Genetic Variation of Major Histocompatibility Complex and Microsatellite Loci: A Comparison in Bighorn Sheep

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ABSTRACT

Examining and comparing genetic variation for major histocompatibility complex (MHC) and microsatellite (MS) loci in the same individuals provides an opportunity to understand the forces influencing genetic variation. We examined five MHC and three MS loci in 235 bighorn sheep (*Ovis canadensis*) from 14 populations and found that both types of loci were highly variable and were in Hardy-Weinberg proportions. Mean F_{ST} values for both markers were very similar and MHC and MS genetic variability was predominantly distributed within rather than among populations. However, analyses of genetic distances and tree topologies revealed different spatial patterns of variation for the two types of loci. Collectively, these results indicated that neutral forces substantially influenced MS and MHC variation, and they provided limited evidence for selection acting on the MHC.

MOLECULAR genetic markers have been of great significance in understanding the extent and pattern of genetic variation within and between taxa (KIMURA 1983; LEWONTIN 1991). Generally, it is assumed that the amount of differential selection operating on these molecular markers is small (they are neutral) so that variation is primarily determined by nonselective evolutionary factors such as genetic drift, gene flow, and mutation (NEI 1987). On the other hand, loci under selection, either directional or balancing selection, may have amounts or patterns of variation that primarily reflect past selective events and are not necessarily consistent with population history or structure of the taxa.

Variation in the genes in the major histocompatibility complex (MHC) is universally thought to be maintained by some sort of balancing selection (NEI and HUGHES 1991; HEDRICK 1994a). In fact, the patterns of variation within and between taxa are consistent with balancing selection having a major role influencing nucleotide sequences, allele frequencies, and linkage disequilibrium at MHC loci (HEDRICK and THOMSON 1983; KLITZ and THOMSON 1987; HUGHES and NEI 1988; HEDRICK *et al.* 1991). Pathogen resistance, negative assortative mating, and maternal-fetal interaction have been proposed as the mechanisms driving balancing selection. Although there is controversy regarding the relative importance of these mechanisms, the role of MHC in pathogen resistance appears likely since MHC molecules present peptides from pathogens to initiate the immune response (BROWN and EKLUND 1994; HEDRICK 1994b).

On the other hand, variation in microsatellite (MS) loci is thought to be primarily influenced by nonselective mechanisms (with the exception of the trinucleotide repeats causing diseases in humans, SUTHERLAND and RICHARDS 1995). Microsatellite loci typically exhibit high variability due to high mutation rates, a large number of unlinked loci, and codominance. These characteristics have made them a nuclear marker of choice for determining within population variation and relationships between closely related taxa (ASHLEY and DOW 1994; QUELLER *et al.* 1994).

In this study, we investigated the factors influencing genetic variation by examining and comparing the extent and pattern of genetic variation for MHC and MS loci in the same individuals and populations of bighorn sheep (*Ovis canadensis*). Infectious disease has been a major cause of mortality among bighorn sheep populations since at least the early 1800s (BUECHNER 1960), suggesting that there has been opportunity for selection at MHC loci. By comparing the pattern of genetic variation for MS and MHC loci in the same individuals, we can determine if selection is, or has been, acting on the MHC loci.

MATERIALS AND METHODS

Samples: We collected blood samples and isolated DNA by standard methods (MILLER *et al.* 1988) from 235 bighorn sheep from 14 populations (Figure 1). These animals were captured from 1992 to 1994 as part of herd-health surveys

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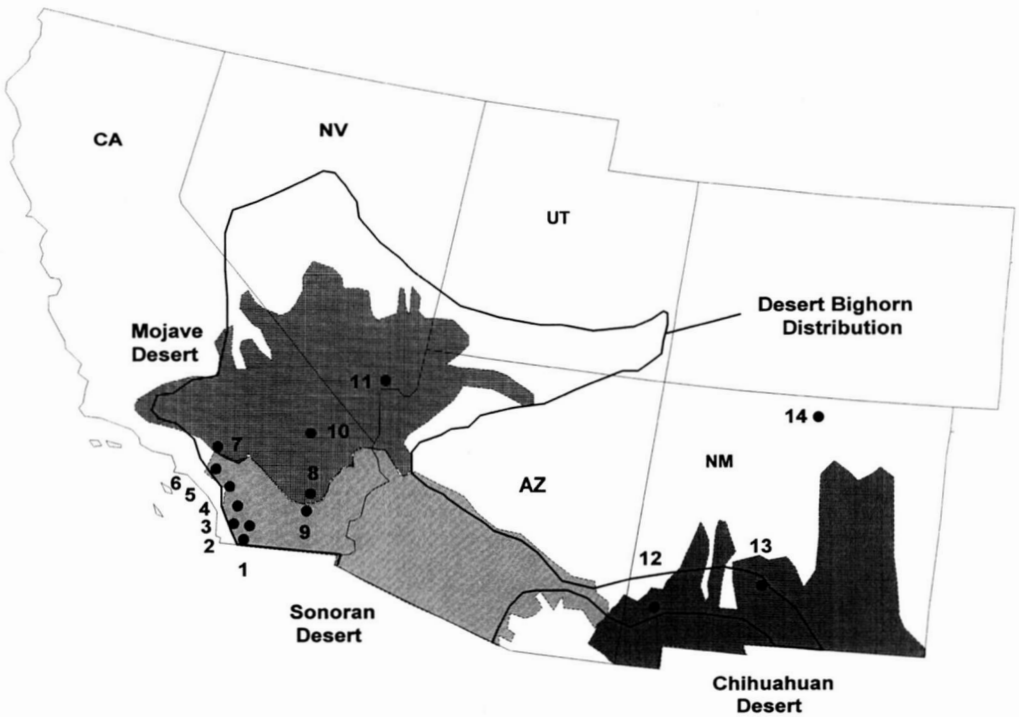

FIGURE 1.—Location of bighorn sheep populations, and estimated population size and proportions sampled (in parentheses). Peninsular Ranges, Sonoran Desert: 1, Carrizo Canyon (50–100, 0.22–0.44); 2, Vallecito Mountains (25–50, 0.24–0.48); 3, San Ysidro Mountains (50–100, 0.22–0.44); 4, Coyote Canyon (50–100, 0.10–0.20); 5, Santa Rosa Mountains (100–150, 0.19–0.29); 6, San Jacinto Mountains (25–50, 0.18–0.36); Mojave Desert: 7, San Gorgonio Mountain (100–150, 0.13–0.19); 8, Eagle Mountains (50–100, 0.25–0.50); 9, Orocopia Mountains (100–150, 0.09–0.13); 10, Old Dad Peak (200–300, 0.08–0.12); 11, Muddy Mountains (100–200, 0.10–0.19); Chihuahuan Desert: 12, Red Rock Refuge (100–150, 0.12–0.18); 13, San Andres Mountains (25–50, 0.16–0.32); Rocky Mountains: 14, Wheeler Peak (50–100, 0.23–0.46).

or translocation efforts, and, for many of the populations, a substantial proportion (≥20%) of the total population was sampled. Desert bighorn populations were sampled in the Peninsular Ranges in the Sonoran Desert in southern California (Carrizo, Vallecito, San Ysidro, Coyote, Santa Rosa, and San Jacinto), the Mojave Desert in California and Nevada (San Gorgonio, Eagle, Orocopia, Old Dad, and Muddy), and the Chihuahuan Desert in New Mexico (San Andres and Red Rock). Based on demographic studies, the six populations in the Peninsular Ranges comprise a single metapopulation with significant movement of animals (rams) between populations. The Mojave populations are a more heterogeneous group and may belong to several different metapopulations. The Orocopia population, although technically located in the Sonoran Desert, was included in the Mojave group because of its close proximity to the Eagle Mountains and the likelihood of historic interpopulation movements. The Red Rock population is a large captive herd that was derived primarily from animals in the San Andres Mountains. For comparative purposes, we also included one population of Rocky Mountain bighorn sheep from Wheeler Peak, NM, that was exclusively derived from Rocky Mountain bighorn transplanted from Banff, Alberta, Canada.

