# MANAGING WILDLIFE IN THE SOUTHWEST: New Challenges for the 21st Century

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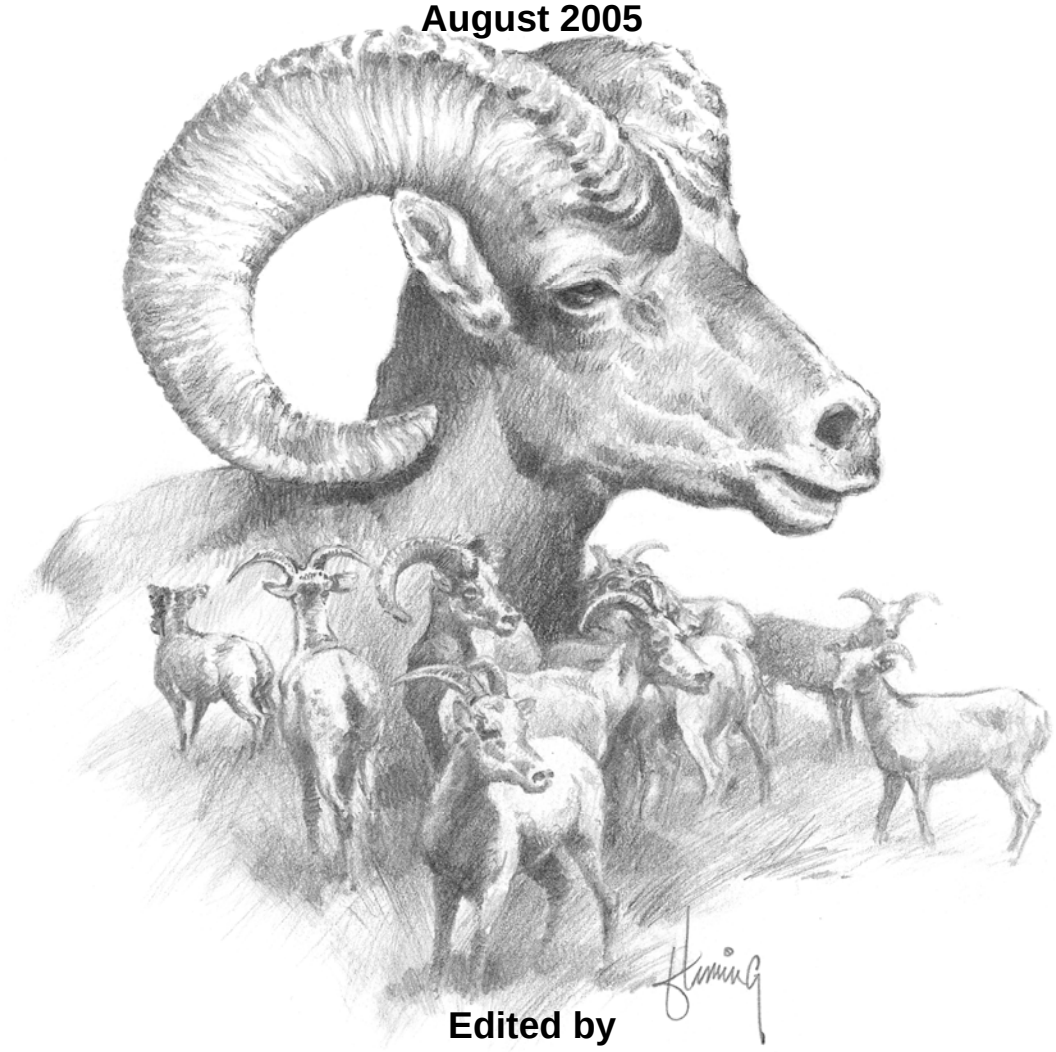

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# GENETIC SUBSPECIES IDENTIFICATION OF A RECENTLY COLONIZED BIGHORN SHEEP POPULATION IN CENTRAL ARIZONA

