The Prairie Naturalist 47:30–39; 2016

Assessing Genetic Variation of Rocky Mountain Bighorn Sheep at Elk Mountain

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ABSTRACT The bighorn sheep (*Ovis canadensis canadensis*) herd occupying the Elk Mountain Region of South Dakota and Wyoming was established in 2001 with 20 individuals. An additional 7 ewes were released in 2004. The population increased to approximately 120 individuals in 2009, but subsequently declined to an estimated 80 individuals. Because of the low population size used to establish the herd and relative isolation of the Elk Mountain Region, potential factors associated with the population decline included reduced genetic diversity. Our objectives were to: 1) assess genetic diversity, 2) measure effective population size (N_e), and 3) compare the genetic diversity of Elk Mountain bighorn sheep to other native and translocated herds throughout the United States. Genetic analysis was conducted on DNA from 43 unique samples collected from bighorn sheep on Elk Mountain between 2012 and 2014, using 15 microsatellite loci. When compared to other populations, our results indicated the Elk Mountain bighorn sheep herd had levels of genetic variation similar to healthy native herds. Additionally, N_e/N for the herd fell within values reported for other healthy bighorn sheep populations. Nevertheless, further genetic evaluation is recommended for all Black Hills bighorn sheep herds.

KEY WORDS bighorn sheep, effective population size, Elk Mountain, genetic variation, translocate.

Bighorn sheep (*Ovis canadensis canadensis*) once numbered in the millions across North America (Buechner 1960, Mattern 1999). Similar to bison (*Bison bison*), bighorns were hunted by Native Americans as a source of food, clothing, and tools (Mattern 1999). Encroachment and uncontrolled hunting, along with the introduction of domestic livestock and their associated diseases, caused bighorn sheep numbers to plummet in the early to mid-1900s (Buechner 1960).

Decline of wildlife populations across their entire distribution in concurrence with the absence of immigrants (most often young males) moving between populations may result in isolated populations with concomitant loss of genetic diversity (Schwartz et al. 1986, Thompson and Jenks 2010). For long-lived, low-fecundity species, such as bighorn sheep, declines in population size coupled with decreased gene flow via population isolation are detrimental (DeForge et al. 1979, Berger 1990, Miller and Waits 2003, Hogg et al. 2006, Hedrick 2014).

Within South Dakota, bighorn sheep were historically abundant in the Black Hills and Badlands regions (South Dakota Game, Fish and Parks [SDGFP] 2007). In the late 1800s, uncontrolled hunting resulted in a sharp decline in populations (SDGFP 2007), and by the early 1900s, bighorn sheep were extirpated from the Black Hills and Badlands (Zimmerman 2008). Reintroduction efforts to South Dakota began in Badlands National Park, in south-central South Dakota in 1964 (Fig. 1); restoration was initiated by SDGFP. In 1991, 26 bighorns (*O. c. canadensis*) from Georgetown, Colorado were released in Spring Creek Canyon in the Black Hills; an additional 5 sheep from the Badlands herd (*O. c. canadensis*) were released into Spring Creek a year later (Fig. 1; SDGFP 2007). The Elk Mountain herd was established in 2001 when SDGFP relocated 20 bighorn sheep (3 rams, 11 ewes, and 6 lambs) from the Spring Creek population into Hell Canyon, located in the southern Black Hills. These sheep subsequently moved to Elk Mountain, along the South Dakota-Wyoming state line (Fig. 1). In 2004, SDGFP released 7 bighorn ewes (*O. c. canadensis*) from Wheeler Peak, New Mexico on Elk Mountain to increase the genetic diversity of the herd. By 2009 casual observations and classification counts conducted by both SDGFP and Wyoming Game and Fish Department (WGFD) indicated the herd had grown to approximately 120 individuals. In 2010, these same counts revealed a decline in population size to 100, and in 2011, the herd decreased to an estimated 80 individuals.

