
CV	0.1129	0.1036	0.0855	0.0913	0.0893	0.0743	0.0877	0.0898	0.1011		0.00071	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024
Stir Up	0.0509	0.0638	0.0552	0.0611	0.0402	0.0391	0.0473	0.0493	0.0762	0.0534		0.00048	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024
BM	0.1028	0.0828	0.1	0.0786	0.0809	0.0824	0.1029	0.0956	0.1215	0.0969	0.0695		0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024
LS	0.118	0.0994	0.075	0.0666	0.0768	0.0801	0.0857	0.0828	0.1257	0.0697	0.0669	0.0761		0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024
El Sol	0.1347	0.1115	0.1031	0.1004	0.0937	0.1084	0.1208	0.1149	0.1452	0.1075	0.1019	0.0861	0.089		0.00024	0.00024	0.00024	0.00024	0.00024	0.00024	0.00024
MWD	0.1846	0.1645	0.1392	0.1528	0.1388	0.1561	0.1703	0.1575	0.1986	0.1504	0.15	0.1864	0.155	0.1665		0.00024	0.00024	0.00024	0.00024	0.00024	0.00024
AV	0.1656	0.1557	0.1138	0.1073	0.1163	0.1157	0.1384	0.1106	0.1763	0.1482	0.1207	0.1204	0.1133	0.1428	0.1915		0.00024	0.00024	0.00024	0.00024	0.00024
CP	0.2142	0.2196	0.2013	0.1988	0.1799	0.1813	0.1819	0.1826	0.1975	0.2131	0.1927	0.2463	0.2263	0.1983	0.2982	0.257		0.00024	0.00024	0.00024	0.00024
Fall	0.1228	0.1272	0.1126	0.1274	0.1151	0.1182	0.1314	0.1304	0.1413	0.124	0.1055	0.1424	0.1395	0.1527	0.1934	0.1886	0.1672		0.00024	0.00024	0.00024
LHWS	0.1924	0.1674	0.1481	0.149	0.158	0.1678	0.1655	0.1673	0.1865	0.1542	0.1618	0.1758	0.1471	0.1972	0.2143	0.1958	0.3183	0.2031		0.00024	0.00024
RAGU	0.1977	0.1955	0.1703	0.1804	0.1605	0.1741	0.1697	0.1604	0.2228	0.1922	0.1816	0.2047	0.1751	0.2073	0.2373	0.2393	0.261	0.2051	0.2847		0.00024
RG	0.2423	0.2408	0.232	0.2555	0.2347	0.234	0.2263	0.2248	0.2687	0.2205	0.2389	0.2501	0.2379	0.2513	0.3169	0.2723	0.3397	0.2343	0.3016	0.2768	

Results from the isolation by distance analysis indicate a significant and positive correlation between geographic distance and genetic distance (R^2= 0.186; p < 0.001) (Figure 8). However, the R-squared value is relatively low, suggesting a slight positive association.

Figure 8. Isolation by distance plot. Axes represent ln(geographic distance) (x) and genetic distance (y) transformed to (F/(1-F)). Approximate Bayesian computation results for slope [95% confidence interval] is: 0.0531 [0.0453, 0.0606]. P-value < 0.001.



Discussion

This project establishes a baseline understanding of Stephens' kangaroo rat population genetics across the species geographic range. To date, it is the most comprehensive study on SKR landscape genetics with the largest sample size reflecting the most range-wide collection sites.

The results of this study show the highest genetic variation in terms of allelic richness primarily in northern populations (i.e. Lake Perris, San Jacinto Wildlife Area, March, Sycamore Canyon, Lake Mathews, etc.) and the lowest in the southern most populations (i.e. Ramona Grasslands, Rancho Guejito, Camp Pendleton) suggesting that the species may have expanded southward from an ancestral population in the north of the current range.

We used two types of genetic markers to assess both historical and more recent genetic structuring across the species range. The results from this study do not support the geographic structuring of the species into 3 subregions as articulated by Metcalf et. al. 2001. Rather, the results from this study suggest a historic continuous range inhabited by SKR that has undergone recent habitat fragmentation. The constructed phylogenetic trees show reciprocal monophyly for the species and we found high haplotype diversity with most range-wide sites containing private haplotypes. The SKR haplotypes are fairly closely related with few mutational changes and there was no obvious trend associated with locality. This was further supported by the observation that the most frequently observed haplotype (Contig2) was wide-spread throughout the range. The inability to resolve any phylogenetic relationships using this marker supports a historically continuous range inhabited by SKR that has since then undergone recent habitat fragmentation. This can lead to isolated populations established by simultaneous divergence and random colonization. A recent study by (Jezkova et al. 2014) also using the mitochondrial control region in *D. merriami* and *D. deserti* was able to find genealogical structure in haplotypes; therefore, our lack of pattern is unlikely due to the resolution of the marker.

