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Genetic variation and population structure in desert bighorn sheep: implications for conservation

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Abstract

Bighorn sheep populations experienced a drastic reduction in both distribution and abundance until the advent of modern wildlife management, where improving viability of extant populations and translocating animals into historical habitat range have been the most important management policies. The fact that subspecies relationships among bighorn are ambiguous, together with the importance of selecting appropriate source stock and the expense of translocation projects, makes an understanding of subspecies relationships and genetic variation, within and between populations, important for the management and conservation of this species. In this study, genetic variation in 279 bighorn sheep from 13 study sites in Arizona, California, New Mexico and Alberta, Canada were examined by analyzing ten microsatellite loci to determine interpopulation differentiation and relationships between closely related taxa. All populations contained a substantial amount of genetic variation. Genetic differences between populations were large and roughly proportional to geographic distance. The significance of this to desert subspecies relationships and management is discussed.

Introduction

With the westward spread of Europeans in North America, desert bighorn sheep (*Ovis canadensis*) populations, which occupied desert mountain ranges in California, Arizona, New Mexico, and Nevada in the United States and Sonora and Baja California in Mexico were greatly reduced in both distribution and abundance (Buechner 1960). Although overhunting, habitat loss and modification, inbreeding depression, competition for food and water, and predation have all been suggested as factors that could have contributed to the population decrease, disease transmission from livestock is considered the single most important factor reducing bighorn sheep numbers. There are many examples of bighorn sheep die-offs and overall declines after introduction of domestic sheep, goats, and cattle and their associated diseases (Brown 1993).

Bighorn sheep populations continued to decline until the advent of modern wildlife conservation and management which focused on improving the viability of existing populations, (e.g., isolating domestic animals from bighorn sheep and developing water holes) and reintroducing (translocating) bighorn sheep into their previous range (Brown 1993). These efforts have been generally successful, but the expense of such projects and the significance of selecting appropriate source stock, makes an understanding of the genetic variation within and between bighorn sheep populations and the subspecies relationships between them critical.

Table 1. Estimated number of desert bighorn sheep in 1978 (Monson 1990) and 1998 (Lee 1998) in the United States and Mexico

Location	1978	1998
United States		
Arizona	2,100–2,600	6,500
California	3,250–3,750	3,500
Nevada	3,700–4,200	5,500
New Mexico	350–390	200
Texas (Transplants)	50	300
Utah	350–500	2,500
Colorado	0	500
Total	9,800–11,490	19,000
Mexico		
Baja California Norte and Sur	4,560–7,800	3,500
Sonora	900	2,500
Chihuahua and Coahuila	100	0
Total	5,560–8,800	6,000
United States and Mexico	15,360–20,290	25,000

Concern for desert bighorn sheep has attracted national attention in both the United States and Mexico and national wildlife refuges have been established for them in Nevada, New Mexico and Arizona. These actions were deemed essential if bighorn sheep are to be saved from extinction (Brown 1993). The decline in desert bighorn sheep in some areas appears to have stabilized over the last 20 years (Table 1). However, the bighorn sheep that inhabit the Peninsular Ranges in southern California have declined in recent years from about 1,200 in 1971 and 600 in 1991 to only 280 in 1998 (Rubin et al. 1998). As a result, in March, 1998, bighorn in the Peninsular Ranges were added to the federal endangered species list (the only large mammal that has been listed in southern California). Also, desert bighorn sheep in New Mexico continue to be listed as a state-endangered species.

The traditional subspecies designations of bighorn sheep were based on comparisons of a limited number of skull measurements (Cowan 1940). Cowan proposed the existence of six subspecies; Rocky Mountain (*O. c. canadensis*), California (*O. c. californiana*) and four desert subspecies – *O. c. nelsoni*, *O. c. mexicana*, *O. c. cremnobates*, and *O. c. weemsi*. However, the importance of these designations to the management and conservation of bighorn sheep has led to several re-evaluations of Cowan's conclusions, and some authors have challenged the validity of these subspecies designations. For example, Wehausen and Ramey (1993), Ramey (1995) and Jesup and Ramey (1995) examined skull morphology, mitochondrial DNA (mtDNA), and allozyme variation, respectively, and found low genetic variation within, and no significant differentiation between, the four desert subspecies. They suggested that these four subspecies should be recognized as a single polytypic subspecies (*O. c. nelsoni*). Further, Boyce et al. (1997) examined variation at three microsatellite and five major histocompatibility complex loci in populations of bighorn sheep in California and New Mexico and found a complex set of relationships between populations of *O. c. nelsoni* and other putative subspecies of desert bighorn sheep.

Although traditional conservation policies have been oriented toward protecting species and their natural habitat, in recent years attention has also focused on applying genetic techniques that can give new insight into conservation problems (e.g. Hedrick and Miller 1992; Smith and Wayne 1996). Genetic data can address two issues pertinent to bighorn sheep management. First, a genetic survey can directly measure the amount of genetic variation in sheep populations. There has been concern that population isolation might lead to inbreeding and population decline (DeForge et al. 1979). For example, Bleich et al. (1995) showed that modern bighorn sheep populations have lost historic connections to adjacent populations, Ramey (1995) showed bighorn sheep populations have low levels of mtDNA diversity, Sausman (1984) showed inbreeding increased lamb mortality in captivity, and Berger (1990) found that small populations of bighorn sheep have had a high extinction rate. Quantifying the amount of genetic variation in populations will help evaluate how likely these factors are, and permit genetic information to be used in sheep management.