Numerous studies have assessed genetic variation of both native and translocated herds of bighorn sheep across the western United States. Some have focused on genetic drift, inbreeding, and translocation efforts and their effects on the genetic diversity of bighorn sheep herds (Ramey et al. 2000, Whittaker et al. 2004, Hogg et al. 2006, Hedrick 2014). Luikart et al. (2008) detected an association between genetic variability of an isolated bighorn sheep population and the herds' susceptibility to parasitism. Others have examined the viability of small populations persisting through time (Berger 1990, Hogg et al. 2006). Within South Dakota, studies on bighorn genetics have only been completed on two herds: Badlands and Custer State Park (CSP) located in the southeastern Black Hills (Fig. 1). The study on the Badlands population focused on the effects of a bottleneck and subsequent effects of augmentation to the herd (Ramey et al. 2000, Zimmerman 2008), while the study on the CSP herd com-

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pared the level of genetic variation of the introduced herd to the source population (Fitzsimmons et al. 1997).

The population decline of the Elk Mountain herd provided the impetus for our study to investigate several aspects and potential causes of the decline simultaneously: population size, disease prevalence, survival of adult and neonatal sheep, and genetic diversity of the herd. Here we focus on the genetic aspects of a previously unstudied population; specifically, our objectives were to: 1) assess genetic diversity, 2) measure effective population size (N_e), and 3) compare the genetic diversity of Elk Mountain's bighorn sheep herd to other native and translocated herds throughout the United States.

STUDY AREA

Our study area, Elk Mountain, is located in the southern Black Hills in western South Dakota and eastern Wyoming, USA (Fig. 1). The study area encompasses approximately 18,600 ha. Elevations range from 1,132 to 1,728 m above mean sea level, and topography consists of rock outcrops, rolling hills, steep ridges, and gulches (Froiland 1990). Herbaceous cover (*Bromus spp, Poa annua*) dominated the landscape at 54.7% (USDA GeoSpatialDataGateway 2014), while shrub/scrub (*Artemisia* spp.) covered 26.8%, and combined with evergreen forest (17.7%; *Pinus ponderosa*) comprised the majority of the remaining landscape. Average annual pre-