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***Abstract:*** Two subspecies of bighorn sheep currently occur in Arizona: the desert bighorn sheep (*Ovis canadensis mexicana, O. c. nelsoni*) and Rocky Mountain bighorn sheep (*O. c. canadensis*). In central Arizona (Game Management Unit 23 [GMU 23]), bighorn sheep colonized an area along the Salt River; however, the source of this population was enigmatic. Although the nearest desert bighorn sheep herd is <30 km to the southwest of the herd in Unit 23, no obvious movement corridors were evident between them. Rocky Mountain bighorn sheep from an earlier translocation occur about 160 km east of the herd in Unit 23, and these animals could have used the Salt River drainage as a movement corridor to colonize this new area. In an effort to clarify the subspecies affinity of bighorn sheep in the colonized area, we obtained mitochondrial DNA sequences (473-bp of the control region) from bighorn sheep in GMU 23 ($n$ = 5), Rocky Mountain bighorn sheep as a reference ($n$ = 8), and desert bighorn sheep references ($n$ = 58). Our data provided strong support for the hypothesis that bighorn sheep in GMU 23 were of Rocky Mountain origin, suggesting that these sheep have moved about 160 km west along the Salt River drainage over the last 25 years. These data will facilitate effective management of this herd to minimize its impact on neighboring native desert bighorn sheep populations. The future growth of this population could jeopardize the integrity of subspecific classifications in central Arizona. Given documented long-distance movements of males, sheep populations (including translocation programs) should be managed to maintain subspecific separation.

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***Key words:*** Arizona, bighorn sheep, colonization, mitochondrial DNA, *Ovis canadensis*, population genetics, subspecies.

Although the concept and application of subspecies is controversial (Mayr 1982, Ryder 1986, Moritz 1994, Paetkau 1999), there is no argument that different geographic forms of the same species exist as a result of adaptation to local environmental conditions. Bighorn sheep are no exception and were historically classified into 7 subspecies (Cowan 1940). Historically, subspecies descriptions were sometimes based on vague morphological characters measured for a few individuals. These subspecies names are then perpetuated for decades because of a

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lack of clarifying analyses. In recent years, more extensive morphological analyses and the advent of high-resolution genetic markers has led to a fuller understanding of phylogeographic differentiation in many species of large mammals (Cronin 1992, Lee et al. 1994, Cronin and Bleich 1995, Cronin et al. 1995, Lou 1998, Hundertmark et al. 2002, Williams et al. 2004, Stephen et al. 2005). In bighorn sheep, a more sophisticated analysis of skull morphology combined with genetic techniques revealed subspecies classifications different from Cowan's (1940) analysis (*Ovis canadensis auduboni* is extinct and thus excluded from analysis; Ramey 1993). Ramey's (1993) analyses did not support the recognition of separate desert bighorn sheep subspecies in the Southwest (i.e., *O. c. nelsoni, O. c. cremnobates, O. c. mexicana, and O. c. weemsi*; Ramey 1993), and only weakly supported differentiation between this desert complex and *O. c. californiana* in the Sierra Nevada. However, this same analysis found pronounced differences between desert bighorn sheep subspecies (collectively) and the Rocky Mountain bighorn sheep (Ramey 1993).

A common management tool for bighorn sheep and other game species is translocation, either for restoration or augmentation of populations or for increased variety in hunting opportunities. Often, these translocations have mixed subspecies that traditionally were separated, creating the opportunity for hybridization to occur. Biologically, hybridization may result in the loss of unique genetic, morphological, behavioral, or ecological characteristics that have evolved in local populations over time. Groups of genes that have evolved to work together (i.e., locally adapted gene complexes) may be disrupted, leaving hybrid populations poorly adapted to local environments (Dobzhansky 1970), and potentially leading to extinction of naturally-occurring types (Rhymer and Simberloff 1996). The administrative implications of hybridization also are critical, particularly when dealing with game species. Management recommendations, hunting regulations and record-keeping, and hunter enthusiasm are often subspecies-specific, and will be seriously confounded if populations are composed of hybrid individuals or individuals of unknown subspecies affinity.

