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Final Report

Genetic Variation In Desert Bighorn Sheep

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I. Overview of project and report

The major goal of this project was to evaluate genetic differences between bighorn sheep in northern and southern Arizona. To do this we compared genetic markers at three locations in northern Arizona with three locations in southern Arizona. The evolutionary significance of the difference that we found was evaluated by including four populations of desert bighorn sheep in California, one population of desert bighorn sheep in New Mexico, and two populations of Rocky Mountain bighorn sheep in our analysis. Although we could not conclusively resolve the evolutionary relationships of bighorn sheep in the Southwest, we can make recommendations for management of desert bighorn sheep in Arizona.

In the main body of this report we will summarize our findings and their implications for management of bighorn sheep in Arizona. Appendix 1 discusses our methods and results in detail. Appendixes 2 and 3 contain the data that we collected.

II. Summary of Findings

1. We found substantial amounts of neutral genetic variation at all study locations. These locations included native populations in the Black Mountains (Mt. Davis, Lost Cabin, Mt. Nutt), native populations in southern Arizona (Kofa, Castle Dome), and a population transplanted from the Kofa Mountains (Stewart).
2. We found substantial amounts of potentially immunologically important major histocompatibility (MHC) locus variation at all study locations in Arizona.
3. Adjacent locations within northern and southern Arizona had very similar gene frequencies, indicating that migration between locations is common. The three locations

in northern Arizona and two locations in southern Arizona can probably be best described as metapopulations.

4. We found substantial genetic differences between sheep in northern and southern Arizona.
5. Analysis of the rate of genetic differentiation with geographic distance suggests that the genetic differences between bighorn sheep in northern Arizona and southern Arizona are consistent with the distance between the two regions. In other words, we found no evidence for rapid genetic differentiation across a subspecies boundary.

III. Implications for Desert Bighorn Sheep Management in Arizona

1. The five populations of native sheep in Arizona that we analyzed (Mt. Davis, Lost Cabin, Mt. Nutt, Kofa, and Castle Dome) all have sufficient genetic variation for use as source stock for translocations.
2. Bighorn sheep transplanted from the Kofa mountains to Stewart Mountain have retained most of their genetic variation.
3. We recommend that bighorn sheep within the Black Mountains be managed as a metapopulation. We recommend that bighorn sheep within the Kofa-Castle Dome region be managed as a metapopulation.
4. We recommend that bighorn sheep from southern Arizona be considered genetically distinct from bighorn sheep in northern Arizona.
5. We recommend replacing the subspecies paradigm for bighorn sheep in Arizona with a differentiation by distance paradigm.

Publicity

This research has been described at three meetings: the 1998 Desert Bighorn Council, the 1998 California Population and Evolutionary Genetics meeting, and the 1998 Conservation Biology meeting. In addition, two out of four planned manuscripts have been completed and submitted to journals. These two manuscripts include a book chapter, "Population structure in desert bighorn sheep: implications for conservation in Arizona", and "Genetic variation in desert bighorn sheep" for the *Desert Bighorn Sheep Council Transactions*. We are working on a manuscript for *Conservation Biology* describing the microsatellite data and a manuscript for *Molecular Biology and Evolution* describing the MHC data.

APPENDIX 1 : Manuscript submitted to *Desert Bighorn Sheep Council Transactions*

Introduction

Bighorn sheep occupied most of the desert mountain ranges in California, Arizona, and New Mexico until human settlement led to the extirpation of sheep from much of their historic range. Existing bighorn sheep populations occupy fragments of historic range (Bleich 1995). Bighorn sheep management has focused on improving the viability of existing populations and reintroducing sheep to their previous range, and these efforts have become increasingly successful. This success has been due, in part, to research that has improved our understanding of sheep populations. Now, recent advances in molecular genetic methods offer a view of the evolution and genetic differentiation of bighorn sheep in the Southwest that should be valuable for sheep management.

Genetic data can address two issues pertinent to 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), and each component in this scenario has received some support. For example, Bleich (1995) showed that modern bighorn sheep populations have lost historic connections to adjacent populations. Ramey (1995) showed bighorn sheep populations have low levels of mitochondrial DNA diversity. Sausman (1984) showed inbreeding increased lamb mortality in captivity. And lastly, Berger (1990) showed 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 this scenario is, and will permit management to use genetic information during sheep management. Second, recognizing historic patterns of genetic variation in 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 lower the fitness of populations.