Molecular techniques and analysis: Hybridization probes (DRB3-B2, DQB1-TM) that contain the second domain of the bovine BoLA-DRB3 gene and the transmembrane region of the bovine BoLA-DQB1 gene, respectively, were used to investigate *Taq*I restriction fragment length polymorphisms (RFLPs) in the bighorn sheep MHC class II region (BURKE *et al.* 1991; STONE and MUGGLI-COCKETT 1992). MS DNA typing was performed for markers DRB3 (herein designated MDRB3, ELLEGREN *et al.* 1993), D5S2 (STEFFEN *et al.* 1993), and OARFCB11 (herein designated FCB11, BUCHANAN *et al.* 1993). From these references, D5S2 and FCB11 are both simple GT dinucleotide repeats while MDRB3 has a more complicated repeat pattern and is linked to the MHC class II region.

Allele frequencies were determined using BIOSYS-I (SWOFFORD and SELANDER 1989), and deviations from Hardy-Weinberg proportions were examined using exact probability calculations (LEVENE 1949). F_{ST}, modified by weighting according to sample size, and Nei's standard genetic distance values (D) were calculated using the formulas described in NEI (1977 and 1972, respectively). We also calculated the equivalent genetic distance values D_1 of GOLDSTEIN *et al.* (1995) and S_B of SLATKIN (1995) for the MS loci.

Population variability and genetic structure were examined at different geographic scales by determining F_{ST} values and genetic distances within and across regions (Peninsular, Mojave, Chihuahuan, and Rocky Mountain). To examine the proportion of F_{ST} values explained by sampling alone, we pooled the observed samples in the group under consideration (*e.g.*, the six Peninsular samples) and then drew 1000 random samples of the same size and calculated a F_{ST} value for each set of random samples. We tested for correlations between the genetic distances (D) obtained for MHC and MS loci and genetic distances for both groups of loci and geographic distance by calculating Mantel correlation coefficients (e.g., SOKAL and ROLHF 1995). Phenetic (unweighted pair-group method, UPGMA) and phylogenetic (neighbor-joining, NJ) methods were used to infer relationships among populations (KUMAR *et al.* 1993).

RESULTS

Extent of genetic variation: Examination of the intensity and distribution of the RFLP banding patterns for the bovine probes identified five polymorphic MHC

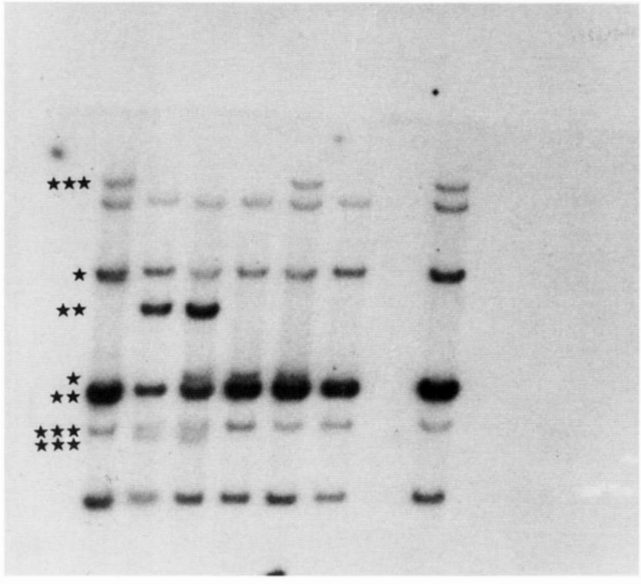

FIGURE 2.—*Taq*I restriction fragment patterns for seven bighorn sheep after hybridization with the bovine MHC class II DRB3-B2 probe. *DRB3-1 locus, **DRB3-2 locus, ***DRB3-3 locus.

loci. The DQB1-TM probe hybridized strongly (dark bands) to a bighorn sheep DQB1-like gene (DQB1-1 with four alleles) very similar to itself, and it also cross-hybridized (light bands) to a second, less similar gene (DQB1-2 with three alleles). We infer that these are two separate loci because the bands (alleles) at each locus occurred in codominant patterns that were independent of patterns at the other locus. Likewise, the DRB3-B2 probe cross-hybridized with three different DRB3-like loci (DRB3-1 with four alleles; DRB3-2 with three alleles; DRB3-3 with three alleles), and each locus had a set of codominant alleles that was independent of the other two loci (Figure 2).

PCR amplification resulted in fragment length polymorphisms for each of the three MS markers consistent with amplification of multiple alleles at each locus (APPENDIX A). The three MS loci were polymorphic in all populations with the exception of MDRB3, which was fixed for a single allele in the San Andres population. The average number of alleles ranged from 2.3 to 5.0 for the MS loci (weighted mean of 3.6 alleles per locus). Three alleles were restricted to a single population (MDRB3*1 in Eagle, MDRB3*6 in Red Rock, D5S2*9 in Muddy), and two additional alleles were limited to populations in a single region (D5S2*7 in five Peninsular populations, D5S2*3 in two Mojave populations).

Examination of MS allele frequencies (weighted by sample size) as a function of allele size showed that the three alleles for FCB11 differed by a only single dinucleotide repeat (Figure 3). The three same size alleles were found by FORBES *et al.* (1995) in Rocky Mountain bighorn sheep. D5S2 had a wider distribution of allele sizes, but every dinucleotide repeat size between 201 and 219 was represented in at least one population except for 215. On the other hand, the distribution of allele sizes for MDRB3 was extremely wide ranging from 153 to 227. In sequencing alleles for this locus in cattle, which had a very similar size distribution, ELLEGREN et al. (1993) found a combination of three repeat motifs rather than a simple dinucleotide repeat pattern.

The five MHC loci were polymorphic, but to a lesser extent than the MS loci (Table 1). The average number of alleles ranged from 1.6 to 2.8 for the MHC loci (weighted mean of 2.1 alleles per locus). DQB1-1 and DRB3-1 were polymorphic in all 14 populations, DQB1-2 and DRB3-3 were polymorphic in 12 populations, while DRB3-2 was polymorphic in six populations (APPENDIX A). There were several MHC alleles that were specific to the Wheeler Peak population (DQB1-2*3, DRB3-1*2, DRB3-1*4). The rest of the MHC alleles were present in more than one population except for one low-frequency allele (DRB3-2*3, 0.042) found only in the Eagle population in the Mojave Desert.