Two subspecies of bighorn sheep currently reside in Arizona: the desert bighorn sheep and the Rocky Mountain bighorn sheep (Cowan 1940). Rocky Mountain bighorn sheep from Alberta were released in New Mexico near Arizona in 1971 and currently occupy areas in east-central Arizona (Hoffmeister 1986, Heffelfinger et al. 1995). Desert bighorn sheep occur in scattered populations throughout the southern and western halves of Arizona (Fig. 1). Over the last decade, groups of bighorn sheep have been reported periodically along the Salt River Canyon in the southern portion of GMU 23 (Fig. 1). Some observers concluded that these sheep appear more like Rocky Mountain bighorn than desert bighorn with heavier musculature, larger bodies, and darker pelage (Fig. 2). However, if the sheep in this newly colonized area were of Rocky Mountain origin, individuals would have had to utilize the Salt River drainage as a movement corridor from the nearest source population over 160 km to the east. The nearest desert bighorn sheep herd is <30 km to the southwest, however, no evident movement corridor exists between these herds.

The mixing of genetic stock from Alberta with endemic Arizona desert bighorn sheep has legal and administrative repercussions, and could have negative biological consequences for the resulting population. Our objective was to use available genetic tools to determine subspecific affinity of the sheep in the southern part of GMU 23. With such information, we can minimize potentially detrimental interbreeding between distinct subspecies of bighorn sheep in Arizona, and design management strategies to maximize hunting opportunities in the state.

## METHODS

In December 2004, we captured and radiocollared 4 bighorn sheep in the Black Mesa area of southern GMU 23 to monitor their movements, survival, and habitat use. We took blood samples from these 4 individuals and collected muscle tissue from a fifth sheep that died during attempts to capture it. These 5 sheep were compared to bighorn sheep of known subspecies affiliation from throughout Arizona. The reference collection (58 desert and 8 Rocky Mountain bighorn sheep) came from known subspecies from checking stations operated by the