We will address these issues by examining genetic variation in bighorn sheep from across the Southwest. Of the many genetic markers now available, microsatellite loci are best suited for these questions (e.g. Ashley and Dow 1994, Jarne and Lagoda 1996). Microsatellites are DNA sequences composed of a variable number (typically 5 to 60) of tandem repeats, such as ...CACACACA. Specific loci (locations in the genome) are defined by unique DNA sequences that flank the repeated units, and individuals are characterized by the number of repeats at that location. Sequences with more than 40 repeat units are uncommon (Valdes et al. 1993), but the mechanism constraining the number of repeated units is not known. Microsatellite loci have become popular genetic markers for evolutionary studies because they have a high mutation rate and are considered selectively neutral. Mutation changes the number of repeats at a locus, and the high mutation rate creates variation quickly. This allows recent evolutionary events to be detected. Neutrality ensures that the number of repeats at a particular locus will not affect the fitness of the individual. As a result, the amount of genetic differentiation at microsatellite loci for two isolated populations is proportional to the length of time they have been separated. If two populations have been exchanging members, the amount of genetic differentiation will be inversely proportional to the migration rate between the populations.

Study Area

We obtained blood, tissue, or DNA samples from 279 sheep at the 13 locations in California, Arizona, New Mexico, and Canada listed in Table 1. Arizona Game and Fish Department provided 98 blood samples from sheep captured in Arizona, including samples from the Kofa Mountains, Castle Dome Mountains, Stewart Mountain, Mt. Davis, Lost Cabin, Mt. Nutt. In addition, Arizona Game and Fish provided four liver samples from the Kofa Mountains collected by hunters. This study also includes 122 DNA samples previously analyzed at three microsatellite loci by Boyce et al. (1997) from sheep at San Ysidro, San Geronio, Eagle, Old Dad, and Wheeler Peak. Lastly, Stephen Forbes provided data and DNA from 55 bighorn sheep in Alberta, Canada. Figure 1 shows the location of the nine study sites in California and Arizona.

These study sites are composed of native sheep except for the Stewart Mountain, Wheeler Peak, and Red Rock Refuge study sites. The Stewart Mountain sheep were transplanted from the Kofa Mountains of Arizona; the Wheeler Peak sheep were transplanted from Alberta, Canada; and the Red Rock Refuge sheep were captured in the San Andres Mountains of New Mexico.

Methods

We genotyped all individuals at ten dinucleotide microsatellite loci: FCB11, FCB128, FCB266, FCB304, MAF33, MAF 36, MAF48, MAF65, MAF209, and DS52 (Buchanan et al. 1993; Crawford et al. 1994; Steffen et al. 1993). We chose these loci because they have been informative in previous studies of genetic variability in bighorn sheep (Forbes et al. 1995; Boyce et al. 1997).

We began our data analysis by testing whether the data at each study site was consistent with random mating with respect to the ten genetic markers in our analysis. This was

accomplished by testing the data for agreement with Hardy-Weinberg proportions using GENEPOP 3.0 (Raymond and Rousset, 1995). We tested each locus, each study site, and each locus at each study site, using the Bonferroni adjustment for multiple comparisons as criteria for statistical significance (Sokal and Rohlf 1995). Next, we calculated two 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. Second, we calculated the genetic distance of Nei (1977) between each pair of study sites. This statistic is a measure of genetic differentiation for pairs of populations and equals zero when the two populations are identical and infinity when the two populations share no genetic markers. Randomization was used to test for the statistical significance of each genetic distance.

Genetic distances between study sites were summarized with two methods. First, we used PHYLIP (Felsenstein 1993) to construct a UPGMA phylogenetic tree of the 13 sampling sites. The significance of the nodes in the tree was tested by bootstrapping over loci using the DISPAN software package (Ota 1993). 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 (E. S. R. I. 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 (Sokal and Rohlf 1995) to test for correlation between genetic and geographic distances.

Results

There was considerable genetic variation at all of the sampling locations (see Table 1). There was variation at each locus and in each population. With only a few exceptions, each sample contained more than one microsatellite variant at each locus. None of the loci or study sites differed significantly from Hardy-Weinberg proportions, indicating mating apparently was random with respect to these loci. 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. The three most genetically variable sampling locations were Eagle, Kofa, and Alberta; the three least genetically variable sampling locations were Red Rock, Mt. Nutt, and Old Dad. The confidence intervals for the gene diversity at each location indicate that the lowest heterozygosities are significantly lower than the highest; however, all 13 study sites have a substantial amount of genetic variation, and none of them can be considered genetically impoverished.