Observed and expected overall heterozygosities for each of the MHC and MS loci were generally in close agreement (APPENDIX A). The mean observed heterozygosity for MHC loci over all samples was 0.325 and ranged from 0.075 to 0.512, while the observed heterozygosity for MS loci was higher (0.571) and ranged from 0.417 to 0.855 (Table 1). Three MHC locus-population combinations showed deviations of the observed from the expected heterozygosity at the 0.05 significance level (exact probabilities for a two-tailed test), while none of the MS had significant deviations. The three MHC combinations were DRB3-1 for Muddy (observed number of heterozygotes, 13; expected number of heterozygotes, 8.2, $P = 0.028$), Eagle for DQB1-2 (obs. # het., 3; exp. # het., 10.7, $P = 0.0012$), and Muddy for DRB3-3 (obs. # het., 3; exp. # het. 8.8, $P = 0.009$). When correction for multiple comparisons was made (58 tests for the MHC data), the level of significance at the table level using a Bonferroni correction (RICE 1989) was 0.00088, lower than the level of significance level for any of the cases. However, because there was linkage disequilibrium between these MHC loci and the associated MDRB3 MS locus (S. KALINOWSKI, P. W. HEDRICK, and W. M. BOYCE, unpublished results), it is not clear how many independent tests there are and how this would influence the level of significance. Therefore, we concluded that there was no evidence of significant deviation from Hardy-Weinberg proportions for any of the MS loci and no evidence for the MHC loci when multiple comparisons were taken into account.

Differences between populations and regions: We examined differentiation among populations and regions in several different ways. Mean F_{ST} values for the MHC and MS loci for different regional groupings of populations were very similar (Table 2). These values were highly significant ($P < 0.001$), indicating that there was substantial subdivision of genetic variability among the samples at all levels. Examination of the influence of sampling indicated that 49 and 55% of the observed F_{ST} values for MHC and MS, respectively, were explained

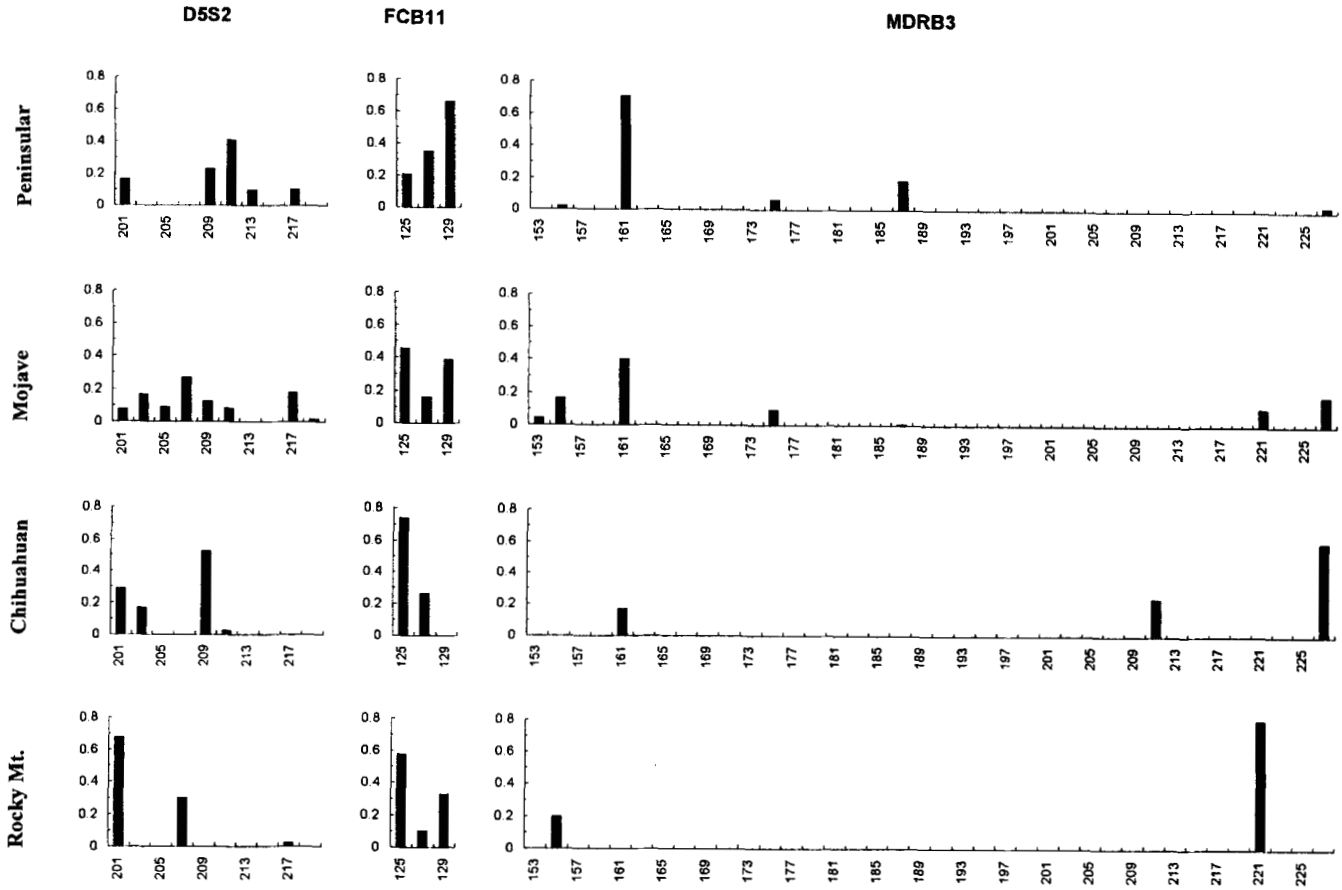

FIGURE 3.—Weighted (by sample size) allele frequencies at the three MS loci for bighorn sheep in four geographic regions as a function of allele sizes in base pairs.

by sampling alone for populations in the Peninsular populations (Table 2). For most of the remaining groups, <25% of the F_{ST} value was explained by the sampling effect. Genetic variability for both markers was predominantly distributed within, rather than among, populations for all regional groupings of populations. When all 14 populations were combined, ~28 and 25% of the maximum possible diversity occurred among populations for MHC and MS loci, respectively.

Evaluation of the equivalent genetic distance values D_1 and S_B indicated that the MS loci D5S2 and FCB11 appeared to be good dinucleotide repeat loci (Table 3). However, the values differed by almost an order of magnitude with D5S2 having much larger distance values than FCB11. As a result, the average values shown in Table 3 primarily reflect the values for only one locus, D5S2. We did not include MDRB3 in this analysis (Table 3) because it appears to be a complicated repeat in cattle and our fragment sizes were very similar to those in cattle (ELLEGREN *et al.* 1993). When we did calculate repeat size-based genetic distances (assuming a dinucleotide repeat) for this locus, values averaged ~400 for comparisons between populations in the Peninsular region and >400 for comparisons between populations in different regions. Since we obtained large differences in genetic distances over loci using these size based-distances, and we had a small number of MS loci, the genetic distances reported below (and used in UPGMA and NJ trees) are Nei's standard genetic distance (D) based on allele frequencies only.

Genetic distances were typically smaller for MHC loci than for MS loci for comparisons between populations (APPENDIX B) and between regions (Table 3). Distances for both markers were markedly higher for inter- than intraregional comparisons, with one exception. In this case, distance values for the comparison within the Mojave region and between the Mojave and Peninsular regions were relatively similar for MHC (0.150, 0.173) and MS (0.596, 0.616) loci. The two genetic measures were highly correlated (0.833) within the Peninsular region, while there was a nonsignificant negative correlation (−0.200) between MS and MHC distances in the Mojave (Table 4). Distances for the two markers were also significantly correlated whenever populations from two or more regions were combined.

