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Assessing temporal genetic variation in a cougar population: influence of harvest and neighboring populations

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Abstract The geography of the Black Hills region of South Dakota and Wyoming may limit connectivity for many species. For species with large energetic demands and large home ranges or species at low densities this can create viability concerns. Carnivores in this region, such as cougars (*Puma concolor*), have the additive effect of natural and human-induced mortality; this may act to decrease long-term viability. In this study we set out to explore genetic diversity among cougar populations in the Black Hills and surrounding areas. Specifically, our objectives were to first compare genetic variation and effective number of breeders of cougars in the Black Hills during three harvest regimes: pre (2003–2006), moderate (2007–2010), and heavy (2011–2013), to determine if harvest impacted genetic variation. Second, we compared genetic structure of the Black Hills cougar population with cougar populations in neighboring eastern Wyoming and North Dakota. Using 20 microsatellite loci, we conducted genetic analysis on DNA samples from cougars in the Black Hills (n = 675), North Dakota (n = 113), and eastern Wyoming (n = 62) collected from 2001–2013. Here we report that the Black Hills cougar population maintained genetic variation over the three time periods. Our substructure analysis suggests that the maintenance of genetic variation was due to immigration from eastern Wyoming and possibly North Dakota.

Keywords Cougar · Genetic variation · Black Hills · Harvest · South Dakota · Microsatellite

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Introduction

By the early 1900s, the North American cougar (*Puma concolor*) population had been displaced from two-thirds of its historic range (Young and Goldman 1946). Populations have since increased, and currently occupy the western United States and part of southern Florida (Beier 1991; Logan and Sweanor 2001). Several studies have suggested habitat barriers (i.e., fragmentation and habitat degradation) can limit gene flow resulting in distinct genetic structure among some cougar populations (Ernest et al. 2003; Mcrae et al. 2005; Loxterman 2011; Andreasen et al. 2012; Holbrook et al. 2012). In contrast, others have argued immigration/emigration can maintain genetic variation (Biek et al. 2006; Anderson et al. 2004) and can limit population subdivision in wild cougar populations (Sinclair et al. 2001; Anderson et al. 2004).

Advances in DNA analyses have enabled genetics to play an increasingly important role in the conservation and management of many wildlife populations (DeYoung and Honeycutt 2005; Culver and Schwartz 2011). Genetic data can allow for estimates of effective population size (N_e), dispersal, inbreeding, and gene flow (Culver and Schwartz 2011), and recently has been applied to many wide-ranging carnivore populations (e.g., Spong et al. 2000; Haag et al. 2010; Coster and Kovach 2012; Croteau et al. 2012). The

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additive effects of human-induced mortality to some North American cougar populations may affect the genetic viability of these populations; particularly those in unique geographic regions, i.e., the Black Hills National Forest.

Historically, cougars were found throughout South Dakota (Young and Goldman 1946), but were thought to have been eradicated by the early 1900s (South Dakota Mountain Lion Management Plan 2010). The Black Hills complex is relatively small (Fig. 1) with an estimated 6703 km^2 of suitable cougar habitat (Fecske 2003), and is largely surrounded by inhospitable cougar habitat (Anderson et al. 2004), i.e., the Northern Great Plains. During the late 1980s to early 1990s, reports of cougar sightings in the Black Hills increased; some of which were eventually verified by state biologists (South Dakota Mountain Lion Management Plan 2010). By 2002, there was an estimated population size of 127–149 cougars inhabiting the Black Hills (Fecske 2003). The cougar population in the Black Hills likely resulted from immigrants from neighboring Wyoming populations, which either founded the current population, or augmented a small remnant population that persisted (Fecske 2003).

Initial genetic data suggested that the Black Hills were founded by immigrants from north-central and southeastern Wyoming, and that sustained gene flow had occurred between regions (Anderson et al. 2004). By 2006, a concomitant study showed that cougars in the Black Hills had maintained genetic variation, and the population was