The genetic distance between each pair of sampling locations, ranged from a minimum of 0.020 between Mt. Davis and Lost Cabin to a maximum of 0.870 between San Ysidro and Alberta (Gutiérrez-Espeleta et al. 1998). All of genetic distances were highly statistically significant ($p < 0.001$), except for the two smallest genetic distances ($D_{Davis,Cabin} = 0.02$, $\hat{p} = 0.06$; $D_{Kofa,Castle} = 0.04$, $\hat{p} = 0.05$). This indicates that each pair of study sites is genetically different, except for the two pair just mentioned.

The phylogenetic tree depicted in Figure 2 provides one method of summarizing the genetic differences between study sites. The values shown at the nodes of the tree estimate the probability of obtaining the indicated clusters of study sites if the study was repeated with ten randomly chosen loci. As can be seen, only two clusters of study sites received reasonable support from the data: the three study sites in the Black Mountains of 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, and both of these well supported clusters are at the tips of the phylogenetic tree. The major structure of the tree can not be considered reliable.

Genetic differentiation between study sites is generally proportional to geographic separation (Figure 3). A Mantel test found this relationship to be significantly different from random ($\hat{p} < 0.001$). The relationship is roughly linear for distances up to 300 kilometers, and then appears to asymptote, with genetic distances between 0.25 and 0.75 for study sites more distantly separated. If currently recognized subspecies definitions have a biological basis, we would expect a higher rate of genetic differentiation with distance when comparing locations across subspecies lines than within subspecies. This expectation is not met.

Discussion

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 et al. (1995) and Forbes and Hogg (1998) in five populations of Rocky Mountain bighorn sheep (*O. c. canadensis*). Comparing the two data sets reveals that the rate of genetic differentiation as a function of geographic distance is much steeper among desert bighorn than Rocky Mountain bighorn. This could be explained by larger population sizes for Rocky Mountain sheep, higher migration rates, or by similarities between populations in the Rockies remaining from post-Pleistocene colonization. If desert populations have historically been more isolated than Rocky Mountain populations, we would expect to find less genetic variation in the desert populations than in the Rockies. This expectation is 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 average gene diversity in the 11 desert locations in this current study of 0.49 at the eight loci in common.

This data set complements the mitochondrial data of Ramey (1995) to provide a comparison between male and female migration rates. Ramey examined mitochondrial DNA (mtDNA) sequences at 26 locations in the Southwest (including Old Dad, Eagle, San Gorgonio, Kofa, and Red Rock) and found lower levels of genetic variation and greater differences between adjoining populations than in our study. Because mtDNA is only inherited maternally, mtDNA variation reflects only the evolutionary history of females. The low levels of mtDNA variation and high level of population differentiation indicate that the dispersal rate for ewes has been low. In contrast to mitochondrial DNA, microsatellite DNA is inherited both maternally and paternally. The higher level of genetic variation and less extreme genetic differentiation at microsatellite loci probably reflects higher dispersal rates among rams.

The relatively high gene diversity in desert bighorn population shows that desert sheep populations have been large and/or well connected during recent evolutionary history. These gene diversities, however, probably do 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 dispersal rates to and from other populations and minimized by low dispersal rates. Fortunately, dispersal rates as low as one migrant per generation are effective in preventing loss of genetic variation caused by fragmentation. Schwartz et al. (1986) have used this reasoning to argue that excessive loss of genetic variation is unlikely for large metapopulations of sheep. We agree. The recommendation

(Bleich et al. 1990) that corridors between sheep populations be protected for sheep movement is also sound.

Assigning biological significance to the genetic differences between populations found in this study is difficult. Populations with similar microsatellite variation may still have adaptively important differences maintained by natural selection. In addition, populations with differing microsatellite markers may share adaptively important traits. Evidence has been found for both of these situations (Karhu et al. 1993; Scheffer et al. 1998). Microsatellite differentiation only reflects the opportunity for other traits to evolve independently in each population. So, very similar populations such as Mt. Davis and Lost Cabin have had virtually no opportunity for independent evolution. In fact, these two locations practically constitute a single population. They, perhaps, should be considered sub-units of a metapopulation. The same is true for the Kofa and Castle Dome locations. 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 between these regions with opportunity for independent evolution and adaptation to local environments. Because we can not 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.

A significant characteristic of the relationship between genetic differentiation and geographic separation is that the relationship is only linear for distances up to 500 kilometers. More distantly separated populations are not noticeably more genetically differentiated. This is likely an artifact of mutational restrictions of microsatellite loci that do not permit increased differentiation beyond a certain point.

