Journal of Mammalogy, 86(4):000–000, 2005

POPULATION GENETIC ANALYSIS OF SONORAN PRONGHORN *(ANTILOCAPRA AMERICANA SONORIENSIS)*

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The endangered Sonoran pronghorn (*Antilocapra americana sonoriensis*) consists of only 2 small populations, 1 in Arizona and 1 in Mexico. Mitochondrial DNA sequence data and 5 microsatellite loci were used to compare levels of genetic diversity and differentiation between the 2 Sonoran pronghorn populations and between this subspecies and other selected pronghorn populations, both proximate and distant to the Sonoran pronghorn's current range. The data support a history of recent isolation of Sonoran pronghorn populations from those in Arizona, New Mexico, and Texas. Loss of genetic diversity due to bottlenecks and drift has been severe in both populations of the Sonoran pronghorn, but is most pronounced in the Arizona population. These results are discussed in terms of conservation management strategies.

Key words: *Antilocapra*, microsatellites, mitochondrial DNA, phylogeography, population genetics, pronghorn antelope, subspecies

Four extant subspecies of pronghorns inhabit the open plains of western North America (Lee 1992; Lee et al. 1994). The American pronghorn (*Antilocapra americana americana*) occupies the greatest range in North America, extending southward from Canada into northern Arizona, northern New Mexico, and northern Texas. The Mexican pronghorn (*A. a. mexicana*) is thought historically to have occurred throughout northeastern Mexico northward into southeastern Arizona, southern New Mexico, and the Big Bend region of western Texas. The Peninsular pronghorn (*A. a. peninsularis*) is limited to the Baja peninsula of Mexico. Finally, the Sonoran pronghorn (*A. a. sonoriensis*) once occurred throughout southwestern Arizona, extreme southeastern California, northeastern Baja California, and northwestern Sonora, Mexico (Arizona Game and Fish Department 1981) and is now limited to 2 small populations. These subspecific delineations have had an effect on both management and conservation efforts for the North American pronghorn.

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In 1967, the Sonoran pronghorn subspecies was listed as Endangered by the United States Fish and Wildlife Service, after having severely declined in number and geographic range because of overgrazing, agricultural development, human development, and poaching (Arizona Game and Fish Department 1981). Legal harvest of Sonoran pronghorns in both the United States and Mexico has been restricted for at least 80 years. A Sonoran Pronghorn Recovery Plan was adopted in 1982, and by 1988 the Cabeza Prieta National Wildlife Refuge in Arizona was designated as the lead office for recovery efforts. Currently, there are 2 populations of the Sonoran subspecies, both of which are legally protected. One occupies a small portion of the Sonoran Desert in southwestern Arizona and the other occurs from southwestern Arizona southward through Sonora, Mexico, to the Gulf of California (United States Fish and Wildlife Service 1998). These populations currently are unable to exchange migrants because of the fencing at the border of the United States and Mexico. In 2000, survey data indicated that there were 99 Sonoran pronghorns north of the United States–Mexico boundary (Bright et al. 2001). However, recent drought conditions have caused high adult mortality, and it is likely that little, if any, recruitment of fawns occurred in 2002, resulting in a further reduction in the population (J. L. Bright, pers. comm.). The Sonoran pronghorn

population in Mexico also has been reduced and recently was estimated at approximately 300 individuals (United States Fish and Wildlife Service 1998). Both populations of the Sonoran pronghorn currently occupy an extremely harsh desert habitat, with high temperatures, little moisture, and minimal forage (Carr 1981). Consequently, there is a high potential that this subspecies persists under strong environmental selection and constitutes a very unique component of the species.

Conservation efforts for the Sonoran pronghorn are shadowed by uncertainty surrounding the distinctness of this subspecies. Ideally, the characterization of a subspecies would employ rigorously collected sets of data (ideally from multiple, independent sources) to evaluate the characteristics of a population that render it distinct from its conspecifics (Cronin 1993). Unfortunately, few such data are available with which to evaluate the uniqueness of the Sonoran subspecies. In the original subspecies description, Goldman (1945: 3) believed that subspecific status was warranted because of ''smaller size; paler color; and a smaller skull that differed in detail.'' However, this designation of the Sonoran subspecies was based on only 2 specimens, both collected long before Goldman (1945) examined them. Unfortunately, additional morphometric examinations of skulls from the range of the Sonoran pronghorn (the 2 from Goldman and 4 others) by Paradiso and Nowak (1971) and Hoffmeister (1986) failed to clarify the taxonomic status of pronghorns from this region. Thus, morphological evidence underpinning the designation of the Sonoran pronghorn as a distinguishable subspecies is weak at best.

Recent genetic analysis of mitochondrial DNA (mtDNA) sequence data also called the taxonomic status of the Sonoran pronghorn into question (Lou 1998). He evaluated genetic variation in pronghorns throughout much of their geographic range (196 pronghorns in 14 populations), including a sample of 9 Sonoran pronghorns from Arizona, by using a 282-base-pair segment of the mtDNA control region. Two genetically differentiated groups of pronghorn populations were recovered; a northern group (represented by samples from Colorado, Kansas, Montana, Idaho, Wyoming, and Oregon) and a southern group (comprised of samples of *A. a. sonoriensis* from Texas and southwestern Arizona). A north–south differentiation of pronghorn populations also was supported in an earlier study that used allozyme and mtDNA fragment data (Lee et al. 1994). The study of Lou (1998) did not provide support for a taxonomic distinction between Sonoran pronghorns and those sampled from Texas. However, that study remained inconclusive because of small sample sizes in the pertinent populations and it lacked samples of *A. a. americana* from Arizona and samples of *A. a. sonoriensis* from Mexico.