The highly evolving microsatellite markers provided more resolution for inferring recent population structure. Results show high genetic structure in association with demarcation between range-wide populations. Microsatellites were also able to detect significant isolation by distance. Particularly in the southern part of the range, populations appear to be more genetically differentiated from each other, with the exception of Rancho Guejito and Ramona Grassland which fell into one cluster. Overall connectivity between genetic clusters was low, with little admixture with the exception of Canyon Lake, Crown Valley, Stirrup, and Bachelor Mountain which share similar genetic signatures.

We found no evidence of evolutionary divergence events within the species. Our study implies that recent effects of habitat fragmentation and population isolation in SKR have created a metapopulation-like structure in the species across its current range. Limited potential for long-range dispersal and genetic drift are potential and likely sources of the phylogeographic divergence noted here, but the evidence is also consistent with this being a relatively recent phenomenon.

CONCLUSIONS, MANAGEMENT RECOMMENDATIONS AND FUTURE DIRECTIONS

Our results indicate that, historically, there was no geographic genetic structuring across the species range. However, a recent loss of connectivity across the species range due to contemporary urbanization may have driven the genetic structuring that is currently present between populations. Thus, SKR populations across the range are becoming increasingly isolated due to limited dispersal and associated gene flow. Should this trend of fragmentation continue, populations in isolation can be at risk of extirpation. Based on these results, in order to mitigate for effects of fragmentation, we recommend minimizing further fragmentation and enhancing connectivity across the species range. The results of this study indicate that gene flow has been especially restricted to the southernmost populations and connectivity in that region will be important to the continuing existence of SKR populations and maintaining the extent of existing SKR occupied habitat. Further analyses are required to determine which populations to prioritize for mitigation. For example, recent fragmentation can be assessed using GIS and mapping state and interstate highways from the California Major Roads layer (California Spatial Information Library; http:gis.ca.gov/data/epi). This information can be combined with Population Viability Analyses to determine population vulnerability by site in response to urbanization. Incorporating urban development through the valleys into this modeling will be critical for understanding the effects of fragmentation on SKR gene flow as this species inhabits valley floors and is likely restricted by availability of open habitat on gentle to no slopes. Though these analyses are beyond the scope of this contract, some general recommendations can be made for range-wide SKR management.

Restoration of historic levels of gene flow could improve range-wide genetic diversity, decrease inbreeding in isolated populations and thus stochastic extinctions and improve fitness. Two primary methods have been used to restore gene flow within metapopulations that are affected by anthropogenic impacts. Assisted gene flow (Aitken and Whitlock 2013) or human mediated dispersal (Akçakaya et al. 2007) through translocations has been used to successfully manage small populations via simulation of historic gene flow. For example, in African wild dogs (*Lycaon pictus*) in which populations are isolated by hard fragmentation (i.e. wildlife fencing), metapopulation modelling suggested that periodic, managed gene flow through translocations should be implemented to reduce inbreeding and the resultant effects of extinction (Akçakaya et al. 2007). Alternatively or in addition, dispersal within the already fragmented landscape matrix could be increased through the establishment of habitat corridors. Corridors can increase movement between habitat patches by more than 50% (Hilty et al. 2006, Gilbert-Norton et al. 2010), but natural corridors (those existing in landscapes) show more movement than manipulated corridors (Gilbert-Norton et al. 2010) . Recent data indicate that Heteromyids will use wildlife culverts that are designed for them (Shier, unpublished data), thus reestablishing corridors through fragmented habitat may improve dispersal and gene flow between isolated populations of SKR. But more information on SKR habitat selection and movement behavior is needed to provide direction for determining dispersal corridors that are critical for landscape connectivity. Current technologies (e.g. GPS telemetry) used for the study of space use and movement can simultaneously measure attributes of the environment with space use on scales ranging from cm to km (reviewed in Kerr and Ostrovsky 2003, Turner et al. 2003). To date, space use and habitat selection studies for SKR are limited, thus more detailed

studies on SKR space use and habitat selection behavior will improve facilitate management and recovery of this listed species.