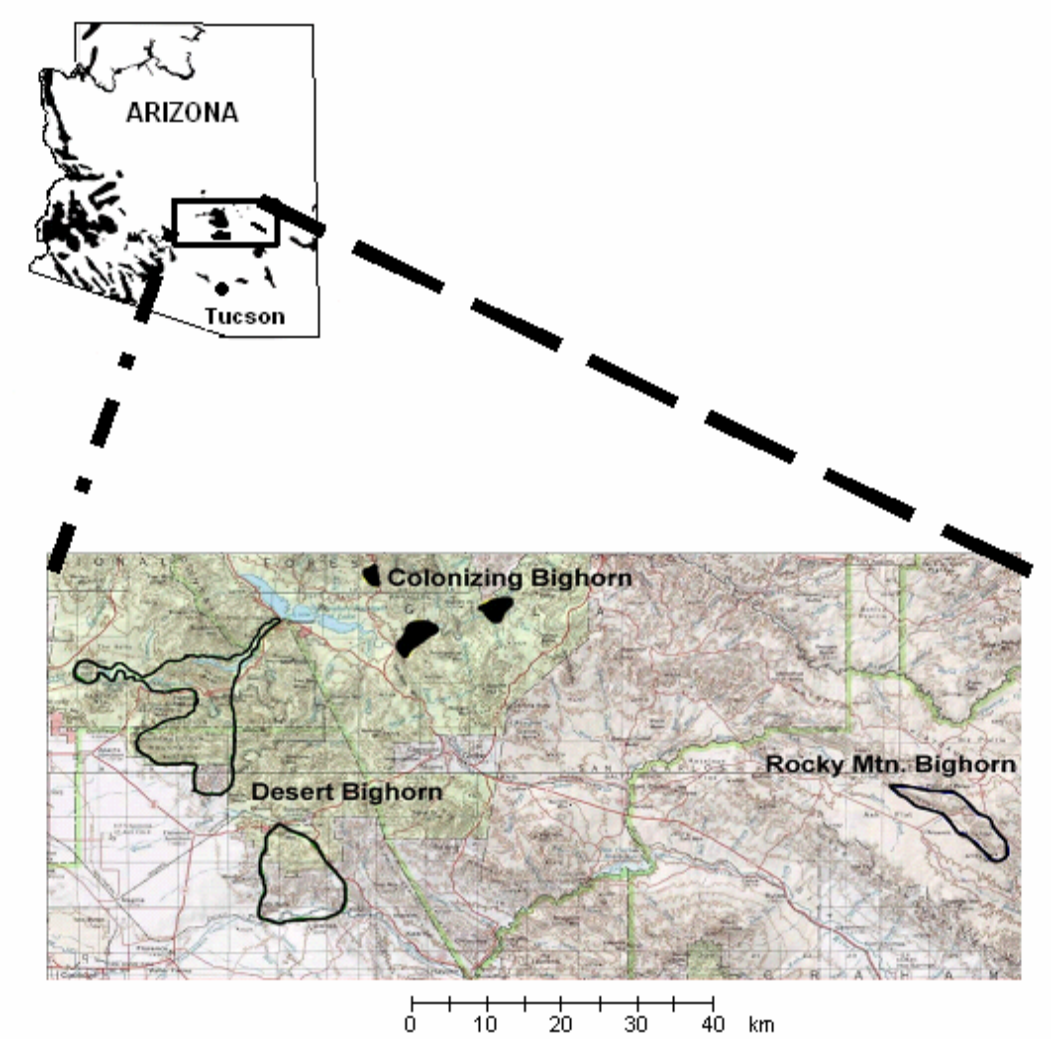


Fig.1 Location of desert bighorn and Rocky Mountain bighorn sheep populations in relation to the recently colonized area in the southern portion of Game Management Unit 23, central Arizona, 2004

Arizona Game and Fish Department. The reference desert bighorn sheep samples represented most populations in the western and southern half of Arizona (Fig. 1). Rocky Mountain bighorn samples were collected in the same manner from GMU 27 and 28 in east central Arizona.

To prepare blood samples for DNA extraction, we added 900 µL of 20 mM Tris-HCl to each sample, mixed by vortexing, allowed to sit at room temperature for 10 minutes, then centrifuged at 14,000 rpm for 20 seconds. We repeated this procedure 2 additional times using the pellet from the previous spin to ensure removal of most of the red blood cells, which in mammals do not contain DNA. For tissue samples and prepared blood samples, we extracted DNA using a modified sodium acetate precipitation protocol (modified from the PUREGENE kit; Gentra Systems, Minneapolis, Minnesota). We assessed the quantity and quality of extracted DNA via electrophoresis through an agarose gel stained with ethidium bromide, and diluted each sample to approximately 10 ng/µL in TLE (10 mM Tris-HCl, 0.1 mM EDTA).

We amplified a 473 base pair portion of the mitochondrial control region using PCR primers from Epps et al. (2005*a*, *b*). We gener-

Fig. 2. Ram showing phenotypic resemblance to Rocky Mountain bighorn sheep (right) seen in a desert bighorn sheep population (desert bighorn ram on left; GMU 22) <30 km southwest of the colonizing Rocky Mountain bighorn sheep in central Arizona, October 2004.

ated amplicons using the following PCR thermocycler profile: an initial denaturation step of 5 minutes at 94° C, followed by 35 cycles of 94° C for 60 seconds, 61° C for 70 seconds, and 72° C for 90 seconds, and a final extension step at 72° C for 5 minutes. We estimated the quality and relative quantity of PCR products by electrophoresis through agarose gels stained with ethidium bromide. We cleaned PCR products using a low sodium precipitation protocol, in which we precipitated the DNA with a sodium acetate solution (0.12 mM NaOAc in 100% ethanol), centrifuged to form a pellet, washed with 70% ethanol, and resuspended in water.

Ten microliter sequencing reactions contained approximately 30 ng PCR product (as determined by agarose gel electrophoresis), 5 pmol forward or reverse primer, and 1 µL ABI Big Dye Terminator version 3.1 cut with 3 µL 5X buffer (Applied Biosystems, Foster City, California, USA). Sequencing reactions were carried out as follows: 98° C for 5 minutes, followed by 26 cycles of 98° C for 30 seconds, 50° C for 15 seconds, and 60° C for 2 minutes. We cleaned sequenced products using the low sodium precipitation protocol described above, and the Purdue University Core Genomics Center ran these products on an ABI 3730 automated DNA sequencer. We edited sequences using Sequencher version 4.1 software (Gene Codes Corp., Ann Arbor, Michigan, USA). We sequenced all individuals in the forward and reverse directions to ensure consistency.