The genetic relationships estimated by this analysis, unfortunately, can not easily be used to produce a taxonomy of desert bighorn sheep. Fairly strong genetic differentiation exists in southwestern populations of bighorn sheep. However, genetic differences appear to be associated with geographic distance rather than any specific 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 we have no support for current subspecies designation. Rather, this analysis appears to support the view of Ramey (1995) that of desert bighorn sheep are a polytypic subspecies.

However, we emphatically acknowledge that this study has not provided a strong test of the existing taxonomy. For example, we have included only one location (San Ysidro) from the Peninsular Ranges. 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 kilometers, but apparently within the range expected for that 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 decrease the width of confidence intervals for genetic distances (data not shown) and increase the statistical significance of clusters in the phylogenetic tree. However, the inability of this analysis to establish a taxonomy for desert bighorn sheep may reflect the inappropriateness of the subspecies concept (Wilson and Brown 1954) as much as limitations in our data.

Acknowledgements

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Table 1. Location, currently recognized subspecies, number of individuals sampled (N), and gene diversity (\hat{H} and 95% confidence interval for \hat{H}) of the 13 study sites included in this study.

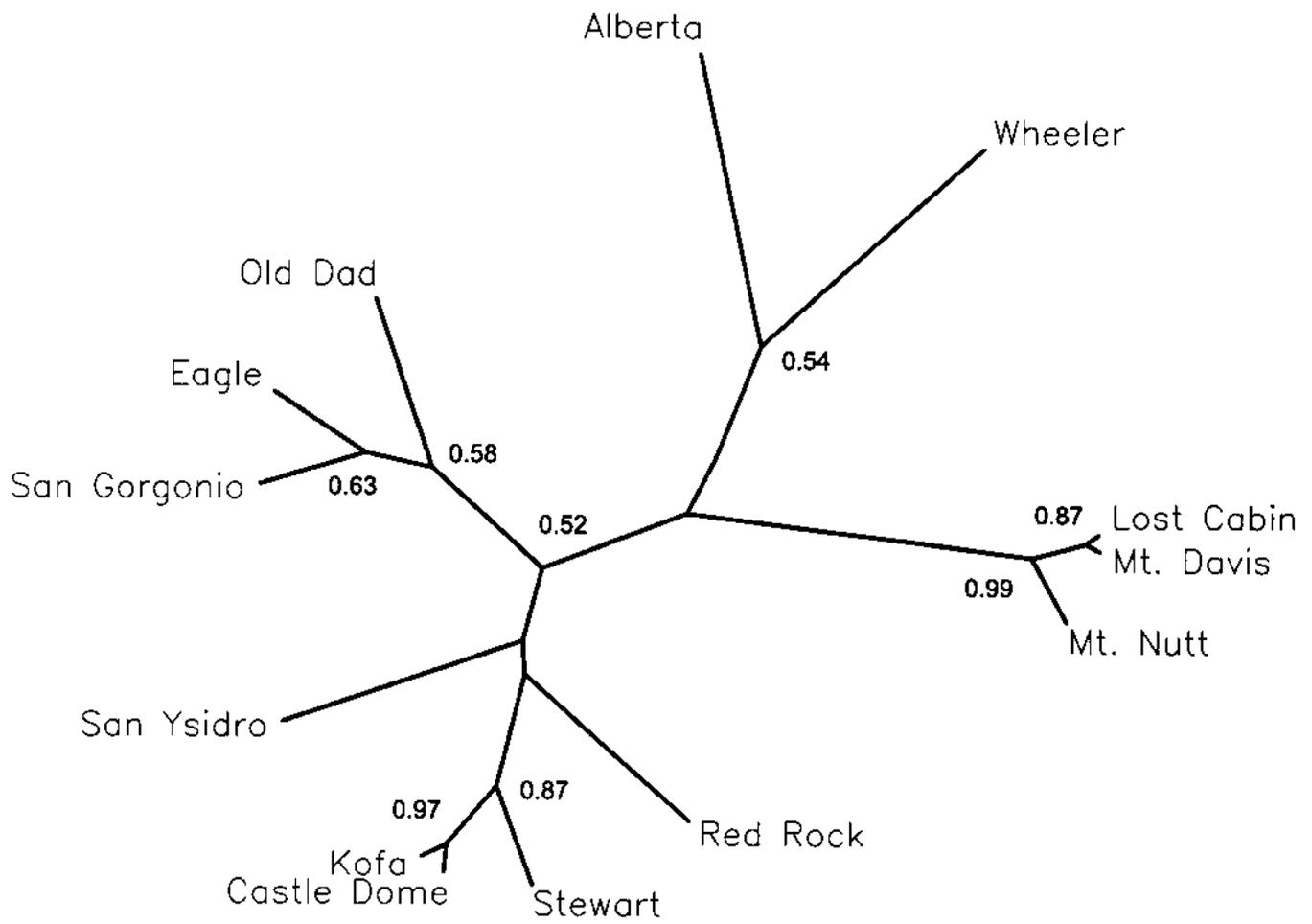
Location	Subspecies	N	\hat{H} (min, max)
Northern Arizona			
Mt. Davis	<i>O. c. nelsoni</i>	15	0.54 (0.49, 0.59)
Lost Cabin	<i>O. c. nelsoni</i>	16	0.55 (0.51, 0.58)
Mt. Nutt	<i>O. c. nelsoni</i>	28	0.44 (0.39, 0.49)
Southern AZ			
Kofa Mountains	<i>O. c. mexicana</i>	9	0.60 (0.55, 0.64)
Stewart Mountain	<i>O. c. mexicana</i>	14	0.54 (0.50, 0.58)
Castle Dome Mountains	<i>O. c. mexicana</i>	20	0.58 (0.55, 0.62)
Southern California			
Old Dad Mountains	<i>O. c. nelsoni</i>	23	0.45 (0.41, 0.50)
Eagle Mountains	<i>O. c. nelsoni</i>	23	0.63 (0.60, 0.66)
San Gorgonio	<i>O. c. nelsoni</i>	22	0.46 (0.41, 0.51)
San Ysidro	<i>O. c. cremnobates</i>	22	0.49 (0.45, 0.53)
New Mexico			
Red Rock Refuge	<i>O. c. mexicana</i>	25	0.36 (0.30, 0.42)
Rocky Mountains			
Wheeler Peak, N.M.	<i>O. c. canadensis</i>	7	0.55 (0.51, 0.58)
Alberta, Canada	<i>O. c. canadensis</i>	55	0.59 (0.56, 0.63)