ACKNOWLEDGEMENTS

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APPENDIX A TRAPPING DATA FROM SITES COMPLETED AS PART OF THIS CONTRACT

A single asterisk indicates that the samples did not amplify. A double asterisk indicates that the samples came from the same individual. A triple asterisk indicates that the sample spilled in the field. Finally boxes indicate individuals for which genetics showed misidentification. These individuals were DKR rather than SKR. Data on Rancho Guejito are not included.

Date	Site	ID	Sex	Age	Weight (g)	Reproductive Condition	Lat	Long
8/22/2011	Anza ValleySilverado	1	F	Adult	60	Visible Nipples	33° 28' 50.617" N	116° 41' 29.305" W
8/22/2011	Anza ValleySilverado	2	F	Adult	62	Visible Nipples	33° 28' 50.617" N	116° 41' 29.305" W
9/6/2015	Anza ValleySilverado	3	F	Adult	57	Visible Nipples	33°29'17.20"N	116°41'28.81"W
9/6/2015	Anza ValleySilverado	4	F	Adult	56.5	Visible Nipples	33°29'17.20"N	116°41'28.81"W
9/6/2015	Anza ValleySilverado	5	M	Adult	72	Scrotal	33°29'17.20"N	116°41'28.81"W
9/6/2015	Anza ValleySilverado	6	F	Adult	64	Visible Nipples	33°29'17.20"N	116°41'28.81"W
9/6/2015	Anza ValleySilverado	7	M	Adult	68	Scrotal	33°29'17.20"N	116°41'28.81"W
9/6/2015	Anza ValleySilverado	8	M	Adult	66	Scrotal	33°29'17.20"N	116°41'28.81"W
9/6/2015	Anza ValleySilverado	9	F	Adult	57	Visible Nipples	33°29'17.20"N	116°41'28.81"W
9/6/2015	Anza ValleySilverado	10	F	Juv	52	No Visible Nipples	33°29'17.20"N	116°41'28.81"W
9/6/2015	Anza ValleySilverado	11	F	Adult	64	Visible Nipples	33°29'17.20"N	116°41'28.81"W
9/6/2015	Anza ValleySilverado	12	M	Adult	67	Scrotal	33°29'17.20"N	116°41'28.81"W
9/6/2015	Anza ValleySilverado	13	M	Adult	64	Scrotal	33°29'17.20"N	116°41'28.81"W
9/6/2015	Anza ValleySilverado	14	F	Adult	65	Visible Nipples	33°29'47.16"N	116°41'04.82"W
9/6/2015	Anza ValleySilverado	15	F	Adult	57	Visible Nipples	33°29'47.16"N	116°41'04.82"W
9/6/2015	Anza ValleySilverado	16	F	Juv	47	No Visible Nipples	33°29'47.16"N	116°41'04.82"W
9/6/2015	Anza ValleySilverado	17	F	Adult	64	Visible Nipples	33°29'47.16"N	116°41'04.82"W
9/6/2015	Anza ValleySilverado	18	M	Adult	67	Scrotal	33°29'47.16"N	116°41'04.82"W
9/6/2015	Anza ValleySilverado	19	M	Adult	57	Scrotal	33°29'47.16"N	116°41'04.82"W
9/6/2015	Anza ValleySilverado	20	F	Juv	41	No Visible Nipples	33°29'47.16"N	116°41'04.82"W
19-Jun-12	Canyon Lake	1*		Adult			33° 42' 00.73"N	117° 15' 50.11" W
19-Jun-12	Canyon Lake	2*		Adult			33° 42' 00.73"N	117° 15' 50.11" W