The geographic distance between populations provides at least a partial explanation for the correlation between MS and MHC distances. Although there was a positive correlation between genetic distance (MS and MHC) and geographic distance in both the Peninsular and Mojave regions, the relationship did not become significant ($P < 0.05$) until these two regions were combined (Table 4). The strength of the relationship between genetic and geographic distance for both mark-

TABLE 1

Average number of alleles and observed and expected heterozygosity in 14 bighorn sheep populations for five MHC and three MS loci

Region Population	Sample size	Average no. of alleles		MHC heterozygosity		MS heterozygosity	
		MHC	MS	H_O	H_E	H_O	H_E
Peninsular ranges							
Carrizo	22	2.0	3.7	0.305	0.250	0.667	0.543
Vallecito	12	2.2	3.3	0.364	0.361	0.667	0.574
San Ysidro	22	2.6	4.0	0.380	0.304	0.470	0.507
Coyote	10	1.8	3.3	0.160	0.175	0.476	0.502
Santa Rosa	29	2.2	4.0	0.336	0.303	0.571	0.552
San Jacinto	9	2.2	3.0	0.328	0.390	0.630	0.636
Mean		2.2	3.7	0.324	0.294	0.587	0.549
Mojave Desert							
San Gorgonio	19	1.6	3.3	0.137	0.142	0.574	0.544
Eagle	25	2.8	5.0	0.512	0.573	0.855	0.766
Orocopia	13	1.8	3.7	0.292	0.329	0.718	0.629
Old Dad	23	2.4	3.3	0.384	0.384	0.507	0.533
Muddy	19	1.2	4.0	0.371	0.400	0.491	0.516
Mean		2.1	3.9	0.357	0.384	0.586	0.600
Chihuahuan Desert							
San Andres	8	1.6	2.3	0.075	0.195	0.444	0.359
Red Rock	18	1.6	2.7	0.242	0.286	0.622	0.544
Mean		1.6	2.6	0.187	0.256	0.628	0.498
Rocky Mountain							
Wheeler	23	2.4	2.7	0.361	0.422	0.417	0.454
Mean (all regions)		2.1	3.6	0.325	0.335	0.571	0.557

ers then increased as more populations, located further apart, were combined in the analysis (*i.e.*, 0.795 and 0.600 for all regions combined, $P < 0.001$).

Examination of genetic and geographic distances between adjacent populations clearly delineated similarities and differences in the patterns of MS and MHC variability (Figure 4). MHC genetic distances were fairly similar and relatively low for nearest-neighbor comparisons throughout the Peninsular and Mojave regions. MHC genetic distances then increased sharply and remained high (>0.5) for comparisons between populations >500 km apart. MS genetic distances were also fairly similar and relatively low within the Peninsular region, and values tended to increase with increasing

TABLE 2

F_{ST} values for MHC and MS loci among bighorn sheep populations in different regions

Locus	Peninsular	Mojave	Peninsular and Mojave	Peninsular, Mojave, Chihuahuan	All regions
MHC					
DQB1-1	0.143 (0.388)	0.213 (0.166)	0.198 (0.243)	0.223 (0.225)	0.223 (0.221)
DQB1-2	0.169 (0.351)	0.297 (0.165)	0.229 (0.263)	0.342 (0.186)	0.442 (0.143)
DRB3-1	0.088 (0.581)	0.129 (0.382)	0.194 (0.242)	0.187 (0.294)	0.272 (0.201)
DRB3-2	0.064 (0.739)	0.162 (0.324)	0.236 (0.246)	0.247 (0.243)	0.234 (0.261)
DRB3-3	0.134 (0.380)	0.134 (0.308)	0.221 (0.233)	0.251 (0.219)	0.235 (0.230)
Mean	0.120 (0.489)	0.187 (0.366)	0.216 (0.251)	0.250 (0.238)	0.281 (0.211)
MS					
MDRB3	0.140 (0.398)	0.182 (0.226)	0.224 (0.239)	0.267 (0.220)	0.324 (0.179)
D5S2	0.131 (0.420)	0.208 (0.197)	0.231 (0.232)	0.241 (0.242)	0.260 (0.223)
FCB11	0.067 (0.829)	0.266 (0.151)	0.226 (0.234)	0.263 (0.225)	0.253 (0.229)
Mean	0.113 (0.549)	0.219 (0.191)	0.227 (0.235)	0.257 (0.229)	0.251 (0.207)

Values in parentheses are the proportion of F_{ST} explained by sampling alone.

TABLE 3

Mean genetic distances (Nei's *D*) within and between regional groupings of bighorn sheep for MHC and MS loci

Loci	Region	Peninsular	Mojave	Chihuahuan
MHC (*D*)	Peninsular	0.084 (0.200)		
	Mojave	0.173 (0.176)	0.150 (0.162)	
	Chihuahuan	0.221 (0.169)	0.370 (0.191)	0.049 (0.559)
	Rocky Mountain	0.493 (0.040)	0.581 (0.051)	0.627 (0.049)
MS (*D*)	Peninsular	0.202 (0.258)		
	Mojave	0.616 (0.131)	0.596 (0.122)	
	Chihuahuan	1.283 (0.070)	0.834 (0.186)	0.197 (0.312)
	Rocky Mountain	1.258 (0.052)	0.839 (0.098)	0.946 (0.071)
MS (D_1, S_B)[a]	Peninsular	26.0 (47.5, 4.5)		
	Mojave	28.2 (50.0, 6.5)	32.0 (56.7, 7.2)	
	Chihuahuan	29.4 (49.9, 8.9)	28.2 (50.2, 6.2)	14.8 (28.1, 1.5)
	Rocky Mountain	39.5 (71.5, 7.5)	37.9 (69.0, 6.9)	18.5 (32.0, 5.1)

Proportion explained by sampling alone are in parentheses.

[a] The equivalent average genetic distances (D_1, S_B) for MS loci D5S2 and FCB11 are given, with the individual values for each locus in parentheses.

geographic distance across all regions. However, in contrast to the MHC pattern, large MS genetic distances were obtained for some nearest-neighbor comparisons (San Jacinto-San Gorgonio and Old Dad-Muddy) but not for others (Eagle-Orocopia).

We calculated genetic distance measures for each of the MS loci for the adjacent population comparisons shown in Figure 4. Nearly all of the effect for the San Jacinto-San Gorgonio comparison was due to locus D5S2, which had a very high genetic distance of 4.3. In fact, these two adjacent samples share only one low-frequency allele (D5S2*5), which had a frequency of 0.111 in San Jacinto and 0.056 in San Gorgonio. For the other high MS distance values (Figure 4, APPENDIX B), two, and usually all three, of the MS loci contributed to the observed genetic distances.

Tree analysis for the MS loci using UPGMA (Figure 5) and NJ (not shown) both clustered the six Peninsular samples together. In both trees, the Muddy sample from the Mojave region also clustered with these samples. By examining the allele frequencies from the different samples (APPENDIX A), this clustering appears to occur because the Muddy population had alleles in relatively high frequency at both MDRB3 (allele 2) and D5S2 (allele 3) that were not common in the other Mojave samples, and this population had an allele at high frequency at FCB11 (allele 3) that was also in high frequency in the Peninsular samples. The other four Mojave samples cluster together in the UPGMA tree but not the NJ tree. In both trees, the two Chihuahuan samples cluster together.