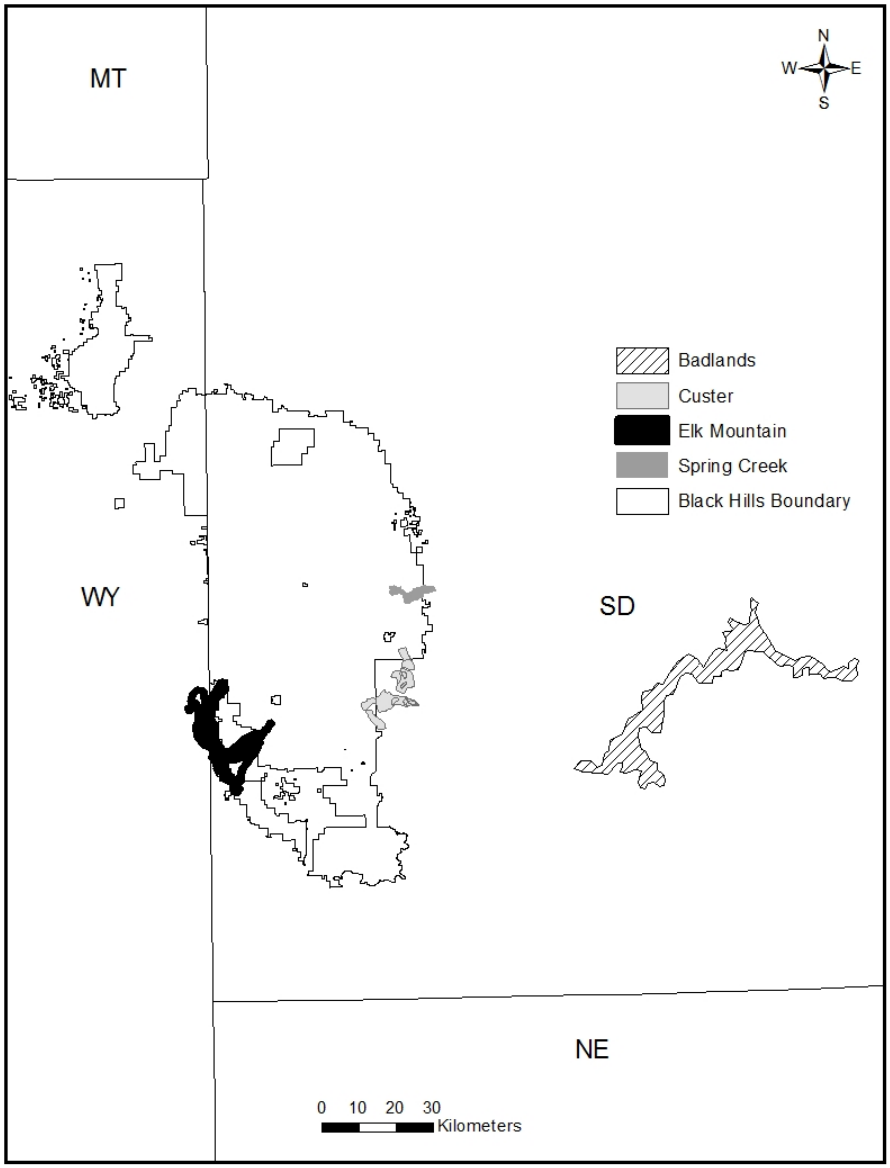

Figure 1. The Elk Mountain bighorn sheep study area in black, located in the southern Black Hills of western South Dakota and eastern Wyoming, USA. Other South Dakota bighorn sheep herds also are shown.

cipitation was 42 cm; mean temperatures ranged from a low of –11° C in January to a high of 32° C in July (National Oceanic and Atmospheric Administration [NOAA] 2014). Climate values were based on data collected at the Newcastle, Wyoming weather station from 1981–2010 (NOAA 2014). Other wild ungulates in the study area included mule deer (*Odocoileus hemionus*), white-tailed deer (*O. virginianus*), elk (*Cervus elaphus*), and pronghorn (*Antilocapra americana*); domestic sheep (*O. aries*) and goats (*Capra hircus*) were present on the perimeter of the study area located on private lands. Domestic cattle (*Bos spp*.) were grazed in the study area during the summer months. The Badlands bighorn sheep herd was located approximately 113 km, the Spring Creek herd was approximately 65 km, and the CSP herd was approximately 53 km from Elk Mountain.

METHODS

From March 2012–February 2014, we captured adult bighorn sheep inhabiting the Elk Mountain region of South Dakota and Wyoming via drop net (Jessup et al. 1984, Kock et al. 1987) and helicopter net gun (Jacques et al. 2009). We collected blood samples from all captured sheep for genetic analysis. Additionally, hunters provided a small tissue sample from harvested rams occupying Elk Mountain during the course of this study. Blood and tissue samples were frozen following collection. All animal handling procedures were approved by the South Dakota State University Animal Care and Use Committee (Approval Number 12-090A) and followed recommendations of the American Society of Mammalogists (Sikes et al. 2011).

We conducted DNA extraction and genetic analysis at the National Genomics Center for Wildlife and Fish Conservation, United States Forest Service, Rocky Mountain Research Station (Missoula, Montana, USA). We analyzed samples at 15 microsatellite loci developed for bighorn sheep (Maudet et al. 2004, Luikart et al. 2008): *MAF36, MAF48, FCB304, AE16, HH62, MAF209, MAF33, FCB266, KRT2, KERA, SOMA, ADCYAP, TCRG4, MMP9, and OLADRBps*. We extracted genomic DNA from blood and tissue with the Dneasy Tissue Kit (Qiagen, Inc., Valencia, CA, USA). Polymerase chain reaction (PCR) volume (10 ml) contained 1.0 mL DNA, 1x reaction buffer (*Applied Biosystems*, Foster City, CA, USA), 2.0 mM MgCl2, 200 mM of each dNTP, 1 mM reverse primer, 1 mM dye-labeled forward primer, 1.5 mg/ml BSA, and 1 U Taq polymerase (*Applied Biosystems*). The PCR profile was 94°C/5 min, [94°C/1 min, 55°C/1 min, 72°C/30s] × 36 cycles. The resultant products were visualized on a LI-COR DNA analyzer (LI-COR Biotechnology). We tested for genotyping error using program DROPOUT (McKelvey and Schwartz 2005) following Schwartz et al. (2006). Genotyping error was assessed via both positive and negative controls and two people independently scoring alleles.