Second, recognizing historic patterns of genetic variation among desert bighorn sheep populations is required to preserve evolutionary relationships during translocation programs. Translocation of sheep has been a valuable part of sheep recovery effort and should not disrupt natural patterns of genetic differentiation. Combining genetically different populations of bighorn sheep could alter adaptations to local environments and subsequently lower the fitness of populations.

Of the many genetic markers now available, microsatellite loci are best suited for answering these questions (e.g. Ashley and Dow 1994; Jarne and Lagoda 1996) because of their high variability, high

Table 2. Location, putative subspecies, number of individuals sampled (*N*), average number of alleles (*n*), and gene diversity ($\hat{H}$) with 95% confidence interval in parentheses for the 13 study sites included in this study

Location	Putative subspecies	*N*	*n*	$\hat{H}$ (95% interval)
Northern Arizona				
Mt. Davis	*O. c. nelsoni*	15	3.3	0.54 (0.49, 0.59)
Lost Cabin	*O. c. nelsoni*	16	3.4	0.55 (0.51, 0.58)
Mt. Nutt	*O. c. nelsoni*	28	2.9	0.44 (0.39, 0.49)
Southern Arizona				
Kofa Mountains	*O. c. mexicana*	9	3.7	0.60 (0.55, 0.64)
Stewart Mountain	*O. c. mexicana*	14	3.1	0.54 (0.50, 0.58)
Castle Dome Mountains	*O. c. mexicana*	20	3.9	0.58 (0.55, 0.62)
Southern California				
Old Dad Mountains	*O. c. nelsoni*	23	3.1	0.45 (0.41, 0.50)
Eagle Mountains	*O. c. nelsoni*	23	4.1	0.63 (0.60, 0.66)
San Gorgonio	*O. c. nelsoni*	22	3.4	0.46 (0.41, 0.51)
San Ysidro	*O. c. cremnobates*	22	3.6	0.49 (0.45, 0.53)
New Mexico				
Red Rock Refuge	*O. c. mexicana*	25	2.4	0.36 (0.30, 0.42)
Rocky Mountains				
Wheeler Peak, N.M.	*O. c. canadensis*	7	3.2	0.55 (0.51, 0.58)
Sheep River, Alberta	*O. c. canadensis*	55	4.4	0.59 (0.56, 0.63)

mutation rate, large number, distribution throughout the genome, codominant inheritance, and neutrality with respect to selection. In this paper, we will quantify the amount of genetic variation in desert bighorn sheep populations to determine both intrapopulation genetic variation and interpopulation differentiation and relationships between closely related taxa, information that is essential for the management and conservation of this species. In particular, we will examine the relationship of genetic distance and both subspecific status and geographic distance between populations. This is achieved by characterizing ten microsatellite loci in 279 individuals within and among populations throughout the range of desert bighorn sheep, including populations from Arizona, California, and New Mexico. For comparative purposes, we also included two populations of Rocky Mountain bighorn sheep.

Materials and methods

We studied 279 bighorn sheep from 13 different populations and the location, putative subspecies, and sample sizes are shown in Table 2. Arizona Game and Fish Department (AGFD) provided 98 blood samples from sheep captured in Arizona from the Kofa Mountains, Castle Dome Mountains, Stewart Mountain, Mt. Davis, Lost Cabin, and Mt. Nutt. In addition, AGFD provided four liver or spleen samples from the Kofa Mountains collected by hunters. Our study also includes 122 DNA samples from Boyce et al. (1997) from sheep at Eagle, Old Dad, San Gorgonio, and San Ysidro, California, and Red Rock and Wheeler Peak, New Mexico. Two of the microsatellite loci used in Boyce et al., D5S2 and OarFCB11, were also analyzed in this survey. Lastly, S. Forbes (Forbes et al. 1995) provided data and DNA from 55 Rocky Mountain bighorn sheep from Sheep River, Alberta, Canada which we used for comparative purposes.

Figure 1 shows the location of the nine study sites in California and Arizona. These populations are composed of native sheep except for the Stewart Mountain, Wheeler Peak, and Red Rock Refuge populations which were transplanted from the Kofa Mountains, AZ, Banff National Park, Alberta, Canada, and the San Andres Mountains, NM, respectively.

DNA isolation and characterization of microsatellites

DNA was isolated from Arizona blood samples using two different methods of DNA extraction: standard proteinase K digestion, followed by phenol/chloroform extraction and ethanol precipitation