19-Jun-12	Canyon Lake	3*		Adult			33° 42' 00.73"N	117° 15' 50.11" W
19-Jun-12	Canyon Lake	4*		Adult			33° 42' 00.73"N	117° 15' 50.11" W
19-Jun-12	Canyon Lake	5*		Adult			33° 42' 00.73"N	117° 15' 50.11" W
13-Oct-14	Canyon Lake	6	F	Adult	63	No Visible Nipples	33° 41' 47.7744"N	117° 17' 32.6826"W
13-Oct-14	Canyon Lake	7	M	Adult	67	Non-scrotal	33° 41' 48.8364"N	117° 17' 33.1404"W
13-Oct-14	Canyon Lake	8	M	Adult	64	Non-scrotal	33° 41' 49.023"N	117° 17' 31.9374"W
13-Oct-14	Canyon Lake	9	M	Adult	61	Non-scrotal	33° 41' 49.5132"N	117° 17' 31.9986"W
13-Oct-14	Canyon Lake	10	F	Adult	59	No Visible Nipples	33° 41' 49.347"N	117° 17' 32.046"W
13-Oct-14	Canyon Lake	11	M	Adult	66	Non-scrotal	33° 41' 27.1314"N	117° 16' 34.6146"W
13-Oct-14	Canyon Lake	12**	M	Adult	63	Non-scrotal	33° 41' 26.5734"N	117° 16' 34.7664"W
13-Oct-14	Canyon Lake	13	M	Adult	67	Non-scrotal	33° 41' 27.5346"N	117° 16' 36.087"W
13-Oct-14	Canyon Lake	14	M	Adult	62	Non-scrotal	33° 41' 27.5388"N	117° 16' 36.0294"W
13-Oct-14	Canyon Lake	15	F	Adult	61	No Visible Nipples	33° 41' 47.5146"N	117° 17' 34.533" W
13-Oct-14	Canyon Lake	16	F	Adult	65	No Visible Nipples	33° 41' 47.7132"N	117° 17' 33.0894"W
14-Oct-14	Canyon Lake	17	M	Adult	61	Non-scrotal	33° 41' 49.8012"N	117° 17' 32.283"W
14-Oct-14	Canyon Lake	18	F	Adult	57	No Visible Nipples	33° 41' 49.3902"N	117° 17' 34.0398" W
14-Oct-14	Canyon Lake	19	M	Adult	63	Non-scrotal	33° 41' 49.3902"N	117° 17' 34.0398" W
14-Oct-14	Canyon Lake	20	M	Adult	65	Non-scrotal	33° 41' 48.5016"N	117° 17' 34.695" W
14-Oct-14	Canyon Lake	21	F	Adult	61	No Visible Nipples	33° 41' 49.347"N	117° 17' 32.046"W
14-Oct-14	Canyon Lake	22**	M	Adult	63	Non-scrotal	33° 41' 25.9476"N	117° 16' 35.3778"W
14-Oct-14	Canyon Lake	23	M	Adult	67	Non-scrotal	33° 41' 27.5388"N	117° 16' 36.0294"W
1/21/2013	Lake Henshaw	1	F	Adult	61	Non-scrotal	33° 17' 27.07"N	116° 42 '12.67"W
6/24/2014	Lake Henshaw	1	n/a	Juv	50	Non-reproductive	33° 17' 27.07"N	116° 42 '12.67"W
6/24/2014	Lake Henshaw	2	F	Adult	62	Visible Nipples	33° 17' 27.07"N	116° 42 '12.67"W
6/24/2014	Lake Henshaw	3	n/a	Juv	48	Non-reproductive	33° 17' 27.07"N	116° 42 '12.67"W
6/24/2014	Lake Henshaw	4	F	Adult	65	Visible Nipples	33° 17' 27.07"N	116° 42 '12.67"W
6/24/2014	Lake Henshaw	5	n/a	Juv	39	Non-reproductive	33° 17' 27.07"N	116° 42 '12.67"W
6/24/2014	Lake Henshaw	6	F	Adult	63	Lactating	33° 17' 27.07"N	116° 42 '12.67"W
6/24/2014	Lake Henshaw	7	M	Adult	61	Scrotal	33° 14' 42.67"N	116° 42' 12.21"W