We calculated observed (H_O) and expected (H_E) heterozygosity, allelic diversity (A), effective alleles (A_E), and tested for deviations from Hardy-Weinberg equilibrium (HWE) via Chi-square tests, using GenAlEx 6.5 (Peakall and Smouse 2006, 2012). We estimated effective population size (N_e) using ONeSAMP 1.2 (Tallmon et al. 2008). Overall population size in 2012 and 2013 was estimated following methods described in Parr (2015). The N_e/N ratio was calculated using the 2013 population estimate.

We evaluated levels of genetic diversity from Elk Mountain to other populations found in the literature. Populations were delineated as "native" or "translocated" for analysis based on clear descriptions of the population's history either within the study or based on descriptions of the same population in other studies.

RESULTS

We successfully genotyped and analyzed 43 unique samples collected from Elk Mountain bighorn sheep. We calculated an H_O of 0.59 (SE=0.03), and an H_E of 0.58 (SE=0.03); heterozygosity by locus ranged from 0.35–0.75 (Table 1). Allelic richness (A) ranged from 3–7 ($\bar{x}$ =4.33; SE=0.30), and average number of effective alleles (A_E) was 2.55 (SE=0.18; Table 1). We found no deviations from HWE. Population estimates for 2012 and 2013 were 80 and 100 sheep, respectively. Effective population size was 24 (19–32; 95% CL). The N_e/N ratio was 0.24. Genetic diversity of other bighorn sheep herds (native and translocated) reported in Forbes et al. (1995), Boyce et al. (1997), Gutiérrez-Espeleta et al. (2000), Whittaker et al. (2004), and Zimmerman (2008) ranged from 0.32–0.63 (H_E) and 2.0–4.6 (A; Table 2). Ne/N ratios for bighorn sheep herds (native and translocated) reported in Fitzsimmons et al. (1995, 1997), Johnson et al. (2011), and Buchalski et al. (2015) ranged from 0.10–0.50.

DISCUSSION

Our results indicated that genetic diversity was not a population limiting factor for the Elk Mountain bighorn sheep herd. While we found high levels of heterozygosity and allelic diversity, as well as an N_e analogous to other native populations, we recognize that a *direct* comparison to these populations cannot be made, as microsatellite loci used in these studies varied with the exception of Zimmerman (2008). When compared to other herds in South Dakota, we determined the genetic variation of the Elk Mountain herd was similar to the bighorn sheep herd located in and surrounding Badlands National Park (Zimmerman 2008). Although the distance between these herds (~113 km) greatly limits direct gene flow via immigration/dispersal, translocation events in 2001 and 2004 likely account for a large portion of the genetic similarity between the two herds.

Table 1. Genetic variation of the Elk Mountain bighorn sheep, southern Black Hills of western South Dakota and eastern Wyoming, 2012–2014.

Locus	N[a]	A[b]	A_E[c]	H_O[d]	H_E[e]
MAF36	43	4	1.58	0.35	0.37
MAF48	43	4	2.18	0.58	0.54
FCB304	43	3	2.32	0.49	0.57
AE16	42	7	3.74	0.71	0.73
HH62	43	5	1.74	0.47	0.42
MAF209	43	5	3.37	0.67	0.70
MAF33	43	4	3.36	0.67	0.70
ADCYAP	42	3	2.31	0.64	0.57
TCRG4	43	3	2.56	0.58	0.61
MMP9	43	4	3.02	0.67	0.67
KRT2	39	4	1.83	0.49	0.45
KERA	41	3	1.79	0.39	0.44
OLADRBps	39	5	3.11	0.74	0.68
SOMA	40	5	3.18	0.75	0.69
FCB266	43	6	2.11	0.58	0.53
Mean	*42.00*	*4.33*	*2.55*	*0.59*	*0.58*
SE	*0.39*	*0.30*	*0.18*	*0.03*	*0.03*

[a]Number of samples; [b]Allelic richness; [c]Number of effective alleles per locus; [d]Observed heterozygosity; [e]Expected heterozygosity.

