

## Population structure and genetic variation in the endangered Giant Kangaroo Rat (*Dipodomys ingens*)

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### Abstract

Populations of the endangered giant kangaroo rat, *Dipodomys ingens* (Heteromyidae), have suffered increasing fragmentation and isolation over the recent past, and the distribution of this unique rodent has become restricted to 3% of its historical range. Such changes in population structure can significantly affect effective population size and dispersal, and ultimately increase the risk of extinction for endangered species. To assess the fine-scale population structure, gene flow, and genetic diversity of remnant populations of *Dipodomys ingens*, we examined variation at six microsatellite DNA loci in 95 animals from six populations. Genetic subdivision was significant for both the northern and southern part of the kangaroo rat's range although there was considerable gene flow among southern populations. While regional gene diversity was relatively high for this endangered species, hierarchical F-statistics of northern populations in Fresno and San Benito counties suggested non-random mating and genetic drift within subpopulations. We conclude that effective dispersal, and therefore genetic distances between populations, is better predicted by ecological conditions and topography of the environment than linear geographic distance between populations. Our results are consistent with and complimentary to previous findings based on mtDNA variation of giant kangaroo rats. We suggest that management plans for this endangered rodent focus on protection of suitable habitat, maintenance of connectivity, and enhancement of effective dispersal between populations either through suitable dispersal corridors or translocations.

### Introduction

The extent of population subdivision and the distribution of genetic variation are indicative of past historical events, current social and dispersal behavior, and future probability of survival. Low genetic diversity at neutral loci may be correlated with and therefore indicative of a low potential for

adaptation to changing environmental conditions, which may pose particular problems for the survival of endangered species (Lynch et al. 1995; Loew 2000; Frankham et al. 2002). At the same time, the coexistence of numerous subpopulations connected by limited amounts of gene flow has been shown to maintain much more genetic variation across the entire population than a randomly

mating population of the same size (Lacy 1987). Baseline data on the fine-scale genetic population structure of endangered species are important for identifying and protecting populations that serve as a source for future colonizations, and to assess the potential risk of local extinctions of genetically depauperate satellite populations (Crozier 1997).

This study was designed to provide baseline information on the genetic population structure of the giant kangaroo rat, *Dipodomys ingens*, a federally listed endangered rodent (US Fish and Wildlife Service 1987), and to inform the sampling design of future studies. The giant kangaroo rat is endemic to the western Tulare Basin and adjacent areas in South-central California (US Fish and Wildlife Service 1987), where it once was very abundant and widespread until the 1960s. Agricultural development of the San Joaquin Valley (SJV) has severely reduced natural habitats, and giant kangaroo rats are currently restricted to the western edge of the Tulare Basin and its adjacent foothills in two disjunct areas: western Kern and eastern San Luis Obispo counties, and western Fresno and eastern San Benito counties. Three other small, isolated subpopulations are found on private lands near the southern subpopulations (Williams 1992). This distribution of *D. ingens* represents less than 3% of their historical range, and remaining habitats are highly fragmented and mostly located on suboptimal terrain (Grinnell 1932; Williams 1992; Williams et al. 1993, 1995; Goldingay et al. 1997).

Giant kangaroo rats are restricted to grasslands-dominated landscapes on sandy-loam soils that are not subject to frequent flooding, and are typically found in nearly flat or gently sloping terrain. Habitat for *D. ingens* is mostly dominated by exotic grasses and forbs, although communities of saltbushes (*Atriplex* spp.) and California ephedra (*Ephedra californica*) characterize the southern part of their geographic range (Williams 1992). Compared to their historical range, the current distribution of giant kangaroo rats includes habitat less ideally suited to sustain healthy population growth and to ensure long-term survival of populations (Goldingay 1997; Germano et al. 2001).

Historical populations of giant kangaroo rats in the San Joaquin Valley were contiguous across large areas of suitable habitat, but increased fragmentation probably resulted in a metapopulation structure (Grinnell 1932; Williams 1992; Williams

et al. 1993). Such a metapopulation structure will result in major population fluctuations in many ephemeral satellite populations, whose existence depends entirely on recolonization from source populations and, therefore, effective dispersal. Based on preliminary capture-mark/recapture data (Williams unpublished data), male giant kangaroo rats on average disperse ~122 m and females ~99 m, and both sexes occasionally exhibit long-distance dispersal events greater than 700 m. While such dispersal behavior may result in sufficient gene flow to maintain healthy metapopulations, Williams (1992) pointed out that few of the remaining populations are sufficiently stable to act as possible source populations for dispersal and colonization. Further, much extant habitat might even act as a dispersal sink during unfavorable weather conditions resulting in local extinctions and an impoverished metapopulation structure.

Our pilot study is an extension of Good et al.'s (1997) research, which examined genetic evidence for the spatial heterogeneity and historical demography of giant kangaroo rat populations based on mitochondrial DNA polymorphism. We examine microsatellite DNA variation for a subset of those same *D. ingens* populations to evaluate fine-scale population structure and, to a more limited extent, social structure of *D. ingens*; our results will inform future studies on giant kangaroo rats (e.g., behavioral studies) with respect to appropriate sampling design given kangaroo rat population substructure. Microsatellite DNA tends to be neutral and therefore does not provide a direct measure of the genetic diversity relevant to natural selection and adaptation (Frankham et al. 2002). However, microsatellite DNA variation reflects the impact of all other evolutionary forces, i.e., mutation, migration, genetic drift and inbreeding that affect overall genetic variation, and the high rates of change at microsatellite loci makes them suitable to reconstruct recent changes in population structure (Goldstein and Schlötterer 1999). In addition, highly variable microsatellite loci are particularly useful genetic markers when conducting a pilot project on a species, such as the giant kangaroo rat, with little background information on genetic substructuring of populations, effective dispersal and potential for inbreeding (Baverstock and Moritz 1996). First, we examine whether the northern part of the *D. ingens* range shows a greater amount of subdivisions and

genetic diversity than the southern populations as was postulated by Good et al. (1997) based on mtDNA control region sequence variation. Secondly, we investigate the hypothesis that the current fragmentation of *D. ingens* populations has resulted in genetic substructuring and loss of neutral genetic diversity. Thirdly, we attempt to infer effective dispersal between populations and to gain insights into social structure from F-statistics. We discuss all our results in the context of genetic management of remnant populations.