6/24/2014	Lake Henshaw	8	M	Adult	66	Scrotal	33° 14' 42.67"N	116° 42' 12.21"W
6/24/2014	Lake Henshaw	9	F	Adult	68	Visible Nipples	33° 14' 42.67"N	116° 42' 12.21"W
6/24/2014	Lake Henshaw	10	F	Adult	64	Lactating	33° 14' 42.67"N	116° 42' 12.21"W
6/24/2014	Lake Henshaw	11	M	Adult	65	Scrotal	33° 14' 42.67"N	116° 42' 12.21"W
6/24/2014	Lake Henshaw	12	M	Adult	66	Scrotal	33° 14' 42.67"N	116° 42' 12.21"W
6/24/2014	Lake Henshaw	13	n/a	Juv	42	Non-reproductive	33° 14' 42.67"N	116° 42' 12.21"W
6/25/2014	Lake Henshaw	14	F	Adult	59	Lactating	33° 14' 42.67"N	116° 42' 12.21"W
6/25/2014	Lake Henshaw	15	M	Juv	44	Scrotal	33° 17' 27.07"N	116° 42 '12.67"W
6/25/2014	Lake Henshaw	16	F	Adult	60	Lactating	33° 17' 27.07"N	116° 42 '12.67"W
6/25/2014	Lake Henshaw	17	F	Adult	62	Visible Nipples	33° 17' 27.07"N	116° 42 '12.67"W
6/25/2014	Lake Henshaw	18	M	Adult	66	Scrotal	33° 17' 27.07"N	116° 42 '12.67"W
6/25/2014	Lake Henshaw	19	F	Adult	63	Visible Nipples	33° 14' 42.67"N	116° 42' 12.21"W
6/25/2014	Lake Henshaw	20	M	Adult	64	Scrotal	33° 14' 42.67"N	116° 42' 12.21"W
15-Oct-14	Ramona Grassland	1	F	Adult	63	No visible nipples	33° 2' 2.6304"N	116° 57' 1.9722"W
16-Oct-14	Ramona Grassland	2	F	Adult	61	No visible nipples	33° 2' 2.6196"N	116° 57' 1.6518"W
16-Oct-14	Ramona Grassland	3	M	Adult	65	Non-scrotal	33° 2' 2.8644"N	116° 57' 2.3508"W
19-Oct-14	Ramona Grassland	4	F	Adult	67	No visible nipples	33° 2' 3.9222"N	116° 57' 2.055"W
20-Oct-14	Ramona Grassland	5	M	Adult	63	Non-scrotal	33° 2' 4.5486"N	116° 56' 59.3586"W
20-Oct-14	Ramona Grassland	6	M	Adult	62	Non-scrotal	33° 2' 3.2022"N	116° 57' 0.1764"W
7/13/2015	Ramona Grassland	7	F	Adult	73	Lactating	33° 02' 3.57"N	116° 57' 01.55"W
7/13/2015	Ramona Grassland	8	M	Adult	65	Scrotal	33° 02' 03.48"N	116° 57' 04.48"W
7/14/2015	Ramona Grassland	9	M	Adult	67	Lactating	33° 02' 3.27"N	116° 57' 01.66"W
7/14/2015	Ramona Grassland	10	F	Adult	69	Scrotal	33° 02' 03.67"N	116° 57' 06.25"W
7/14/2015	Ramona Grassland	11	F	Adult	63	Lactating	33° 02' 04.64"N	116° 57' 01.63"W
10/17/2015	Ramona Grassland	12	F	Adult	54.5	Visible Nipples	33° 02' 19.39"N	116° 57' 17.49"W
10/17/2015	Ramona Grassland	13	F	Juv	49	Non-reproductive	33° 02' 17.43"N	116° 57' 18.86"W
10/17/2015	Ramona Grassland	14	M	Adult	61	Scrotal	33° 02' 12.80"N	116° 57' 2.54"W
10/17/2015	Ramona Grassland	15	F	Adult	57	Lactating	33° 02' 10.19"N	116° 56' 58.03"W
10/17/2015	Ramona Grassland	16***	M	Adult	62	Scrotal	33° 02' 11.14"N	116° 56' 55.70"W