The question of which approach is best for identifying and characterizing units for conservation is an area of healthy debate (Crandall et al. 2000; King and Burke 1999; Moritz 2002). However, it generally is agreed that it is important and desirable to conserve evolutionarily distinct lineages, the unique diversity therein, and the potential to respond to future changes in selection pressures (Crandall et al. 2000; Grady and Quattro 1999; Ling et al. 2001; Paetkau 1999; Taylor and Dizon 1999; Waples 1998). Subspecific nomenclature historically has been used to sort and classify groups (or individuals in some cases) that were in some way morphologically, geographically, or ecologically variant (Mayr 1982; Smith et al. 1997). By definition, subspecies are not evolutionary independent entities, yet the subspecific designation has a valid and important role in systematics when applied judiciously (Smith et al. 1997). Unfortunately, the uneven application of subspecific designations often makes it difficult to depend upon this nomenclature in prioritizing conservation efforts and making management decisions (Ball and Avise 1992; Moritz 2002). Additionally, such designations can mask important components of a species' evolutionary history because the nomenclature is often discordant with historical population structure (Birungi and Arctander 2000; Burbrink et al. 2000; Cronin 1993; Eizirik et al. 2001; Rhymer et al. 2001). ?2

Unfortunately, as debates over subspecific nomenclature and appropriate definitions of intraspecific conservation units continue, many populations continue to plummet in number. Past morphological analyses of the Sonoran pronghorn have not been accomplished in the rigorous manner demanded by current taxonomic practice, and recent genetic analyses of this subspecies have not addressed the question of population distinctness in a comprehensive manner. Therefore, lack of diagnosable characters unique to *A. a. sonoriensis* may be due to the paucity of attention directed at this question. Additionally, the dynamics between the larger Sonoran pronghorn population in Mexico and its smaller counterpart in Arizona are wholly unexplored. Ideally, the taxonomic validity of the Sonoran pronghorn will be resolved by examining the combination of a suite of molecular markers, ecological data, and more sophisticated morphological analyses.

Toward this goal, our objective was to clarify the genetic distinctness of the Sonoran pronghorn by comparing levels of genetic diversity and differentiation between this subspecies and selected pronghorn populations, both proximate and distant to the Sonoran pronghorn's current range. In addition, we compared levels of genetic diversity within and among the 2 remaining populations of the Sonoran subspecies. By using this information, we evaluate the distinctness of the Sonoran pronghorn subspecies and its characterization as an important unit of conservation within the North American pronghorn species complex.

Materials and Methods

Sampled populations.—Samples from Sonoran pronghorn populations in Arizona and Mexico as well as samples from key populations distributed throughout the southern, central, and northern portion of the pronghorn's range were used in this study. As far as records show, the Sonoran pronghorn populations have never received translocated animals. Non-Sonoran samples included populations from the central and eastern Arizona regions; Brewster County, Texas; Yellowstone National Park, Wyoming; and Oregon. All populations sampled are reported to have received few if any translocated animals. Within the central and eastern Arizona regions we acquired samples from 24 designated game management units (as defined by the Arizona Game and Fish Department). However, only a limited number of samples

TABLE 1.—Localities, collection dates, tissue, and collectors of tissues of *Antilocapra americana* included in analyses.

Population	Localities	Year	Tissue	Collector
Sonoran (Arizona)	Arizona game management units 40b, 40be, 40bw, 43a, 46a, and 46b	1998	Blood, ear punch	Arizona Game and Fish Department
Sonoran (Mexico)	Northwestern Sonora, Mexico	1996, 2000	Blood, ear punch	Arizona Game and Fish Department
Arizona central	Arizona game management units 5a, 5b, 6a, 6b, 5bn, 7, 8, 9, 10, 17, 17a, 19a1, 19an, 19b, and 21	1996, 1997	Liver, muscle	Erin Reat, Arizona Game and Fish Department
Arizona eastern	Arizona game management units 1, 2a, 2b, 2c, 3a, 3b, 3c, 4a, and 4b	1996, 1997	Liver, muscle	Erin Reat, Arizona Game and Fish Department
Texas	Brewster County	1987	Liver, muscle	Lee et al. (1994)
Oregon	Crook County, Deschutes County, and Lake County	2000, 2001	Liver, muscle	Oregon Department of Fish and Wildlife
Wyoming	Yellowstone National Park	1988	Liver, muscle	Lee et al. (1994)

were available from most game management units. Dates and localities of samples used in this study are detailed in Table 1. All capture and handling methods were consistent with animal care and use guidelines of the American Society of Mammalogists (Animal Care and Use Committee 1998).

Analytical groupings within the central and eastern Arizona regions.—In an effort to make valid population-level comparisons among diversity measures (i.e., number of alleles or haplotypes per locus, haplotypic and allelic diversity, expected heterozygosity, and so on) we report the results of 4 Arizona populations within the central and eastern regions. Groupings were based on geographic proximity and evidence of genetic homogeneity from allele frequency comparisons as follows: Arizona central population 1 (game management units 8, 6, and 19a), Arizona central population 2 (game management unit 7), Arizona eastern population 1 (game management units 1, 2a, and 2b), and Arizona eastern population 2 (game management unit 2c). Conversely, analyses of population differentiation (i.e., G_{ST}, F_{ST}, and assignment tests) were executed by using the overall pooled samples from each of the central and eastern Arizona regions. Fig. 1 is a detailed map of all sample localities and analytical groupings.

DNA extraction.—Tissues were kept in field storage buffer (100 mM Tris, 100 mM Na_2ethylenediaminetetraacetic acid at pH 8, 10 mM NaCl, 1% sodium dodecyl sulfate) at ambient temperature until they could be stored at −80°C. DNA was isolated from all samples by using either standard phenol extraction (Sambrook et al. 1989) or by means of Qiagen's Qiamp DNA extraction kit (Qiagen Inc., Valencia, California).