## Materials and methods

### Study locations and populations

We sampled most of the extant populations of giant kangaroo rats in the northern part of their range and most populations in the south with the exception of western Kern County and three, small populations in Santa Barbara and San Luis Obispo counties on private land. In the northern part of their geographic range, we sampled tissue from animals at Monocline Ridge (MR,  $n = 13$ ), Tumey Hills (TH,  $n = 22$ ) and the southern Ciervo Hills (CH,  $n = 14$ ) in Fresno and San Benito counties (Figure 1a). Pairwise linear distances between these sample locations range from  $\sim 12$  to 26 miles

(Figure 1b). We collected samples from the southern part of the range on the Carrizo Plain National Monument at sites in the Elkhorn Plain Ecological Reserve (EPER,  $n = 15$ ), Painted Rock (PR,  $n = 13$ ), and Soda Lake (SL,  $n = 18$ ) (Figure 1a). Animals from populations at EPER and PR were translocated in July 1989 to found the colonies at Soda Lake. These southern sample locations are  $\sim 99$  miles south of CH, and pairwise linear distances among them range from  $\sim 4$  to 18 miles. Good et al. (1997) had sampled additional northern populations in Panoche Valley (PV) and SJV for their study on large-scale population structure of *D. ingens*. Based on the distributions of precincts, many of the northern and one of the southern sample locations (i.e., populations) are apparently divided into separate subpopulations (i.e., colonies) of varying population sizes (Williams et al. 1995). We collected a limited number of individuals from all subpopulations in an effort to maximize complete sampling of the population substructure while minimizing the impact on populations of these endangered kangaroo rats (Table 1). Sizes of populations and subpopulations were censused at the time of sample collection for genetic analyses. These were rough estimates based on burrow and cache counts, as well as capture-mark/recapture methods (Williams 1992). We always used subpopulations as the

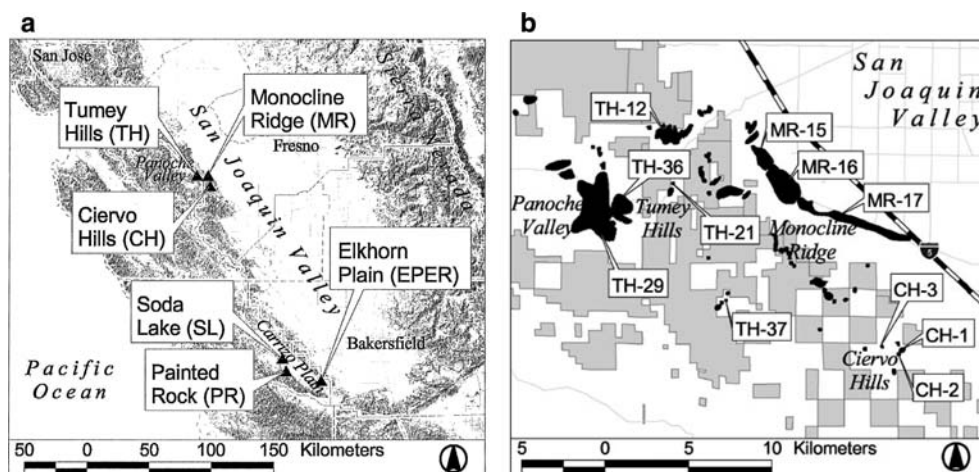


Figure 1. (a) Locations of giant kangaroo rat subpopulations sampled for this project. (b) Approximate geographic extent of subpopulations of the northern subpopulations of giant kangaroo rats (CH2 was excluded from our analyses due to small sample size). Sampling transects (not shown) were used to estimate population size in larger subpopulations. Smaller subpopulations were directly enumerated by counting all burrow systems and assuming one animal per burrow system. Southern populations are much less structured and are not depicted.

Table 1. Identity of populations (sample size per population), estimated census size per population, identity of subpopulations (sample size per subpopulation), and estimated census sizes per subpopulation (*n/a*= not available)

Populations-North	Census size	Sub-population	Census size
Ciervo Hills (CH, <i>n</i> = 14)	80	CH1 (n = 6)	60
		CH3 (n = 8)	20
Tumey Hills (TH, <i>n</i> = 22)	6815	TH12 (n = 5)	1115
		TH21 (n = 6)	79
		TH29 (n = 6)	5486
		TH36 (n = 3)	~135
		TH37 (n = 2)	(TH36&37)
Monocline Ridge (MR, <i>n</i> = 13)	5480	MR15 (n = 3)	<i>n/a</i>
		MR16 (n = 7)	<i>n/a</i>
		MR17 (n = 3)	<i>n/a</i>
Populations-South			
Elkhorn Plain (EPER, <i>n</i> = 15)	20,000	EPER1 (n = 9)	<i>n/a</i>
		EPER2 (n = 6)	<i>n/a</i>
Painted Rock (PR, <i>n</i> = 18)	500	PR (n = 18)	500
Soda Lake, translocated (SL, <i>n</i> = 13)	1300	SL (n = 13)	1300

smallest population unit in our analyses, although for simplicity's sake, we refer to EPER1 and EPER2 as populations when discussing the less structured southern populations. To obtain genetic samples, animals were captured in baited Sherman live traps that had been placed along transects throughout *D. ingens* habitat. We collected tail hairs and ear wedges from each animal, which was subsequently released at the capture location (US Fish and Wildlife Services Permit #WILLDF 1–9 and MOU with California Department of Fish and Game).

#### Microsatellite DNA analysis

DNA was extracted from 2 to 4 mm<sup>2</sup> ear wedges and purified according to standard protocols (Thomas et al. 1990; Good et al. 1997). Quantity and quality of high-molecular weight DNA were assessed by electrophoresis on 0.8% agarose gels in combination with molecular weight standards. Reliability of estimates of genetic summary statistics (e.g., heterozygosity, genetic distance and  $F_{ST}$ ) is more dependent on the number of loci than on the number of sampled individuals (Slatkin and Barton 1989; Baverstock and Moritz 1996). In light of our limited sample sizes for subpopulations, we tried to maximize the number of loci to reconstruct population substructure of *D. ingens*. We analyzed eight microsatellite loci using primers

developed for either *D. ingens* or *D. spectabilis* (Fleischer and Loew 1996; Davis et al. 2000). DNA was amplified in a reaction mix of 1.5 mM MgCl<sub>2</sub>, 0.5 units of Taq Gold DNA polymerase, 200 μM of each dNTP, and 0.5 μM of each primer. Polymerase chain reaction (PCR) conditions were as follows: 94 °C for 10 min, 30 cycles of 94 °C denaturing for 50 s, annealing at 56 °C for 80 s and extension at 72 °C for 30 s. We used a model 377 DNA Sequencer to resolve the PCR products and GENESCAN and GENOTYPER (Applied Biosystems Inc.) software to size the fragments and infer genotypes.