10/17/2015	Ramona Grassland	17	F	Adult	65	Visible Nipples	33° 02' 6.04"N	116° 56' 57.61"W
10/17/2015	Ramona Grassland	18	M	Adult	82	Scrotal	33° 02' 5.52"N	116° 56' 57.58"W
10/17/2015	Ramona Grassland	19	F	Adult	65	Lactating	33° 02' 7.28"N	116° 56' 55.51"W
10/17/2015	Ramona Grassland	20	F	Adult	60	Visible Nipples	33° 02' 7.76"N	116° 56' 56.98"W
10/17/2015	Ramona Grassland	21	F	Juv	34	Visible Nipples	33° 02' 7.62"N	116° 56' 58.92"W
10/17/2015	Ramona Grassland	22	M	Adult	55	Non-scrotal	33° 02' 19.79"N	116° 57' 17.00"W
10/17/2015	Ramona Grassland	23	M	Juv	36	Non-reproductive	33° 02' 19.27"N	116° 57' 17.97"W
9-Nov-14	San Jacinto Wildlife Area	1	F	Adult	63	Visible Nipples	33° 51' 23.0868"N	117° 8' 13.4298"W
9-Nov-14	San Jacinto Wildlife Area	2	M	Adult	67	Non-scrotal	33° 52' 13.8576"N	117° 7' 45.1524"W
9-Nov-14	San Jacinto Wildlife Area	3	F	Adult	64	Visible Nipples	33° 52' 14.379"N	117° 7' 45.0876"W
9-Nov-14	San Jacinto Wildlife Area	4	M	Adult	61	Non-scrotal	33° 52' 14.8542"N	117° 7' 44.8464"W
9-Nov-14	San Jacinto Wildlife Area	5	M	Adult	59	Non-scrotal	33° 52' 14.9016"N	117° 7' 44.0976"W
9-Nov-14	San Jacinto Wildlife Area	6	M	Adult	66	Non-scrotal	33° 52' 21.9036"N	117° 7' 38.9928"W
9-Nov-14	San Jacinto Wildlife Area	7	M	Adult	63	Non-scrotal	33° 52' 23.5236"N	117° 7' 39.0756"W
9-Nov-14	San Jacinto Wildlife Area	8	F	Adult	67	No visible nipples	33° 52' 24.852"N	117° 7' 39.0684"W
9-Nov-14	San Jacinto Wildlife Area	9	F	Adult	62	No visible nipples	33° 51' 13.4058"N	117° 8' 3.0876"W
9-Nov-14	San Jacinto Wildlife Area	10	M	Adult	61	Non-scrotal	33° 51' 12.9234"N	117° 8' 3.3432"W
9-Nov-14	San Jacinto Wildlife Area	11	F	Adult	65	No visible nipples	33° 52' 13.188"N	117° 7' 45.7926"W
10-Nov-14	San Jacinto Wildlife Area	12	M	Adult	61	Non-scrotal	33° 52' 14.379"N	117° 7' 45.0876"W
10-Nov-14	San Jacinto Wildlife Area	13	M	Adult	57	Non-scrotal	33° 52' 16.251"N	117° 7' 43.8168"W
10-Nov-14	San Jacinto Wildlife Area	14	M	Adult	63	Non-scrotal	33° 52' 24.5244"N	117° 7' 39.0858"W
10-Nov-14	San Jacinto Wildlife Area	15	M	Adult	65	Non-scrotal	33° 51' 15.4692"N	117° 8' 1.8594"W
10-Nov-14	San Jacinto Wildlife Area	16	F	Adult	63	No visible nipples	33° 51' 12.7944"N	117° 8' 3.5304"W
10-Nov-14	San Jacinto Wildlife Area	17	M	Adult	67	Non-scrotal	33° 51' 12.3984"N	117° 8' 3.8292"W
10-Nov-14	San Jacinto Wildlife Area	18	M	Adult	64	Non-scrotal	33° 50' 39.5232"N	117° 8' 25.8282"W
10-Nov-14	San Jacinto Wildlife Area	19	M	Adult	61	Non-scrotal	33° 52' 14.9016"N	117° 7' 44.0976"W
10-Nov-14	San Jacinto Wildlife Area	20	M	Adult	59	Non-scrotal	33° 52' 21.9036"N	117° 7' 38.9928"W
11-Nov-14	San Jacinto Wildlife Area	21	M	Adult	63	Non-scrotal	33° 52' 16.251"N	117° 7' 43.8168"W
11-Nov-14	San Jacinto Wildlife Area	22	F	Adult	67	No visible nipples	33° 52' 24.5244"N	117° 7' 39.0858"W

11-Nov-14	San Jacinto Wildlife Area	23	M	Adult	62	Non-scrotal	33° 51' 15.1302"N	117° 8' 2.2194"W
11-Nov-14	San Jacinto Wildlife Area	24	M	Adult	61	Non-scrotal	33° 52' 24.06"N	117° 7' 37.6674"W
11-Nov-14	San Jacinto Wildlife Area	25	M	Adult	65	Non-scrotal	33° 50' 39.9042"N	117° 8' 47.4246"W
12-Nov-14	San Jacinto Wildlife Area	26	F	Adult	61	No visible nipples	33° 50' 40.0488"N	117° 8' 47.331"W
10-Nov-14	San Jacinto Wildlife Area	27	M	Adult	66	Non-scrotal	33° 52' 12.954"N	117° 7' 45.9546"W
12-Nov-14	San Jacinto Wildlife Area	28	F	Adult	57	No visible nipples	33° 52' 14.6388"N	117° 7' 45.1344"W