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*Research Article*

# Genetic Outcomes of Translocation of Bighorn Sheep in Arizona

**DAPHNE A. GILLE,** *Genetics Research Laboratory, California Department of Fish and Wildlife, Rancho Cordova, CA 95670, USA; and Department of Population Health and Reproduction, University of California, Davis, CA 95616, USA*

**MICHAEL R. BUCHALSKI,** *Genetics Research Laboratory, California Department of Fish and Wildlife, Rancho Cordova, CA 95670, USA*

**DAVE CONRAD,** *Arizona Game and Fish Department, Phoenix, AZ 85086, USA*

**ESTHER S. RUBIN,** *Arizona Game and Fish Department, Phoenix, AZ 85086, USA*

**AMBER MUNIG,** *Arizona Game and Fish Department, Phoenix, AZ 85086, USA*

**BRIAN F. WAKELING,** *Nevada Department of Wildlife, Reno, NV 89511, USA*

**CLINTON W. EPPS,** *Department of Fisheries and Wildlife, Oregon State University, Corvallis, OR 97331, USA*

**TYLER G. CREECH,** *Center for Large Landscape Conservation, Bozeman, MT 95771, USA*

**RACHEL CROWHURST,** *Department of Fisheries and Wildlife, Oregon State University, Corvallis, OR 97331, USA*

**BRANDON HOLTON,** *National Park Service, Grand Canyon National Park, Grand Canyon, AZ 86023, USA*

**RYAN MONELLO,** *National Park Service, Biological Resources Division, Fort Collins, CO 80525, USA*

**WALTER M. BOYCE,** *Wildlife Health Center, University of California, Davis, CA 95616, USA*

**MARIA CECILIA T. PENEDO,** *Veterinary Genetics Laboratory, University of California, Davis, CA 95616, USA*

**HOLLY B. ERNEST**,[1] *Wildlife Genomics and Disease Ecology Laboratory, Department of Veterinary Sciences, University of Wyoming, Laramie, WY 82070, USA*

**ABSTRACT** Translocation is an important management tool that has been used for >50 years in Arizona, USA, to increase bighorn sheep (*Ovis canadensis*) population densities and to restore herds to suitable habitat throughout their historical range. Yet, translocation can also alter the underlying genetic diversity and spatial structure of managed wildlife species in beneficial or detrimental ways. To evaluate the long-term effect of translocation actions on bighorn sheep, we characterized statewide genetic structure and diversity using microsatellite and mitochondrial DNA data in 16 indigenous and translocated (supplemented or reintroduced) Arizona populations sampled between 2005 and 2012. Populations that were recipients of translocated animals showed no reduction in genetic diversity with allelic richness and heterozygosity estimates equivalent to, and in some cases greater than, indigenous source populations. The indigenous population occupying the Silver Bell Mountains population displayed the lowest indices of genetic diversity but shared mitochondrial DNA haplotypes with the Mohawk Mountains, Sierra Pinta, and Cabeza Prieta populations, indicating past connectivity and potential opportunities for genetic management if warranted. Bayesian clustering on genetic similarity and genetic divergence estimates corroborated previous work differentiating Rocky Mountain bighorn sheep (*O. c. canadensis*) and 2 desert lineages corresponding with Nelson's (*O. c. nelsoni*) and Mexican desert bighorn sheep (*O. c. mexicana*). In northern Arizona, assignment tests confirmed the presence of 2 indigenous metapopulations of Nelson's desert bighorn sheep in the Black Mountains and Grand Canyon and indicated that gene flow from the Grand Canyon population has likely played a role in maintaining genetic diversity and mitigating founder effects among multiple translocated populations in the area. In southern Arizona, we detected genetic structure consistent with 2 metapopulations of Mexican desert bighorn sheep representing a departure from current management practices that consider this lineage to be a single genetic unit. Several lines of genetic evidence presented in this study suggest that the Bill Williams River area is the contemporary contact zone for the 2 desert lineages; however, the degree to which translocation has enhanced introgression is unknown. Despite relative isolation from other herds, the translocated Rocky Mountain bighorn sheep population in eastern Arizona had high levels of allelic richness and heterozygosity and a negative inbreeding coefficient, conceivably as a result of multiple translocation events from sources in Colorado and New Mexico, USA. Although translocation management has successfully contributed to the reestablishment of bighorn sheep populations in Arizona without diminishing genetic diversity, future translocation should proceed with caution to preserve the genetic integrity and potential local adaptation within the Nelson's and Mexican desert bighorn sheep metapopulations identified in this study. © 2019 The Wildlife Society.

**KEY WORDS** Arizona, bighorn sheep, genetic diversity, management, microsatellite, mitochondrial DNA, *Ovis canadensis*, translocation.

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[1]*E-mail: holly.ernest@uwyo.edu*

Translocation is a tool in wildlife management that involves the intentional, human-mediated movement of individuals, populations, or species from one area with release in another (International Union for Conservation of Nature 2013). Translocations have been employed to achieve a number of management and conservation goals such as increasing population abundance of threatened species, restoring species to their historical range, preserving biodiversity, mitigating the effects of climate change, enhancing genetic diversity, and preventing inbreeding depression (Griffith et al. 1989, Seddon and Armstrong 2016). One species that has derived substantial benefit from translocations is bighorn sheep (*Ovis canadensis*) in North America. Bighorn sheep were numerous and widespread in suitable habitat throughout the southwestern United States and northern Mexico until the arrival of European settlers mainly in the early nineteenth century but as early as the sixteenth century (Buechner 1960). Introduction of livestock diseases, unregulated hunting, competition with livestock for forage and water, water diversion and appropriation, and habitat fragmentation have reduced both bighorn sheep population density and occupied range (Buechner 1960). The establishment of wildlife refuges and strict hunting regulations in the mid-twentieth century allowed bighorn sheep populations to stabilize and begin to modestly increase in numbers (Monson 1990, Lee 1998, Gutiérrez-Espeleta et al. 2000). However, bighorn sheep typically exhibit high levels of philopatry to natal mountain ranges, a behavior that limits the rate of repopulation within available habitat and necessitates human intervention if restoring populations beyond the range of dispersal (Geist 1971, Bleich et al. 1990). Current management programs that focus on reintroducing animals into unoccupied habitat located within their historical range, and supplementing existing populations via translocation, have proven to be effective in promoting population persistence (Bailey 1990, Jessup et al. 1995, Singer et al. 2000).

Translocation ultimately affects genetic variation within the focal populations (Avise 1994, Storfer 1999). Furthermore, the genetic outcomes cannot be fully anticipated and may not be favorable. In bighorn sheep reintroduction, a subset of animals is translocated from a source herd and used to repatriate historically occupied habitat. Because only a fraction of the genetic diversity of the source population is represented in the founder group, these newly established populations are susceptible to genetic drift, founder effects, bottlenecks, and inbreeding depression potentially leading to reduced fitness and higher extinction probability, all of which have been well documented in bighorn sheep (Ramey et al. 2000, Hedrick et al. 2001, Whittaker et al. 2004, Rioux-Paquette et al. 2010). In population supplementation, source animals are translocated into an existing bighorn sheep population. Although the outcome is less clear, population supplementation generally enhances genetic diversity in the recipient population, and thus may also increase evolutionary and adaptive potential and improve population fitness (i.e., genetic rescue; Tallmon et al. 2004, Hogg et al. 2006, Hedrick and Fredrickson 2010, Weeks et al. 2011, Frankham 2015). However, population supplementation also carries theoretical risks, such as the swamping of locally adapted alleles and the disruption of co-adapted gene complexes, potentially leading to outbreeding depression and reduced fitness of hybrid offspring (Storfer 1999, Edmands 2007, Hedrick and Frederickson 2010). To our knowledge, outbreeding depression has not been detected in bighorn sheep (Miller et al. 2012) but remains a noteworthy management concern. Additionally, with both reintroduction and population supplementation, there is the risk that source animals may not be well adapted to local conditions (Wiedmann and Sargeant 2014).

As an iconic game animal, bighorn sheep have a lengthy history of active management and translocation in Arizona. Much like in the rest of the southwestern United States, bighorn sheep were extirpated throughout most of Arizona in the late nineteenth century (Lee 1993), but large herds managed to persist in the Black and Kofa mountains with numerous populations of unknown sizes distributed statewide (Arizona Game and Fish Department [AZGFD], unpublished data). Translocation programs in Arizona began in 1958 (Wild Sheep Working Group 2015) initially to augment numbers and maintain genetic integrity among populations of 2 desert bighorn sheep lineages: Nelson's (*O. c. nelsoni*) and Mexican desert bighorn sheep (*O. c. mexicana*). Later, translocation management included augmentation of the Rocky Mountain bighorn sheep (*O. c. canadensis*) lineage after Rocky Mountain bighorn sheep immigrated from New Mexico to areas near Morenci Mine (AZGFD, unpublished data). The 2 desert lineages are commonly distinguished by putative geographic location and male horn morphology. Nelson's desert bighorn sheep are generally found north of the Bill Williams River in northwestern Arizona and males have wide, flared horns, whereas Mexican desert bighorn sheep are believed to inhabit areas south of the Bill Williams River and males have heavy, tightly curled horns (Cowan 1940; AZGFD, unpublished data). Rocky Mountain bighorn sheep are generally much larger than desert bighorn sheep and are found in eastern and central Arizona (Buechner 1960). Buchalski et al. (2016) further confirmed the presence of these 3 ancestral lineages in Arizona using microsatellite and mitochondrial DNA data.