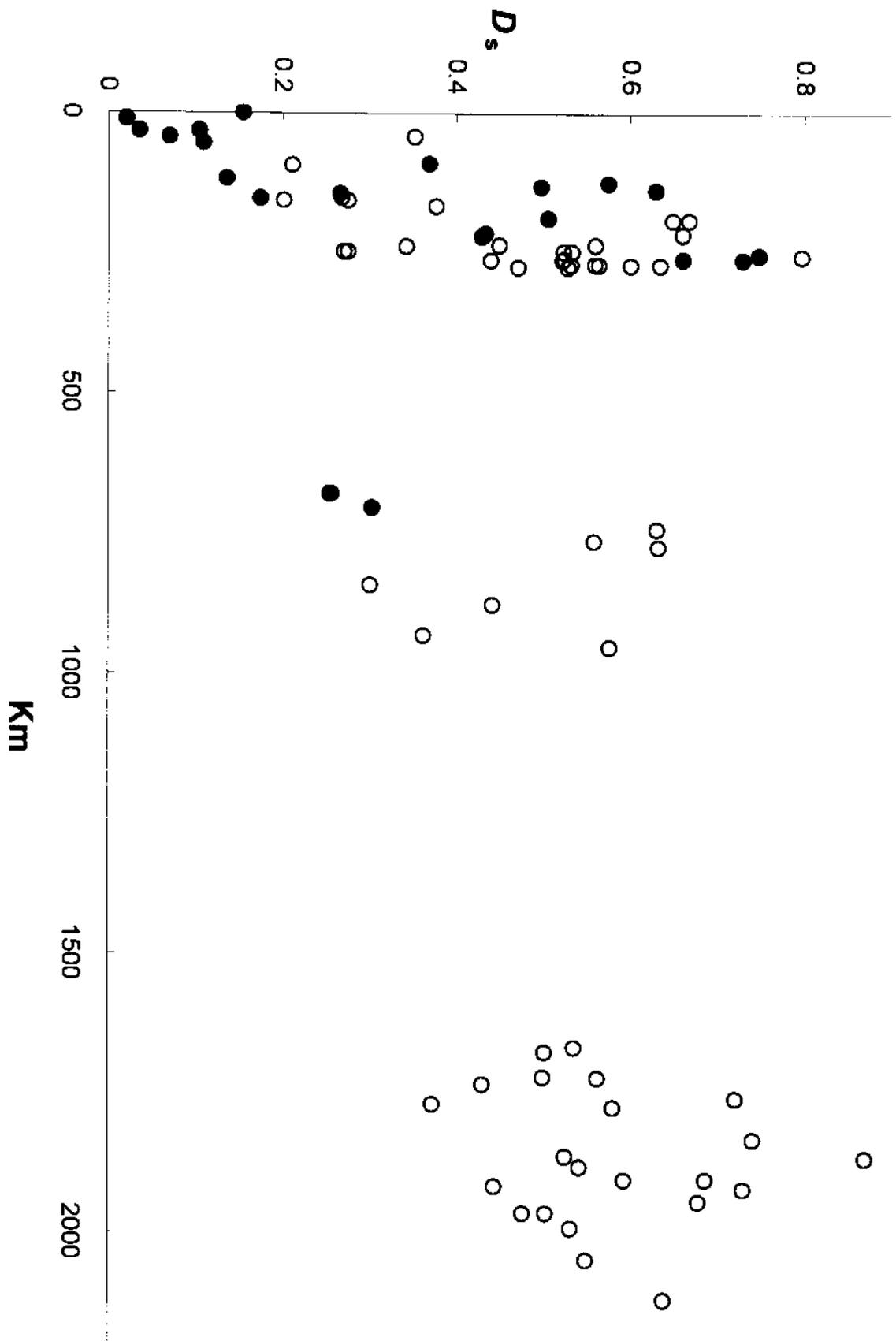
Figure Legends

Figure 1. Location of study sites in Arizona and California. Not shown are the locations of Stewart Mountain, AZ, Wheeler Peak, NM, Red Rock, NM, and Sheep River, Canada.

Figure 2. UPGMA phylogenetic tree. Number indicates the percentage of bootstrap replicates sharing the labeled node.

Figure 3. Genetic distance (Nei, 1977) plotted against geographic distance. Comparisons between and within currently accepted subspecies are indicated by filled and open symbols, respectively.





APPENDIX 2: Observed allele frequencies (loci are listed from most to least heterozygous).

	<i>N</i>	Alleles at locus <i>D5S2</i>								
		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.	8	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.	22	0.159	0.114	-	0.364	0.136	0.068	-	0.159	-
S. Gorgonio	22	-	-	-	0.636	0.045	-	-	0.318	-
San Ysidro	21	-	-	-	-	0.429	0.286	0.214	0.071	-
Red Rock	25	0.340	0.100	-	0.080	0.480	-	-	-	-
Alberta	48	0.396	0.021	0.010	0.490	-	0.083	-	-	-
Wheeler Pk.	7	0.714	-	-	0.286	-	-	-	-	-

	<i>N</i>	Alleles at locus <i>MAF 65</i>									
		115	117	119	121	123	125	127	129	131	133
Mt. Davis	15	0.233	-	-	-	-	-	0.500	-	0.267	-
Lost Cabin	16	0.125	-	-	-	-	0.031	0.469	-	0.375	-
Mt. Nutt	28	0.214	-	-	-	-	0.054	0.268	-	0.464	-
Kofa Mtns.	9	0.056	-	-	-	0.500	0.111	0.111	0.222	-	-
Stewart Mtn.	14	0.250	-	-	-	0.643	0.036	0.071	-	-	-
Castle Dome	20	0.225	-	-	0.025	0.500	0.175	0.075	-	-	-
Old Dad	23	0.500	0.087	-	-	0.196	-	0.174	-	-	0.043
Eagle Mtns.	22	0.455	-	-	-	-	-	0.159	0.364	0.023	-
S. Gorgonio	21	0.786	-	-	-	0.190	-	0.024	-	-	-
San Ysidro	22	0.159	0.568	-	-	0.136	-	0.023	0.114	-	-
Red Rock	24	0.479	-	-	-	0.521	-	-	-	-	-
Alberta	55	0.009	-	0.264	-	0.200	0.182	0.236	0.109	-	-
Wheeler Pk.	7	0.286	-	-	-	0.643	0.071	-	-	-	-