Mitochondrial DNA sequencing and analysis.—Polymerase chain reaction (PCR) was used to amplify approximately 550 bases of the control region of the mtDNA. Primers used to amplify the target region were LGL283 (5′ TACACTGGTCTTGTAACC 3′) and LGL1115 (5′ ATAGACCCTGAAGAA(A/G)GAACCAG 3′), originally used in *Antilocapra* by Lou (1998). PCR conditions were as follows: 96°C (2min.) then 35 cycles of 96°C (30 s), 52°C (20 s), 72°C (2 min 30 s), with a final 10-min extension of 72°C. PCR products were cleaned by using a QIAquick PCR purification kit (Qiagen Inc.). Cycle sequencing was performed in the forward and reverse direction by using a Big Dye Ready Reaction kit (Applied Biosystems, Foster City, California) following the manufacturer's standard protocol and products were cleaned by using ethanol precipitation. Sequencing products were analyzed with an ABI9600 DNA sequencer (Applied Biosystems), aligned against a published pronghorn sequence (Genbank accession number AJ235314), and edited in Sequencher version 4.1 (Gene Code Corp., Ann Arbor, Michigan). Haplotypes were submitted to GenBank under accession numbers AY856385–AY856410. Additionally, 12 haplotypes from a concurrent study in our laboratory were included in all analyses (GenBank accession numbers AY786159–AY786163, AY786166–AY786170, AY786172, and AY786173—Stephen et al., in press).

Phylogenetic analysis of the mtDNA sequence data utilized PAUP* version 4.0b2a (Swofford 2000). Default search parameters were used unless otherwise specified. The data set was analyzed under maximum parsimony criteria and each site was considered an unordered character with 5 possible states (1 of 4 nucleotides or a gap). The weights of all characters were set equally to 1, excepting positions 333 through 337, which were each downweighted to 1/5, because these positions involved insertions or deletions (indels). In this way, indels were not ignored, but the various-length indels were not allowed to outweigh a single nucleotide change at other sites in the sequence, thereby avoiding artificial grouping of unrelated individuals. Because the phylogenetic relationships among the ruminants are unclear, 2 outgroups, each representing different ruminant families, were used in the analyses; sequence data from *Bos* (Bovidae) and *Capreolus* (Cervidae) were obtained from GenBank (accession numbers AB079365 and Z70318, respectively).

Parsimony trees were generated by using heuristic search routines employing 1,000 random addition sequences, swapping on multiple trees (MULPARS option), and using tree-bisection-reconnection branch swapping. Support for nodes was assessed by using

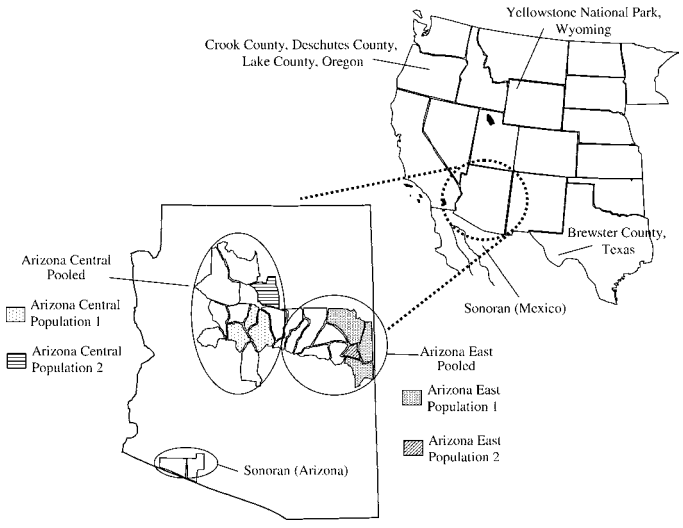

FIG. 1.—Sample localities of *Antilocapra americana* in the United States and Mexico. Heirarchically defined regions within Arizona are delineated on the insert.

TABLE 2.—Primer sets, annealing temperatures (T_a), and allele size ranges for microsatellite loci used in the genetic analysis of populations of American pronghorns (*Antilocapra americana*).

	Primer set	T_a	Size range (base pairs)	Source
Aam2f	5′ CCTGCCCCCTTGCATGATTAT 3′	60	129–159	Carling et al. (2003)
Am2r	5′ TAGCCACCTAACTCCCCTTCCATT 3′			
Aam4f	5′ GAGCCTGGCAGGTTACAGTCTA 3′	60	224–271	Carling et al. (2003)
Aam4r	5′ TCCCCTCCCAATAAAAAAAGAT 3′			
Aam5f	5′ CGGGAGGGTTCTTTACCACT 3′	55	162–170	Carling et al. (2003)
Aam5r	5′ GAAGGGCTTTTCAGATAGTTTCTTT 3′			
ADCYCf	5′ AAAGTGACACAACAGCTTCTCCAG 3′	58	258–282	Lou (1998)
ADCYCr	5′ AACGAGTGTCCTAGTTTGGCTGTG 3′			
PrM6506f	5′ TAGCAACTTGAGCATGGCAC 3′	57	114–140	Stephen et al. (in press)
PrM6506r	5′ GAAGCTTCAGCCTAGCCAGT 3′			

Felsenstein's (1985) bootstrapping method with 2,000 replicates. Consensus trees were constructed by using all equally parsimonious trees under both the majority-rule and strict criteria. The software DnaSP (Rozas and Rozas 1999) was used to calculate pairwise population (pooled for central and east Arizona regions) differentiation statistics (G_{ST}—Weir 1996) based on haplotype frequency distributions and the result was visualized in a phenogram.

Genetic diversity of the mtDNA control region was calculated within each of the sampled populations (nonpooled for Arizona) by using a variety of indices. These include the number of haplotypes (A), the average nucleotide difference within populations (k), and the population-level haplotype diversity (h, where $h = n(1 - Sp_i^2)/n - 1$). When calculating these measures, insertion or deletion events (indels) were counted as a single event, rather than the actual numbers of nucleotides inserted or deleted.