The 3 bighorn sheep lineages in Arizona are currently managed as distinct metapopulations made up of multiple local populations that occupy discrete mountain ranges. Nelson's desert bighorn sheep from the Black Mountains have been used as a source for translocation to reestablish populations within historical habitat in northern Arizona (Fig. 1; Wild Sheep Working Group 2015). Translocations of Mexican desert bighorn sheep in central and southern Arizona are much more complex. Multiple source populations in the Kofa, Plomosa, New Water, and Castle Dome mountains among others (Fig. 1) have been tapped for reintroduction and population supplementation (Wild Sheep Working Group 2015). The original source of Rocky Mountain bighorn sheep in Arizona is not well understood and it is uncertain if the subspecies occurred naturally in the state prior to immigration from New Mexico and transplant from Colorado and New Mexico to the Morenci Mine area

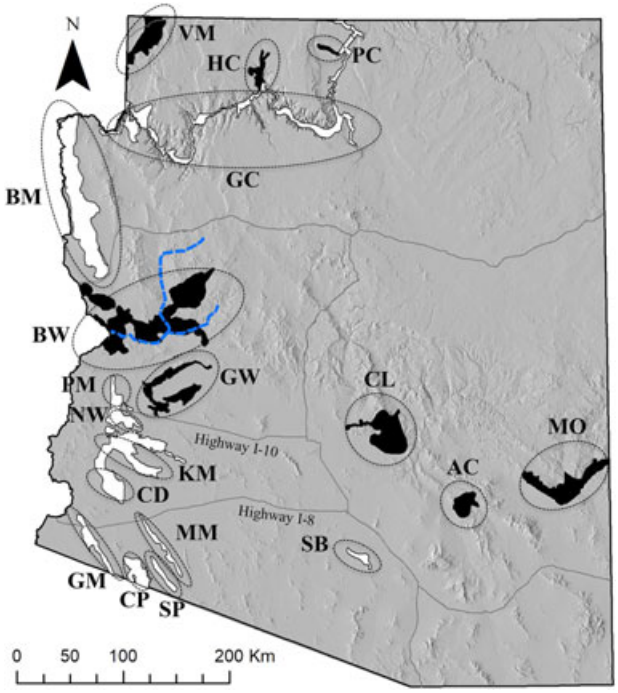


**Figure 1.** Ranges of indigenous (white shading) and translocated (black shading) bighorn sheep populations in Arizona, USA, sampled in this study. Populations are circled. BM = Black Mountains, PC = Paria Canyon, HC = Hack Canyon, VM = Virgin Mountains, BW = Bill Williams River National Wildlife Refuge, SB = Silver Bell Mountains, GM = Gila Mountains, MM = Mohawk Mountains, PM = Plomosa Mountains, NW = New Water Mountains, KM = Kofa Mountains, CD = Castle Dome Mountains, CL = Canyon Lake, AC = Aravaipa Canyon, GW = Granite Wash and Harquahala mountains, MO = Morenci Mine (above collected between 2005–2012), GC = Grand Canyon (2011–2013), CP = Cabeza Prieta, and SP = Sierra Pinta (2002). Gray lines denote major highways. Current management policy treats desert bighorn sheep north of the Bill Williams River (dashed blue line) as Nelson's desert bighorn sheep and those to the south as Mexican desert bighorn sheep. Samples of Rocky Mountain bighorn sheep analyzed in the study were collected from the Morenci Mine population.

(Fig. 1; Wild Sheep Working Group 2015). There have been no intentional translocations of Rocky Mountain bighorn sheep within Arizona to areas that overlap with desert bighorn sheep to preserve separation of ancestral lineages. Bighorn sheep abundance in Arizona has increased over time (Monson 1990, Lee 1998, Gutiérrez-Espeleta et al. 2000) and is presently stable (AZGFD, unpublished data) but the genetic effects of >50 years of translocations are largely unknown.

Published surveys of genetic variation among bighorn sheep populations in Arizona following translocations have been performed on too coarse (Gutiérrez-Espeleta et al. 2000, Buchalski et al. 2016) or too fine (Hedrick 2014) of a spatial scale to inform effective statewide species management. Previous research suggests large source populations of bighorn sheep will have greater genetic diversity than reintroduced populations that originate from a small group of founders (Wright 1931, Hedrick et al. 2001). The genetic diversity of bighorn sheep populations that have been supplemented via translocation is more difficult to predict, but generally we expect supplementation to introduce genetic variation to recipient populations; although the retention of that variation depends upon the success of supplementation, population size, and other factors. Olson et al. (2013) reported that genetic diversity was greatest in mixed bighorn sheep populations in Oregon, USA, that were established and supplemented from multiple sources; genetic diversity declined going from mixed to first order (established from a single source) to second order (established from a first-order source) populations. Further, reintroductions can place distinct lineages in close geographic proximity, facilitating introgression for the first time, or after long periods of separation, which has potentially significant fitness consequences.

We present a descriptive study of microsatellite and mitochondrial DNA diversity in indigenous and translocated (i.e., recipient of translocated animals for reintroduction or supplementation) populations of bighorn sheep in Arizona to assess the genetic outcomes of over half a century of translocation management. Our specific objectives were to compare genetic diversity in indigenous and translocated bighorn sheep populations to determine whether translocated populations are as diverse as their source population(s) or if there is clear evidence of a founder event; identify geographic areas where translocation may have resulted in introgression events, either between lineages or with adjacent indigenous populations; and recommend management actions that will maintain the integrity of the 3 ancestral lineages and enhance the genetic diversity of bighorn sheep herds in Arizona.

## STUDY AREA

We performed a statewide survey of genetic diversity within bighorn sheep populations (samples collected during 2005–2012) in Arizona. The state of Arizona is 295,000 $km^2$ in size with diverse topography including desert valleys and basins, high plateaus, and extensive mountain ranges. The study area encompassed the majority of occupied bighorn sheep habitat in the state and spanned 3 distinct desert ecosystems: the Mojave Desert in the northwest, the Sonoran Desert in the southwest, and the Colorado Plateau in the east. Elevation of these areas ranges from sea level to approximately 2,000 m on average (Hansen 1990). Although much of the state is arid with temperatures exceeding 40°C during summer, some portions regularly experience cold winters with temperatures around 7°C (Hansen 1990, Byrkit et al. 2018). There is a bimodal precipitation pattern in the form of winter rains and summer thundershowers that bring an average annual rainfall of 2.5 cm to 13.0 cm (Hansen 1990, Sheppard et al. 2002, Byrkit et al. 2018). Arizona has a rich floral community with over 3,000 native plant species that are found in a wide range of vegetation communities including forests, grasslands, deserts, chaparral, scrub, and riparian habitats (Lehr and Pinkava 1980, 1982; Brown 1982). Dominant flora varies with topography and elevation, but common species are creosote bush (*Larrea tridentate*), mesquite (*Prosopis* spp.), palo verde (*Parkinsonia* spp.), Joshua tree (*Yucca brevifolia*), pinyon pine (*Pinus* spp.), and juniper (*Juniperus* spp.; Bates and Workman 1983, Hansen 1990). Dominant fauna also varies with habitat type and includes bighorn sheep, deer

(*Odocoileus* spp.), elk (*Cervus elaphus*), fox (*Vulpes* spp.), coyote (*Canis latrans*), mountain lion (*Felis concolor*), black bear (*Ursus americanus*), peccary (*Pecari tajacu*), jackrabbit (*Lepus* spp.), rattlesnake (e.g., *Crotalus* spp.), Gila monster (*Heloderma suspectum*), and roadrunner (*Geococcyx californianus*; Byrkit et al. 2018). Land ownership is comprised primarily of tribal lands (27.8%), private lands (17.3%), Bureau of Land Management (16.6%), United States Forest Service (15.3%), and state lands (12.7%; Arizona State Land Department 2018).

**Desert Bighorn Sheep**

We examined 4 mountain ranges in northern Arizona and the Grand Canyon to represent the Nelson's desert bighorn sheep lineage (Fig. 1; Table 1). The Black Mountains population, which includes the Lake Mead National Recreation Area, has historically served as the translocation source for the lineage and is considered indigenous. Bighorn sheep populations in Paria Canyon, Hack Canyon, and the Virgin Mountains in northern Arizona were believed extirpated prior to reintroductions and subsequent population supplementations from the Black Mountains that occurred between 1979 and 2005 (Wild Sheep Working Group 2015). Each of those translocated populations lies close to the north rim of the Grand Canyon, one of the largest indigenous populations of bighorn sheep persisting in Arizona. Thus, it is possible that bighorn sheep were transiently present in Paria Canyon, Hack Canyon, and the Virgin Mountains at the time of reintroduction; current levels of gene flow between these translocated populations and the Grand Canyon animals are undescribed.

We focused on 10 mountain ranges in central and southern Arizona to represent the Mexican desert bighorn sheep lineage (Fig. 1; Table 1). Indigenous populations located in the Plomosa, New Water, Kofa, and Castle Dome mountains (i.e., the Kofa Mountains suite) are frequently used as sources for translocation within the lineage. Multiple reintroductions from a variety of sources established populations in historical habitat at Canyon Lake and Aravaipa Canyon between 1958 and 1995; according to management records, no bighorn sheep were present in these areas at the time of the first reintroduction (AZGFD, unpublished data; Wild Sheep Working Group 2015). Translocations into Granite Wash and Harquahala mountains from numerous sources occurred between 1994 and 2001 and served to supplement the existing population of bighorn sheep (AZGFD, unpublished data; Wild Sheep Working Group 2015). Indigenous populations of bighorn sheep also inhabit mountain ranges in southern Arizona, namely the Gila, Mohawk, and Silver Bell mountains. Highways are known barriers to connectivity and gene flow in bighorn sheep (Epps et al. 2005) and the presence of 2 major interstate highways (I–8 and I–10; Fig. 1) within the native range of Mexican desert bighorn sheep suggests the potential for genetic substructure.

Patterns of post-glacial expansion for the 2 desert bighorn sheep lineages and their historical contact zone prior to extirpation throughout much of Arizona, are unknown. Such patterns are unlikely to be discernible because Nelson's desert bighorn sheep were repeatedly translocated to the Bill Williams River area (BW) from the Black Mountains between 1986 and 1995 (Wild Sheep Working Group 2015). Management records indicate that an indigenous population of bighorn sheep likely occupied portions of BW prior to translocation; however, classification of the herd as either Nelson's or Mexican desert bighorn sheep is uncertain

**Table 1.** Bighorn sheep from Arizona, USA, 2005–2012, tissue sample information; $n$ = number of samples.

| Population identification | $n$ | Location | Lineage | Indigenous or translocated? | Origin |
|---|---|---|---|---|---|
| BM | 42 | Black Mountains, Lake Mead | Nelson's | Indigenous | |
| PC | 10 | Paria Canyon | Nelson's | Translocated | BM |
| HC | 17 | Hack Canyon | Nelson's | Translocated | BM |
| VM | 14 | Virgin Mountains | Nelson's | Translocated | BM |
| BW | 13 | Bill Williams River National Wildlife Refuge | Nelson's and Mexican | Translocated | BM, PM, and KM |
| SB | 6 | Silver Bell Mountains | Mexican | Indigenous | |
| GM | 12 | Gila Mountains | Mexican | Indigenous | |
| MM | 6 | Mohawk Mountains | Mexican | Indigenous | |
| PM | 21 | Plomosa Mountains | Mexican | Indigenous | |
| NW | 5 | New Water Mountains | Mexican | Indigenous | |
| KM | 31 | Kofa Mountains | Mexican | Indigenous | |
| CD | 7 | Castle Dome Mountains | Mexican | Indigenous | |
| CL | 37 | Canyon Lake | Mexican | Translocated | PM, NW, KM, CD, and Trigo Mountains |
| AC | 7 | Aravaipa Canyon | Mexican | Translocated | KM, NW, PM, Crater, Sand Tank, and Sauceda Mountains |
| GW | 5 | Granite Wash and Harquahala mountains | Mexican | Translocated | KM, CD, Maricopa, Gila Bend, and Eagle Tail Mountains |
| MO | 27 | Morenci Mine | Mexican | Translocated | Colorado: Rocky Mountain National Park, Tarryall Mountains, Rampart Range, and Almont Triangle State Wildlife Area; New Mexico: Wheeler Peak, and Pecos Wilderness |

(AZGFD, unpublished data). Mexican desert bighorn sheep were concurrently reintroduced to habitat south of BW from the Kofa and Plomosa mountains in 1985 and 1986 (Wild Sheep Working Group 2015). The proximity of these translocation efforts in combination with an abundance of suitable habitat provided dispersal and interbreeding opportunities between the 2 desert lineages. Radio-collar data indicate several individuals from reestablished populations of Mexican desert bighorn sheep crossed the Bill Williams River and remained in BW (AZGFD, unpublished data). Further, admixture between the 2 lineages has been detected among individuals sampled from BW (Buchalski et al. 2016), but a detailed genetic examination of bighorn sheep at this modern contact zone is needed and included in this study (Fig. 1 and Table 1).