Analysis for the MHC loci using UPGMA (Figure 6) and NJ (not shown) indicated that the Wheeler population was differentiated from the other populations. Using both tree-building techniques, the Chihuahuan samples cluster together, but they also cluster with the Vallecito sample from the Peninsular region. By examining the allele frequencies from the different samples (APPENDIX A), this clustering appears to occur because the Vallecito sample had alleles at DQB1-1 (allele 3) and DQB1-2 (allele 2) that were closer in frequency to the Chihuahuan samples than the other Peninsular samples. Four of the Peninsular samples (Carrizo, Coyote, Santa Rosa, and San Ysidro) tightly cluster together in the UPGMA tree but not in the NJ tree.

TABLE 4

Correlation coefficients for genetic distances (*D*) between MHC loci, MS loci, and the geographic distances between regional groupings of bighorn sheep

	MHC-MS	MHC-geographic	MS-geographic
Peninsular	0.833**	0.375	0.421
Mojave	−0.200	0.448	0.537
Peninsular and Mojave	0.365*	0.423*	0.365*
Peninsular, Mojave, and Chihuahuan	0.511**	0.518**	0.680***
All regions	0.492***	0.795***	0.600***

Significance level determined using the Mantel test; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

DISCUSSION

Factors influencing genetic variation: Examining and comparing genetic variation for MHC and MS loci in the same individuals provides an opportunity to evaluate the neutral and selective forces influencing genetic variation. In this study, there did not appear to be any deviations from Hardy-Weinberg proportions for either of the two types of loci (Table 1, APPENDIX A). In some Amerindian populations, an excess of heterozygotes of 20–30% for MHC loci has been attributed to the effect of balancing selection (BLACK and SALZANO 1983; MARKOW *et al.* 1993). However, unlike the present study, in these other studies, there were no neutral loci evaluated in the same individu-

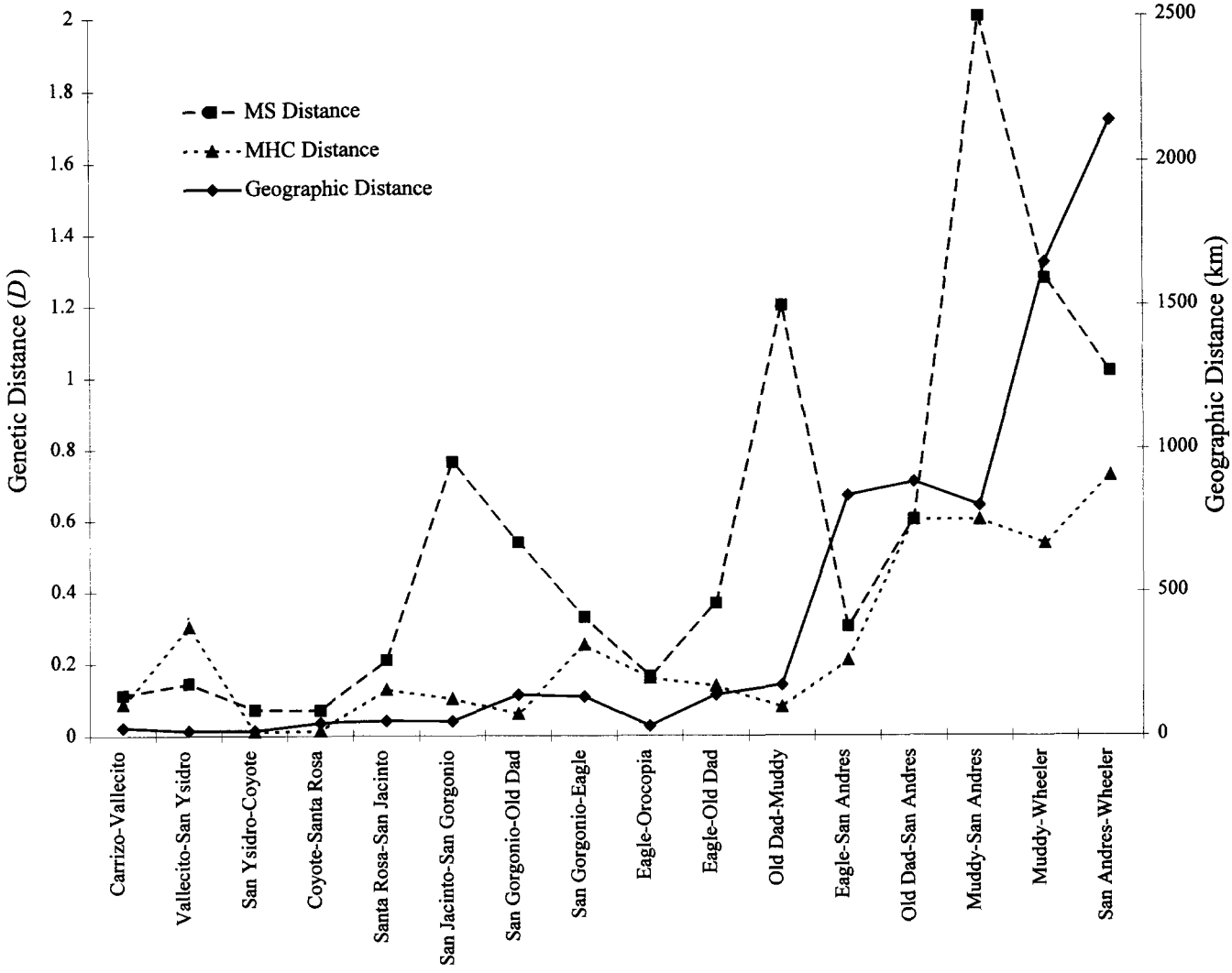

FIGURE 4.—Nei's standard genetic distance (D) between adjacent bighorn sheep populations for three MS and five MHC loci. Also given is the geographic distance (in kilometers) between the pairs of samples.

als to determine if other evolutionary factors may have contributed to the observed excess of heterozygotes over expectations. If balancing selection acted on the bighorn sheep examined here, this effect appears to be small enough that other evolutionary factors have masked it in our analysis, or it acted in such a way as to not increase the number of heterozygotes over Hardy-Weinberg expectations. The presence of small population sizes and genetic drift, as well as the influence of sample size, may make it difficult to determine the effects of selection from genotypic proportions. However, if the selective difference between heterozygotes and homozygotes was as high in bighorn sheep as has been observed in some Amerindian groups, then these differences should have been easily detected with the given sample sizes and population structure in our study.

Variation at MHC and MS loci should be influenced by nonselective forces, but if the selective factors (*i.e.*, infectious diseases) acting on MHC have been of significantly greater magnitude than the nonselective factors, then the extent and patterns of variation for MHC and MS loci may differ greatly. There was a significant correlation between genetic distance and geographic distance for both types of loci when populations were examined across geographic regions (Table 4). Furthermore, mean F_{ST} values were quite similar for both markers for comparisons within and across regions (Table 2). These results indicated that neutral forces such as drift and gene flow substantially influenced differentiation of both MS and MHC loci.