We combined forward and reverse sequences together to make a consensus sequence for each individual using Sequencher version 4.1 and exported these consensus sequences into PAUP* version 4.0b10 software (Swofford 2000). We performed a heuristic search for the most parsimonious phylogenetic tree that best described the relationships among our sequences. We then computed a consensus tree to collapse any nonsignificant branch nodes, and generated confidence values for branch nodes using 100 bootstrap replicates. Thus, our final bootstrapped consensus tree describes the relationships among individual control region sequences in our dataset. Each individual is represented by a horizontal 'branch,' all of which are the same length. Individuals that are connected by a 'node' (represented by a vertical line) are genetically more similar to each other than they are to other individuals to which

they are not connected. Bootstrap numbers represent the confidence in that particular branching pattern; higher values indicate that the data provide stronger support for the given branching pattern than lower values.

**RESULTS**

We aligned 473 bases of mitochondrial control region sequence across 71 individual bighorn sheep (58 desert, 8 Rocky Mountain, and 5 unknown). Forty nucleotide sites were variable within this portion of the mtDNA, resulting in the detection of 18 distinct haplotypes. We identified 14 haplotypes in desert bighorn sheep and 4 haplotypes in Rocky Mountain sheep (Fig. 3). The discrepancy in the numbers of haplotypes may not reflect a lack of genetic diversity in Rocky Mountain bighorn sheep, but may be an artifact of the small sample size for this subspecies. None of the 18 haplotypes were shared between the 2 subspecies (Fig. 3). We identified 6 bases within the mtDNA sequence that were diagnostic between subspecies; in other words, these sites did not vary within subspecies, only between them. All colonizing sheep had haplotypes that were identical to one of the 4 haplotypes found in Rocky Mountain bighorn sheep (Fig. 3), suggesting that the colonizing sheep were of Rocky Mountain origin.

The phylogenetic tree provided additional support for the hypothesis that the colonizing sheep in Unit 23 were of Rocky Mountain origin. We found that Rocky Mountain sheep and the colonizing sheep clustered together with strong bootstrap support, and that these sheep were genetically differentiated from all desert bighorn sheep (Fig. 4). We found evidence for genetic structuring within subspecies, as indicated by bootstrap-supported branching within subspecies; however, in nearly every instance this was uncorrelated to geographic location (Fig. 4).

**DISCUSSION**

Given the geographic distribution of subspecies in Arizona and the results of our analysis, Rocky Mountain bighorn sheep apparently moved westward along the Salt River drainage and into the southern part of GMU 23 during the last 25 years. Perhaps these results should not be surprising in light of the history of Rocky Mountain bighorn sheep in Arizona. The Rocky Mountain subspecies colonized eastern Arizona by movements west from a translocated bighorn sheep population in New Mexico (Heffelfinger et al. 1995). This Rocky Mountain bighorn sheep population in New Mexico was established near the Arizona border with a 1964 translocation of animals from Banff National Park, Alberta and a supplemental release of sheep that previously originated from Banff (Larsen 1971, Ogren 1957). As animals moved west from this population along the San Francisco River they entered Arizona as early as 1971 (Apache County Independent News 1971). The Arizona Game and Fish Department supplemented Rocky Mountain bighorn sheep with 1979 and 1980 translocations into Bush Creek in east-central Arizona (Heffelfinger et al. 1995). The sheep in Bush Creek came from Rocky Mountain National Park (2M:6F) and near Tarryall (5M:7F), Colorado.

The Rocky Mountain bighorn sheep now occupying southern GMU 23 are geographically close to native desert bighorn herds. Marked desert bighorn sheep in Arizona may travel distances of several hundred km; 1 bighorn sheep traveled 110 km from the Superstition Mountains east of Phoenix to the Catalinas near Tucson (Arizona Game and Fish Department,

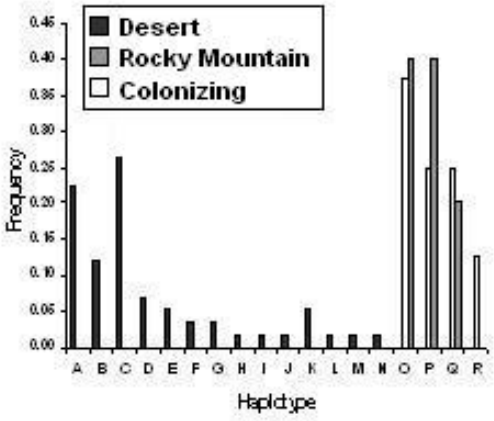


Figure 3. Mitochondrial DNA haplotype frequency distribution for desert (*n* = 58), Rocky Mountain (*n* = 8), and colonizing Game Management Unit 23 (*n* = 5) bighorn sheep, based on 473 bases of control region sequence.