### Rocky Mountain Bighorn Sheep

It is uncertain whether Rocky Mountain bighorn sheep were ever indigenous to Arizona prior to immigration from New Mexico; however, individuals were introduced on multiple occasions to mountain ranges near Morenci Mine from Rocky Mountain National Park and Gunnison, Colorado, and from Taos and the Pecos Wilderness, New Mexico, between 1979 and 2005 (Wild Sheep Working Group 2015). Local management records explain that bighorn sheep of unknown lineage once inhabited the Morenci Mine area but were extirpated long before Rocky Mountain bighorn sheep translocation (AZGFD, unpublished data). The Morenci Mine population (Fig. 1; Table 1) is isolated from nearest-neighboring herds of Mexican desert bighorn sheep by inhospitable terrain; however, genetic introgression between Mexican desert bighorn sheep and Rocky Mountain bighorn sheep was discovered by Buchalski et al. (2016).

## METHODS

### Sample Collection and DNA Extraction

Bighorn sheep samples from Arizona ($n = 260$) were collected between 2005 and 2012 as part of a study plan approved by AZGFD personnel (Table 1). Tissue samples consisted of skin punches, muscle biopsies, and blood from AZGFD-captured or hunter-killed adult bighorn sheep. Global positioning system (GPS) coordinates of each bighorn sheep sample were recorded at the time of capture or estimated based upon hunter descriptions of the kill site locations. We considered desert bighorn sheep samples either putative Nelson's or Mexican desert bighorn sheep based upon their location north or south of the Bill Williams River, respectively (AZGFD, unpublished data; Buchalski et al. 2016). We considered bighorn sheep samples from Morenci Mine in eastern Arizona to be Rocky Mountain bighorn sheep given the translocation history of this area (Wild Sheep Working Group 2015) and previous genetic analyses (Buchalski et al. 2016). In addition, we obtained reference Rocky Mountain bighorn sheep samples ($n = 70$) collected between 1993 and 2005 from 5 locations (Table S1, available online in Supporting Information). We held samples received for this project under the University of California Davis Institutional Animal Care and Use Committee protocol number 16271 (H. B. Ernest). We extracted DNA using QIAmp® Blood and Tissue Kits (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions.

We also incorporated pre-existing data into our analyses from the indigenous population of Nelson's desert bighorn sheep that inhabits the Grand Canyon (C. W. Epps, Oregon State University, unpublished data) and indigenous populations of Mexican desert bighorn sheep located at Sierra Pinta and Cabeza Prieta (J. W. Cain, United States Geological Survey New Mexico Cooperative Fish and Wildlife Research Unit, unpublished data; Fig. 1). Data from the Grand Canyon population consisted of microsatellite genotypes ($n = 259$) collected under research permit numbers GRCA-2011-SCI-018 and GRCA-2012-SCI-0020 (C. W. Epps) approved by the National Park Service between 2011 and 2013 and generated from fecal pellets, tissue, and blood; DNA was extracted as described in Creech et al. (2017). We added existing mitochondrial DNA sequence data to our study to increase geographic coverage of indigenous Mexican desert bighorn sheep populations that inhabit southern Arizona. These sequence data were generated from blood samples from bighorn sheep from Sierra Pinta ($n = 6$) and Cabeza Prieta ($n = 7$; Fig. 1) collected as part of a study plan approved by the United States Fish and Wildlife Service in 2002; DNA was extracted according to Epps et al. (2005).

### Microsatellite Genotyping

We performed genotyping of bighorn sheep from Arizona and reference Rocky Mountain individuals in duplicate at 40 microsatellite loci according to the methods of Buchalski et al. (2015); locus BMS2639 could not be reliably scored and we excluded it from further analysis. We genotyped each Grand Canyon individual at 7 (AE129, FCB193, FCB304, MAF36, MAF48, MAF65, and MAF209) of the 39 total microsatellite loci using the protocol outlined in Creech et al. (2017). We achieved consistency in allele scoring between each method by allele call conversions generated from a shared dataset ($n = 8$) that was independently genotyped at each of the originating laboratories.

### Mitochondrial DNA Sequencing

We amplified a 515-base pair (bp) fragment located within the mitochondrial control region in all Arizona bighorn sheep individuals as per Epps et al. (2005). We performed Sanger sequencing in the forward and reverse direction using ABI BigDye Terminator version 3.1 Cycle Sequencing chemistry on an ABI 3730 Capillary Electrophoresis Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). We edited the resulting sequences and generated contigs using Sequencher version 4.7 (GeneCodes Corporation, Ann Arbor, MI, USA) software and resolved discrepancies between forward and reverse sequences by eye. Bighorn sheep have multiple copies of a 75-bp repetitive sequence (RS) within the mitochondrial control region; all individuals in this study had $\geq 2$ copies of this RS and several had 3 copies (Buchalski et al. 2016). If 3 RSs were present, we generated homologous 515-bp sequences by trimming the

second RS copy (as written in Buchalski et al. 2016) prior to any subsequent analysis.

## Molecular Analyses

*Microsatellites.*—We computed indices of genetic diversity for indigenous and translocated bighorn sheep populations in Arizona. We tested deviations from Hardy-Weinberg equilibrium and linkage equilibrium at microsatellite loci across populations in GENEPOP version 4.2 (Raymond and Rousset 1995, Rousset 2008) using 10,000 dememorization steps, 100 batches, and 1,000 iterations/batch. We adjusted significance values for multiple comparisons with a sequential Bonferroni correction (Holm 1979, Rice 1989). We calculated the average number of alleles across loci ($A$), number of private alleles ($A_P$), and expected ($H_E$) and observed ($H_O$) heterozygosity in GENALEX version 6.502 (Peakall and Smouse 2012). We estimated allelic richness ($A_R$) and the inbreeding coefficient ($F_{IS}$) in FSTAT version 2.9.3.2 (Goudet 1995). We obtained pairwise $F_{ST}$ values, as defined by Weir and Cockerham (1984), among populations of indigenous and translocated bighorn sheep from Arizona using ARLEQUIN version 3.5.12 (Excoffier and Lischer 2010) with 10,000 random permutations and we adjusted $P$-values via the sequential Bonferroni method (Holm 1979, Rice 1989).

We applied the Bayesian clustering method implemented in STRUCTURE version 2.3.4 (Pritchard et al. 2000) to evaluate inter- and intra-population genetic structure of bighorn sheep in Arizona. With this approach, individuals are assigned to a genetic cluster ($K$) based upon allele frequencies that are used to compute the probability of membership to that cluster. We performed 3 separate STRUCTURE analyses. First, to characterize statewide structure of bighorn sheep, we used microsatellite data from all samples (Table 1), including the 5 reference populations of Rocky Mountain bighorn sheep (Table S1) to aid in the identification of Rocky Mountain bighorn sheep genetic clusters in Arizona. To detect local structure that may be obscured by more ancient divergence events, we performed separate nested STRUCTURE runs on the Nelson's and Mexican desert bighorn sheep lineages. For each analysis, we conducted 6 replicate runs of each $K$ with a burn-in period of 500,000 and 1,000,000 Markov chain Monte Carlo repetitions. We selected the population admixture model because we expected gene flow between populations and sampling location was given as a prior (LOCPRIOR). For all 3 analyses, $K$ varied between 1 and the number of mountain ranges because each range could represent a distinct genetic population: statewide analysis including admixed BW and Rocky Mountain reference samples ($K=1$ to $K=21$), Nelson's desert bighorn sheep nested analysis ($K=1$ to $K=4$), and Mexican desert bighorn sheep nested analysis ($K=1$ to $K=10$). We inferred the optimal $K$ for each analysis from the $\Delta K$ statistic of Evanno et al. (2005) calculated in STRUCTURE HARVESTER version 0.6.93 (Earl and vonHoldt 2012). We obtained average coefficients of membership across the 6 replicate runs with CLUMPP version 1.1.2 (Jakobsson and Rosenberg 2007); we visualized the graphical output using DISTRUCT (Rosenberg 2004).

As recommended by Blair et al. (2012), we also used a second independent method to test for hierarchical population structure within the Nelson's and Mexican desert bighorn sheep lineages; these lineages retain a greater number of indigenous local populations, which may exhibit natural substructure. We implemented principal component analysis (PCA) and plots to visualize relationships among sample groups created using the adegenet package (Jombart 2008) in R (R Development Core Team 2017).

We used assignment tests to examine the origin of individual bighorn sheep in translocated populations for which we had samples from every source population (i.e., every documented source of genetic input). Using this approach, we tested the genetic similarity of bighorn sheep in BW to a single Nelson's desert bighorn sheep source population (Black Mountains) and multiple Mexican desert bighorn sheep source populations in the Kofa Mountains suite. We also examined genetic similarity among indigenous Nelson's desert bighorn sheep in the Black Mountains and all 3 translocated populations (Paria Canyon, Hack Canyon, and the Virgin Mountains). Similarly, we compared reintroduced populations of Nelson's desert bighorn sheep in northern Arizona against their source population (the Black Mountains) and the pre-existing genotype data from the adjacent Grand Canyon population. We implemented population assignment using the Bayesian assignment criterion of Rannala and Mountain (1997), which has high assignment accuracy for samples as small as ours ($n \sim 10$; Cornuet et al. 1999). We graphically present the assignment results using the GeneCharts methodology developed by Russell et al. (2010), with an update for accommodating individuals with partially missing data (Veale et al. 2013). GeneCharts are scatterplots of the distribution of log posterior probabilities of genotypes for a set of reference populations with values from a focal population superimposed. The individual points represent the posterior probability of finding that bighorn sheep's genotype in one of the reference populations. We calculated the 1% and 99% quantiles of the posterior distribution of log-genotype probabilities for each reference population to approximate the posterior distribution of the fit to that population. We performed analyses in R (R Development Core Team 2017) using code provided by Veale et al. (2013).

*Mitochondrial DNA.*—We collapsed redundant haplotypes among all bighorn sheep populations from Arizona, including those from Sierra Pinta and Cabeza Prieta in FABOX version 1.41 (Villesen 2007). We compared unique haplotypes to published sequences in GenBank with the basic local alignment search tool (BLAST; Altschul et al. 1990). We obtained the number of haplotypes ($H_T$), the number of private haplotypes ($H_P$), haplotype diversity ($H_D$), and nucleotide diversity ($\pi$) in each Arizona population using DNASP version 5.10.01 (Librado and Rozas 2009). We calculated pairwise $F_{ST}$ among populations of indigenous and translocated bighorn sheep in Arizona in ARLEQUIN version 3.5.12 (Excoffier and Lischer 2010). We assessed significance based upon 10,000 permutations and adjusted

resulting $P$-values using a sequential Bonferroni correction (Holm 1979, Rice 1989).