#### Statistical analyses

We estimated numbers of homozygotes and heterozygotes with null alleles using the EM algorithm of Dempster (1977), as applied in the program GENEPOP version 3.2a (Raymond and Rousset 1995). We used the program Genetic Data Analysis (GDA, Weir 1996; Lewis and Zaykin 1999) to estimate descriptive statistics for each population, such as the proportion of polymorphic loci, and observed and expected heterozygosity. We calculated allele frequencies per locus for each population using GENEPOP version 3.2a (Raymond and Rousset 1995), and assessed differences in allele frequency distributions between populations for each locus individually and across all loci

with chi-square contingency analyses. We also analyzed departures from Hardy–Weinberg equilibrium and linkage disequilibrium with GENEPOP.

We evaluated population substructure and heterozygote deficits with FSTAT version 2.9.3 (updated from Goudet 1995). Specifically, the degree of population subdivision ( $F_{ST}$ ), random allelic association within populations ( $F_{IS}$ ) and the global deficit of heterozygotes ( $F_{IT}$ ) were estimated using Weir and Cockerham's (1984)  $F$ -statistic estimators. Given the large variation in our sample sizes across populations, Weir and Cockerham's estimators are more appropriate for our analysis than Nei's  $F$ -statistic, because they weight allele frequencies according to sample sizes (FSTAT version 2.9.3, updated from Goudet 1995). In addition, we used FSTAT to calculate pairwise  $F_{ST}$  values for the northern and southern populations, without assuming that the populations were in Hardy–Weinberg equilibrium. We also estimated gene diversity averaged across (sub)populations for the northern and southern group (i.e., region) using FSTAT. All significance tests in FSTAT are randomization tests, and we used the suggested, 1000 permutations for fewer than 10 loci;  $P$ -values were obtained after Bonferroni correction for multiple comparisons (Goudet 1995). We examined recent interpopulation dispersal based on assignment tests (i.e., Doh assignment test calculator; Paetkau et al. 1995, 1997; Brzustowski 2002). Assignment tests use individual genotypes and population level allele frequencies to identify potential migrants and to assign them to source populations (Paetkau et al. 1995, 1997; Davies et al. 1999; Brzustowski 2002). The population-level assignment matrix  $A_{x,y}$ , estimates the likelihood of genotypes belonging to the sample population  $x$  compared to another population  $y$  (Brzustowski 2002). The assignment matrix of distances between each pair of populations is calculated as  $d_{x,y} = (A_{x,y} + A_{y,x})/2$ . The distance matrix for the northern subpopulations was expressed as a neighbor-joining tree constructed in PHYLIP NEIGHBOR (Felsenstein 1989).

Using the GDA software package, we also estimated  $F$ -statistics using a 3-level hierarchy for the subdivided populations in the north (Weir 1996; Lewis and Zaykin 1999). As  $F$ -statistics derived from microsatellite data might underestimate the degree of population differentiation

because of homoplasy (Slatkin 1995), we calculated  $Rho_{(ST)}$  according to the technique of Michalakis and Excoffier (1996) using GENEPOP.  $Rho_{(ST)}$  is a measure of correlation in allele size among populations suitable for genetic markers, such as microsatellite loci, that undergo a step-wise mutation model (Slatkin 1995; Rousset 1996). Given the relatively recent fragmentation of the giant kangaroo rat populations,  $F_{ST}$  is probably the better estimator in this analysis (Slatkin 1995), and was presented here. As  $Rho_{(ST)}$  may be more sensitive to isolation by distance in the more distant past, we performed a pairwise  $t$ -test to compare  $F_{ST}$  and  $Rho_{(ST)}$  values, and to maximize our potential to detect genetic population structure. Goldstein et al.'s (1995) genetic distance measure for microsatellite loci,  $(\Delta\mu)^2$  was calculated using the program RSTCALC (Goodman 1997). We tested isolation by distance based on the matrices of  $(\Delta\mu)^2$  and linear distances in miles using Mantel's test as performed in GENEPOP.

We examined the relationship between log-transformed census size and observed heterozygosity, and between sample size and various measures of diversity (e.g., expected heterozygosity,  $F_{IS}$ ) using a Pearson Correlation Coefficient (Sokal and Rohlf 1995; SAS 2001). To assess average relatedness of individuals in subdivided populations, we calculated Queller and Goodnight's (1989) identity-by-descent estimator of  $r$ , and for comparison, Pamilo's (1984, 1985) relatedness index – both estimates were generated using FSTAT. The Queller and Goodnight method estimates average relatedness as  $r = 2F_{ST}/(1 + F_{IT})$  (Wright 1921; Hamilton 1971), using a group-specific correction factor. Pamilo's relatedness index is corrected for potential inbreeding and removes the increase in relatedness due to population substructure (Goudet 1995).

## Results

In this pilot study we determined genetic diversity for 95 animals from 14 subpopulations (Table 1) using eight polymorphic loci (Ds1, Ds3, Ds19, Ds28, Ds30, Ds 46, Di5, Di12E) characterized by Davis et al. (2000). We excluded loci Ds1 and Di12E from analysis of northern populations and loci Ds3 and Ds28 from analysis of the southern

populations due to evidence of null alleles associated with these loci in the north and south, respectively (Dempster et al. 1977; Raymond and Rousset 1995). In addition, we only analyzed females for locus Ds19 because of x-linkage. None of the comparisons between loci showed any significant linkage disequilibrium across populations. The direct comparison of genetic diversity between northern and southern populations was based on only those four loci (i.e., Ds19, Ds30, Ds 46, Di5) suitable for both geographic regions.

#### *Polymorphism and allele frequency distributions*

Loci Ds1, Ds3, Ds28, Ds30, Ds 46 and Di12E were polymorphic for each subpopulation, whereas CH3 was monomorphic for locus Di5 and Ds19 and subpopulation MR17 was fixed for Di5. The latter is not surprising, as Di5 only shows two alleles in *D. ingens* (Davis et al. 2000). The maximum number of alleles detected per polymorphic locus was 14 for the southern (i.e., locus Ds1 and Ds30) and 15 for the northern (i.e., locus Ds30) populations. The mean number of alleles per locus ( $A$ ) ranged from 3 to 5 in the northern populations and from 3 to 7 in southern populations (Table 2, 3, Appendix 1). However, this measure of allelic diversity is correlated with the sample size of our (sub) population (Pearson product-moment coefficient  $r=0.85$ ,  $P=0.0001$ ) and therefore needs to be interpreted with caution.