Heterozygosity and allelic diversity are important measures of genetic variation for populations (Fitzsimmons et al. 1995, Whittaker et al. 2004). Heterozygosity is a measure of allelic pairing at specific loci and reflects recent breeding history (Whittaker et al. 2004). Allelic diversity, or allelic richness, however, is representative of the number of alleles found at specific loci on chromosomes (Whittaker et al. 2004). Decreases in allelic diversity often occur more rapidly than decreases in heterozygosity and is considered a greater indication of loss of genetic variation; both are likely to take place following a severe decline in overall population size (Nei et al. 1975, Leberg 1992). Zimmerman (2008) documented this trend: allelic diversity of bighorn sheep within the Badlands decreased more rapidly than heterozygosity over a 12-year time period. Additionally, as translocated herds often originate from a small number of founders, lower genetic diversity in these populations is often expected (Fitzsimmons et al. 1997) in relation to native herds (Table 2).

H_E and A for extant viable native populations of bighorn sheep ranged from 0.44–0.63 and 2.9–4.6, respectively (Forbes et al. 1995, Boyce et al. 1997, Gutiérrez-Espeleta et al. 2000), while translocated populations had lower H_O ranging from 0.32–0.57 and lower A (2.1–3.8; Boyce et al. 1997, Gutiérrez-Espeleta et al. 2000, Whittaker et al. 2004, Hogg et al. 2006, Zimmerman et al. 2008). Zimmerman (2008) reported an H_E of 0.39 and an A of 2.0 for a historic herd of bighorn sheep in the Badlands of South Dakota using turbinate bone samples from historic samples; however, this particular herd became extinct in the early 1920s. Although not all studies used the same methods or microsatellite loci to assess genetic diversity, they demonstrated an overall trend of higher genetic diversity in native herds than translocated populations. The genetic diversity of the Elk Mountain herd was similar to average values reported for native bighorn sheep herds and higher than average values reported for translocated herds (Table 2). These results are likely because the Elk Mountain herd was established from two separate source populations (Spring Creek Canyon, South Dakota, USA *n*=20; Wheeler Peak, New Mexico, USA, *n*=7), one of which was established from two source populations (Spring Creek Canyon: Georgetown, Colorado, USA, *n*=26 and Badlands, South Dakota, USA, *n*=5), likely accounting for the higher levels of genetic diversity.

The N_e/N ratio has been reported to vary between 0.10-0.33 of the total population (Bartley et al. 1992, Frankham 1995, Hedrick et al. 1995, Frankham 1996, Lacy 1997). Prior to more intensive studies on bighorn sheep, an acceptable N_e/N ratio for sheep populations varied with the managing

Table 2. Genetic diversity of bighorn sheep at Elk Mountain and other native and translocated herds of bighorn sheep (*Ovis* spp) across the western United States and Canada, 1995–2015.