## RESULTS

### Microsatellite Analyses

We successfully genotyped 260 bighorn sheep from Arizona and 70 reference Rocky Mountain bighorn sheep at 39 microsatellite loci. All microsatellite loci incorporated in this study were polymorphic with the possible exception of locus INRA063, which was monomorphic in all but 1 individual. After applying a sequential Bonferroni correction, only loci BM203 and ETH152 deviated significantly from Hardy–Weinberg equilibrium in the BW population. Thirteen pairs of loci showed significant departures from linkage equilibrium, but for each pair this was only the case for a single population. Therefore, we retained all loci for downstream analyses.

Allelic richness and expected and observed heterozygosity were moderate and similar across populations (Table 2). Moreover, genetic diversity in translocated recipient populations was similar or greater than the indigenous source populations that we tested (Table 2). One noticeable outlier was the indigenous Silver Bell population, which exhibited the lowest allelic richness value of 1.84 and the lowest expected and observed heterozygosity of 0.24 and 0.26, respectively. We detected the highest allelic richness value and heterozygosities in the translocated Hack Canyon population. Private alleles were present in both indigenous and translocated populations of bighorn sheep from Arizona. We found the greatest number of private alleles, not surprisingly, in the translocated Rocky Mountain bighorn sheep population in Morenci Mine ($A_P = 27$). Among desert bighorn sheep, the indigenous Nelson's desert bighorn sheep Black Mountains source population possessed the greatest number of private alleles ($A_P = 7$). We observed private alleles in each of the 3 translocated Nelson's desert bighorn sheep populations. Two of these 3 populations also displayed negative inbreeding coefficients ($F_{IS} = -0.01$ in both cases), indicative of slight outbreeding, whereas the inbreeding coefficient of the indigenous Black Mountains source population was positive (0.05), demonstrative of heterozygote deficiency. The highest inbreeding coefficient was in the translocated BW population ($F_{IS} = 0.16$).

We obtained significant pairwise $F_{ST}$ values from microsatellite data for 94% of the 120 population comparisons (Table 3). The previous classification of Nelson's and Mexican desert bighorn sheep as distinct ancestral lineages by Buchalski et al. (2016) was supported here with moderate to very high estimates of $F_{ST}$ (Wright 1978, Hartl and Clark 1997) that ranged from 0.15 to 0.39 for between-lineage comparisons. Similarly, there was great genetic differentiation between Rocky Mountain bighorn sheep and the 2 desert lineages with $F_{ST}$ values between 0.21 and 0.37. In general, genetic differentiation was lower among populations of Nelson's desert bighorn sheep (mean $F_{ST} = 0.05$) than among populations of Mexican desert bighorn sheep (mean $F_{ST} = 0.13$). As expected for connected populations, comparisons of indigenous source populations and their translocated recipient populations (Table 3) revealed minimal genetic structure ($F_{ST} = 0.02$ to 0.11). The low genetic diversity within the Silver Bell Mountains population was highlighted by $F_{ST}$ values between 0.23 and 0.39. The mean $F_{ST}$ value was lower between the Kofa Mountains suite and the Plomosa Mountains (0.07; separated by highway I-10) than between the Kofa Mountains suite and the Gila Mountains (0.15) or the Mohawk Mountains (0.16) populations (both separated from the Kofa Mountains suite by highway I-8 and the Gila River). Genetic differentiation between the Gila Mountains and the Mohawk Mountains

**Table 2.** Indices of microsatellite genetic diversity of indigenous and translocated populations of each bighorn sheep lineage, Arizona, USA, 2005–2012; $n$ = number of samples, $A$ = average number of alleles per locus, $A_R$ = allelic richness, $A_P$ = number of private alleles, $H_E$ = expected heterozygosity, SE = standard error, $H_O$ = observed heterozygosity, and $F_{IS}$ = inbreeding coefficient.

| Population[a] | Lineage | Indigenous or translocated | $n$ | $A$ | $A_R$ | $A_P$ | $H_E$ | SE | $H_O$ | SE | $F_{IS}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| BM | Nelson's | Indigenous | 42 | 4.62 | 3.21 | 7 | 0.58 | 0.03 | 0.56 | 0.03 | 0.05 |
| PC | Nelson's | Translocated | 10 | 3.49 | 3.06 | 1 | 0.55 | 0.03 | 0.59 | 0.04 | −0.01 |
| HC | Nelson's | Translocated | 17 | 4.85 | 3.63 | 4 | 0.63 | 0.03 | 0.66 | 0.03 | −0.01 |
| VM | Nelson's | Translocated | 14 | 4.41 | 3.46 | 4 | 0.60 | 0.03 | 0.58 | 0.03 | 0.06 |
| BW | Nelson's and Mexican | Translocated | 13 | 4.26 | 3.36 | 2 | 0.56 | 0.04 | 0.50 | 0.04 | 0.16 |
| SB | Mexican | Indigenous | 6 | 1.90 | 1.84 | 2 | 0.24 | 0.04 | 0.26 | 0.04 | 0.02 |
| GM | Mexican | Indigenous | 12 | 3.77 | 3.03 | 2 | 0.51 | 0.04 | 0.52 | 0.04 | 0.01 |
| MM | Mexican | Indigenous | 6 | 2.95 | 2.85 | 0 | 0.46 | 0.04 | 0.55 | 0.06 | −0.10 |
| PM | Mexican | Indigenous | 21 | 3.85 | 2.96 | 3 | 0.53 | 0.03 | 0.54 | 0.04 | 0.02 |
| NW | Mexican | Indigenous | 5 | 3.15 | 3.15 | 0 | 0.51 | 0.04 | 0.54 | 0.05 | 0.06 |
| KM | Mexican | Indigenous | 31 | 4.67 | 3.27 | 2 | 0.57 | 0.03 | 0.59 | 0.04 | −0.01 |
| CD | Mexican | Indigenous | 7 | 3.36 | 3.09 | 0 | 0.52 | 0.03 | 0.57 | 0.05 | −0.02 |
| CL | Mexican | Translocated | 37 | 4.23 | 3.15 | 2 | 0.57 | 0.03 | 0.56 | 0.03 | 0.03 |
| AC | Mexican | Translocated | 7 | 3.21 | 2.95 | 0 | 0.51 | 0.04 | 0.52 | 0.04 | 0.05 |
| GW | Mexican | Translocated | 5 | 3.13 | 3.13 | 0 | 0.51 | 0.03 | 0.57 | 0.05 | −0.02 |
| MO | Rocky Mountain | Translocated | 27 | 4.46 | 3.33 | 27 | 0.60 | 0.03 | 0.66 | 0.03 | −0.07 |

[a] BM = Black Mountains, PC = Paria Canyon, HC = Hack Canyon, VM = Virgin Mountains, BW = Bill Williams River National Wildlife Refuge, SB = Silver Bell Mountains, GM = Gila Mountains, MM = Mohawk Mountains, PM = Plomosa Mountains, NW = New Water Mountains, KM = Kofa Mountains, CD = Castle Dome Mountains, CL = Canyon Lake, AC = Aravaipa Canyon, GW = Granite Wash and Harquahala mountains, MO = Morenci Mine.

**Table 3.** Pairwise $F_{ST}$ values for microsatellite (below diagonal line) and mitochondrial sequence (above diagonal line) data from desert bighorn sheep in Arizona, USA, 2005–2012, organized by lineage (Mtn = Mountain) and population; * = values are non-significant following Bonferroni correction, and ‡ = comparison between a source and recipient population.

| | Nelson's | | | | Nelson's and Mexican | Mexican | | | | | | | | | | Rocky Mtn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Population[a] | BM | PC | HC | VM | BW | SB | GM | MM | PM | NW | KM | CD | CL | AC | GW | MO |
| BM | | −0.05*‡ | 0.09*‡ | 0.04*‡ | 0.39 | 0.93 | 0.87 | 0.93 | 0.83 | 0.87 | 0.63 | 0.81 | 0.64 | 0.90 | 0.88 | 0.94 |
| PC | 0.08‡ | | 0.02* | −0.01* | 0.26* | 1.00 | 0.83 | 0.97 | 0.77 | 0.83 | 0.51 | 0.70 | 0.54 | 0.88 | 0.84* | 0.93 |
| HC | 0.06‡ | 0.03 | | −0.02* | 0.09* | 0.69 | 0.60 | 0.69 | 0.64 | 0.55 | 0.43 | 0.47 | 0.44 | 0.67 | 0.53* | 0.87 |
| VM | 0.03‡ | 0.06 | 0.04 | | 0.17* | 0.84 | 0.73 | 0.83 | 0.72 | 0.70 | 0.49 | 0.60 | 0.51 | 0.79 | 0.69* | 0.90 |
| BW | 0.14 | 0.15 | 0.12 | 0.11 | | 0.62 | 0.51 | 0.62 | 0.58 | 0.45 | 0.34 | 0.32* | 0.37 | 0.59 | 0.36* | 0.86 |
| SB | 0.36 | 0.39 | 0.33 | 0.36 | 0.30 | | 0.83 | 0.40* | 0.76 | 0.79* | 0.53 | 0.65* | 0.59 | 0.85 | 0.76* | 0.93 |
| GM | 0.23 | 0.24 | 0.19 | 0.21 | 0.14 | 0.23 | | 0.81 | 0.67 | 0.61 | 0.45 | 0.48* | 0.48 | 0.71 | 0.50* | 0.91 |
| MM | 0.23 | 0.23 | 0.18 | 0.21 | 0.14 | 0.24 | 0.04 | | 0.76 | 0.76* | 0.54 | 0.63* | 0.59 | 0.82 | 0.72* | 0.93 |
| PM | 0.21 | 0.22 | 0.19 | 0.19 | 0.12 | 0.31 | 0.17 | 0.17 | | 0.00* | 0.09* | 0.04* | 0.28 | 0.18*‡ | 0.08* | 0.88 |
| NW | 0.20 | 0.21 | 0.17 | 0.18 | 0.09 | 0.34 | 0.14 | 0.17 | 0.10 | | −0.03* | −0.14* | 0.07* | 0.13*‡ | −0.10* | 0.88 |
| KM | 0.19 | 0.20 | 0.17 | 0.17 | 0.08 | 0.25 | 0.12 | 0.13 | 0.06 | 0.03 | | −0.10* | 0.07* | 0.19*‡ | 0.00*‡ | 0.80 |
| CD | 0.20 | 0.21 | 0.17 | 0.17 | 0.09 | 0.33 | 0.16 | 0.19 | 0.06 | 0.05* | 0.01* | | 0.05* | 0.14 * | −0.15*‡ | 0.86 |
| CL | 0.20 | 0.22 | 0.19 | 0.19 | 0.11 | 0.28 | 0.16 | 0.16 | 0.08 | 0.06 | 0.04 | 0.05 | | 0.39 | 0.17* | 0.83 |
| AC | 0.21 | 0.23 | 0.17 | 0.19 | 0.14 | 0.29 | 0.14 | 0.14 | 0.10‡ | 0.10‡ | 0.08‡ | 0.12 | 0.10 | | −0.02* | 0.90 |
| GW | 0.18 | 0.20 | 0.16 | 0.15 | 0.08* | 0.33 | 0.13 | 0.14 | 0.05 | 0.05* | 0.02*‡ | 0.04*‡ | 0.06 | 0.05* | | 0.88 |
| MO | 0.22 | 0.24 | 0.21 | 0.21 | 0.22 | 0.37 | 0.26 | 0.26 | 0.25 | 0.23 | 0.23 | 0.22 | 0.23 | 0.26 | 0.23 | |

[a] BM = Black Mountains, PC = Paria Canyon, HC = Hack Canyon, VM = Virgin Mountains, BW = Bill Williams River National Wildlife Refuge, SB = Silver Bell Mountains, GM = Gila Mountains, MM = Mohawk Mountains, PM = Plomosa Mountains, NW = New Water Mountains, KM = Kofa Mountains, CD = Castle Dome Mountains, CL = Canyon Lake, AC = Aravaipa Canyon, GW = Granite Wash and Harquahala mountains, MO = Morenci Mine.

populations was also low ($F_{ST} = 0.04$) and consistent with connectivity (Epps et al. 2010). Non-significant estimates of $F_{ST}$ may indicate substantial gene flow between populations or that the sample sizes were too small to detect population structure and should be interpreted with caution.