Microsatellite loci genotyping and analysis.—Amplification of 5 microsatellite loci was performed by using fluorescently labeled primers following a standard PCR regime. Primer sets, annealing temperatures, and PCR conditions for the 5 loci are detailed in Table 2. Amplified products were electrophoresed through a polyacrylamide gel by using an ABI377 automated DNA sequencer (Applied Biosystems). In addition to the internal lane standard, a marker-specific ladder, representing the range of allele sizes, was constructed by combining the amplified products of known individuals and this pooled allelic ladder was loaded onto each gel to facilitate accurate scoring. The software program Genotyper V. (Perkin Elmer, Foster City, California) was used in analyzing gels and scoring loci.

For all analyses, polymorphisms at the microsatellite loci were assumed to be neutral. To check the data set for the presence of null alleles, the discrete populations in Texas (Brewster County), Wyoming (Yellowstone National Park), and Arizona (Arizona central populations 1 and 2 and Arizona eastern populations 1 and 2) were tested at each locus for conformation to Hardy–Weinberg expectations by using the exact test of Guo and Thompson (1992) using Genepop version 3 (Raymond and Rousett 1995). All loci also were examined for the possibility of linkage disequilibrium within these populations by using the same program.

We used the 2001 Genetic Data Analysis software program of Lewis and Zaykin (http://lewis.eeb.uconn.edu/lewishome/software.html, last date accessed 29 April 2005) to estimate partitioning of genetic variance among populations (*F*-statistics—Weir and Cockerham 1984). In addition, we performed an assignment test (http://www.biology.ualberta.ca/jbrzusto, last date accessed 29 April 2005), which used a maximum-likelihood approach to reassign each individual to a population based on the specific allelic composition of the originally defined populations (Cornuet et al. 1999; Paetkau 1995; Waser and Strobeck 1998). Genotype frequencies of zero were computed as frequencies of 0.01, following Paetkau et al. (1998).

Allele frequencies and observed heterozygosities (H_O) were calculated for all populations by using Genepop version 3 (http://wbiomed.curtin.edu.au/genepop/, last date accessed 29 April 2005). Genetic diversity of each population was assessed by using the Shannon–Weaver index (D, $\Sigma p_i(\ln p_i)$—Malone et al. 2003; Shannon and Weaver 1964; Surridge et al. 1999), which takes into account allelic variation and evenness in allele distribution. Pairwise comparisons of allelic frequency distributions between populations (nonpooled for Arizona) were executed by using Genepop version 3. Statistical differences in allele frequency distributions among population pairs were assessed by using Fisher's exact test.

RESULTS

Mitochondrial DNA.—Mitochondrial DNA sequencing resulted in 493 readable nucleotides in the control region. Thirty-eight haplotypes within *A. americana* were recovered from 269 individuals, including 34 variable characters (Table 3). Little phylogenetic resolution was recovered at this locus. Maximum parsimony analysis resulted in more than 15,000 equally parsimonious trees of 87 steps. Only a few nodes received bootstrap support greater than 80%. The majority-rule consensus tree (Fig. 2) showed clades recovered in at least 60% of the most parsimonious trees. Geographically defined groupings were sparse and tended to show small haplotype clusters that differed by 1 base change or an indel event.

The Sonoran pronghorn population in Mexico contained 2 unique haplotypes at low frequencies but otherwise displayed haplotype distributions similar to that of the Sonoran pronghorn population in Arizona (Table 3). This similarity was reflected in a low measure of population differentiation between the 2 Sonoran pronghorn populations ($G_{ST} = 0.009$; Table 4). Conversely, both Sonoran pronghorn populations were highly differentiated from pronghorn populations in the northern United States (G_{ST} ranging from 0.27 to 0.42), exhibited an intermediate range of differentiation from individuals in the Texas population (G_{ST} ranging from 0.117 to 0.196), and were least differentiated from pronghorns in the central and eastern Arizona regions (G_{ST} ranging from 0.025 to

Table 3.—Mitochondrial DNA haplotype frequency distributions in 8 populations and 2 regions of *Antilocapra americana*. The number of individuals in each group is indicated by *n*. Summary statistics are included at the end of the table. These include total number of haplotypes (TH), average number of base-pair differences between individuals (k), and haplotypic diversity (h) for each sampled population and region (NA, not available).

	Sonoran (Arizona) $n = 24$	Sonoran (Mexico) $n = 12$	Arizona central (pooled) $n = 83$	Arizona central population 1 $n = 20$	Arizona central population 2 $n = 5$	Arizona eastern (pooled) $n = 71$	Arizona eastern population 1 $n = 30$	Arizona eastern population 2 $n = 13$	Texas $n = 42$	Oregon $n = 16$	Wyoming $n = 21$
A	—	—	—	—	—	—	—	—	0.048	0.312	0.286
A2	—	—	—	—	—	—	—	—	0.048	0.125	—
A3	—	—	—	—	—	—	—	—	—	—	0.190
A4	—	—	—	—	—	—	—	—	0.024	—	—
AC	—	—	0.060	—	—	0.113	0.133	0.077	—	—	—
AC1	—	—	0.024	—	—	—	—	—	—	—	—
AC2	—	—	—	—	—	—	—	—	0.048	—	—
AC3	—	—	—	—	—	—	—	—	0.024	—	—
AE	0.042	—	0.241	—	—	0.296	0.167	0.461	—	—	—
AE1	—	—	0.072	0.500	0.200	0.056	—	0.154	—	—	—
AG	—	—	0.036	—	0.200	—	—	—	—	—	—
AI	—	—	—	—	—	0.028	—	—	—	—	—
AJ	—	—	0.012	—	—	—	—	—	—	—	—
AJ1	—	—	0.145	0.150	0.200	0.028	0.033	—	0.024	—	—
C1	—	—	—	—	—	—	—	—	—	0.063	—
AQ	—	—	—	—	—	0.014	0.033	—	—	—	—
B	0.417	0.417	0.084	0.050	—	0.338	0.500	0.308	0.667	—	0.048
B1	—	0.083	—	—	—	—	—	—	—	—	—
B2	—	—	0.012	—	—	—	—	—	—	—	—
C	—	—	—	—	—	—	—	—	—	0.125	0.095
UA	—	—	—	—	—	—	—	—	—	0.187	—
P	—	—	—	—	—	—	—	—	0.024	—	—
E	—	—	0.012	0.050	—	—	—	—	—	—	—
E1	—	—	—	—	—	—	—	—	—	0.063	—
E2	—	—	—	—	—	—	—	—	0.071	—	—
UL	—	—	—	—	—	—	—	—	—	0.063	—
F	—	—	0.012	—	—	—	—	—	—	—	0.048
H	0.542	0.417	0.012	—	—	—	—	—	—	—	—
H1	—	0.083	—	—	—	—	—	—	—	—	—
HO	—	—	0.012	—	—	—	—	—	—	—	—
K	—	—	—	—	—	0.014	—	—	—	—	—
L2	—	—	—	—	—	—	—	—	—	—	0.048
L3	—	—	—	—	—	—	—	—	—	—	0.048
X	—	—	0.012	—	—	—	—	—	—	—	—
Y	—	—	0.241	0.250	0.400	0.113	0.100	—	—	—	—
Y2	—	—	0.012	—	—	—	—	—	—	—	—
Z1	—	—	—	—	—	—	—	—	0.024	—	0.048
Z6	—	—	—	—	—	—	—	—	—	0.063	0.190
TH	3	4	16	5	NA	9	5	4	10	8	9
k	1.09	1.42	3.08	3.04	NA	2.12	1.62	1.64	1.96	1.92	2.84
h	0.55	0.70	0.85	0.78	NA	0.78	0.72	0.72	0.55	0.89	0.87