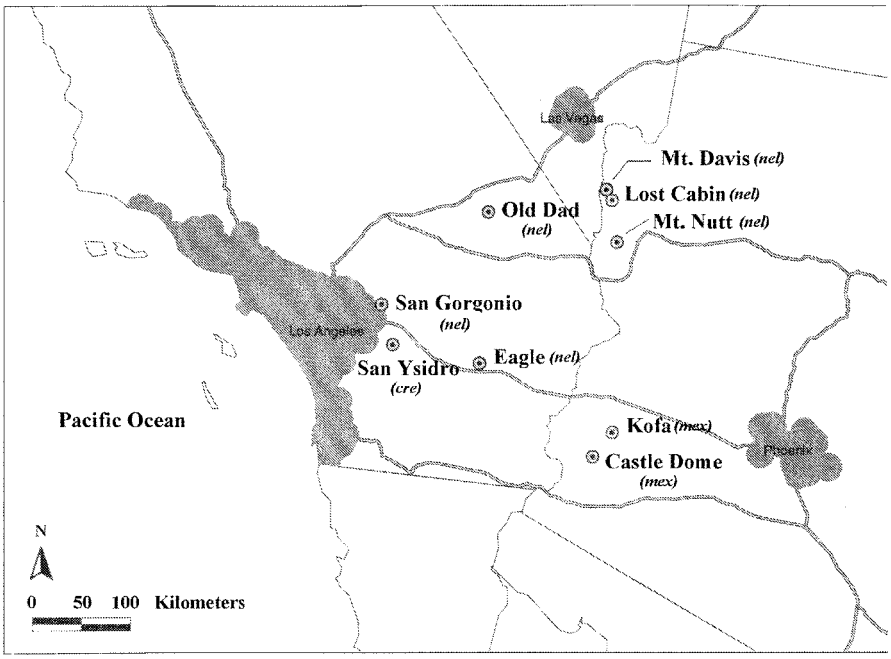

Figure 1. Location of natural populations in Arizona and California The putative subspecies are indicated as: *O. c. nelsoni* (nel), *O. c. mexicana* (mex), and *O. c. cremnobates* (cre). Not shown are the locations of Stewart Mountain, AZ (a transplant population from Kofa Mountains, AZ), Red Rock, NM (a captive population from San Andres Mountains just north of Las Cruces, NM), Wheeler Peak, NM (a transplant population from Banff National Park, Alberta, Canada), and Sheep River, Alberta, Canada.

(Sambrook et al. 1989) and MasterPure Genomic DNA Purification Kit (Epicentre Technologies). Alternatively, whole blood was centrifuged at 8000 rpm for 10 min. and separated in three phases and the buffy coat (white cells) was used for DNA extraction using the QIAmp tissue Kit (Qiagen). This same kit was used to purify genomic DNA from the tissue samples.

All 279 sheep were genotyped with nine dinucleotide microsatellite loci (OarFCB11, OarFCB128, OarFCB266, OarFCB304, MAF33, MAF36, MAF48, MAF65 and MAF209) isolated from domestic sheep (*Ovis aries*) (Buchanan et al. 1993; Crawford et al. 1994) and one dinucleotide locus (DS52) isolated from cattle (*Bos taurus*) (Steffen et al. 1993). These loci were selected because of their high polymorphism and high number of alleles previously detected in sheep and cattle.

Primer pairs were initially tested for amplification using a Perkin Elmer 9600 Thermocycler. Polymerase chain reaction (PCR) reactions (10 μl) contained 50 ng of purified genomic DNA, 50 mM KCl, 10 mM Tris-HCl pH 8.3, 1.2 or 3 mM $MgCl_2$, 0.2 mM each dNTP, 10 pmol of each unlabeled primer, and 1 U Taq DNA polymerase. All amplifications included an initial denaturation step of 3 min at 94 °C; followed by 30 cycles of 30 s at 94 °C, 30 s at the appropriate annealing temperature (Buchanan et al. 1993; Steffen et al. 1993) and 22 s at 72 °C. Final extension was for 5 min at 72 °C. PCR products were electrophoresed in 2% agarose gels and visualized after staining with ethidium bromide (1.5 μg/ml) against a standard marker (100bp). To genotype individuals, 1 μCi of α^{32}P dATP was directly incorporated in a new 5-μl reaction volume, under identical conditions. Amplification products were mixed with 4 μl of sequencing loading buffer (95% formamide, 20 mM EDTA, 0.05% bromophenol blue and 0.05% xylene cyanol), heated to 85 °C for 3 min and then put in ice for 2 min. Three microliters of this mix were then run on 6% denaturing polyacrylamide gels. Allele sizes were determined by comparison against the pBSMB-sequencing control from the Amplicycle sequencing kit (Perkin Elmer). The amplification products were electrophoresed for approximately 2.5 h and the gels were fixed by soaking in 5% methanol / 5% acetic acid for 8 min. The gels were dried under vacuum at 94 °C and exposed to X-ray film overnight at room temperature.

Data analysis

Because microsatellite loci are codominant, traditional population genetic methods can be used for calculating allelic and genotype frequencies and standard population genetic models can be applied. We began our data analysis by testing whether the genotypic frequencies at each study site were consistent with Hardy-Weinberg proportions for the ten genetic markers in our analysis using GENEPOP 3.0 (Raymond and Rousset 1995). We tested each locus in each sample, each locus across samples, and each sample across loci and used Bonferroni adjustment for multiple comparisons as criteria for statistical significance (Rice 1989). GENEPOP uses a randomization test to calculate the probability for each locus in each sample and these probabilities can be combined to provide test for one population across loci or one locus across populations. Next, we calculated three sets of summary statistics. First, we calculated an unbiased estimate of the gene diversity (mean expected heterozygosity), $\hat{H}$, at each study site (e.g. Nei 1987). This statistic is a measure of the amount of genetic variation present at each location and is independent of sample size. Confidence intervals for estimates of gene diversity were obtained using the *t* distribution (Nei 1987). We also calculated the mean numbers of alleles per population for the ten loci but realize that this measure is highly sample-size dependent. Second, we measured differentiation between the groups in two ways, using both the standard genetic distance of Nei (1977) and F_{ST} values (Nei 1987). Randomization was used to test the statistical significance of the genetic distance and F_{ST} values.

Genetic distances between study sites were summarized with two methods. First, we used PHYLIP (Felsenstein 1993) to construct a UPGMA (unweighted pair-group method using arithmetic averages) phylogenetic tree of the 13 sampling sites. Bootstrapping over loci (1000 replicates) using the DISPAN software package (Ota 1993) tested the reliability of the nodes in the tree. Second, we compared the genetic distance between each pair of study sites with the geographic distance measured in kilometers. Geographic distances were obtained from the geographic information system program ARCVIEW 3.0 (ESRI 1998). For the three study sites of transplanted sheep (Stewart Mountain, Wheeler Peak, and Red Rock), we used the original location of their sheep to calculate geographic distances. We used a Mantel test (Mantel 1967; Sokal and Rohlf 1995) to test for correlation between genetic and geographic distances.