Bayesian cluster analysis using all Arizona and reference Rocky Mountain bighorn sheep samples showed the greatest support for 3 genetic clusters ($K = 3$; Fig. 2a) corresponding to the Nelson's desert bighorn sheep, Mexican desert bighorn sheep, and Rocky Mountain bighorn sheep lineages identified in Arizona by Buchalski et al. (2016). Admixture between the 2 desert lineages was evident in the BW population and in a few individuals in the Hack Canyon and Virgin Mountains populations. Also, 2 bighorn sheep in the Morenci Mine population had mixed membership to the Mexican desert bighorn sheep and Rocky Mountain bighorn sheep genetic clusters. The low levels of admixture with Mexican desert bighorn sheep in the British Columbia and Alberta Rocky Mountain bighorn sheep reference populations represents uncharacterized genetic substructure within Rocky Mountain bighorn sheep and is irrelevant to this study.

The most probable $K$ value was 2 for Nelson's and Mexican desert bighorn sheep nested STRUCTURE analyses (Fig. 2b,c). However, the Evanno method cannot compute the likelihood of $K = 1$ so the possibility that each lineage could be a single, interbreeding genetic population cannot be ruled out based upon these data alone. In the Nelson's desert bighorn sheep analysis, individuals from the indigenous Black Mountains population assigned to 1 cluster and the second cluster consisted of individuals from the translocated Paria Canyon, Hack Canyon, and Virgin Mountains populations. Some admixture was evident between the 2 clusters, a finding that may reflect both gene flow between the translocated populations and an unsampled population, or the persistence of animals in these areas prior to reintroduction. In contrast, there was a very clear division of the 2 Mexican desert bighorn sheep clusters. Individuals from the indigenous Silver Bell, Gila, and Mohawk mountains populations made up 1 cluster located in southern Arizona. Individuals from the indigenous Plomosa, New Water, Kofa, and Castle Dome Mountains populations and translocated Canyon Lake, Aravaipa Canyon, and Granite Wash and Harquahala mountains populations formed a second cluster that represented the western-central portion of the state. We identified a single migrant individual, or offspring of a migrant, in the Gila Mountains population that had allele frequencies more consistent with bighorn sheep in the Kofa Mountains suite (Fig. 2c).

Principal components analysis of Nelson's and Mexican desert bighorn sheep populations corroborated nested STRUCTURE analyses and identified 2 genetic clusters within each lineage (Fig. 3a,b). In the Nelson's desert bighorn sheep analysis, we observed 2 genetic clusters along the first principal component axis (8.7% variance explained), 1 corresponding to the indigenous Black Mountains populations and 1 that included the translocated Paria

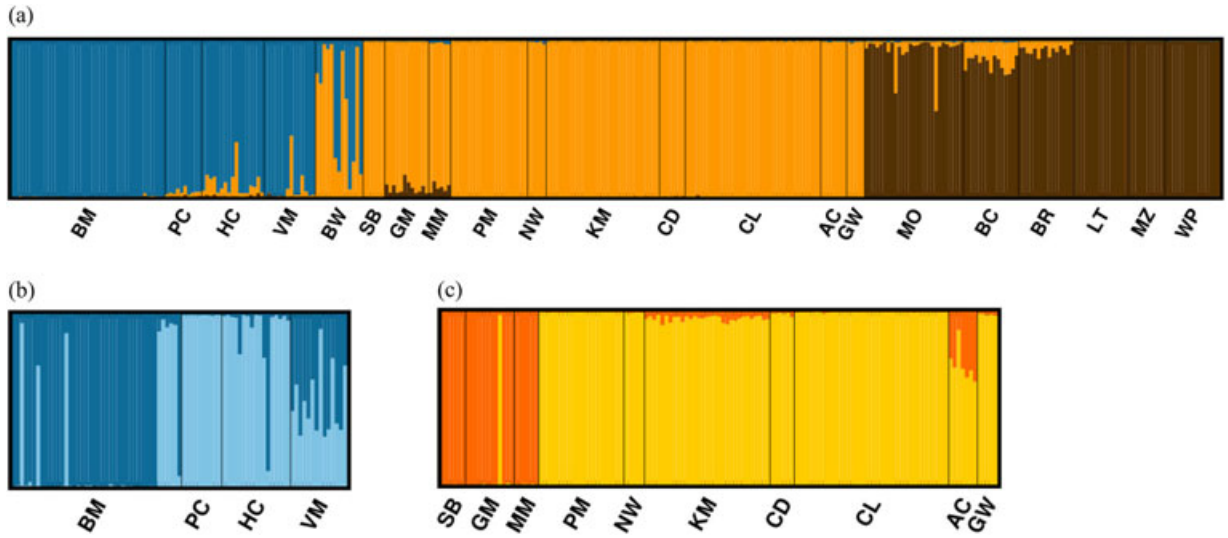


**Figure 2.** Bar plots generated by STRUCTURE showing 3 genetic clusters ($K = 3$) consistent with 2 desert (Nelson's [blue] and Mexican desert bighorn sheep [orange]) and 1 Rocky Mountain (brown) ancestral lineages by population (a), 2 nested genetic clusters ($K = 2$) of Nelson's desert bighorn sheep by population (b), and 2 nested genetic clusters ($K = 2$) of Mexican desert bighorn sheep by population (c), in Arizona, USA, 2005–2012. Each line represents the proportion of assignment of an individual to a particular cluster (a single immigrant, or offspring of an immigrant, is evident in the Gila Mountains population). Bighorn sheep populations from Arizona: BM = Black Mountains, PC = Paria Canyon, HC = Hack Canyon, VM = Virgin Mountains, BW = Bill Williams River National Wildlife Refuge, SB = Silver Bell Mountains, GM = Gila Mountains, MM = Mohawk Mountains, PM = Plomosa Mountains, NW = New Water Mountains, KM = Kofa Mountains, CD = Castle Dome Mountains, CL = Canyon Lake, AC = Aravaipa Canyon, GW = Granite Wash and Harquahala mountains, and MO = Morenci Mine. Reference Rocky Mountain bighorn sheep populations (1993–2005): BC = British Columbia, Canada, BR = Alberta, Canada, LT = Pecos Wilderness, New Mexico, USA, MZ = Manzano Mountains, New Mexico, WP = Wheeler Peak, New Mexico.

and Hack canyon populations; the Virgin Mountains population appeared admixed. As before, the indigenous Silver Bell, Gila, and Mohawk mountains populations of Mexican desert bighorn sheep clustered together and individuals from the indigenous Plomosa, New Water, Kofa, and Castle Dome mountains and the translocated Canyon Lake, Aravaipa Canyon, and Granite Wash and Harquahala mountains comprised the second cluster along the first principal component axis (9.7% variance explained). The migrant, or migrant offspring, from the western-central cluster in the Gila Mountains population was also evident in the Mexican desert bighorn sheep PCA scatter plot.

Genotypes of approximately half of the bighorn sheep from BW fell within the 99% quantiles in the GeneChart of the known Nelson's and Mexican desert bighorn sheep source populations for that area (Black Mountains and Kofa Mountains suite), consistent with the presence of a contact zone between the lineages (Fig. 4). The genotypes of 6 bighorn sheep fell outside the distribution for Mexican desert bighorn sheep from the Kofa Mountains suite, suggesting that a remnant indigenous population of this lineage may have occupied the area prior to translocations.

Exploratory assignment tests of Nelson's desert bighorn sheep populations revealed that genotypes of several animals from Paria Canyon, Hack Canyon, and the Virgin Mountains fell outside the 99% quantile in the GeneChart for the Black Mountains, their documented translocation source (Fig. S1). This result indicates gene flow from an untested population or that bighorn sheep were present in these areas but unknown to managers prior to translocation; subsequent assignment tests that included pre-existing genotypes of bighorn sheep from the Grand Canyon were informative on this point (Fig. 5a–c). Among the 3 translocated Nelson's desert bighorn sheep populations, several individuals still showed a high probability of assignment to their source population in the Black Mountains (i.e., within the 99% quantile in the GeneChart and above the diagonal line). However, many others assigned with higher probability to the Grand Canyon population (i.e., outside the source population distribution and below the diagonal line). The distribution of genotypes from Hack Canyon and, to a lesser extent, in Paria Canyon, indicates substantial gene flow with the Grand Canyon population. Bighorn sheep from the Virgin Mountains, the translocated population at the greatest geographic distance from the Grand Canyon, showed the highest probability of assignment to the Black Mountains population with the exception of a few individuals. In addition, several of the alleles that appeared private to Hack Canyon, Paria Canyon, and the Virgin Mountains were shared with the Grand Canyon (data not shown) providing further evidence of genetic connectivity with this indigenous population.