Allele at locus *MAF 48*

	<i>N</i>	<i>120</i>	<i>122</i>	<i>124</i>	<i>126</i>	<i>128</i>	<i>130</i>	<i>132</i>	<i>134</i>
Mt. Davis	15	-	-	0.600	0.100	0.300	-	-	-
Lost Cabin	16	-	-	0.656	-	0.344	-	-	-
Nutt	28	-	-	0.232	-	0.768	-	-	-
Kofa Mtns.	8	0.375	0.063	0.438	-	0.125	-	-	-
Stewart Mtn.	14	0.179	0.393	0.179	-	0.250	-	-	-
Castle Dome	20	0.450	0.375	0.075	-	0.100	-	-	-
Old Dad	23	-	0.435	0.087	0.174	0.304	-	-	-
Eagle Mtns.	23	-	0.457	0.152	0.087	0.304	-	-	-
S. Gorgonio	22	0.409	0.091	0.023	0.273	0.205	-	-	-
San Ysidro	22	0.091	0.386	0.295	0.227	-	-	-	-
Red Rock	25	-	0.340	-	0.520	0.140	-	-	-
Alberta	55	-	0.136	0.182	-	-	0.109	0.545	0.027
Wheeler Pk.	7	-	0.071	0.643	-	-	0.143	0.143	-

Alleles at locus *MAF 209*

	<i>N</i>	<i>109</i>	<i>111</i>	<i>113</i>	<i>115</i>	<i>117</i>	<i>119</i>	<i>121</i>	<i>123</i>
Mt. Davis	15	-	-	0.600	-	0.400	-	-	-
Lost Cabin	16	-	-	0.625	-	0.281	-	0.031	0.063
Mt. Nutt	28	-	-	0.536	-	0.375	-	0.089	-
Kofa Mtns.	8	-	-	-	0.063	0.375	0.188	0.188	0.188
Stewart Mtn.	14	-	-	-	0.107	0.071	0.464	-	0.357
Castle Dome	20	0.100	-	-	-	0.375	0.175	0.075	0.275
Old Dad	23	0.283	0.065	-	-	-	-	0.652	-
Eagle Mtns.	23	-	-	-	0.087	0.283	0.130	0.500	-
S. Gorgonio	22	-	-	-	-	0.114	0.023	0.864	-
San Ysidro	22	-	-	0.295	-	0.114	0.045	0.545	-
Red Rock	25	-	-	0.580	-	-	0.280	0.140	-
Alberta	55	0.027	-	0.418	0.045	0.345	0.009	-	0.155
Wheeler Pk.	7	0.429	-	-	-	0.071	-	-	0.500

Alleles at locus *MAF 36*

	<i>N</i>	93	95	99	101	103	105	107	109
Mt. Davis	15	0.167	-	-	0.100	0.100	0.300	-	0.333
Lost Cabin	16	0.250	-	-	-	0.063	0.156	-	0.531
Mt. Nutt	28	-	-	-	0.036	-	0.071	-	0.893
Kofa Mtns.	9	0.389	-	-	-	0.111	0.389	0.056	0.056
Stewart Mtn.	14	0.786	-	-	-	-	0.214	-	-
Castle Dome	20	0.350	-	-	-	0.125	0.300	0.175	0.050
Old Dad	22	0.773	-	-	-	-	0.227	-	-
Eagle Mtns.	23	0.348	0.065	-	0.087	0.109	0.261	0.043	0.087
S. Gorgonio	22	0.091	0.068	-	-	0.250	0.318	-	0.273
San Ysidro	22	0.727	-	-	0.091	0.068	-	0.068	0.045
Red Rock	25	0.960	-	-	-	-	-	-	0.040
Alberta	55	0.145	-	0.527	-	-	-	-	0.327
Wheeler Pk.	7	0.071	-	0.286	-	0.571	-	-	0.071

Alleles at locus *FCB 266*

	<i>N</i>	87	89	91	93	95	97	99	101
Mt. Davis	15	-	-	-	-	0.800	-	0.133	0.067
Lost Cabin	16	-	-	-	-	0.500	-	0.313	0.188
Mt. Nutt	28	-	-	-	0.018	0.446	-	0.304	0.232
Kofa Mtns.	9	0.111	-	-	0.111	-	-	0.778	-
Stewart Mtn.	14	-	-	-	-	0.250	-	0.750	-
Castle Dome	20	0.050	-	-	0.100	0.125	-	0.725	-
Old Dad	23	-	-	-	-	0.196	-	0.804	-
Eagle Mtns.	22	0.091	-	-	0.136	0.159	-	0.614	-
S. Gorgonio	21	0.048	-	0.024	-	0.262	-	0.667	-
San Ysidro	22	-	-	-	-	-	0.227	0.773	-
Red Rock	25	0.220	0.200	-	-	-	-	0.580	-
Alberta	55	0.409	0.218	-	0.027	0.064	-	0.282	-
Wheeler Pk.	7	-	0.143	-	0.143	0.143	-	0.571	-