Results

Amount of genetic variation

The allelic frequencies for all sample and locus combinations are given in the Appendix. Of the 130 locus-population combinations, 127 (98%) were polymorphic and the only monomorphic exceptions were MAF33 at Red Rock and OarFCB128 at Mt. Nutt and Mt. Davis. D5S2 had the highest observed gene diversity for all the loci ($\hat{H}$ = 0.732) and the highest average number of alleles per population (4.38), while OarFCB128 had the lowest gene diversity ($\hat{H}$ = 0.184) and the lowest average number of alleles per population (2.00). MAF65 had the most alleles overall with the 10 alleles representing every dinucleotide number between 115 and 133 present in at least one population, while OarFCB11 had just three alleles overall.

All populations had substantial levels of genetic variation as shown by the average number of alleles and gene diversity (Table 2). The average number of alleles per locus ranged from 2.4 in Red Rock to 4.4 in Alberta, with a mean of 3.4 alleles per locus overall. The average gene diversity was 0.51 for the 11 desert study sites, 0.57 for the two Rocky Mountain sites, and 0.52 overall. It ranged from 0.36 in Red Rock to 0.63 in Eagle. Some of the alleles were unique to a single population (MAF65-121 in Castle Dome, OarFCB128-112 in San Ysidro, MAF65-133 and MAF209-111 in Old Dad and OarFCB128-118, MAF48-134 and MAF65-119 in Alberta). None of the loci or study sites differed significantly from the Hardy–Weinberg proportions.

The confidence intervals for the gene diversity at each location indicate that the lowest heterozygosities were significantly lower than the highest; however, all 13 populations had a substantial amount of genetic variation, and none of them can be considered genetically impoverished. The Red Rock sample had the lowest estimated heterozygosity while Eagle, Kofa and several other samples had high and similar levels of genetic variation.

Genetic differences between populations and regions

The genetic distance between each pair of sampling locations ranged from a minimum of 0.020 between

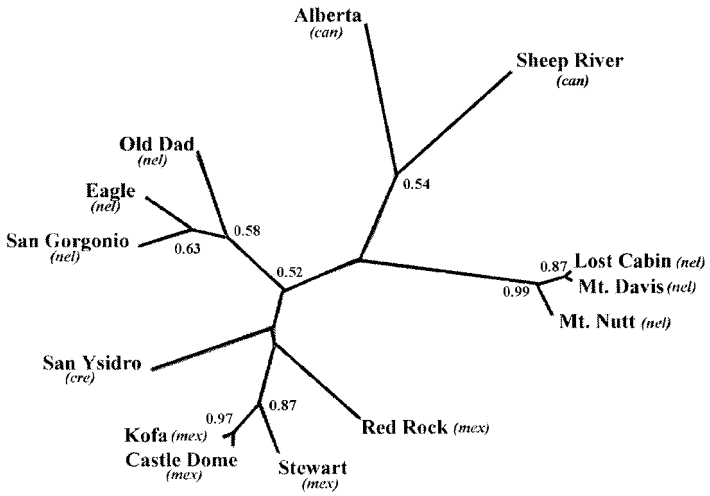

Figure 2. UPGMA phylogenetic tree based on Nei (1978) genetic distance. Numbers indicate the proportion of bootstrap replicates sharing the labeled node. The putative subspecies are indicated as: *O. c. nelsoni* (nel), *O. c. mexicana* (mex), *O. c. cremnobates* (cre), and *O. c. canadensis* (can).

Mt. Davis and Lost Cabin to a maximum of 0.870 between San Ysidro and Alberta (Table 3). All the genetic distances were highly statistically significant ($p < 0.001$), except for the two smallest genetic distances ($D_{\text{Davis,Cabin}} = 0.02$, $D_{\text{Kofa,Castle}} = 0.04$).

We used the pairwise D values to build the phylogenetic tree in Figure 2, which provides one method of summarizing the genetic relationships between study sites. The values shown at the nodes of the tree estimate the probability of obtaining the indicated clusters of populations if the study were repeated with ten randomly chosen loci (only values greater than 0.50 are given). As can be seen, only two population clusters received strong support from the data: the three study sites in the Black Mountains of North Arizona (Lost Cabin, Mt. Davis, and Mt. Nutt) and the three study sites with sheep in or from Southern Arizona (Kofa, Castle Dome, and Stewart). These two clusters are composed of neighboring locations (see Figure 1), and both of these well-supported clusters are at the tips of the phylogenetic tree. Although the phylogenetic tree clustered together the two Rocky Mountain bighorn sheep populations (Alberta and Wheeler) and is also consistent with a metapopulation structure for populations in the Mojave Desert (San Gorgonio, Eagle and Old Dad) (Boyce et al. 1997), the major structure of this tree is not strongly supported.

We also made comparisons among regional grouping of populations using D values. When we compared distances between northern Arizona samples, D was 0.094, while between southern Arizona samples, D was 0.162. When northern and southern Arizona samples were compared (boldface in Table 2), the average pairwise D value was 0.644. The two highest D values for the Arizona samples were obtained when southern Arizona was compared to Alberta ($D = 0.668$) and northern Arizona was compared to San Ysidro ($D = 0.786$).