### Mitochondrial DNA Analyses

Twenty of the 260 bighorn sheep samples from Arizona did not amplify by polymerase chain reaction or produced poor sequences and were excluded from the data set. After sequence trimming to remove RSs (Buchalski et al. 2016), we identified 30 unique haplotypes among 16 populations of bighorn sheep from Arizona (Tables 4 and S3). All haplotypes were previously published in GenBank (Table S2). The 2 desert lineages had no haplotypes in common; however, we observed

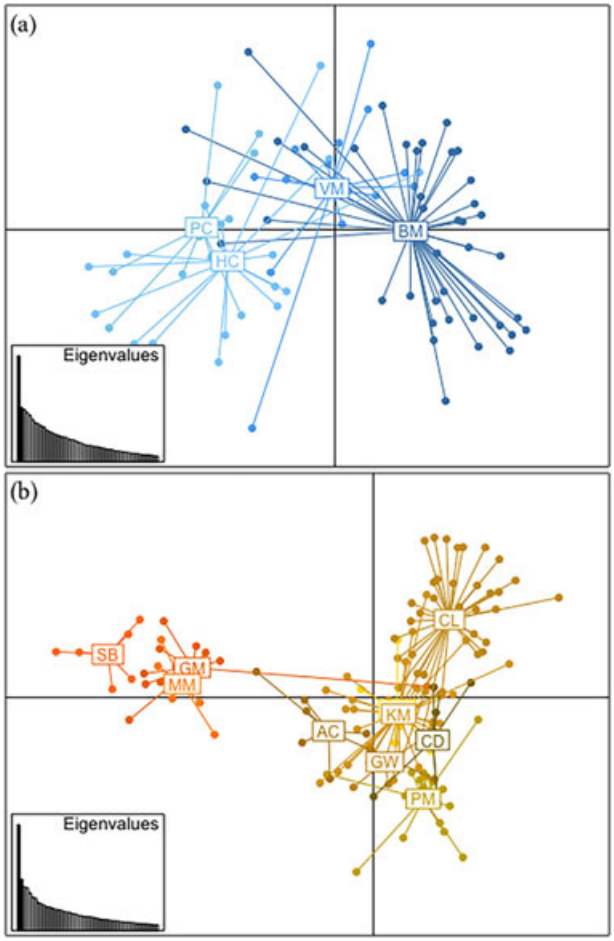


**Figure 3.** Two-dimensional plots of the results of principal component (PC) analyses ($x$-axis = PC1, $y$-axis = PC2) of Nelson's (a) and Mexican desert bighorn sheep (b), from Arizona, USA, 2005–2012. Genotypes of individual bighorn sheep within each population are denoted by points. Colors correspond to genetic clusters identified by STRUCTURE. The indigenous Black Mountains (BM) cluster is shown in dark blue and the translocated Paria Canyon (PC), Hack Canyon (HC), and Virgin Mountains (VM) populations are shown in shades of light blue. The southern Mexican desert bighorn sheep cluster is shown in shades of orange, SB = Silver Bell Mountains, GM = Gila Mountains, MM = Mohawk Mountains, and the western-central Mexican desert bighorn sheep cluster is shown in shades of yellow and gold, PM = Plomosa Mountains, NW = New Water Mountains, KM = Kofa Mountains, CD = Castle Dome Mountains, CL = Canyon Lake, AC = Aravaipa Canyon, and GW = Granite Wash and Harquahala mountains.

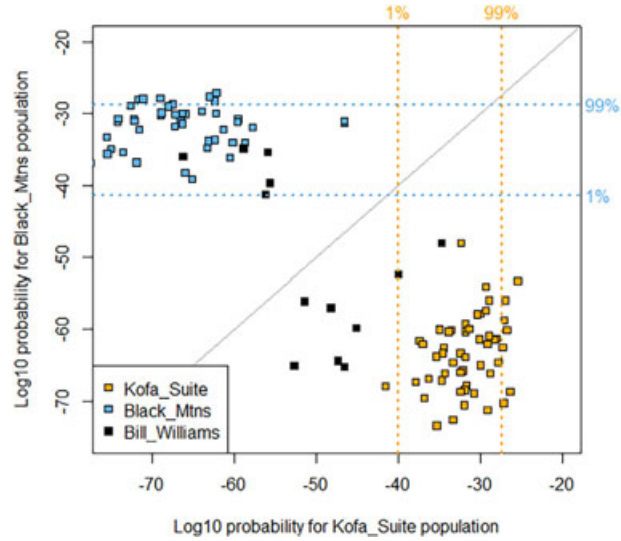


**Figure 4.** Assignment test results displayed as GeneCharts showing the genetic similarity of the admixed Bill Williams River area population to the indigenous Nelson's desert bighorn sheep source population (Black Mountains [Mtns]; above diagonal line) and to the indigenous Mexican desert bighorn sheep source populations (the Kofa Mountains suite; below diagonal line) in Arizona, USA, 2005–2012. Each axis of the scatter plot shows the estimated log posterior probability of finding an individual bighorn sheep genotype within a population of interest. A high log posterior probability indicates that a genotype has been confidently assigned to a particular population. Each dot represents an individual. Dotted lines demarcate the 99% quantiles.

both Nelson's and Mexican desert bighorn sheep haplotypes in the admixed BW population. The most common haplotype found among sampled populations of Nelson's desert bighorn sheep was GC1. Haplotype CP5, which differs from GC1 by a single bp, was private among BW bighorn sheep. This observation is incongruent with our microsatellite GeneChart results and suggests that Nelson's desert bighorn sheep may have also occupied this area prior to translocations. Despite high levels of genetic diversity at 39 microsatellite loci, we found only 2 haplotypes in the indigenous Black Mountains population, with 1 haplotype present in just a single individual (Fig. 6). As would be expected, we also observed GC1 in each of the 3 translocated populations putatively established from the Black Mountains population, yet we detected several private haplotypes in both the translocated Hack Canyon and the Virgin Mountains populations (Fig. 6). No mitochondrial DNA sequences were available from the Grand Canyon samples. Although haplotype E was private to the Hack Canyon population in this study, it was also the dominant haplotype among bighorn sheep in Zion National Park, Utah, USA, in Buchalski et al. (2016). Such a result points to potential gene flow between northern Arizona and southern Utah populations of bighorn sheep. The distribution of haplotypes among Mexican desert bighorn sheep populations provided further evidence for the 2 distinct groups within the lineage. We did not observe haplotype sharing among bighorn sheep from southern Arizona and those in the western-central part of the state, with the exception of the single migrant individual, or migrant offspring, previously identified by STRUCTURE analysis. The addition of pre-existing mitochondrial DNA sequences from Sierra Pinta and Cabeza Prieta revealed gene flow among indigenous bighorn sheep populations in southern Arizona, as evident by the distribution of haplotypes (Fig. 6; Table 4). Similar to microsatellite results, individuals from the Silver Bell Mountains population had the least amount of mitochondrial DNA diversity, sharing a single haplotype (U) that also occurred in the Mohawk Mountains, Sierra Pinta, and Cabeza Prieta populations (Fig. 6). The mitochondrial DNA sequences from Sierra Pinta and Cabeza Prieta also revealed gene flow among these populations and the Mohawk Mountains and the Gila Mountains populations (Fig. 6). Haplotypes were largely shared among the populations comprising the Kofa Mountains suite (Table 4). The presence of multiple private haplotypes in the translocated

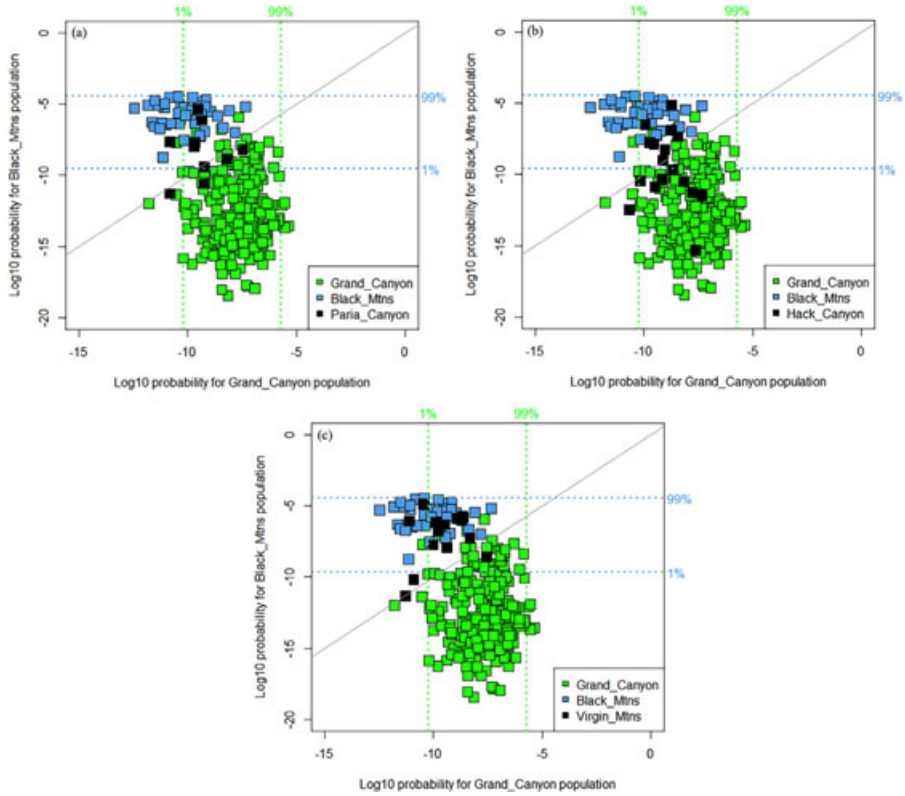


**Figure 5.** Assignment test results displayed as GeneCharts showing the genetic similarity of Nelson's desert bighorn sheep individuals from the translocated Paria Canyon (a), Hack Canyon (b), and Virgin Mountains (c) populations to the putative Black Mountains (Mtns) source (above diagonal line; 2005–2012) and the indigenous Grand Canyon (below diagonal line; 2011–2013) populations in Arizona, USA. Each axis of the scatter plot shows the estimated log posterior probability of finding an individual bighorn sheep genotype within a population of interest. A high log posterior probability indicates that a genotype has been confidently assigned to a particular population. Each dot represents an individual. Dotted lines demarcate the 99% quantiles.

Canyon Lake and Aravaipa Canyon populations are consistent with translocation records (i.e., unsampled source populations). The 3 Rocky Mountain bighorn sheep haplotypes identified in the Morenci Mine population were private to the lineage. Unfortunately, the 2 individuals with admixed genotypes as indicated by STRUCTURE did not sequence successfully, so it was not possible to evaluate whether mitochondrial DNA introgression has occurred between the Rocky Mountain bighorn sheep and desert lineages.

Results from pairwise $F_{ST}$ tests using mitochondrial data tended to agree with the microsatellite analysis. Comparisons between populations of the Nelson's and Mexican desert bighorn sheep lineages were generally significant and ranged from 0.43 to 1.00 (Table 3). Genetic differentiation between Rocky Mountain bighorn sheep at Morenci Mine and all other populations of desert bighorn sheep was consistently high with $F_{ST}$ values from 0.80 and 0.94 (Table 3). Such high estimates of pairwise $F_{ST}$ computed from maternally inherited mitochondrial DNA sequences may suggest that female groupings or philopatry have influenced genetic population structure among bighorn sheep in Arizona (Geist 1971, Festa-Bianchet 1986, Boyce et al. 1999). Alternatively, the difference in magnitude between $F_{ST}$ values obtained from mitochondrial DNA sequences and microsatellite data could also reflect the disparate modes of inheritance, effective population size, and mutation rates inherent to each type of genetic marker (Weber and Wong 1993, Slatkin 1995, Jorde et al. 1998, Scheffler 1999, Ballard and Whitlock 2004). Few of the $F_{ST}$ values for within-lineage comparisons were significant and may again indicate genetic connectivity among bighorn sheep populations or that the sample sizes were too small to discern population structure.