0.047). One of the 2 high-frequency haplotypes in the Sonoran pronghorns (haplotype "h" at ~50%) was found in only 1 other individual from central Arizona. In contrast, the other high-frequency haplotype in the Sonoran pronghorn population (haplotype "b" at ~40%) was found at high frequency in both the eastern Arizona region and the Texas population. Differentiation among populations is illustrated as a phenogram generated from a neighbor-joining analysis of pairwise G_{ST} values (Fig. 3A).

Genetic diversity of the mtDNA control region was lowest in the Arizona Sonoran pronghorn population ($n = 24$), which contained only 3 haplotypes (Table 3). All populations, including those with much smaller sample sizes, were found to contain more haplotypes than the Arizona Sonoran pronghorn population. For example, populations with similar sampling effort, such as Arizona central population 1, Arizona eastern population 1, and Wyoming contained 5, 5, and 9 haplotypes, respectively. Although it contained only one-half of the number of individuals surveyed in the Arizona Sonoran pronghorn population, the Sonoran pronghorn population in Mexico ($n = 12$) exhibited 4 haplotypes.

Almost all populations had higher levels of mtDNA haplotype diversity than the Arizona Sonoran pronghorn population, as indicated by the haplotype diversity index (h), the only exception

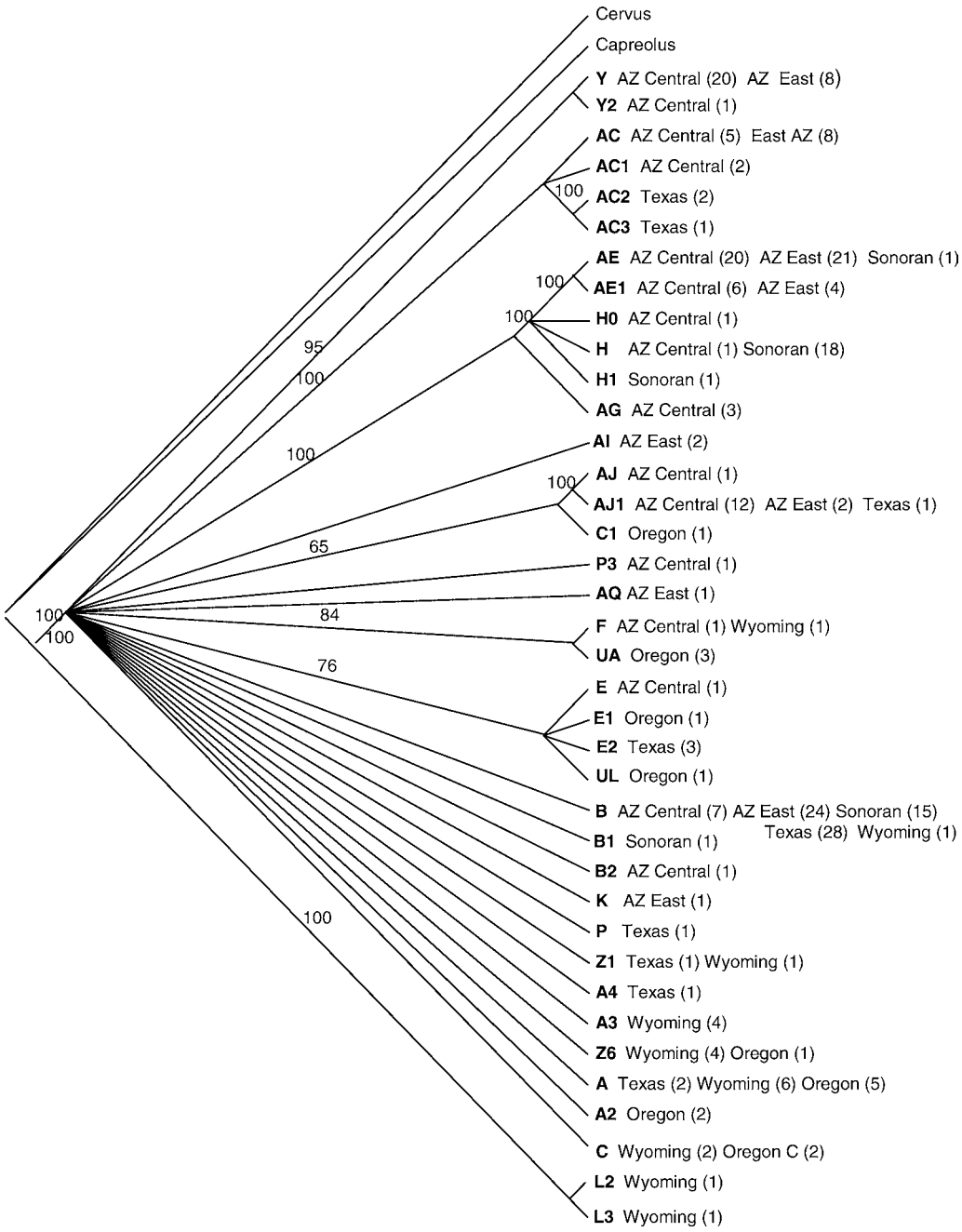

FIG. 2.—Majority-rule consensus topology (>60%) generated from 15,000 most-parsimonious trees. Each haplotype (terminal branch) is labeled in bold along with the localities where the haplotype has been recovered (AZ = Arizona). Numbers in parentheses refer to numbers of sampled individuals of *Antilocapra americana* with that haplotype.

being the Texas population (Table 3). For instance, populations such as Arizona central population 1, Arizona eastern population 1, and Wyoming had diversity indices (0.715–0.867) that were approximately 50% larger than that observed in the Arizona Sonoran pronghorn population (h = 0.55). Examination of the average nucleotide difference within populations revealed that both of the Sonoran pronghorn populations consisted of a very closely related group of individuals with few base differences between haplotypes (k = 1.09 and 1.42), whereas the other discrete populations surveyed (nonpooled) had a more diverse array of haplotypes (average k = 2.04).

Microsatellite loci.— Hardy–Weinberg expectations were met at all loci in 5 of the 6 populations that were tested. The Texas population had a consistent deficiency of heterozygotes suggesting population-level phenomena, such as nonrandom mating or a Wahlund effect, rather than null alleles. Additionally, the pairwise tests between microsatellite loci showed no evidence of linkage disequilibrium. The 5 microsatellite loci yielded an overall average of 6.5 alleles per locus and a total of 58 allelic states across all populations.

Pairwise F_{ST} values between all population pairs (pooled for Arizona regions) ranged from 0.034 to 0.205 and indicated that

TABLE 4.—Pairwise measures of population differentiation of American pronghorns (*Antilocapra americana*). G_{ST} values, calculated from mitochondrial DNA sequence data, are in the upper diagonal. F_{ST} values, calculated from microsatellite data, are in the lower diagonal.

	Sonoran (Arizona)	Sonoran (Mexico)	Arizona central (pooled)	Arizona eastern (pooled)	Texas	Oregon	Wyoming
Sonoran (Arizona)	—	0.009	0.046	0.047	0.196	0.345	0.421
Sonoran (Mexico)	0.073	—	0.025	0.026	0.117	0.271	0.382