Figure 3 plots both the genetic and geographic distances for all the populations. A Mantel test found this relationship to be significantly different from random ($\hat{p} < 0.001$). Beyond 300 km, although the geographic distances cluster either around 700 km (California–New Mexico comparisons) and 1700 km (California–Canada comparisons) the genetic distance appears to asymptote (plateau), with values ranging between 0.25 and 0.75 for study sites more distantly separated than 300 km apart.

Also in Figure 3, comparisons within putative subspecies (solid circles, $N = 22$) and between subspecies (open circles, $N = 56$) are presented. If currently recognized subspecies definitions had a biological basis, we would expect a higher rate of genetic differentiation with distance when comparing locations across subspecies lines than within subspecies. Focusing on the pairs of populations between 50 and 300 km apart, this figure shows no relationship between genetic distance and currently recognized subspecies. The relationship is roughly linear and very similar as shown by the slopes of linear regression for distances up to ∼300 km for both comparisons within and between putative subspecies. The slopes for the

Table 3. The genetic distance between each pair of study sites with comparisons between northern and southern Arizona in bold face. Samples 1-3, 7–9 are *O. c. nelsoni*, 4–6, 11 are *O. c. mexicana*, 9 is *O. c. cremnobates*, and 12 and 13 are *O. c. canadensis*

	2	3	4	5	6	7	8	9	10	11	12	13
Mt. Davis (1)	0.020	0.108	**0.533**	**0.559**	**0.523**	0.575	0.660	0.600	0.429	0.632	0.500	0.535
Lost Cabin (2)		0.069	**0.449**	**0.523**	**0.560**	0.497	0.729	0.634	0.433	0.558	0.430	0.502
Mt. Nutt (3)			**0.667**	**0.660**	**0.649**	0.629	0.748	0.797	0.505	0.631	0.580	0.562
Kofa Mtns. (4)				0.035	0.154	0.522	0.471	0.270	0.201	0.254	0.476	0.594
Castle Dome (5)					0.104	0.563	0.531	0.342	0.267	0.303	0.531	0.679
Stewart Mt. (6)						0.439	0.528	0.275	0.275	0.256	0.503	0.687
Old Dad (7)							0.265	0.376	0.174	0.441	0.372	0.721
S. Gorgonio (8)								0.351	0.135	0.576	0.542	0.741
SanYsidro (9)									0.210	0.361	0.730	0.870
Eagle Mtns. (10)										0.300	0.444	0.525
Red Rock (11)											0.638	0.549
Wheeler Pk. (12)												0.368
Sheep River (13)												

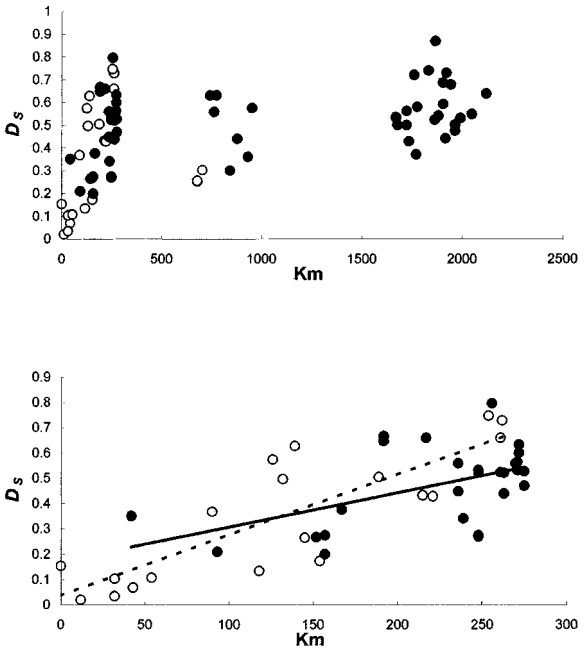

Figure 3. Pairwise genetic distances (D_S) plotted against geographic distance (km) for all comparisons. Comparisons within and between populations from putative subspecies are indicated by open and filled symbols, respectively. The linear regression slopes are given for the populations < 300 km apart for within putative subspecies comparisons (broken line) and between subspecies comparisons (solid line) in the lower graph.

within and between putative subspecies comparisons were not statistically significantly different and explained 70% and 27% of the variance, respectively, for these comparisons. If there were a difference, then a higher slope for the between subspecies comparisons might be expected. For the group further apart (around 700 km), the two within subspecies comparisons, Red Rock-Kofa and Red Rock-Castle Dome, do have slightly lower genetic distances than the seven between subspecies comparisons.

We calculated F_{ST} values for the ten loci for different regional grouping of populations. Genetic differentiation among the three northern Arizona populations and among the three southern Arizona populations was low (F_{ST} = 0.043 and 0.038, respectively), while it was higher among the six Arizona populations (F_{ST} = 0.204). The F_{ST} for all desert populations was 0.267 and when all 13 populations were combined, the F_{ST} was 0.264.

Discussion

All the bighorn sheep populations studied had significant amounts of genetic variation based on their gene diversity and average number of alleles per locus. To illustrate this we can make a comparison with Rocky Mountain bighorn sheep or domestic sheep using the data of Forbes et al. (1995). For eight of the microsatellite loci we examined, they found an observed heterozygosity of 0.57 for Rocky Mountain

bighorn sheep and 0.66 for domestic sheep. For our 10 population samples of desert bighorn of Arizona and California for the same eight loci, the observed heterozygosity was 0.50. In other words, even though the genetic variation in desert bighorn sheep is somewhat lower than either Rocky Mountain bighorn sheep or domestic sheep, the populations are not depauperate in genetic variation as has been suggested. The Red Rock population had the lowest average number of alleles and gene diversity, while Eagle, Kofa and several other samples had high and similar values. The Red Rock population is a large captive herd (100–200) that was derived primarily from animals in the San Andres Mountains, NM, while the Eagle population is in the Mojave desert where there may be several different metapopulations (Boyce et al. 1997). The Sheep River population was already known to have high gene diversity and a high average number of alleles per locus (Forbes et al. 1995).