## DISCUSSION

This investigation examined the effects of decades of translocation management and natural dispersal events on genetic variation in indigenous and translocated populations of bighorn sheep in Arizona. Contrary to published accounts of genetic bottlenecks following bighorn sheep reintroductions (Fitzsimmons et al. 1997, Ramey et al. 2000, Hedrick et al. 2001, Whittaker et al. 2004) and predictions of low genetic diversity generated from simulated pedigrees (Hedrick 2014), we found substantial genetic variation in translocated populations of desert bighorn sheep in Arizona. The most direct comparisons of genetic diversity between indigenous and translocated populations could be made for populations of Nelson's desert bighorn sheep in northern Arizona because there was only a single source (Black Mountains). Between 12 and 24 animals were initially translocated to re-establish the Paria Canyon, Hack Canyon, and Virgin Mountains populations (Wild Sheep Working Group 2015). Despite this small number of founders,

**Table 4.** Mitochondrial haplotype distribution among populations of bighorn sheep in Arizona, USA, 2005–2012; Pop = population, I = indigenous, T = translocated, * = private haplotypes, and ‡ = haplotype is not private because it was also observed in pre-existing sequence data from either the Sierra Pinta or Cabeza Prieta populations.

| Pop[a] | Lineage | I or T | GC1 | A | E | CP1 | CP2 | CP3 | CP4 | U | W | SD1 | CS1 | T | SC1 | SD4 | SD8 | SD6 | SD7 | SC2 | SD11 | SD9 | SD10 | SD12 | SD14 | SD13 | SD15 | CS2 | CP5 | RM1 | RM2 | RM3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BM | Nelson's | I | 38 | 1 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| PC | Nelson's | T | 10 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| HC | Nelson's | T | 12 | 1 | 1* | 2* | | | | | | | | | | | | | | | | | | | | | | | | | | |
| VM | Nelson's | T | 11 | | | | 1* | 1* | 1* | | | | | | | | | | | | | | | | | | | | | | | |
| BW | Nelson's & Mexican | T | 5 | | | | | | | | | | 1 | | | | | | | | | | | | | | | 4 | 2* | | | |
| SB | Mexican | I | | | | | | | | 6 | | | | | | | | | | | | | | | | | | | | | | |
| GM | Mexican | I | | | | | | | | | 5‡ | 2* | 1 | | | | | | | | | | | | | | | | | | | |
| MM | Mexican | I | | | | | | | | | 3 | | | 3‡ | | | | | | | | | | | | | | | | | | |
| PM | Mexican | I | | | | | | | | | | | | | 3 | 16 | 1 | 1 | | | | | | | | | | | | | | |
| NW | Mexican | I | | | | | | | | | | | | | | 1 | 1 | | 2 | 1 | | | | | | | | | | | | |
| KM | Mexican | I | | | | | | | | | | | 3 | | 5 | 8 | | 1 | 6 | 3 | 2 | 2* | 1* | | | | | | | | | |
| CD | Mexican | I | | | | | | | | | | | 1 | | 1 | 2 | | | 1 | | | | | | | | | | | | | |
| CL | Mexican | T | | | | | | | | | | | 2 | | 2 | 1 | | | 17 | | 1 | | | 9* | 2* | 2* | | | | | | |
| AC | Mexican | T | | | | | | | | | | | | | | 1 | | | | 5 | | | | | | | 1* | | | | | |
| GW | Mexican | T | | | | | | | | | | | | | | | | 1 | | 1 | | | | | | | | 1 | | | | |
| MO | Rocky Mountain | T | | | | | | | | | | | | | | | | | | | | | | | | | | | | 18* | 2* | 1* |

[a] BM = Black Mountains, PC = Paria Canyon, HC = Hack Canyon, VM = Virgin Mountains, BW = Bill Williams River National Wildlife Refuge, SB = Silver Bell Mountains, GM = Gila Mountains, MM = Mohawk Mountains, PM = Plomosa Mountains, NW = New Water Mountains, KM = Kofa Mountains, CD = Castle Dome Mountains, CL = Canyon Lake, AC = Aravaipa Canyon, GW = Granite Wash and Harquahala mountains, MO = Morenci Mine.

subsequent population supplementation events, along with natural dispersal of bighorn sheep from the Grand Canyon, have resulted in genetically diverse populations. In fact, allelic richness, heterozygosity, and haplotype diversity for all 3 translocated Nelson's desert bighorn sheep populations were similar to or greater than their Black Mountains source. Similarly, there was also no reduction in genetic diversity in translocated Mexican desert bighorn sheep populations compared to the indigenous sources that we tested. Translocation from multiple sources, including the large and genetically diverse Kofa Mountains suite of populations, has likely been important in sustaining genetic diversity within translocated Mexican desert bighorn sheep populations. This is particularly true for Canyon Lake and Aravaipa Canyon, both of which were surrounded by large areas unoccupied by sheep and therefore isolated from other populations at the time of initial translocation. Excluding the Silver Bell Mountains population, overall allelic richness and heterozygosity levels were moderate to high and were consistent with values previously reported for desert bighorn sheep in Arizona, California, Nevada, and New Mexico (Boyce et al. 1996, Gutiérrez-Espeleta et al. 2000, Epps et al. 2005). Despite being isolated from other Rocky Mountain bighorn sheep herds, it was not surprising that allelic richness and heterozygosity of the translocated Morenci Mine population were similarly high and the inbreeding coefficient was negative given that multiple and potentially diverse sources from New Mexico were used to establish this population. Furthermore, the recent translocation of Rocky Mountain bighorn sheep to the Morenci Mine area in 2003–2005 from New Mexico may have introduced genetic variation sufficient to counteract any potential effects of genetic drift predicted for small, isolated populations (Frankham 1996). Finally, it is also possible that Rocky Mountain bighorn sheep from New Mexico may occasionally disperse across the state border and interbreed with Morenci Mine resident bighorn sheep (AZGFD, unpublished data).

Allelic richness and heterozygosity were notably low in the indigenous Silver Bell Mountains population. Historically, the Silver Bell Mountains population was part of a larger complex of bighorn sheep endemic to the neighboring Santa Rita, Catalina, and Rincon mountains. More recently, the population has experienced a marked decline in density likely as a result of urban development, population fragmentation, and disease (AZGFD, unpublished data, Jansen et al. 2007). At the time tissue samples were collected for this investigation (2005–2012), approximately 50 bighorn sheep remained in the Silver Bell Mountains (AZGFD, unpublished data). It is unclear whether reduced genetic diversity was a contributor to or a result of population decline. Such low values may also be a reflection of vacant historical habitat in nearby mountains and potential isolation of the Silver Bell Mountains population from other herds. All individuals sampled from the Silver Bell Mountains population possessed a single haplotype. This haplotype was also observed in half of the bighorn sheep in the Mohawk Mountains population and in the

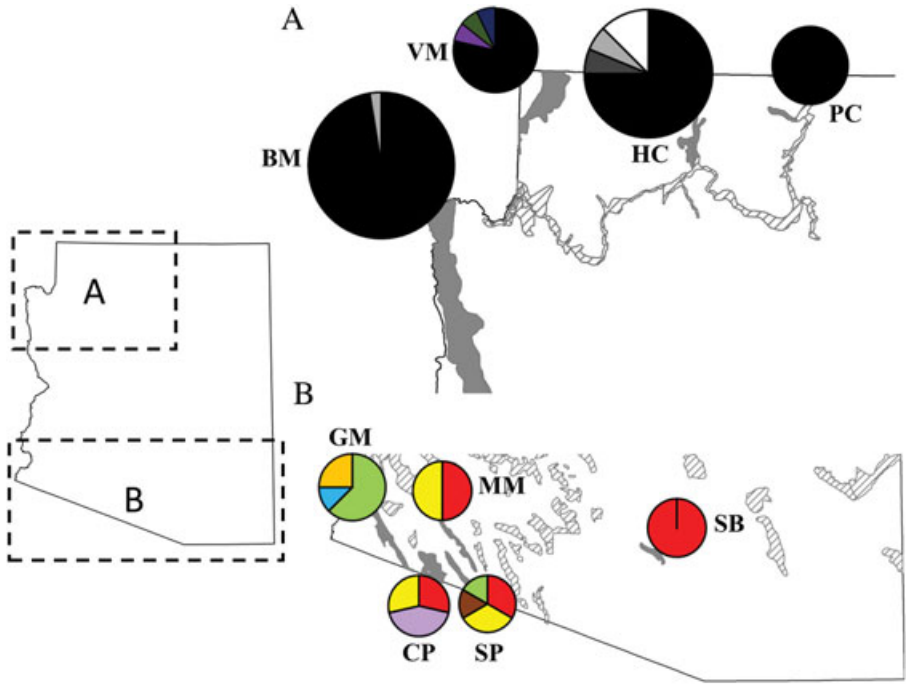


**Figure 6.** Haplotype frequency and distribution in Nelson's desert bighorn sheep populations (A) and select Mexican desert bighorn sheep populations from southern Arizona (B), USA, 2005–2012. Each circle represents a population. Circle size is indicative of sample number. Individual haplotypes are displayed as unique colors. BM = Black Mountains, PC = Paria Canyon, HC = Hack Canyon, VM = Virgin Mountains, SB = Silver Bell Mountains, GM = Gila Mountains, MM = Mohawk Mountains, CP = Cabeza Prieta (2002), and SP = Sierra Pinta (2002). Ranges for each of the select populations are shaded in gray.

Sierra Pinta and Cabeza Prieta populations. In the past 3–5 years, the census size of the Silver Bell Mountains population has steadily increased (AZGFD, unpublished data). Further demographic and genetic investigation is warranted to determine whether dispersal from neighboring herds and subsequent natural genetic rescue (Hedrick and Frederickson 2010) has contributed to population recovery. However, should translocation be necessary in the future to augment the genetically depauperate Silver Bell Mountains population, our results provide preliminary guidance suggesting that the Gila and Mohawk mountains, Sierra Pinta, or Cabeza Prieta may be appropriate, closely related source populations. In this sense, the recommendations made by Wehausen (1991) and Ramey (1995) hold and selecting nearby sources that are genetically similar and that occupy similar habitat as a recipient population remains the best approach for minimizing the translocation of maladapted animals.