Arizona central (pooled)	0.104	0.105	—	0.016	0.085	0.155	0.127
Arizona eastern (pooled)	0.103	0.100	0.034	—	0.077	0.154	0.141
Texas	0.205	0.178	0.109	0.114	—	0.139	0.140
Oregon	0.198	0.191	0.052	0.096	0.107	—	0.061
Wyoming	0.175	0.156	0.067	0.080	0.094	0.059	—

although the 2 Sonoran pronghorn populations had detectable differences in genotypic distributions ($F_{ST} = 0.073$), they were more alike one another than they were to any of the other populations sampled (Table 4). Unsurprisingly, the least-differentiated paired comparison was that consisting of the central and eastern Arizona regions ($F_{ST} = 0.034$). A phenogram reflecting the relative genetic distances between the populations is shown in Fig. 3B. The vast majority of individuals were correctly assigned to their source populations in the assignment test (Table 5). The few Sonoran pronghorn individuals from Arizona and Mexico not assigned to their source populations were either assigned to the alternate Sonoran pronghorn population or to the central Arizona region. The vast majority of individuals from the remaining populations also were assigned to their correct source populations. When misassignments did occur, they tended to be between populations in similar geographic regions, rarely assigning northern individuals into southern populations, or vice versa.

The nuclear diversity data followed the pattern observed for the mtDNA data, with the 2 populations of Sonoran pronghorns sharing a large proportion of their alleles (73%) and exhibiting less overall diversity than the other sampled populations (Table 6). Unlike the results of the mtDNA data, nuclear data revealed that the Sonoran pronghorn population in Mexico was genetically less diverse than the Arizona Sonoran pronghorn population. However, this could be a result of the large difference in sample sizes between the Arizona and Mexican Sonoran pronghorn populations. Genetic diversity in terms of the number of alleles and evenness of allele frequency distributions (Shannon–Weaver diversity index, D) was lower in the Sonoran pronghorn populations from Arizona (D = 0.954) and Mexico (D = 0.879), than for all of the remaining populations (D values ranged from 1.271 to 1.709). Both of the Sonoran pronghorn populations had numbers of alleles per locus similar to the Arizona eastern population 2 but exhibited lower values than the other discrete populations sampled (ranging from 5.8 to 8.6 alleles per locus). All pairwise comparisons of populations (nonpooled for Arizona) detected significant differences ($P < 0.05$) in allelic frequency distributions for at least 4 of the 5 microsatellite loci tested.

DISCUSSION

The results of this study indicate that the Sonoran pronghorn is not highly distinct as compared to other populations of its conspecifics. However, it is clear at both the mitochondrial and nuclear loci that Sonoran pronghorns have differentiated from the other populations surveyed and have likewise begun to differentiate into a Mexican population and an Arizona population. It does not appear that differentiation between the Sonoran pronghorn and the other conspecific populations surveyed occurred as a result of prolonged separation and a subsequent accumulation of unique genetic diversity. Rather, examination of our data suggests that the observed differentiation is likely a result of isolation by distance in historically contiguous populations, loss of genetic diversity due to genetic bottleneck in the 2 Sonoran pronghorn populations, and the cumulative effects of genetic drift.