Herd	Subspecies	H_E[a]	A[b]	Source
Elk Mountain	*O. c. canadensis*	0.58	4.3	Parr 2015
Native				
Peninsular Ranges, California	*O. c. nelsoni*	0.55	3.7	Boyce et al. 1997
Mojave Desert, California/Nevada	*O. c. nelsoni*	0.60	3.9	Boyce et al. 1997
Sun River, Montana	*O. c. canadensis*	0.59	4.5	Forbes et al. 1995
Whiskey Basin, Wyoming	*O. c. canadensis*	0.60	4.0	Forbes et al. 1995
Tarryall, Colorado	*O. c. canadensis*	0.55	3.4	Forbes et al. 1995
Mt. Davis, Arizona	*O. c. nelsoni*	0.54	3.3	Gutiérrez-Espeleta et al. 2000
Lost Cabin, Arizona	*O. c. nelsoni*	0.55	3.4	Gutiérrez-Espeleta et al. 2000
Mt. Nutt, Arizona	*O. c. nelsoni*	0.44	2.9	Gutiérrez-Espeleta et al. 2000
Kofa Mountains, Arizona	*O. c. mexicana*	0.60	3.7	Gutiérrez-Espeleta et al. 2000
Castle Dome Mountains, Arizona	*O. c. mexicana*	0.58	3.9	Gutiérrez-Espeleta et al. 2000
Old Dad Mountains, California	*O. c. nelsoni*	0.45	3.1	Gutiérrez-Espeleta et al. 2000
Eagle Mountains, California	*O. c. nelsoni*	0.63	4.1	Gutiérrez-Espeleta et al. 2000
San Gorgonio, California	*O. c. nelsoni*	0.46	3.4	Gutiérrez-Espeleta et al. 2000
San Ysidro, California	*O. c. cremnobates*	0.49	3.6	Gutiérrez-Espeleta et al. 2000
Sheep River, Alberta	*O. c. canadensis*	0.59	4.6	Forbes et al. 1995
Sheep River, Alberta	*O. c. canadensis*	0.59	4.4	Gutiérrez-Espeleta et al. 2000
Badlands National Park, South Dakota (historic)	*O. c. auduboni*	0.39	2.0	Zimmerman 2008
Native Average[c]		0.55	3.7	
Translocated				
Chihuahuan Desert, New Mexico	*O. c. nelsoni*	0.50	2.6	Boyce et al. 1997
Bison Range, Alberta	*O. c. canadensis*	0.43	2.1	Forbes et al. 1995
Stewart Mountain, Arizona	*O. c. mexicana*	0.54	3.1	Gutiérrez-Espeleta et al. 2000
Red Rock Refuge, New Mexico	*O. c. mexicana*	0.36	2.4	Gutiérrez-Espeleta et al. 2000
Wheeler Peak, New Mexico	*O. c. canadensis*	0.55	3.2	Gutiérrez-Espeleta et al. 2000
Hart Mountain, Oregon	*O. c. californiana*	0.35	2.2	Whittaker et al. 2004
Aldrich Mountain, Oregon	*O. c. californiana*	0.35	2.2	Whittaker et al. 2004
John Day River, Oregon	*O. c. californiana*	0.39	2.4	Whittaker et al. 2004
Steens Mountain, Oregon	*O. c. californiana*	0.32	2.2	Whittaker et al. 2004
Leslie Gulch, Oregon	*O. c. californiana*	0.34	2.3	Whittaker et al. 2004
Santa Rosa Mountains, Nevada	*O. c. californiana*	0.57	3.8	Whittaker et al. 2004
Badlands National Park, South Dakota	*O. c. canadensis*	0.37	2.2	Zimmerman 2008
Badlands National Park, South Dakota[d]	*O. c. canadensis*	0.63	4.9	Zimmerman 2008
Translocated Average[e]		0.42	2.6	

[a]Expected heterozygosity; [b]Allelic richness; [c]Averages of native herds excluding the extinct Badlands population; [d]Immediate post-translocated values; [e]Average of translocated herds excluding immediate post-translocated herds.

agency, with reports ranging between 0.33-0.50 of overall population size (Wakelyn 1987, Fitzsimmons et al. 1995, 1997). Fitzsimmons et al. (1997) later demonstrated N_e/N varied with individual herds. In three translocated herds of bighorn sheep in Wyoming, N_e/N ranged between 0.10-0.45 of the total population, while the source herd had an N_e/N of 0.23 (N_e=244, N=1070; Fitzsimmons et al. 1997). More contemporary N_e/N ratios for bighorn sheep ranged from 0.10-0.50 (Johnson et al. 2011; Buchalski et al. 2015). Effective population size for bighorn sheep on Elk Mountain was 24 animals. Despite the use of various methods to estimate N_e among multiple species, the N_e/N estimate for bighorn sheep at Elk Mountain fell within reported ranges for bighorn sheep. Furthermore, the estimated ratio for N_e/N was similar to that reported for the native Whiskey Basin bighorn sheep herd (0.24 vs 0.23; Fitzsimmons 1997). As N_e/N was comparable in size to those of successful native herds, and Elk Mountain had high levels of H_O and A, this population's growth did not seem to be limited due to genetic diversity.