Our data has interesting similarities and differences to comparable data in Rocky Mountain bighorn sheep. Eight of the ten loci included in this study were previously analyzed by Forbes and Hogg (1999) in populations of Rocky Mountain sheep. A comparison of the two data sets reveals that the rate of genetic differentiation as a function of geographic distance is much steeper among desert sheep than Rocky Mountain sheep. This could be explained by larger population sizes or higher rates of gene flow for Rocky Mountain sheep, or by similarities between populations in the Rocky Mountains remaining from post-Pleistocene colonization. If desert populations have historically been smaller than Rocky Mountain populations, we might expect to also find less genetic variation in the desert populations than in the Rocky Mountains. This expectation was not convincingly met. The gene diversity in Rocky Mountain sheep ranged from 0.43 to 0.60 with an average of 0.55 (Forbes et al. 1995) compared to an only slightly lower average gene diversity in the 10 desert Arizona and California locations in this current study of 0.50 at the eight loci in common.

Our data show that F_{ST} values were quite different for comparisons within and across regions. The low F_{ST} values within northern Arizona and within southern Arizona indicate that they are genetically similar within these groups. Furthermore, microsatellite allele frequencies were very similar among adjacent Arizona populations separated by short distances (e.g. Castle Dome and Kofa), indicating that there are not extrinsic barriers to gene flow between them. The F_{ST} for all Arizona populations was greater than within either northern or southern Arizona populations, indicating that northern Arizona populations are genetically different from southern Arizona populations and that there was substantial subdivision of genetic variability among these populations. These high F_{ST} values between northern and southern Arizona populations were largely due to alleles present in one or a few populations and absent in others. The most common allele in northern Arizona was not always the most common allele in southern Arizona. This suggests that neutral forces such as genetic drift have caused substantial differentiation between northern and southern Arizona populations.

We found a positive correlation between genetic and geographic distance (Figure 3). Genetic distances were relatively low for nearest-neighbor comparisons (i.e. Mt. Davis-Lost Cabin), and values tended to increase with increasing geographic distance up to ~300 km. Beyond this geographic distance, D values remained in the range of 0.25 and 0.75. Perhaps constraints on allele size caused genetic distance measures to plateau, with the level of the plateau being determined by the degree of constraint, the mutation rate, and population size (Feldman et al. 1997; Nauta and Weissing 1996).

Conservation genetics of desert bighorn sheep

The relatively high gene diversity in desert bighorn sheep populations show that these populations have been large and/or well connected to each other during recent evolutionary history. These gene diversities, however, may not reflect disturbances associated with human development during the past few centuries. Current population sizes and dispersal rates may or may not be adequate to retain existing genetic variation for an extended period. Retention of genetic variation within populations is maximized by high gene flow rates to and from other populations, and is minimized by low gene flow rates. Gene flow rates as low as one migrant per generation are generally effective in preventing loss of genetic variation caused by fragmentation but will result in some differentiation among groups. Schwartz et al. (1986) have used this reasoning to argue that excessive loss of genetic variation is unlikely for large metapopulations of sheep. If there is continued exchange between populations in a metapopulation, then this appears reasonable. The recommendation (Bleich et al. 1995) that corridors between sheep populations in a metapopulation be

protected for sheep movement is consistent with this strategy.

Assigning biological significance to the genetic differences between populations found in this study is difficult. Populations with similar frequencies at microsatellite loci may still have adaptively important differences maintained by natural selection (Hedrick 1999). In addition, populations with differing frequencies at microsatellite loci may share adaptively important traits. Differentiation at microsatellite loci should reflect the potential for adaptive differences among populations. Therefore, very similar populations such as Mt. Davis and Lost Cabin have had virtually no opportunity for independent evolution. In fact, these two locations appear to constitute a single population or could be considered sub-units of a metapopulation. The same is true for the Kofa and Castle Dome locations. This indicates that the three populations in northern Arizona and the three populations in southern Arizona form discrete groups (metapopulations) with relatively high gene flow within them. In contrast, the large genetic differences between the three Northern Arizona locations (Mt. Davis, Lost Cabin, Mt. Nutt) and the three Southern Arizona locations (Kofa, Stewart, Castle Dome) imply a relatively long separation (or low gene flow) between these regions with opportunity for independent evolution and adaptation to local environments.

From a conservation genetics perspective (Hedrick and Miller 1992), populations should be managed so that enough genetic variability is retained to provide for future adaptation and successful expansion of native and reintroduced free-ranging populations. Because we cannot directly evaluate the biological significance of genetic differences between locations and because genetic differences are roughly proportional to geographic distances, the most conservative method of selecting stock for translocations would be to choose the closest available population to preserve local variation and/or potential adaptations.