In our overall analysis of mitochondrial variation among the study populations, we did not observe deeply separated lineages. Although one does not expect to find a great deal of phylogenetic structure among subspecies, we were struck by the extent to which haplotypes seldom differed by more than a few base substitutions, with an average 0.6% sequence

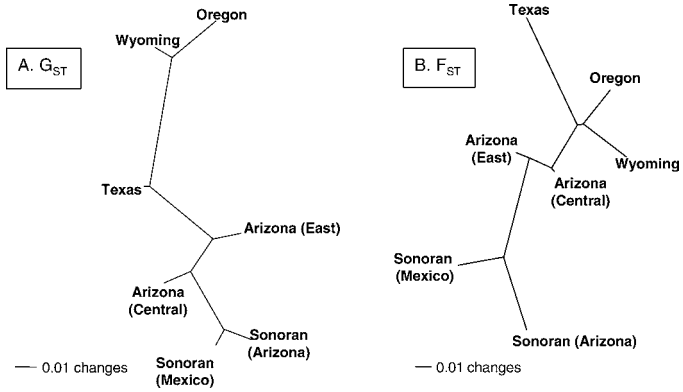

FIG. 3.—Population differentiation of *Antilocapra americana* based on mitochondrial DNA and microsatellite data. A) A neighbor-joining algorithm is used to generate a phenogram based on G_{ST} values calculated from mitochondrial haplotype frequencies. B) Pairwise coancestry values (F_{ST}) were used in a neighbor-joining algorithm to generate a phenogram reflecting average relatedness across 5 nuclear loci.

TABLE 5.—Results of an assignment test based on data from 5 nuclear microsatellite loci in 5 populations and 2 regions of American pronghorns (*Antilocapra americana*). Individuals from each source population (row) are assigned to the population (column) that best fits its overall genotype.

	Sonoran (Arizona)	Sonoran (Mexico)	Arizona central (pooled)	Arizona eastern (pooled)	Texas	Oregon	Wyoming
Sonoran (Arizona)	**30**	1	1	0	1	0	0
Sonoran (Mexico)	3	**11**	1	0	0	0	0
Arizona central (pooled)	3	9	**103**	18	1	3	6
Arizona eastern (pooled)	4	0	12	**59**	1	0	4
Texas	0	0	0	2	**42**	1	1
Oregon	0	0	1	1	0	**13**	5
Wyoming	0	0	2	0	1	1	**21**

divergence between populations. This is quite low when compared to other intraspecific studies using mtDNA control region sequence data. For example, sequence divergence averaged 13% and 9.8% between geographically separated populations of Grant's gazelles (Arctander et al. 1996) and African antelope (Birungi and Arctander 2000), respectively. As well, studies show up to 5% divergence within single populations (buffalo and impala—Birungi and Arctander 2000; and references therein) and approximately 2.5% sequence divergence between subspecies of Eld's deer (Balakrishnan et al. 2003). In fact, these results are quite comparable to the level of intraspecific variation of American bison at the mtDNA control region (mean 0.69%—Ward et al. 1999). Restriction site analysis of the mitochondrial genome has yielded high estimates of divergence within North American mule deer subspecies (6–7%) and lower estimates of divergence within moose, elk, and caribou (<2%—Cronin 1992).

The topology of the mtDNA phylogeny is very shallow across these populations and almost completely unresolved in the majority-rule consensus. The strongly supported nodes that were identified consist of a few, closely related haplotypes that do not have a great deal of geographic structure. The Sonoran pronghorn shares a high frequency of ancestral polymorphisms with populations in Arizona and Texas. These results indicate that the Sonoran pronghorn populations recently were contiguous with the other southern populations or, at the very least, were exchanging many migrants in the recent past. Although Sonoran pronghorns do possess a few haplotypes and alleles not found in other populations, this also is true for every other pronghorn population sampled.

The nuclear microsatellite loci are rapidly evolving markers and thus, our expectation was that these data would readily reveal structure among populations. Although little unique allelic variation was identified within individual populations, allelic frequencies differed significantly among populations. In fact, the Sonoran pronghorn populations easily were differentiated from one another as well as all other pronghorn populations sampled by using the nuclear data. This is illustrated clearly in the results of the assignment test and by the high levels of variance partitioning observed among population pairs. The high level of allele frequency differentiation between the Sonoran pronghorn populations and adjacent populations, with little evidence of unique genetic variation, is likely a reflection of accelerated population differentiation in these small populations due to genetic drift. As a whole, neither the nuclear data nor the mtDNA data support the assertion that the Sonoran pronghorn population is particularly divergent or taxonomically distinct from the other North American pronghorn populations.

Our measures of both nuclear and mitochondrial genetic differentiation among populations indicate that the Sonoran pronghorn populations are most closely related to each other, followed by the Arizona populations and the remaining populations from Texas, Wyoming, and Oregon, respectively. Interestingly, both data types show that the Sonoran pronghorn population in Mexico is as closely related to pronghorns from the central and eastern Arizona regions as is the Arizona Sonoran pronghorn population, although geographically much more distant. This is likely because the Mexican population has remained larger than the Arizona Sonoran population by at

TABLE 6.—Summary of results of microsatellite data analyses in American pronghorns (*Antilocapra americana*). Number of individuals (*n*), average number of alleles per locus (A), observed heterozygosity (H), inbreeding coefficient (F_{IS}) averaged over 5 loci, and the population-level genetic diversity (D) are reported for each population and region.

	Sonoran (Arizona)	Sonoran (Mexico)	Arizona central (pooled)	Arizona central population 1	Arizona central population 2	Arizona eastern (pooled)	Arizona eastern population 1	Arizona eastern population 2	Texas	Oregon	Wyoming
n	33	15	146	34	17	80	22	15	46	22	27
A	4.4	4.4	8	5.8	6	7	5.8	4.6	6.4	8.6	7
H	0.502	0.573	0.630	0.683	0.704	0.583	0.592	0.634	0.658	0.734	0.636
F_{IS}	0.143*	0.019	0.046*	−0.040	−0.057	0.138*	0.006	0.066	0.070*	0.020	0.018
D	0.954	0.879	1.479	1.341	1.351	1.418	1.334	1.241	1.419	1.709	1.366

* $P < 0.05$.

least 3-fold and therefore the effects of genetic drift and bottlenecking have been less severe.