On the other hand, the pattern of variation differed for the two markers. Examination of genetic distances between adjacent populations (Figure 4) showed that MS distances were often much higher than MHC distances, regardless of the geographic distance between the populations (*e.g.*, San Jacinto-San Gorgonio, Old Dad-Muddy, and Muddy-San Andres). These observations may be a function of high mutation rates for MS coupled with relatively low rates of gene flow between some populations (SCRIBNER *et al.* 1994). Alternatively, the uniformly low MHC genetic distances across the Peninsular and Mojave regions (Figure 4) suggest that similar selection pressures (*e.g.*, disease)

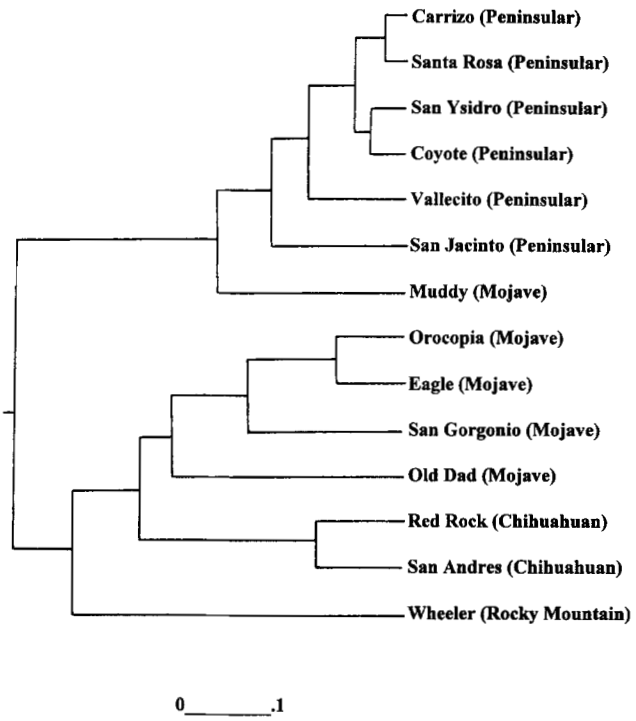

FIGURE 5.—Tree topology for the three MS loci using UPGMA and Nei's standard genetic distance (D).

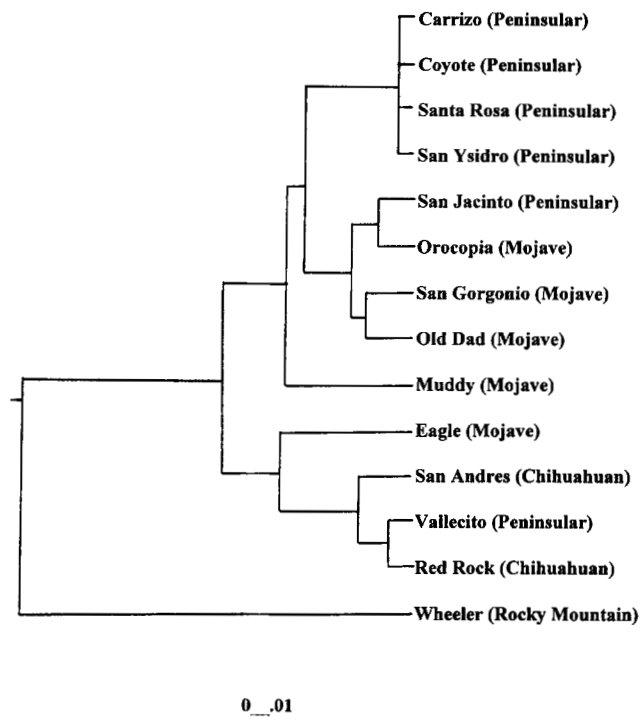

FIGURE 6.—Tree topology for the five MHC loci using UPGMA and Nei's standard genetic distance (D).

may have occurred across all of these populations. The larger MHC distances seen for most of the comparisons between populations >500 km apart (*e.g.*, Old Dad-San Andres and Muddy-Wheeler) were quite similar and may have resulted from a combination of similar selective and nonselective (*e.g.*, drift) pressures. There is some support for this interpretation since all of the bighorn populations in this study have been exposed to a variety of potentially virulent pathogens (ELLIOTT *et al.* 1994; W. M. BOYCE, unpublished data). A detailed analysis of disease/genotype associations for the animals examined in this study is under way (W. M. BOYCE, unpublished results).

Given the well-documented importance of endemic and epidemic disease on bighorn sheep (BEUCHNER 1960; OLDT 1992; JESSUP and BOYCE 1996), we anticipated that we might find stronger evidence than we did for selection acting on the MHC. There are relatively few documented examples of a strong relationship between disease resistance and MHC variation, and HEDRICK and KIM (1997) outlined a number of reasons why it may be difficult to demonstrate the effects of selection. Two of the many factors that may have limited our ability to detect selection include small sample sizes and the extent of stochastic factors operating on bighorn sheep populations.

Conservation genetics: Our analyses revealed relatively high levels of genetic variability for both MHC and MS markers in terms of number of alleles and observed heterozygosities, indicating that there is a large reservoir of previously undescribed nuclear DNA variation in bighorn populations across the southwestern United States (Table 1). These results are in apparent contrast with RAMEY (1995) and JESSUP and RAMEY (1995), who found low overall mtDNA nucleotide diversity and low heterozygosities for allozymes, respectively, in bighorn sheep across the same region. These differences may partly occur because different genetic markers can provide varying degrees of resolution, are subject to different rates of mutation, and are likely to be affected by different evolutionary processes. For example, because mtDNA is maternally inherited and is haploid, the effective population size causing genetic drift is half the female effective population size, a value that may be about one-quarter that for nuclear genes. Furthermore, similarity of mtDNA sequences does not necessarily imply that there are not significant genetic differences for other markers (DOWLING *et al.* 1992). For example, HEDRICK and PARKER (unpublished results) found substantial MHC differences among samples of the endangered Gila topminnow which all appeared to have the same mtDNA haplotype (QUATTRO *et al.* 1996).

Our results are consistent with a metapopulation structure for the six populations in the Peninsular Ranges. Within this region, mean F_{ST} and genetic distance values were relatively small for MS (F_{ST} = 0.113, D = 0.202) and MHC (F_{ST} = 0.120, D = 0.084) loci (Tables 2 and 3), indicating that these populations formed a discrete group with relatively high gene flow between them. On the other hand, the average MS genetic distance between the Peninsular populations and the three nearby Mojave populations (San Gorgonio, Eagle, and Orocopia) was 0.627, more than three times the distance within the Peninsular region. In addition, the MS distance between the two adjacent popula-

tions (San Jacinto, San Gorgonio) at the Peninsular-Mojave boundary was quite large (0.765, Figure 4). At locus D5S2, these two populations shared only one low frequency allele and ~90% of the alleles in each population were not found in the other. These results indicated that there was relatively low gene flow between the Peninsular metapopulation and nearby Mojave populations, a view that is also supported by tree analysis for the MS markers (Figure 5).

Our results are also consistent with a metapopulation structure for populations in the Mojave. For example, based on genetic distances and F_{ST} values, the San Gorgonio, Eagle, and Orocopia populations appeared to belong to one metapopulation. In contrast, the Old Dad and Muddy populations, located further to the north, appeared to be somewhat differentiated from the three southern populations (Figures 1 and 4). However, our sampling of populations in the Mojave region was quite limited relative to the large number of populations within this region. BLEICH *et al.* (1990, 1996) provided a cogent discussion of metapopulation theory relative to bighorn sheep in the Mojave region, and it appears that many more populations would need to be sampled to accurately evaluate the genetic structure of populations across this broad area.