While no current documentation exists on the genetic diversity on other bighorn sheep populations in the Black Hills, it is possible the Spring Creek population has experienced declines in genetic diversity. This population has declined in size since 2006 (Smith et al. 2014). Smith et al. (2014) also reported only 2% of lambs born survived to a year of age over a three year time span. While gene flow is believed to occur between the subpopulations of Spring Creek via ram movements (SDFGP 2007), the lack of lamb recruitment may inevitably result in a loss of genetic diversity in these herds via smaller population sizes and genetic drift (Courchamp et al. 1999). No known movements between the Spring Creek and Elk Mountain herds have occurred (J. Kanta, South Dakota Game, Fish and Parks, personal communication). In contrast, the Elk Mountain herd has seen population growth until recently, and during the course of this study, annual recruitment of lambs was 35% (Parr 2015). Additionally, Luikart et al. (2008) found a positive relationship between population genetic variability and susceptibility to parasitism, and Boyce et al. (2011) found a positive relationship between previous exposure to pathogens and survival against pneumonia. Other bighorn sheep populations in the Black Hills have experienced pneumonia related mortalities during previous years (SDGFP 2007, Smith et al. 2014); the Elk Mountain herd did not experience pneumonia related mortality during the course of our study (Parr 2015). As the genetic variation and previous pathogen exposure of the other bighorn sheep herds in the Black Hills is unknown, it is possible that low levels of variation have led to decreased fitness within individuals and an increase in susceptibility to pneumonia, while the high levels of variation and previous pathogen exposure (Parr 2015) on Elk Mountain may contribute to their defense against the disease.

MANAGEMENT IMPLICATIONS

The multiple relocation events for bighorn sheep in South Dakota, and the augmentation event following the introduction of the bighorn sheep herd on Elk Mountain, have resulted in a high level of genetic diversity, which is likely augmented by successful lamb recruitment documented within the herd. We recommend continued genetic monitoring of this bighorn sheep herd every ten years to quantify genetic diversity. Additionally, we recommend genetic analyses on the other bighorn sheep herds within the Black Hills of South Dakota to determine their genetic variability, particularly as these herds are currently experiencing low recruitment. A continent-wide compilation of genetic measures within all populations of bighorn sheep would be beneficial to managing agencies; such a database would provide baseline data for all herds and could be useful when considering herd reintroductions and/or augmentations. Furthermore, as a single migrant per generation can keep populations from becoming genetically isolated, and most migrants are often young males, we suggest implanting passive integrated transponder (PIT) tags in juvenile males when possible or fitting males with radio collars. In locations where migrants are believed to occur, these chips or collars would provide a more effective means of informing managing agencies of such occurrences.

ACKNOWLEDGMENTS

Financial support for this project was funded by Federal Aid to Wildlife Restoration (Study No. 7545) administered through South Dakota Department of Game, Fish and Parks, the Wyoming Community Foundation, Wyoming Governor's Big Game License Coalition, Bowhunters of Wyoming, Midwest Chapter of the Wild Sheep Foundation, the Dacotah Chapter of Safari Club International, and Wyoming Chapter of the Wild Sheep Foundation. We thank South Dakota Department of Game, Fish and Parks, Wyoming Game and Fish Department, Quicksilver Air, and Native Range Helicopter Services for their assistance in capturing bighorn sheep. We thank the landowners around Elk Mountain for allowing access to their property and access to the mountain via their property. We thank J. Kanta and J. Sandrini for their assistance coordinating sheep captures, providing equipment, and providing assistance throughout the course of this study. We thank K. Pilgrim and M. Schwartz for their assistance with genetic analyses.

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Submitted 13 June 2015. Accepted 20 March 2016. Associate Editor was Chris DePerno.