The small Sonoran pronghorn populations exhibited lower levels of genetic diversity than did the other pronghorn populations surveyed. Furthermore, the Sonoran pronghorn population in Arizona contained less diversity than its Mexican counterpart at the mitochondrial locus. Haplotypes found within the Sonoran pronghorn populations were closely related, often only differing by 1 nucleotide change or an indel. This pattern of reduced genetic diversity also is observed at the nuclear loci, where both diversity and heterozygosity measures clearly demonstrated lower levels of genetic diversity in the 2 Sonoran pronghorn populations compared to other populations sampled at similar intensities. The obligatory accumulation of inbreeding in these isolated populations will erode heterozygosity over time, and as one would expect, seems to have begun to do so more quickly in the Arizona Sonoran pronghorn population than in the Mexican population. Recent evidence from a reintroduced elk population in Pennsylvania provides a clear example of the extreme decreases in allelic diversity and lowered levels of heterozygosity that can result as a consequence of a prolonged bottleneck event (Williams et al. 2002).

There are no commonly accepted criteria for accepting or rejecting subspecific designations based on genetic data. Ball and Avise (1992), among others, argue against subspecific recognition of short-term population separation. Using this as a guideline, examination of our data does not strongly support the subspecific level of nomenclature for the Sonoran pronghorn in that these populations do not show signs of prolonged separation as would be evidenced by highly divergent lineages or striking amounts of unique genetic diversity. Conversely, our data are in line with reports of high numbers of pronghorns in contiguous populations across the southwestern United States until the 1800s, when populations were decimated by overhunting and competition with livestock (Buechner 1950). However, it is plausible that strong selection over a very short time period has enabled these Sonoran pronghorn populations to occupy the marginal habitat and unique environment of the Sonoran Desert. It is already thought that Sonoran pronghorns have altered their social structure in order to persist in the rugged conditions of their current habitats (Arizona Game and Fish Department 1981). It is unlikely that we would detect evidence of such specific adaptations with data from neutral loci. It may be more useful to study the issue of phenotypic adaptation in the Sonoran pronghorn with a comprehensive morphological and ecological data set.

Examination of our data indicates that, although the 2 Sonoran pronghorn populations are not especially distinct, they are differentiated from the other pronghorn populations surveyed in this study and only moderately differentiated from each other. There is no evidence to suggest that differentiation happened over a long period of isolation, but it more likely has occurred rapidly, including 1 or several bottleneck events. Given that microsatellite loci and mtDNA control region sequences are some of the most rapidly evolving markers, it is not likely that further analyses of the pronghorn genome would dramatically change our interpretation of the genetic distinctness of the Sonoran pronghorn. However, additional analysis of microsatellite variation, as more loci become available for this species, might enhance our ability to assess the loss of genetic variation in these declining populations and aid in the formulation of recovery plans.

Neither of the 2 Sonoran pronghorn populations are stable. Numbers of individuals in the Arizona population continue to decline and little information is available on the current status of the Mexican population. Both Sonoran pronghorn populations exhibit low levels of haplotypic and allelic diversity as compared to the other populations surveyed. Additionally, each of the 2 Sonoran pronghorn populations contains a small amount of genetic variability not found in the other population because of recent obstructions to gene flow (highways, fences, and canals) and random genetic drift. Conservation of the remaining genetic diversity in the Sonoran pronghorn populations clearly should be a high priority for managers of this species. Examination of our data indicates that increasing population numbers is critical to maintenance of the remaining genetic diversity and to hinder the deleterious effects of inbreeding (O'Brien and Evermann 1989; Saccherri et al. 1998). This certainly is consistent with the current goals of the recovery plan for this species and wildlife managers are already well aware of the need to increase population size. Examination of our data suggests that augmentation of the Arizona population with individuals from Mexico (through translocation or artificial insemination) might be an acceptable course of action, particularly if the Arizona population of the Sonoran pronghorn continues its precipitous decline. These recommendations are similar to findings in a recent study of Eld's deer (Balakrishnan et al. 2003). Furthermore, it would clearly be advantageous if additional populations were established with a mixture of individuals from both Sonoran pronghorn populations. However, until the habitat available to the Sonoran pronghorn can sustain an increase in population number or the number of populations, the loss of genetic diversity in this species is likely to continue.

Acknowledgments

Thanks to all the volunteers from Arizona Game and Fish Department, Arizona State University, University of Arizona, Northern Arizona University, Yavapai College, Grand Canyon University, Pima Community College, and the Arizona Antelope Foundation who helped operate the sample collection stations, especially R. Barkhurst, J. Beals, D. N. Cagle, A. Fuller, J. Galaway, J. Goodwin, J. Hanna, B. Henry, J. Hervert, C. A. Jones, R. M. Lee, M. A. McKenzie, A. Munig, J. B. Pickrell, P. Shaw, J. Simmons, J. Vassel, B. F. Wakeling, and many others. The Southern Arizona Bowhunting Chapter of Safari Club International provided funding for necessary field supplies. Our appreciation goes to all the Arizona Game and Fish Department employees who went out of their way to collect samples from far-reaching areas. Arizona pronghorn hunters played an integral role in sample collection of non–Sonoran pronghorns, sometimes going far out of their way to return samples in good condition. We also are very grateful to L. Cox for the time and effort she spent helping in the laboratory. We appreciate the many helpful suggestions that graduate students and postdoctoral fellows in the laboratory of O. E. Rhodes Jr. gave after reviewing an earlier draft of the manuscript. This is

a contribution of the Federal Aid in Wildlife Restoration Act Project W-53-M and W-78-R of the Arizona Game and Fish Department.

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Submitted 3 July 2004. Accepted 3 January 2005.

Associate Editor was Robert D. Bradley.