Alleles at locus *FCB 304*

	<i>N</i>	<i>136</i>	<i>138</i>	<i>140</i>	<i>142</i>
Mt. Davis	15	0.467	0.200	0.333	-
Lost Cabin	16	0.625	0.063	0.313	-
Mt. Nutt	28	0.643	0.196	0.161	-
Kofa Mtns.	9	0.611	-	0.222	0.167
Stewart Mtn.	14	0.250	0.357	0.286	0.107
Castle Dome	20	0.625	-	0.250	0.125
Old Dad	23	0.065	-	0.717	0.217
Eagle Mtns.	23	0.348	-	0.522	0.130
S. Gorgonio	22	0.045	-	0.955	-
San Ysidro	22	0.159	-	0.727	0.114
Red Rock	25	0.920	-	0.080	-
Alberta	55	0.391	0.491	0.064	0.055
Wheeler Pk.	7	0.214	0.214	0.357	0.214

Alleles at locus *FCB 11*

	<i>N</i>	<i>127</i>	<i>129</i>	<i>131</i>
Mt. Davis	15	0.233	0.433	0.333
Lost Cabin	16	0.406	0.500	0.094
Mt. Nutt	28	0.429	0.339	0.232
Kofa Mtns.	9	0.333	0.500	0.167
Stewart Mtn.	14	0.107	0.607	0.286
Castle Dome	20	0.050	0.825	0.125
Old Dad	23	0.826	0.130	0.043
Eagle Mtns.	23	0.457	0.217	0.326
S. Gorgonio	22	0.568	0.023	0.409
San Ysidro	22	0.045	0.227	0.727
Red Rock	25	0.760	0.240	-
Alberta	55	0.645	0.200	0.155
Wheeler Pk.	7	0.643	0.143	0.214

Alleles at locus *MAF 33*

	<i>N</i>	<i>121</i>	<i>123</i>	<i>125</i>	<i>127</i>	<i>129</i>	<i>131</i>
Mt. Davis	15	0.600	0.067	-	-	0.033	0.300
Lost Cabin	16	0.781	0.063	-	-	-	0.156
Mt. Nutt	28	0.804	0.018	-	-	-	0.179
Kofa Mtns.	9	-	0.889	-	-	0.111	-
Stewart Mtn.	14	-	0.571	0.214	-	0.214	-
Castle Dome	20	-	0.675	0.175	-	0.150	-
Old Dad	23	0.717	0.065	0.217	-	-	-
Eagle Mtns.	22	0.227	0.591	0.136	0.045	-	-
S. Gorgonio	22	0.136	0.523	0.205	0.136	-	-
San Ysidro	22	0.091	0.818	0.091	-	-	-
Red Rock	25	-	1.000	-	-	-	-
Alberta	55	0.164	0.173	0.009	0.655	-	-
Wheeler Pk.	7	0.786	0.071	-	0.143	-	-

Alleles at locus *FCB 128*

Study Area	<i>N</i>	<i>112</i>	<i>114</i>	<i>116</i>	<i>118</i>
Mt. Davis	15	-	-	1.000	-
Lost Cabin	16	-	0.156	0.844	-
Mt. Nutt	28	-	-	1.000	-
Kofa Mtns.	9	-	0.333	0.667	-
Stewart Mtn.	14	-	0.107	0.893	-
Castle Dome	20	-	0.275	0.725	-
Old Dad	23	-	0.043	0.957	-
Eagle Mtns.	23	-	0.174	0.826	-
S. Gorgonio	22	-	0.114	0.886	-
San Ysidro	22	0.091	0.023	0.886	-
Red Rock	25	-	0.020	0.980	-
Alberta	48	-	0.073	0.875	0.052
Wheeler Pk.	7	-	0.143	0.857	-

APPENDIX 3. Allele frequencies at MHC locus DRB3 in Arizona populations of desert bighorn sheep.

	<i>N</i>	Alleles at MHC locus DRB3										
		1	2	3	4	5	6	7	8	9	10	11
Cabin	15	0.233	0.367		0.233			0.033	0.133			
C. Dome	20	0.200	0.025			0.200	0.175	0.100	0.200	0.100		
Davis	15	0.333	0.200		0.367		0.033		0.033	0.033		
Kofa	4	0.125	0.125	0.125		0.125		0.250		0.125	0.125	
Nutt	28	0.232	0.232		0.214			0.304	0.018			
Stewart	14	0.071	0.036			0.214	0.250	0.107	0.143	0.107		0.071