#### *Hardy Weinberg equilibrium and heterozygosity*

While single-locus estimates of  $F_{IS}$  varied greatly, the multilocus, within-population  $F$ -statistics clearly showed that the observed genotypic proportions were not in Hardy–Weinberg equilibrium (Table 4). This multilocus  $F_{IS}$  statistic was not affected by sample size (Pearson product-moment coefficient  $r = -0.17$ ,  $P=0.56$ ). Many (sub-) populations showed heterozygote deficits; only subpopulations CH1, MR15, MR17 and TH37 in the north, and EPER2 and the translocated population at Soda Lake in the south showed random mating. Small sample sizes for MR15, MR17 and TH37 might have resulted in a Type II error, which would have obscured heterozygote deficiencies in those subpopulations. Neither observed ( $H_o$ ) nor expected heterozygosity ( $H_e$ ) were correlated with sample sizes in our study (Pearson product-moment coefficient  $r=0.01$ ,  $P=0.96$  and

$r=-0.06$ ,  $P=0.84$ , respectively). Expected heterozygosity, which is less sensitive to sampling than  $H_o$ , was generally high and ranged from 0.41 in CH3 to 0.78 in TH36 for northern subpopulation, and from 0.63 in EPER2 to 0.75 in EPER2 for southern populations (Table 3). The average gene diversity for the northern ( $H_s=0.69$ ) and southern group of populations ( $H_s=0.72$ ) did not differ (FSTAT permutation test,  $P=0.75$ ) when based on only those four microsatellite loci (i.e., Ds19, Ds 30, Ds 46, Di5) suitable for analysis of both, the north and south. Similarly, expected heterozygosity ( $H_e$ ) based on six (albeit different) microsatellite loci (Table 3) did not differ between northern subpopulations and southern populations ( $D=0.25$ ,  $P=0.99$ , KS test). Observed heterozygosity ( $H_o$ ) was not significantly correlated with estimates of census size (Pearson product-moment coefficient  $r=0.51$ ,  $P=0.13$ ).

Average relatedness of individuals within subpopulations in the north (Queller and Goodnight 1989) was significantly elevated above the baseline probability of identity-by-state due to the population frequency of alleles (mean  $r=0.135$ ,  $SE=0.028$ , 95%  $CI=0.077-0.173$ ). Within-group  $r$ -values for southern populations were also elevated, but to a lesser degree (mean  $r=0.036$ ,  $SE=0.02$ , 95%  $CI=0.014-0.058$ ). In comparison, Pamilo's estimates of inbreeding corrected relatedness were  $r_c = -0.403$  for the north and  $r_c = -0.375$  for the south.

#### *Population differentiation and genetic distances*

Consistent with the deviation from HW frequencies within populations,  $F_{IS}$  values for single loci were significantly positive for all but two loci (KR3, KR5A) in the northern subpopulations, and two out of six loci in the southern populations (Table 5). The multilocus  $F_{IS}$  was significantly higher than zero at the 1% level for both regions, indicating a major deficit in heterozygotes. The multilocus  $F_{ST}$  values deviated significantly from zero for both, the northern and southern region, despite the fact that only locus KR30 was significantly positive for the single-locus analysis of the southern populations (Table 5). There was no significant difference between  $F_{ST}$  and  $Rho_{ST}$  values based on a paired  $t$ -test ( $P>0.05$ ).

A Mantel test indicated no clear relationship between the matrices of genetic distances (delta

Table 2. Observed allele frequencies by locus and population

Locus: Ds1															
Population	N	169	171	173	175	176	177	193	197	199	201	205	209	213	215
Painted Rock	18	0.000	0.083	0.194	0.083	0.000	0.000	0.000	0.083	0.167	0.028	0.056	0.250	0.028	0.028
Soda Lake	13	0.077	0.269	0.000	0.154	0.077	0.077	0.038	0.15	0.077	0.000	0.000	0.15	0.000	0.000
EPER1	9	0.000	0.167	0.278	0.111	0.000	0.056	0.000	0.000	0.222	0.000	0.000	0.167	0.000	0.000
EPER2	6	0.000	0.333	0.000	0.250	0.000	0.083	0.000	0.000	0.167	0.000	0.000	0.167	0.000	0.000
Locus: Ds19															
Population	N	103	105	107	109	111	113	117	119	125	130	138			
Painted Rock	18	0.111	0.056	0.056	0.056	0.167	0.167	0.222	0.056	0.000	0.111	0.000			
Soda Lake	13	0.167	0.000	0.000	0.000	0.167	0.333	0.000	0.000	0.333	0.000	0.000			
EPER1	9	0.000	0.333	0.000	0.000	0.000	0.167	0.333	0.000	0.000	0.000	0.167			
EPER2	6	0.000	0.000	0.500	0.500	0.000	0.000	0.000	0.000	0.000	0.000	0.000			
Locus: Ds30															
Population	N	220	229	231	237	239	241	243	245	247	249	251	253	255	267
Painted Rock	18	0.000	0.000	0.000	0.056	0.000	0.000	0.194	0.389	0.139	0.056	0.111	0.000	0.056	0.000
Soda Lake	13	0.042	0.125	0.042	0.083	0.000	0.167	0.250	0.167	0.000	0.042	0.000	0.000	0.000	0.083
EPER1	9	0.000	0.000	0.056	0.000	0.222	0.000	0.056	0.333	0.056	0.111	0.111	0.056	0.000	0.000
EPER2	6	0.083	0.000	0.000	0.000	0.000	0.000	0.417	0.333	0.000	0.000	0.000	0.083	0.083	0.000
Locus: Ds46															
Population	N	144	146	148	150	152	154	156	158	160	162	166			
Painted Rock	18	0.028	0.083	0.056	0.139	0.222	0.028	0.139	0.194	0.028	0.056	0.028			
Soda Lake	13	0.042	0.000	0.042	0.167	0.417	0.042	0.042	0.208	0.000	0.042	0.000			
EPER1	9	0.000	0.111	0.056	0.444	0.111	0.222	0.056	0.000	0.000	0.000	0.000			
EPER2	6	0.000	0.167	0.083	0.500	0.250	0.000	0.000	0.000	0.000	0.000	0.000			
Locus: Di5A															
Population	N	179	182												
Painted Rock	18	0.194	0.806												
Soda Lake	13	0.192	0.808												
EPER1	9	0.333	0.667												
EPER2	6	0.083	0.917												
Locus: Di12E															
Population	N	195	205	209	222	225									
Painted Rock	18	0.111	0.528	0.167	0.194	0.000									
Soda Lake	13	0.038	0.500	0.231	0.192	0.038									
EPER	9	0.000	0.222	0.333	0.444	0.000									
EPER2	6	0.000	0.417	0.083	0.500	0.000									

Data are shown for the southern populations only as the data for the northern subpopulations was too complex for depiction. Loci are named according to the serially numbered clone; allele designation is the size of amplification product in bp, N is the number of individuals sampled per population.