It is important to reiterate that for this study, we used the translocated designation to refer to populations that were established either by reintroduction (i.e., animals from a source herd were introduced into unoccupied historical bighorn sheep habitat) or population supplementation (i.e., existing populations were augmented with animals from a source herd). Historical records were insufficient to definitively classify translocations as reintroduction or supplementation events; however, the consensus of all accounts collected by managers is described in the Study Area section. As such, it is possible that we did not observe the classical prediction of low genetic diversity indices (i.e., allelic richness and heterozygosity) associated with reintroduction because most translocations were supplementations. If only a small number of bighorn sheep remained in historical habitat, in some cases unknown to managers, prior to translocation, the genetic effects (e.g., inbreeding, drift, and low heterozygosity) of small population size may have been mitigated with an influx of new alleles. Moreover, the connectivity of populations within each of the 2 desert bighorn sheep lineages has likely also been instrumental in maintaining genetic diversity, as it also has in Nelson's desert bighorn sheep in California (Epps et al. 2005, 2006).

In evaluating the genetic outcomes of translocation, we also must consider the number of generations that have passed since reintroduction or population supplementation. In a finite population, the loss of heterozygosity per generation is expected to be (Hedrick 2000):

$$\frac{H_t}{H_0} = \left(1 - \frac{1}{2Ne}\right)^t \tag{1}$$

where $H_t$ and $H_0$ correspond to the heterozygosity at generation $t$ and 0, respectively, $Ne$ is the effective population size, and $t$ is the number of generations. Thus, if only a few generations have passed between a translocation event and sample collection, we may not yet be able to fully understand the long-term genetic consequences (e.g., heterozygosity loss) of translocation. The time frame of bighorn sheep

translocations in Arizona and the type of translocation is not consistent among the populations we examined. Translocations in this study occurred across a wide range from 1958 to 2005. Assuming a generation time of 6 years (i.e., the mean age of female reproduction; Coltman et al. 2003, Hogg et al. 2006, Johnson et al. 2011), approximately 9 to 0 generations may have passed in some populations between translocation and our sample collection. Furthermore, management records were insufficient to say with certainty whether translocations were true reintroductions or supplementation events and in some populations, there were multiple, repeated translocation events. We, therefore, do not have the data or power to fully resolve the effect of generations passed since translocation on our results. Future studies will be needed to determine whether the high levels of genetic diversity observed here can be maintained over time, and even those investigations will be confounded by the same issues described earlier in this paragraph.

Bayesian clustering on genetic similarity, estimates of pairwise $F_{ST}$, and the distribution of mitochondrial haplotypes corroborated previous work that identified desert bighorn sheep of 2 distinct lineages (Nelson's and Mexican desert bighorn sheep) and the Rocky Mountain lineage in the state. Desert bighorn sheep in Arizona are presently managed as 2 metapopulations: the Nelson's desert bighorn sheep lineage and the Mexican desert bighorn sheep lineage. Under this metapopulation framework, any robust and healthy population of bighorn sheep may serve as a source for translocation provided the source and recipient populations belong to the same lineage. However, our results revealed hierarchical population structure within each desert lineage that should be considered as part of a translocation management strategy (Fig. 7). In northern Arizona, STRUCTURE analysis and assignment test results were congruent and supported the presence of 2 main genetic groups within Nelson's desert bighorn sheep that roughly corresponded to the Black Mountains and translocated populations and Grand Canyon populations. It is also of note that although the translocated Nelson's desert bighorn sheep populations received animals from the Black Mountains, there is also clear evidence of gene flow with the Grand Canyon population. Dispersal between the Grand Canyon and the translocation of managed populations to the north is significant because of the potential for the transmission of novel pathogens. For example, epizootics of bacterial pneumonia have caused numerous large-scale mortality events of bighorn sheep in North America (Monello et al. 2001, Besser et al. 2012). In 2015, bighorn sheep in the Black Mountains tested positive for a strain of *Mycoplasma ovipneumoniae* that has not been detected in Grand Canyon animals (A. E. Justice-Allen, AZGFD, personal communication). Disease surveillance of Nelson's desert bighorn sheep populations therefore remains a critical component of risk assessment prior to translocation.

We also observed 2 distinct genetic groups of Mexican desert bighorn sheep (Fig. 7) consisting of the Kofa Mountains suite plus the translocated Canyon Lake, Aravaipa Canyon, and Granite Wash and Harquahala mountains populations located in western-central Arizona

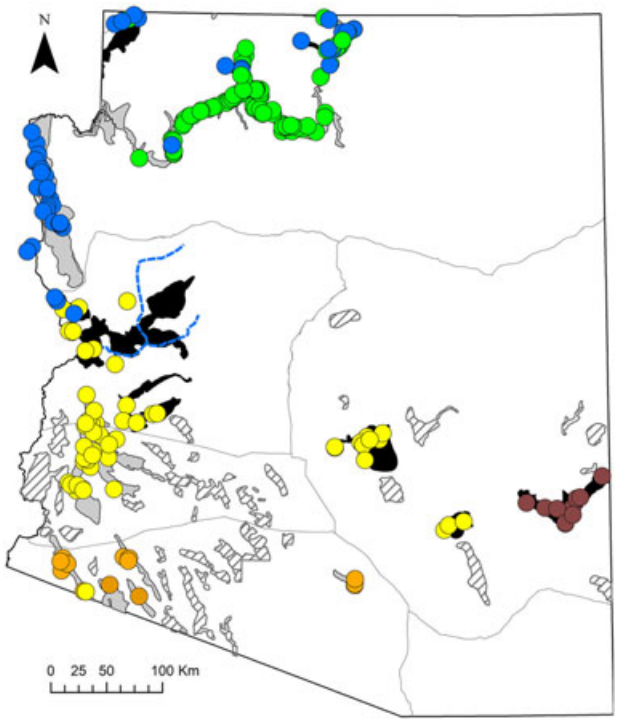


**Figure 7.** Individual sample locations and associated Nelson's desert bighorn sheep (blue = Black Mountains metapopulation and green = Grand Canyon metapopulation) and Mexican desert bighorn sheep (yellow = Kofa metapopulation and orange = southern metapopulation) metapopulations in Arizona, USA, 2002–2013, derived from multiple lines of genetic evidence. The Rocky Mountain bighorn sheep population from Morenci Mine is shown in brown. Gray and black shading denote the ranges of indigenous and translocated populations sampled in this study, respectively. Hatched shading shows ranges of bighorn sheep populations in Arizona from which we had no samples.

(i.e., the Kofa metapopulation) and the Gila, Mohawk, and Silver Bell mountains populations located in southwestern Arizona (i.e., the southern metapopulation). Historically, the Gila River may have bifurcated the Kofa metapopulation and the southern metapopulation. Today, highway I-8 likely reinforces this historical barrier or acts similarly to restrict dispersal and gene flow. Though our results suggest genetic structure consistent with limited gene flow between the Kofa and southern metapopulations, we also have direct evidence that contemporary dispersal still occurs. STRUCTURE analysis, PCA, and haplotype data showed that 1 male sampled from the Gila Mountains had dispersed across the highway from the Kofa metapopulation (Table 4, Fig. 2c). Our data suggest that the indigenous Plomosa Mountains population is genetically most similar to bighorn sheep in the Kofa metapopulation, despite being geographically separated by highway I-10. Investigation of local geography including features associated with the Gila River such as canals, farm fields, fences, and river bottom is needed to determine why highway I-8, or the surrounding area, strongly resists gene flow, but highway I-10 and vicinity does not. A more

comprehensive sampling of all source populations would provide greater resolution of Mexican desert bighorn sheep population substructure. Nonetheless, to preserve genetic diversity and potential local adaptation, it will be prudent for managers to take into account these 2 metapopulations (Fig. 7) when planning future translocation actions. Specifically, the Kofa metapopulation is a large and genetically diverse complex that should be used as a mainstay for translocation in central Arizona. Because the Kofa and southern metapopulations display limited connectivity, future augmentation of herds south of highway I-8 should be performed using animals from within the southern metapopulation.

Our study demonstrates that BW represents the contemporary contact zone between the Nelson's and Mexican desert bighorn sheep lineages. The high inbreeding coefficient ($F_{IS} = 0.16$) calculated for the BW population points to a deficit of heterozygotes and most likely represents a Wahlund effect (Wahlund 1928) resulting from the combination of genotypes from both lineages in our sample. Bayesian clustering analysis in STRUCTURE and assignment tests results presented as GeneCharts further support admixture between the 2 desert lineages in BW. However, which lineage(s) may have historically occupied BW prior to the initiation of translocation as a management tool remains uncertain. GeneChart analysis (Fig. 4) indicated that remnant herds of Mexican desert bighorn sheep may still have inhabited BW prior to translocations from the Kofa Mountains suite. Conversely, the presence of a private mitochondrial haplotype in BW that is 1 bp different from the most common Nelson's desert bighorn sheep haplotype we observed suggests this lineage may have been indigenous to BW. Such data imply that the prehistoric contact zone of Nelson's and Mexican desert bighorn sheep may indeed have been in the BW area although translocation management may still have augmented introgression.

The results of genetic assignment tests in Buchalski et al. (2016), in addition to those presented here clearly show interbreeding between the 2 desert bighorn sheep lineages occupying BW as well as between Rocky Mountain and Mexican desert bighorn sheep in Morenci Mine. It is difficult to say, given insufficient historical records, whether these lineages would have naturally come into contact without translocation management. Genetic analyses of historical samples (e.g., museum specimens) predating bighorn sheep translocations in 1958 for BW and the Morenci Mine area could provide insight into the original range distribution of these lineages and the location of previous contact zones in Arizona. In the absence of such data, managers should assume that introgression will continue and hybrid individuals will become more common on the landscape.

## MANAGEMENT IMPLICATIONS

Our study revealed that it is possible to reestablish populations of large game animals using translocation management without reduction of genetic diversity over the short term and with minimal erosion of ancestral lineage. The current position of AZGFD is that each bighorn sheep lineage (Nelson's desert bighorn sheep, Mexican desert bighorn sheep, and Rocky Mountain bighorn sheep) is managed as a metapopulation. However, given the significant genetic substructure within each desert lineage of unknown cause observed here, we advocate limiting translocation to within each of the 4 genetically differentiated metapopulations designated in this study (Fig. 7). This conservative recommendation is meant to avoid potential adverse consequences (i.e., disruption of local adaptation followed by outbreeding depression and spread of disease) of translocation among the 4 metapopulations until more research can be conducted.

Bighorn sheep that are translocated out of their environmental niche may experience physiological stress leading to heightened predation and disease susceptibility, poor demographic performance and recruitment, and ultimately translocation failure. Furthermore, the translocation of animals to or from an unconnected, immunologically naïve population may cause unintended pathogen transmission and reduce translocation efficacy. According to our data, translocation within each of the 4 metapopulations identified here would reinforce the existing divisions already present among populations, which could be rooted in both postglacial expansion and anthropogenic barriers to movement. Our data also demonstrate that there are enough healthy herds with sufficient standing genetic variation within each cluster to sustain such a strategy of translocation management. Translocation of source animals that are genetically similar to recipient populations as well as the separation of ancestral lineages remains the best means of preserving genetic diversity and potential local adaptation in bighorn sheep. Should the status of existing bighorn sheep populations change (e.g., population decline or loss of genetic diversity), or if new data shed light on the specific origin of genetic divergence among metapopulations, this conservative recommendation may be reevaluated to accommodate new threats to population persistence.

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