unpublished data). Currently the Rocky Mountain sheep in the southern portion of GMU 23 are < 30 km to the northeast of the nearest native bighorn sheep population. The landscape between these 2 subspecies is not conducive to sheep movements, but the 110 km-movement mentioned above occurred through similarly inhospitable terrain.

Our data illustrate the potential for intermixing of these 2 subspecies in central Arizona. Because our data are mitochondrial in origin, at this point we know only that each of the colonizing bighorn sheep we sampled had a Rocky Mountain sheep mother. Since we found no desert sheep haplotypes in our unknown sample, it seems unlikely that there are desert bighorn sheep (or Rocky Mountain bighorn male x desert bighorn female hybrids) in GMU 23 at this time. If desert bighorn males are in GMU 23 but were unsampled, it is possible that they are hybridizing with Rocky Mountain females; such hybrids would not be detectable using our methods (they would have a Rocky Mountain haplotype). Although it seems unlikely that introgression is occurring within the newly colonized population in GMU 23 at this time,

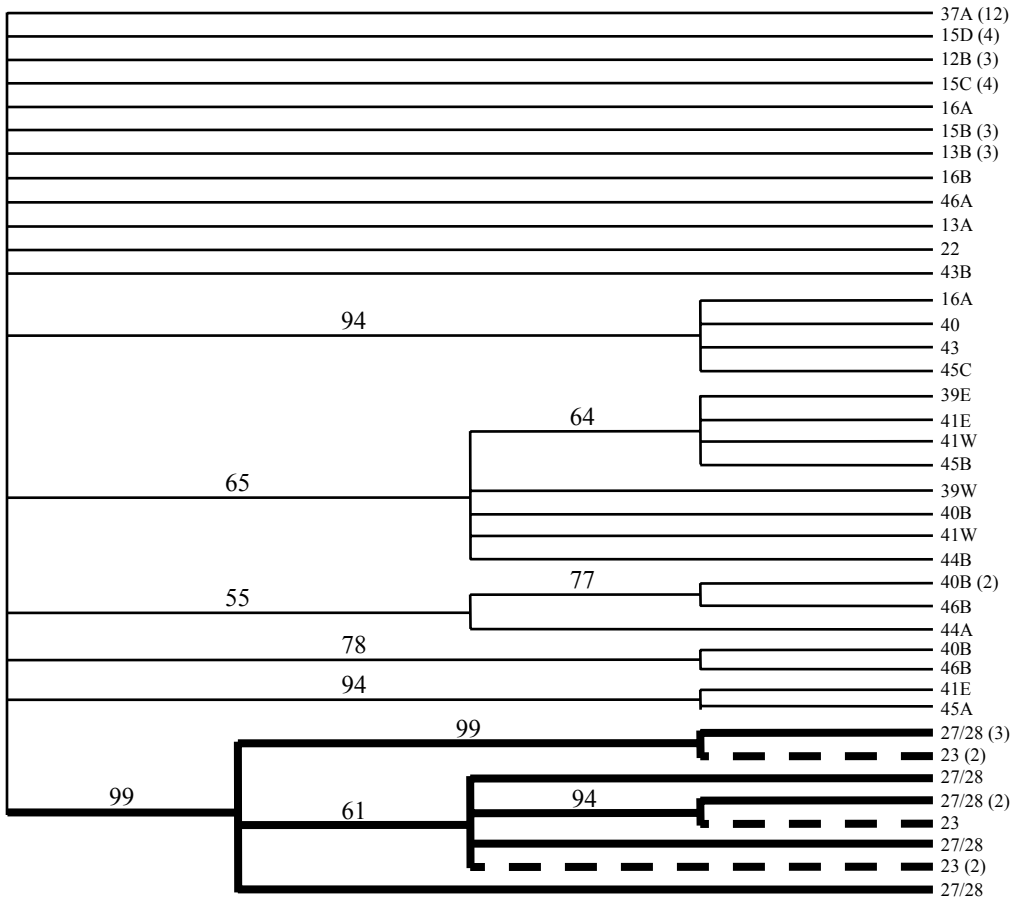


Fig. 4. Maximum parsimony consensus tree depicting genetic relationships among desert (thin lines), Rocky Mountain (bold lines), and colonizing (dashed lines) bighorn sheep. Individuals are identified by the Arizona Game Management Unit from which they were sampled. Individuals possessing the same haplotype and sampled in the same management unit were collapsed into a single branch; the number of individuals represented by each branch is indicated in parentheses. Bootstrap values, based on 100 replicates as implemented in PAUP*, are indicated at nodes.

several observations of a phenotypically Rocky Mountain ram in nearby GMU 22 suggest that Rocky Mountain bighorn sheep may be moving beyond the boundaries of the newly colonized population. This ram has darker pelage and heavier musculature than any of the other rams observed in this population (J. Heffelfinger, personal observation; Fig. 2). Additionally, a few of the sheep radio-collared on Black Mesa north of the Salt River have crossed to the south side of the river near Klondike Butte, showing that the river may not completely prohibit movements. Previous to this study, bighorn sheep have been reported periodically on Klondike Butte (J. Heffelfinger, personal observation).

## MANAGEMENT IMPLICATIONS

Diagnosing an introgressed population of mixed subspecies may not be straight-forward. For example, because males are more prone to long-distance, exploratory movements (Monson and Sumner 1980), they are the most likely to move to a nearby population of a different subspecies. Offspring of a Rocky Mountain bighorn male in an otherwise desert bighorn population will all carry desert bighorn sheep mtDNA and would not be detectable with the methods used here. There are microsatellite loci available for bighorn sheep that would be informative and allow managers to diagnose mixed populations by looking at nuclear DNA (Epps et al. 2005*b*).