The MS and MHC genetic distances were highly correlated (0.833, $P < 0.01$) within the Peninsular region (Table 4). This suggests that evolutionary factors that tie these populations together, such as gene flow or extinction-recolonization dynamics, override any effects of differential selection on MHC variation among them. In contrast, the correlation of MHC and MS genetic distances within the Mojave region was low (−0.200), suggesting that the two sets of genes were influenced by different evolutionary factors over these samples. Interpopulation migration rates between our Mojave samples were undoubtedly lower than migration rates in the Peninsular region since the geographic distances between populations are much larger in the Mojave (Figures 1 and 4). Therefore, low migration rates coupled with high mutation rates for MS could partially account for the lack of MS and MHC correlation in the Mojave. An alternative explanation is that the pattern of MS variation in the Mojave region was dominated by the cumulative effects of genetic drift and extinction-recolonization dynamics while uniform selection was important for the MHC genes.

The regional groupings that we used in our analyses closely approximate the subspecies (Peninsular, *O. c. cremnobates;* Mojave, *O. c. nelsoni;* Chihuahuan, *O. c. mexicana;* and Rocky Mountain, *O. c. canadensis*) recognized by COWAN (1940). WEHAUSEN and RAMEY (1993) and RAMEY (1995) challenged the validity of these subspecies designations based on morphometric and mtDNA analyses and suggested that the desert subspecies *O. c. nelsoni* and *O. c. cremnobates* should be recognized as a single polytypic subspecies (*O. c. nelsoni*). Although our study was not designed to address taxonomic questions, our results using nuclear markers are consistent with the interpretation that genetic variation within desert bighorn sheep is largely apportioned within populations (or metapopulations) rather than among the putative subspecies (Tables 2 and 3). Although MS and MHC genetic distances were correlated with the geographic distances between populations (Table 4), tree topologies were not strictly concordant with regional (subspecies) groupings (Figures 4 and 5). For example, the Muddy (*O. c. nelsoni*) population clustered with the Peninsular (*O. c. cremnobates*) populations (metapopulation) rather than adjacent Mojave (*O. c. nelsoni*) populations in MS UPGMA (Figure 4) and NJ trees. In contrast, the Wheeler population appeared to be a clear outgroup relative to the desert populations based on differentiation of both MS and MHC loci.

Other considerations: Nei's D and F_{ST} values for our three MS loci were very similar to those reported by FORBES *et al.* (1995) for eight MS loci in five populations of Rocky Mountain bighorn sheep. These results suggest that our small number of loci may have provided reasonable estimates of genetic distances across the study area. Since allele-size-based methods (*e.g.*, D_1) are more sensitive than frequency-based methods (Nei's D) to distant historical separations between populations, FORBES *et al.* (1995) suggested that both methods should be used to maximize sensitivity to both between-population and between-species differences. Calculations of the size-based genetic distance values for our MS loci demonstrated two potential problems in using these distance measures. First, one of our loci (MDRB3) had a very wide range in fragment size. Fortunately, ELLEGREN *et al.* (1993) had previously sequenced alleles at this locus in cattle (these alleles are very similar in size to the our alleles in bighorn sheep) and found that it was not a simple dinucleotide repeat. If we did not have this information and used this locus in a size-based distance measure, then it would have contributed nearly all of the genetic distance. Second, when we calculated the size-based distance for the other two MS loci, both of which appeared to be good dinucleotide repeats, the value for one locus was nearly an order of magnitude higher than the other locus. Of course, when these are averaged, then the locus with the much lower value contributes very little to the genetic distance. In other words, even within good repeat loci, size-based methods may be unduly influenced by one or only a few loci.

The five class II MHC loci that we examined are assumed to be closely linked in bighorn sheep as they are in humans (TROWSDALE 1993) and other mammals. As a result, the alleles at different loci are expected to be statistically associated, *i.e.*, in gametic (linkage) disequilibrium. Additional analysis has indicated that the five MHC loci and the linked MS locus (MDRB3) show extensive pairwise disequilibrium. Because the estimation of linkage disequilibrium is quite involved when there are multiple alleles involved, the approaches, results, and discussion of the impact of linkage disequilibrium will be presented separately.

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APPENDIX A

Allele frequencies and heterozygosity for five MHC (DQB1-1, DQB1-2, DRB3-1, DRB3-2, DRB3-3) and three microsatellite (MDRB3, D5S2, FCB11) loci in 14 populations of bighorn sheep

Locus and allele[a]	Population													
	Carrizo	Vallecito	San Ysidro	Coyote	Santa Rosa	San Jacinto	San Gorgonio	Eagle	Orocopia	Old Dad	Muddy	San Andres	Red Rock	Wheeler Peak
DQB1-1														
1 (9.2)	0.762	0.409	0.750	0.900	0.679	0.375	0.921	0.208	0.346	0.682	0.773	0.188	0.533	0.659
2 (5.2)	0.238	0.545	0.091	0.100	0.250	0.125	0.000	0.354	0.000	0.068	0.045	0.813	0.467	0.000
3 (6.5, 4.7)	0.000	0.000	0.091	0.000	0.036	0.500	0.079	0.292	0.269	0.250	0.000	0.000	0.000	0.000
4 (4.7)	0.000	0.045	0.068	0.000	0.036	0.000	0.000	0.146	0.385	0.000	0.182	0.000	0.000	0.341
H_o	0.476	0.545	0.500	0.200	0.500	0.500	0.053	0.667	0.538	0.364	0.091	0.125	0.533	0.318
H_E	0.363	0.543	0.416	0.180	0.474	0.594	0.145	0.725	0.660	0.468	0.368	0.305	0.498	0.449
DQB1-2														
1 (8.5)	0.833	0.409	0.938	0.900	0.750	0.875	1.000	0.548	1.000	0.950	0.964	0.188	0.269	0.200
2 (7.0)	0.167	0.591	0.063	0.100	0.250	0.125	0.000	0.452	0.000	0.050	0.036	0.813	0.731	0.000
3 (4.0)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.800
H_O	0.333	0.455	0.125	0.200	0.357	0.250	0.000	0.143	0.000	0.100	0.071	0.125	0.231	0.200
H_E	0.278	0.483	0.117	0.180	0.375	0.219	0.000	0.495	0.000	0.095	0.069	0.305	0.393	0.320
DRB3-1														
1 (6.0)	0.405	0.364	0.341	0.250	0.268	0.611	0.974	0.583	0.692	0.659	0.531	0.813	0.556	0.024
2 (4.0)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.476
3 (3.5)	0.595	0.636	0.659	0.750	0.732	0.389	0.026	0.417	0.308	0.341	0.469	0.188	0.444	0.000
4 (1.9)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.500
H_O	0.619	0.545	0.682	0.300	0.536	0.556	0.053	0.583	0.462	0.500	0.813	0.125	0.444	0.571
H_E	0.482	0.463	0.449	0.375	0.392	0.475	0.051	0.486	0.426	0.449	0.498	0.305	0.494	0.523
DRB3-2														
1 (4.3)	0.024	0.000	0.068	0.000	0.000	0.000	0.000	0.250	0.000	0.182	0.471	0.000	0.000	0.190
2 (3.3)	0.976	1.000	0.932	1.000	1.000	1.000	1.000	0.708	1.000	0.818	0.529	1.000	1.000	0.810
3 (3.1)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.042	0.000	0.000	0.000	0.000	0.000	0.000
H_O	0.048	0.000	0.136	0.000	0.000	0.000	0.000	0.375	0.000	0.364	0.706	0.000	0.000	0.286
H_E	0.046	0.000	0.127	0.000	0.000	0.000	0.000	0.434	0.000	0.298	0.498	0.000	0.000	0.308
DRB3-3														
1 (9.6)	0.000	0.045	0.159	0.050	0.143	0.444	0.553	0.313	0.538	0.227	0.000	0.000	0.000	0.071
2 (2.9)	0.976	0.864	0.773	0.950	0.857	0.500	0.447	0.375	0.462	0.591	0.500	1.000	1.000	0.690
3 (2.7)	0.024	0.091	0.068	0.000	0.000	0.056	0.000	0.313	0.000	0.182	0.500	0.000	0.000	0.238
H_O	0.048	0.273	0.455	0.100	0.286	0.333	0.579	0.792	0.462	0.591	0.176	0.000	0.000	0.429
H_E	0.046	0.244	0.373	0.095	0.245	0.549	0.494	0.664	0.497	0.566	0.500	0.000	0.000	0.461