$\mu$ )<sup>2</sup> and linear distances between northern ( $P=0.31$ ) or southern (sub)populations ( $P=0.61$ ). Varying evolutionary histories (i.e., time since isolation,  $N_e$ ) for the different (sub)populations could result in such a lack of isolation-by-distance, however, dispersal that is greatly affected by habitat fragmentation and patchy distribution of dispersal corridors is a more likely explanation, especially for the northern populations. For example, pairwise  $F_{ST}$  analysis for northern populations showed significant differentiation be-

tween CH and the populations in Tumey Hills (TH) and MR; the latter two populations, however, were not significantly isolated from each other (Table 6). The assignment test revealed recent dispersal from neighboring Tumey Hill into MR ( $P<0.001$ ) and vice versa ( $P=0.001$ ), and it indicates that Ciervo Hill received recent immigrants from MonoclineRidge ( $P=0.00$ ), but not Tumey Hill ( $P>0.05$ ) (Figure 2). More specifically, the assignment matrix of northern subpopulations in CH, MR and TH (Figure 3) suggests

Table 3. Descriptive statistics for each northern and southern population across loci, including the mean sample size (n), the proportion of polymorphic loci (P), the mean number of alleles per locus (A), the mean number of alleles per polymorphic locus (Ap), the expected heterozygosity (He) under Hardy–Weinberg equilibrium, the observed heterozygosity (Ho) and the number of private alleles

Northern populations	n	P	A	Ap	He	Ho (SE)	Private allele #
CH1	5.33	1.00	3.67	3.67	0.71	0.66 (0.097)	0
CH3	7.17	0.67	3.00	4.00	0.41	0.31 (0.12)	1
MR15	2.80	1.00	3.20	3.20	0.74	0.67 (0.14)	1
MR16	6.33	1.00	5.33	5.33	0.74	0.64 (0.07)	3
MR17	2.83	0.83	2.83	3.20	0.63	0.66 (0.17)	0
TH12	7.50	1.00	4.83	4.83	0.74	0.45 (0.03)	1
TH21	6.17	1.00	4.67	4.67	0.77	0.63 (0.07)	2
TH29	5.33	1.00	4.50	4.50	0.73	0.60 (0.08)	1
TH36	3.33	1.00	3.83	3.83	0.78	0.58 (0.16)	1
TH37	1.83	1.00	2.33	2.33	0.72	0.50 (0.18)	1
Southern populations							
Soda Lake	11.00	1.00	6.17	6.17	0.74	0.73 (0.08)	8
Eper1	8.00	1.00	4.83	4.83	0.75	0.54 (0.11)	2
Eper2	5.33	1.00	3.50	3.50	0.63	0.67 (0.10)	0
Painted Rock	16.50	1.00	7.17	7.17	0.74	0.56 (0.14)	8

that CH3 and TH21 are genetically distant from all other populations, that CH1 and TH36 are moderately connected and that gene flow among the remaining subpopulations (MR15, MR16, MR17, TH12, TH29, TH 37) seems relatively frequent.

Neighboring populations in the south are located within 4 miles of each other along the Carrizo Plain with no obvious geographic barriers to interpopulation dispersal, nevertheless isolation-by-distance is not expected as the Soda Lake population was founded by animals translocated

from other southern populations. Consistent with a lack of barriers to gene flow, pairwise comparisons of southern populations did not reveal any  $F_{ST}$  values that deviated from zero (Table 6). The assignment tests suggests that most recently animals dispersed from EPER ( $P=0.021$ ) and Soda Lake ( $P=0.01$ ) into the smaller population at Painted Rock (Figure 2).

Although there is clear evidence of ongoing gene flow, especially between neighboring, spatially connected populations, genetic drift affects genetic diversity and populations structure of giant

Table 4. Fis per population and locus in the northern and southern (sub)populations of giant kangaroo rats

Population	Ds3	Ds19	Ds28	Ds30	Ds46	Di5	All
Ch1	-0.364	0.600	-0.064	0.216	0.077	0.063	0.103
CH3	-0.191	NA	0.646**	-0.292	0.523*	NA	0.247*
MR15	0.273	NA	-0.143	0.667	-0.333	-0.333	0.111
MR16	0.130	0.182	0.104	0.167	0.200	-0.091	0.143*
MR17	-0.500	-0.333	-0.333	-0.091	0.600	NA	-0.091
TH12	0.364	0.520**	0.486*	0.512*	0.324	0.192	0.416**
TH21	0.000	0.273	0.143	0.143	0.216	0.478	0.199*
TH29	0.000	0.059	0.314	0.407	0.130	0.333	0.194*
TH36	-0.091	1.000	0.273	-0.143	0.667	-0.200	0.317*
TH37	1.000	NA	0.000	0.000	1.000	0.000	0.500
Population	Ds1	Ds19	Ds30	Ds46	Di5	Di12E	All
Painted Rock	0.043	0.526	0.234*	0.314**	0.141	0.165	0.257**
Soda Lake	0.226*	-0.200	0.060	-0.073	0.294	-0.132	0.009
Eper1	0.089	0.667	0.226	-0.018	0.543	0.360	0.308**
Eper2	0.000	-1.000	-0.111	-0.190	0.000	0.487	-0.111

\* $P < 0.05$ , \*\* $P < 0.01$ ; probability that heterozygote frequencies do not differ from those predicted under Hardy–Weinberg equilibrium.