Subspecies designations

Our data do not support current subspecies boundaries and cannot easily be used to determine the relationships among putative subspecies of desert bighorn sheep. Although fairly strong genetic differentiation exists in desert bighorn populations, genetic differences appear to be associated with geographic distance rather than any subspecific boundary. If existing subspecies boundaries have biological meaning, we would expect to find increased genetic differences when comparing populations across subspecies boundaries. Because we find no evidence for this in our data, we conclude that there is little support for the current subspecies designations, e.g. all *O. c. nelsoni* populations did not cluster together. Rather, our analysis appears to generally support the view of Ramey (1995) that desert bighorn sheep are a polytypic subspecies.

However, we emphatically acknowledge that this study has not provided a strong test of the existing subspecies designations. For example, we have included only one location (San Ysidro) from the Peninsular Ranges and no samples of *O. c. weemsi*. The genetic distance between San Ysidro and San Gorgonio to the north was fairly high (0.35), considering the two locations are separated by only 42 km, but apparently within the range expected for that geographic distance. Examining the putative subspecies boundary between the Peninsular Ranges and the adjacent ranges in the Mojave Desert would require more sampling locations in order to detect a potential transition zone. Similarly, examining the putative subspecies boundary between Nelson and Mexican bighorn sheep in Arizona would require study sites closer to the potential boundary. Future research would also benefit from additional loci in order to increase the statistical significance of clusters in the phylogenetic tree. However, the inability of this analysis to establish desert bighorn sheep subspecies relationships may reflect the inappropriateness of the subspecies concept to this species as much as limitations in our data.

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Appendix

Allelic frequencies for the 10 microsatellite loci in the 13 populations of bighorn sheep. Sample size (given for *D5S2*) is the same for all loci

		D5S2								
	N	*203*	*205*	*207*	*209*	*211*	*213*	*215*	*219*	*221*
Mt. Davis	15	0.133	—	0.067	0.100	0.500	0.167	—	—	0.033
Lost Cabin	16	0 156	—	0.094	0.063	0.406	0.250	—	—	0.031
Mt. Nutt	28	0.250	—	0.018	—	0.732	—	—	—	—
Kofa Mtns.	9	0.125	—	—	0.063	0.313	0.438	0.063	—	—
Stewart Mtn.	14	0.179	—	—	0.036	0.571	—	—	0.214	—
Castle Dome	20	0.250	—	—	0.050	0.350	0.175	0.175	—	—
Old Dad	23	0.152	0.500	—	0.152	—	0.196	—	—	—
Eagle Mtns.	23	0.159	0.114	—	0.364	0.136	0.068	—	0.159	—
S. Gorgonio	22	—	—	—	0.636	0.045	—	—	0.318	—
San Ysidro	22	—	—	—	—-	0.429	0.286	0.214	0.071	—
Red Rock	25	0.340	0.100	—	0.080	0.480	—	—	—	—
Alberta	7	0.396	0.021	0.010	0.490	—	0.083	—	—	—
Wheeler Pk.	55	0.714	—	—	0.286	—	—	—	—	—

	AMF 65									
	115	*117*	*119*	*121*	*123*	*125*	*127*	*129*	*131*	*133*
Mt. Davis	0.233	—	—	—	—	—	0.500	—	0.267	—
Lost Cabin	0.125	—	—	—	—	0.031	0.469	—	0.375	—
Mt. Nutt	0.214	—	—	—	—	0.054	0.268	—	0.464	—
Kofa Mtns.	0.056	—	—	—	0.500	0.111	0.111	0.222	—	—
Stewart Mtn.	0.250	—	—	—	0.643	0.036	0.071	—	—	—
Castle Dome	0.225	—	—	0.025	0.500	0.175	0.075	—	—	—
Old Dad	0.500	0.087	—	—	0.196	—	0.174	—	—	0.043
Eagle Mtns.	0.455	—	—	—	—	—	0.159	0.364	0.023	—
S. Gorgonio	0.786	—	—	—	0.190	—	0.024	—	—	—
San Ysidro	0.159	0.568	—	—	0.136	—	0.023	0.114	—	—
Red Rock	0.479	—	—	—	0.521	—	—	—	—	—
Alberta	0.009	—	0.264	—	0.200	0.182	0.236	0.109	—	—
Wheeler Pk.	0.286	—	—	—	0.643	0.071	—	—	—	—

	MAF 48							
	120	*122*	*124*	*126*	*128*	*130*	*132*	*134*
Mt. Davis	—	—	0.600	0.100	0.300	—	—	—
Lost Cabin	—	—	0.656	—	0.344	—	—	—
Nutt	—	—	0.232	—	0.768	—	—	—
Kofa Mtns.	0.375	0.063	0.438	—	0.125	—	—	—
Stewart Mtn.	0.179	0.393	0.179	—	0.250	—	—	—
Castle Dome	0.450	0.375	0.075	—	0.100	—	—	—
Old Dad	—	0.435	0.087	0.174	0.304	—	—	—
Eagle Mtns.	—	0.457	0.152	0.087	0.304	—	—	—
S. Gorgonio	0.409	0.091	0.023	0.273	0.205	—	—	—
San Ysidro	0.091	0.386	0.295	0.227	—	—	—	—
Red Rock	—	0.340	—	0.520	0.140	—	—	—
Alberta	—	0.136	0.182	—	—	0.109	0.545	0.027
Wheeler Pk.	—	0.071	0.643	—	—	0.143	0.143	—