APPENDIX A

Allele frequencies and heterozygosity for five MHC (DQB1-1, DQB1-2, DRB3-1, DRB3-2, DRB3-3) and three microsatellite (MDRB3, D5S2, FCB11) loci in 14 populations of bighorn sheep

Locus and allele[a]	Population													
	Carrizo	Vallecito	San Ysidro	Coyote	Santa Rosa	San Jacinto	San Gorgonio	Eagle	Orocopia	Old Dad	Muddy	San Andres	Red Rock	Wheeler Peak
MDRB3														
1 (153)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.174	0.000	0.000	0.000	0.000	0.000	0.000
2 (155)	0.023	0.000	0.068	0.000	0.000	0.000	0.000	0.065	0.000	0.239	0.474	0.000	0.000	0.200
3 (161)	0.773	0.450	0.773	0.857	0.732	0.444	0.444	0.239	0.346	0.543	0.421	0.000	0.233	0.000
4 (175)	0.000	0.000	0.045	0.000	0.036	0.444	0.056	0.087	0.077	0.217	0.000	0.000	0.000	0.000
5 (187)	0.205	0.550	0.023	0.143	0.196	0.111	0.000	0.000	0.000	0.000	0.026	0.000	0.000	0.000
6 (211)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.333	0.000
7 (221)	0.000	0.000	0.000	0.000	0.000	0.000	0.472	0.087	0.000	0.000	0.000	0.000	0.000	0.800
8 (227)	0.000	0.000	0.091	0.000	0.036	0.000	0.028	0.348	0.577	0.000	0.079	1.000	0.433	0.000
H_O	0.455	0.500	0.455	0.286	0.393	0.778	0.667	0.870	0.615	0.435	0.737	0.000	0.600	0.300
H_E	0.361	0.495	0.387	0.245	0.423	0.568	0.576	0.772	0.541	0.600	0.591	0.000	0.647	0.320
D5S2														
1 (201)	0.045	0.050	0.000	0.357	0.250	0.556	0.000	0.130	0.038	0.152	0.000	0.250	0.300	0.675
2 (203)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.152	0.077	0.500	0.000	0.333	0.100	0.000
3 (205)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.038	0.000	0.421	0.000	0.000	0.000
4 (207)	0.000	0.000	0.000	0.000	0.000	0.000	0.639	0.196	0.308	0.152	0.132	0.000	0.000	0.300
5 (209)	0.159	0.050	0.432	0.357	0.196	0.111	0.056	0.196	0.000	0.000	0.342	0.333	0.600	0.000
6 (211)	0.568	0.600	0.273	0.071	0.429	0.333	0.000	0.130	0.000	0.196	0.026	0.083	0.000	0.000
7 (213)	0.091	0.100	0.227	0.143	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
8 (217)	0.136	0.200	0.068	0.071	0.107	0.000	0.306	0.196	0.538	0.000	0.000	0.000	0.000	0.025
9 (219)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.079	0.000	0.000	0.000
H_O	0.727	0.800	0.682	0.857	0.821	0.778	0.611	0.913	0.692	0.739	0.632	1.000	0.667	0.450
H_E	0.623	0.585	0.683	0.714	0.703	0.568	0.495	0.828	0.607	0.665	0.681	0.708	0.540	0.454
FCB11														
1 (125)	0.159	0.250	0.045	0.071	0.071	0.333	0.556	0.435	0.308	0.826	0.026	0.833	0.700	0.575
2 (127)	0.341	0.150	0.227	0.214	0.286	0.222	0.028	0.239	0.346	0.130	0.105	0.167	0.300	0.100
3 (129)	0.500	0.600	0.727	0.714	0.643	0.444	0.417	0.326	0.346	0.043	0.868	0.000	0.000	0.325
H_O	0.818	0.700	0.273	0.286	0.500	0.667	0.444	0.783	0.846	0.348	0.105	0.333	0.600	0.500
H_E	0.608	0.555	0.417	0.439	0.500	0.642	0.517	0.647	0.666	0.299	0.234	0.278	0.420	0.554

[a] Allele size in kilobases (MHC loci) or base pairs (MS loci).

APPENDIX B

Genetic distances (*D*) between populations of bighorn sheep determined from five MHC and three MS loci

Population	Carrizo	Vallecito	San Ysidro	Coyote	Santa Rosa	San Jacinto	San Gorgonio	Eagle	Orocopia	Old Dad	Muddy	San Andres	Red Rock	Wheeler Peak
Carrizo		0.087	0.018	0.011	0.013	0.139	0.185	0.213	0.186	0.075	0.150	0.264	0.112	0.438
Vallecito	0.113		0.301	0.132	0.065	0.221	0.381	0.141	0.316	0.228	0.337	0.087	0.023	0.541
San Ysidro	0.112	0.145		0.011	0.020	0.098	0.150	0.199	0.125	0.047	0.111	0.398	0.197	0.454
Coyote	0.168	0.342	0.072		0.014	0.161	0.207	0.274	0.203	0.092	0.156	0.390	0.173	0.443
Santa Rosa	0.038	0.143	0.085	0.071		0.128	0.221	0.186	0.181	0.100	0.189	0.272	0.109	0.460
San Jacinto	0.313	0.376	0.409	0.279	0.210		0.102	0.104	0.042	0.051	0.237	0.360	0.264	0.622
San Gorgonio	0.700	0.748	0.697	0.675	0.706	0.765		0.252	0.084	0.060	0.206	0.477	0.357	0.582
Eagle	0.478	0.555	0.466	0.509	0.451	0.417	0.329		0.159	0.137	0.217	0.208	0.183	0.680
Orocopia	0.638	0.749	0.643	0.689	0.619	0.787	0.409	0.164		0.076	0.229	0.471	0.366	0.598
Old Dad	0.652	0.812	0.901	0.844	0.768	0.480	0.538	0.367	0.760		0.078	0.393	0.251	0.510
Muddy	0.494	0.604	0.244	0.311	0.374	0.714	0.771	0.612	0.808	1.201		0.601	0.391	0.534
San Andres	1.721	1.669	1.655	1.758	1.691	1.097	1.188	0.303	0.512	0.602	2.000		0.049	0.724
Red Rock	1.024	1.316	0.920	0.854	0.988	0.707	0.943	0.305	0.653	0.521	1.314	0.197		0.530
Wheeler	1.547	1.348	1.666	1.114	1.214	0.657	0.396	0.602	1.128	0.797	1.274	1.016	0.877	

MHC loci are above diagonal and MS loci below diagonal.