Table 5. Estimates of F-statistics across northern and southern populations of giant kangaroo rats

Locus	$F_{IS}$	$F_{ST}$	$F_{IT}$
<i>All the northern populations</i>			
Ds3	0.04	0.06	0.10
Ds19	0.34**	0.13*	0.42**
Ds28	0.24**	0.12**	0.33**
Ds30	0.17**	0.11**	0.26**
Ds46	0.33**	0.03	0.35**
Di5	0.14	0.03	0.17
All loci	0.22**	0.09**	0.29**
<i>All southern populations</i>			
Ds1	0.10	0.01	0.11**
Ds19	0.36**	0.01	0.36**
Ds30	0.14*	0.03**	0.17**
Ds46	0.10	0.04	0.14*
Di5	0.29	-0.02	0.28*
Di12E	0.16	0.03	0.18*
All loci	0.18**	0.02**	0.20**

\* $P < 0.05$ ,  $P < 0.01$ ; probability that estimate is not different from zero.

kangaroo rats. A 3-level hierarchical analysis revealed that reduced heterozygosity of the northern region was apparently due to inbreeding ( $f = 0.21$ ,  $P < 0.05$ ) and genetic drift within subpopulations (Theta-S = 0.08,  $P < 0.05$ ), while the organization into populations added no additional loss of diversity (Theta-P = 0.01,  $P > 0.05$ ). Consistent with the high  $F_{IS}$  and  $F_{ST}$  values within the northern and southern regions, the overall heterozygote deficit ( $F_{IT}$ ) within each of these regions was significantly different from zero (Table 5).

## Discussion

Alteration of natural communities has resulted in local extinctions and restriction of the overall

Table 6. Pairwise values of  $F_{ST}$  for northern and southern populations of giant kangaroo rats

North		South	
CH × MR	0.07**	PR × EPER1	0.006
CH × TH	0.05**	PR × EPER2	0.04
MR × TH	0.004	SL × PR	-0.0001
		SL × EPER1	0.05
		SL × EPER2	0.08
		EPER × EPER2	0.05

\* $P < 0.05$ , \*\* $P < 0.01$ ; probability that estimate is not different from zero.

distribution of giant kangaroo rats. Remnant populations are fragmented and frequently limited to suboptimal habitat on the western edge of the Tulare Basin and its adjacent foothills (Grinnell 1932; Williams 1992; Williams et al. 1993, 1995; Goldingay et al. 1997). We were interested to learn how such a metapopulation structure affected patterns of genetic variation among giant kangaroo rats. Specifically, our pilot study investigated whether *D. ingens* shows a greater amount of subdivisions and diversity associated with the complex topography of the northern part of the range, and whether the current fragmentation of *D. ingens* populations resulted in genetic substructuring and loss of neutral genetic diversity. Furthermore, we interpreted effective dispersal in the context of topography and we inferred aspects of social structure. We discuss our findings in the light of Good et al.'s (1997) results on the large-scale spatial heterogeneity of those giant kangaroo rat populations, and in the context of genetic management of remnant populations.

Predictions about the expected partitioning of neutral genetic variation within and between populations can be ambiguous as they depend on a combination of factors, such as probability of local extinctions and recolonizations, and the amount and direction of gene flow (Lacy 1987; Gilpin 1991; Hastings and Harrison 1994; Harrison and Hastings 1996). In general, population fluctuations and turnover within a metapopulation accelerate genetic drift, decrease genetic diversity within populations and increase differentiation between populations (McCauley 1991; Harrison and Hastings 1996). However, the extent of population differentiation will vary with the number of migrants between populations (Wade and McCauley 1988), which in turn will be affected by geographic distance, topography and ecological conditions.

Populations and subpopulations of giant kangaroo rats typically vary greatly in size, time, and space, and many of them were below 500 individuals at the time of sampling for this study or during previous bottlenecks, others had gone extinct entirely (Williams 1992; Williams et al. 1995; Good et al. 1997). We documented partitioning of neutral genetic variation for giant kangaroo rats that reflects this complex history of fragmentation, size fluctuations and gene flow among the remnant populations. Consistent with increasing habitat fragmentation, genetic subdivi-