	MAF 209							
	109	*111*	*113*	*115*	*117*	*119*	*121*	*123*
Mt. Davis	—	—	0.600	—	0.400	—	—	—
Lost Cabin	—	—	0.625	—	0.281	—	0.031	0.063
Mt. Nutt	—	—	0.536	—	0.375	—	0.089	—
Kofa Mtns.	—	—	—	0.063	0.375	0.188	0.188	0.188
Stewart Mtn.	—	—	—	0.107	0.071	0.464	—	0.357
Castle Dome	0.100	—	—	—	0.375	0.175	0.075	0.275
Old Dad	0.283	0.065	—	—	—	—	0.652	—
Eagle Mtns.	—	—	—	0.087	0.283	0.130	0.500	—
S. Gorgonio	—	—	—	—	0.114	0.023	0.864	—
San Ysidro	—	—	0.295	—	0.114	0.045	0.545	—
Red Rock	—	—	0.580	—	—	0.280	0.140	—
Alberta	0.027	—	0.418	0.045	0.345	0.009	—	0.155
Wheeler Pk.	0.429	—	—	—	0.071	—	—	0.500

	MAF 36							
	93	*95*	*99*	*101*	*103*	*105*	*107*	*109*
Mt. Davis	0.167	—	—	0.100	0.100	0.300	—	0.333
Lost Cabin	0.250	—	—	—	0.063	0.156	—	0.531
Mt. Nutt	—	—	—	0.036	—	0.071	—	0.893
Kofa Mtns.	0.389	—	—	—	0.111	0.389	0.056	0.056
Stewart Mtn.	0.786	—	—	—	—	0.214	—	—
Castle Dome	0.350	—	—	—	0.125	0.300	0.175	0.050
Old Dad	0.773	—	—	—	—	0.227	—	—
Eagle Mtns.	0.348	0.065	—	0.087	0.109	0.261	0.043	0.087
S. Gorgonio	0.091	0.068	—	—	0.250	0.318	—	0.273
San Ysidro	0.727	—	—	0.091	0.068	—	0.068	0.045
Red Rock	0.960	—	—	—	—	—	—	0.040
Alberta	0.145	—	0.527	—	—	—	—	0.327
Wheeler Pk.	0.071	—	0.286	—	0.571	—	—	0.071

	FCB 266							
	87	*89*	*91*	*93*	*95*	*97*	*99*	*101*
Mt. Davis	—	—	—	—	0.800	—	0.133	0.067
Lost Cabin	—	—	—	—	0.500	—	0.313	0.188
Mt. Nutt	—	—	—	0.018	0.446	—	0.304	0.232
Kofa Mtns.	0.111	—	—	0.111	—	—	0.778	—
Stewart Mtn.	—	—	—	—	0.250	—	0.750	—
Castle Dome	0.050	—	—	0.100	0.125	—	0.725	—
Old Dad	—	—	—	—	0.196	—	0.804	—
Eagle Mtns.	0.091	—	—	0.136	0.159	—	0.614	—
S. Gorgonio	0.048	—	0.024	—	0.262	—	0.667	—
San Ysidro	—	—	—	—	—	0.227	0.773	—
Red Rock	0.220	0.200	—	—	—	—	0.580	—
Alberta	0.409	0.218	—	0.027	0.064	—	0.282	—
Wheeler Pk.	—	0.143	—	0.143	0.143	—	0.571	—

	FCB 304				*FCB 128*			
	136	*138*	*140*	*142*	*112*	*114*	*116*	*118*
Mt. Davis	0.467	0.200	0.333	—	—	—	1.000	—
Lost Cabin	0.625	0.063	0.313	—	—	0.156	0.844	—
Mt. Nutt	0.643	0.196	0.161	—	—	—	1.000	—
Kofa Mtns.	0.611	—	0.222	0.167	—	0.333	0.667	—
Stewart Mtn.	0.250	0.357	0.286	0.107	—	0.107	0.893	—
Castle Dome	0.625	—	0.250	0.125	—	0.275	0.725	—
Old Dad	0.065	—	0.717	0.217	—	0.043	0.957	—
Eagle Mtns.	0.348	—	0.522	0.130	—	0.174	0.826	—
S. Gorgonio	0.045	—	0.955	—	—	0.114	0.886	—
San Ysidro	0.159	—	0.727	0.114	0.091	0.023	0.886	—
Red Rock	0.920	—	0.080	—	—	0.020	0.980	—
Alberta	0.391	0.491	0.064	0.055	—	0.073	0.875	0.052
Wheeler Pk.	0.214	0.214	0.357	0.214	—	0.143	0.857	—

	MAF 33						*FCB 11*		
	121	*123*	*125*	*127*	*129*	*131*	*127*	*129*	*131*
Mt. Davis	0.600	0.067	—	—	0.033	0.300	0.233	0.433	0.333
Lost Cabin	0.781	0.063	—	—	—	0.156	0.406	0.500	0.094
Mt. Nutt	0.804	0.018	—	—	—	0.179	0.429	0.339	0.232
Kofa Mtns.	—	0.889	—	—	0.111	—	0.333	0.500	0.167
Stewart Mtn.	—	0.571	0.214	—	0.214	—	0.107	0.607	0.286
Castle Dome	—	0.675	0.175	—	0.150	—	0.050	0.825	0.125
Old Dad	0.717	0.065	0.217	—	—	—	0.826	0.130	0.043
Eagle Mtns.	0.227	0.591	0.136	0.045	—	—	0.457	0.217	0.326
S. Gorgonio	0.136	0.523	0.205	0.136	—	—	0.568	0.023	0.409
San Ysidro	0.091	0.818	0.091	—	—	—	0.045	0.227	0.727
Red Rock	—	1.000	—	—	—	—	0.760	0.240	—
Alberta	0.164	0.173	0.009	0.655	—	—	0.645	0.200	0.155
Wheeler Pk.	0.786	0.071	—	0.143	—	—	0.643	0.143	0.214

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