Given the documented movements of bighorn sheep in the past, the colonization of Rocky Mountain bighorn sheep we documented could jeopardize the subspecific integrity of bighorn sheep in central Arizona. This is potentially problematic from a biological and administrative perspective. From a biological standpoint, the size difference between the 2 subspecies (Rocky Mountain sheep can be 20-25% larger than desert sheep; J. Heffelfinger, personal observation) could cause reproductive problems such as dystocia, as has been documented in white-tailed deer (*Odocoileus virginianus*, Galindo-Leal and Weber 1994). It is possible that larger Rocky Mountain bighorn sheep males impregnating smaller desert females could result in prepartum lambs that are too large for successful birthing.

There also are several administrative issues. First, hunters in Arizona are allowed to harvest only 1 Rocky Mountain and 1 desert bighorn sheep in a lifetime. A population of sheep that is known or suspected to be a mixture of these 2 subspecies obviously presents an administrative problem for managers and hunters. In a mixed population, it would be necessary to administratively designate which subspecies was represented by the animals harvested from that population. Additionally, some organizations, such as the Boone and Crockett Club, keep records of hunter-harvested animals and have different record-keeping categories for desert and Rocky Mountain bighorn sheep. A mixed population renders any animals taken from that population ineligible for entry or would have to be entered in the larger Rocky Mountain category regardless of outward appearance.

There is considerable interest in the sheep hunting community in collecting a mature specimen from each of the 4 major categories of mountain sheep: Dall's sheep (*Ovis dalli*), Stone's sheep (*O. d. stonei*), desert bighorn, and Rocky Mountain bighorn. A population of compromised subspecific integrity obviously has social and biological implications. Desert bighorn sheep are not as widely distributed or abundant as Rocky Mountain bighorns. Hunting opportunities for desert sheep are quite limited as compared to the other 3 recognized forms of wild mountain sheep. Managers must keep this in mind when dealing with Rocky Mountain bighorn sheep expanding beyond their natural range and into historic desert sheep range.

Once a population becomes a mixture of subspecies, the situation cannot be reversed without depopulation and re-establishment. Because of this, it is imperative that managers consider the consequences of natural movements and use translocation to lessen, rather than hasten, the occurrence of intermingling. Managers should retain geographic buffers between bighorn sheep subspecies.

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