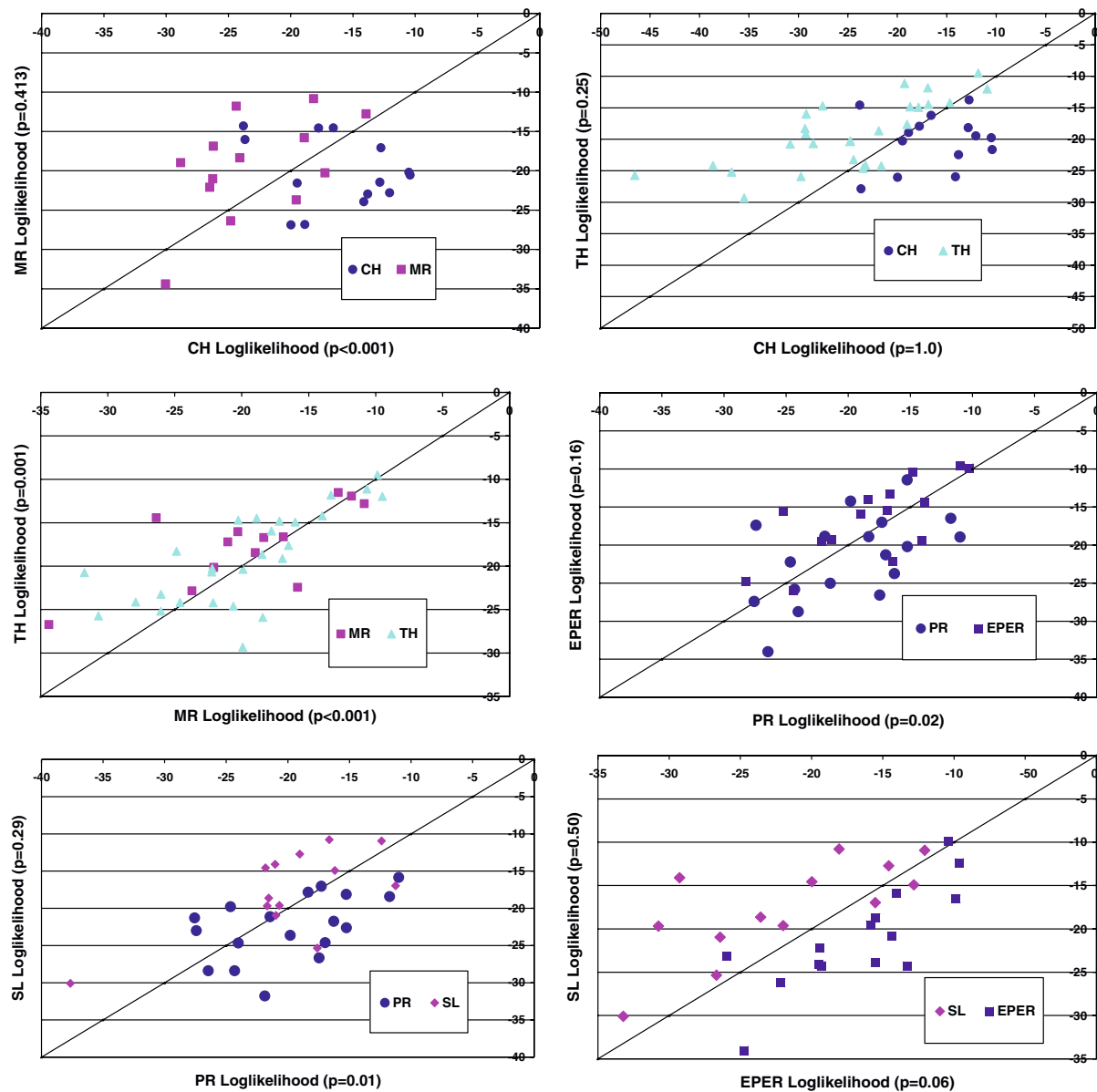


Figure 2. Assignment of individuals to populations by log-likelihood profiles.  $P$ -values associated with each population (e.g., CH) indicate the probability of cross-assignment of animals from that population to the other population (e.g., MR) in each pairwise comparison. For example, a significant proportion of CH animals were assigned to MR ( $P < 0.001$ ), hence there is a high probability that there have been recent migrants from MR into CH populations.

sion was highly significant across the northern and southern part of the range of giant kangaroo rats. Hierarchical  $F$ -statistics of northern populations in Fresno and San Benito counties confirmed a significant decrease in observed heterozygosity that suggested non-random mating and genetic drift within subpopulations, in addition to the sampling drift associated with the higher level population

structure. Similarly, multilocus  $F$ -statistics indicate that the southern part of the giant kangaroo rat range does not contain randomly mating populations, instead those populations also experience significant amounts of genetic drift and inbreeding.

Genetic drift causes random fixation and loss of alleles within population and increases the

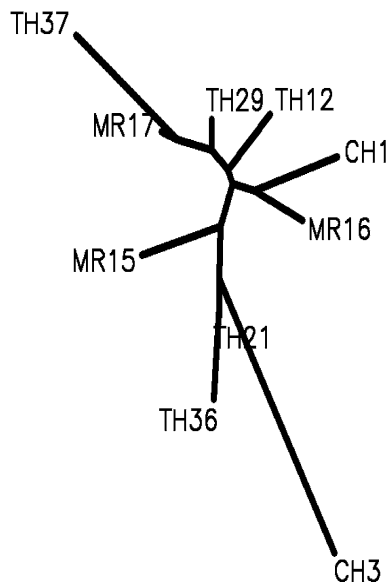


Figure 3. Neighbor-joining tree for northern subpopulations based on assignment distance matrix.

differentiation between populations. However, gene flow between populations can counteract drift and preserve overall genetic diversity within a metapopulation (Lacy 1987; Harrison and Hastings 1996). The fact that gene diversity estimates for the northern and southern range were relatively high compared to other endangered species (Frankham et al. 2002, p. 80) suggests current or relatively recent connectivity (or both) among the remnant kangaroo rat (sub)populations. Indeed, pairwise  $F_{ST}$  analysis showed that the northern populations at MR and TH were not completely isolated from each other. In contrast, the small subpopulations at CH differed greatly from each other and were genetically divided from the other northern subpopulations.

Similarly, pairwise  $F_{ST}$  values of southern populations (i.e., EPER, Soda Lake and Painted Rock) reveal that either historical or ongoing gene flow between southern locations has negated significant isolation of populations. Good et al. (1997) concluded that the southern populations effectively act as one large population, but that they may have experienced significant fluctuations in size. Such population size fluctuations are consistent with our finding that southern populations deviate from Hardy–Weinberg expectations, possibly due to random genetic drift. Heterozygote frequencies at the translocated Soda Lake popu-

lation showed no deviation from Hardy–Weinberg frequencies, which is consistent with its rapid population growth after its establishment with founders derived from EPER and Painted Rock, and the apparent continued gene flow between southern populations. These results suggest that translocation is a promising management tool to maintain viable, genetically diverse population of giant kangaroo rats.

#### *Fine-scale population structure, effective dispersal and topography*

Good et al. (1997) determined the population structure of a subset of our samples of *D. ingens* using mtDNA analysis. Based on the frequency and distribution of tip (i.e., terminal) haplotypes, they determined that the Ciervo Hills' population was probably founded by one or several recent migrations from the northwestern PV, and probably isolated from other populations by distance and geographic barriers to gene flow. Our results are consistent with these ideas. Specifically, CH3 showed the lowest proportion of polymorphic loci, low expected levels of heterozygosity, a significant deficit of heterozygotes and significant genetic isolation, whereas CH1 showed greater genetic diversity and greater connectivity with northern subpopulations.

The topography across the range of *D. ingens* is complex, and remnant populations are frequently separated by unsuitable habitat that may limit dispersal. Consequently, linear geographic distances are less likely to predict effective dispersal than ecological conditions and physical characteristics of the environment, such as topography. For example, the proximity (i.e., approximately 13 miles) of subpopulation TH12 with subpopulations MR15 and MR16 was associated with low values of genetic distance ( $\Delta \mu^2$  values were 3.73 and 1.85 respectively), which would be consistent with recent historical or current gene flow. Indeed closer inspection of the topography reveals a possible northern dispersal corridor between those subpopulations along Panoche Creek. In contrast, the linear geographic distance between TH21 and MR also was approximately 13 miles, but the associated ( $\Delta \mu^2$ ) values were high (34.12 for MR15 and 19.57 for MR16). In this case, gene flow was probably limited by the physical barrier posed by mountain ridges as high

as 1100 feet between TH21 and MR. These results were consistent with Good et al. (1997), who concluded that the topographical complexity of the north results in high levels of genetic diversity and substructure.

In summary, we detected that the populations in the north and south were highly structured although there was considerable gene flow between all southern populations. The northern Ciervo Hills' population was subdivided and genetically separated from other populations in the north. Evidence suggests that subpopulations in CH and TH have been connected to PV via long-distance migrants or stepping stone populations. These conclusions support previous findings by Good et al. (1997), except for their specific prediction that genetic diversity of northern populations might be higher than those in the south due to the higher topographical complexity in the north; we did not detect such differences in gene diversity based on microsatellite variation.

#### *Inference about social structure*

Deviation from Hardy–Weinberg genotype proportions can be attributed to several different factors – either sampling or demographic. For example, undetected null alleles could confound interpretation of  $F_{IS}$  values because they will inflate the proportion of homozygotes within populations (Beaumont and Bruford 1999). However, it is unlikely for several reasons that null alleles played a major role in causing significant heterozygote deficiencies in our giant kangaroo rat populations. First, we tested for and excluded loci that showed clear evidence of null alleles, and secondly allele frequencies in the translocated, growing population of Soda Lake did not deviate from Hardy–Weinberg proportions, and showed no indication of null alleles. Definitive detection of all null alleles may require pedigree data for our focal species (Stoneking et al. 1981; Murphy et al. 1996); however, such data are difficult to attain for this endangered species.

Demographic factors, such as population subdivision and social structure are the more likely causes for the significant  $F_{IS}$  and  $F_{ST}$  values of giant kangaroo rat populations. Inbreeding, or identity-by-descent will result in heterozygote deficit relative to expected Hardy–Weinberg frequencies, and provide positive  $F_{IS}$  values. Simi-

larly, genetic drift in small populations also will decrease observed heterozygote frequencies and compound any potential effects of mating among relatives. In contrast, a high degree of polygyny and male-biased dispersal will cause heterozygote excess and result in measurements of negative  $F_{IS}$  values (Prout 1981; Chesser 1991a, b; Stortz et al. 2001).

Giant kangaroo rats are probably polygynous although we do not know to what extent, and they apparently show a limited sex-bias in natal dispersal, which is typical for kangaroo rats (Jones 1988, 1989; Jones et al. 1988; Waser and Elliott 1991; Price et al. 1994; Williams et al. 1999; Cooper and Randall 2001; Metcalf et al. 2001; Randall et al. 2002). The fact that we obtained no significant negative  $F_{IS}$  values for any population is consistent with a low degree of polygyny and insignificant sex bias in natal dispersal. Any excess of heterozygotes that may have occurred due to dispersal and polygyny is likely to have been counteracted by the prominent effects of genetic drift and possible mating among related individuals across precincts. Mild inbreeding in our populations is supported by the significant average relatedness of individuals within the northern and the southern populations. However, we consider it unlikely that giant kangaroo rats systematically inbreed because such consistent non-random mating should result in significant linkage disequilibrium across loci (Hartl and Clark 1997), which we did not document.

#### *Management conclusions and recommendations for future studies*

Our pilot study shows that giant kangaroo rats have a typical metapopulation structure with varying degrees of differentiation across subpopulations. Gene diversity within populations was relatively high and even small subpopulations make significant contributions to the overall genetic diversity of giant kangaroo rats. Managers should particularly focus on enhancing connectivity and populations size of those subpopulations we identified as genetically distinct and depauperate, and those that are geographically isolated (Lacy 1987; Crozier 1997). For example, the small population size, relatively large heterozygosity and genetic isolation of TH21 make it an important contributor to genetic diversity that is potentially

at risk of extinction. However, its geographic proximity to TH12 and the local topography suggests that habitat along Silver Creek could be managed to provide a suitable dispersal corridor between the two populations, hence reduce genetic isolation of TH21. The kangaroo rat population at Soda Lake was newly founded through translocation in 1989 and subsequently showed significant population growth. In our study we documented that Soda Lake animals harbored the highest level of observed heterozygosity and that there was no evidence of inbreeding. Therefore we consider translocation of animals a viable option to increase genetic diversity and improve population demographics, especially for populations that are geographically very isolated and at risk of extinction. For example, remnant populations at CH are genetically divergent and isolated from northern populations and CH3 in particular shows low genetic diversity. Translocation from and into the populations at CH should be a preferred management strategy to preserve their genetic contributions as their geographic isolation renders dispersal corridors impractical.

Our study clearly emphasizes the importance of understanding the impact of dispersal patterns and population dynamics on population genetic structure in the context of topography and habitat fragmentation. Giant kangaroo rats like other *Dipodomys* species apparently colonize novel or previously occupied habitat through occasional long distance dispersers or in a stepping stone fashion through several shorter dispersal events (Metcalf et al. 2001). The extent of genetic diversity and probability of survival of founder populations will depend on continued interpopulation dispersal, the diversity of source populations that contribute

to the new colony and the extent of population growth after colonization (Harrison and Hastings 1996). As discussed above, topography and habitat distribution will significantly affect dispersal and likely source populations. The translocated Soda Lake population illustrates that genetically diverse founders and subsequent gene flow with neighboring populations can result in rapid population growth and maintenance of high genetic diversity, whereas the Ciervo Hill populations illustrate the loss of diversity if founder populations stay small and geographically isolated.

Further studies on the population genetics, social structure, and natal and breeding dispersal behavior of giant kangaroo rats should ensure sufficient sample sizes and complete sampling of the complex subpopulation and social structure of kangaroo rats. Such studies will be essential to design more comprehensive management plans that maximize long-term survival of this endangered species.

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## Appendix 1

Appendix 1. Observed allele frequencies by locus and population in north

Population	N	1	2	3	4	5	6	7	8	9	10	11	12	13	14
<i>Locus: KR3</i>															
CH1	6	0.000	0.000	0.250	0.417	0.250	0.083	0.000	0.000						
CH3	8	0.000	0.000	0.000	0.750	0.188	0.062	0.000	0.000						
MR15	3	0.000	0.000	0.333	0.167	0.333	0.167	0.000	0.000						
MR16	7	0.071	0.000	0.357	0.286	0.143	0.071	0.000	0.071						
MR17	3	0.000	0.000	0.333	0.167	0.000	0.500	0.000	0.000						
TH12	8	0.000	0.000	0.188	0.062	0.438	0.125	0.188	0.000						



Appendix 1. (Continued)

Population	N	1	2	3	4	5	6	7	8	9	10	11	12	13	14
MR16	7	0.143	0.857												
MR17	3	0.000	1.000												
TH12	8	0.312	0.688												
TH21	7	0.429	0.571												
TH29	6	0.333	0.667												
TH36	4	0.250	0.750												
TH37	2	0.250	0.750												

Loci are named according to the serially numbered clone; allele designations per locus are sequential, N is the number of individuals sampled per (sub)population.

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