Fig. 1 Map of Black Hills National Forest study area (located in western South Dakota and northeastern Wyoming), and of approximate locations of North Dakota and eastern Wyoming sample distribution by county
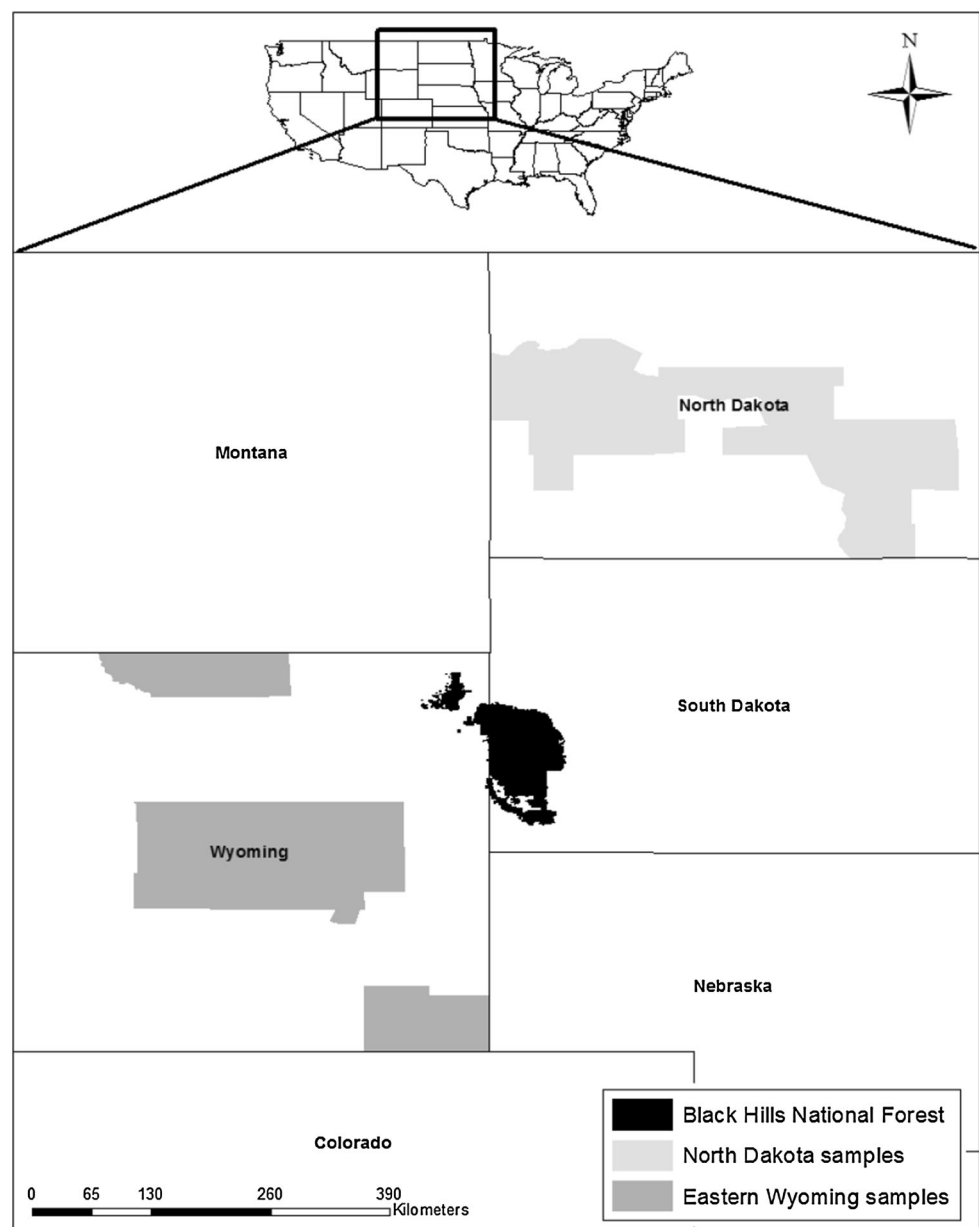
saturated (Thompson 2009) with a conservative modeled population estimate of about 250 individuals (J. A. Jenks, South Dakota State University, unpublished data). As a result, South Dakota Department of Game, Fish and Parks (SDGFP) initiated its first cougar harvest season in 2005 and harvest limits in the Black Hills Fire Protection Area have increased steadily from 2005–2013 (Fig. 2).

The semi-isolated nature of the Black Hills could make the Black Hills cougar population prone to deleterious effects from potential inbreeding and increased genetic drift (Culver and Schwartz 2011). Prolonged and intense harvest has the potential to result in a greater loss of genetic variation and alteration of population structure (Allendorf et al. 2008). Thus, the objectives of this study were to: assess and compare genetic variation (i.e., heterozygosity, allelic richness, effective population size) of cougars in the Black Hills during three different harvest regimes: pre (2001–2006), moderate (2007–2010), and heavy (2011–2013), and examine the impact of increased harvest on population substructure. Additionally, while previous and current telemetry data verify cougar dispersal out of the Black Hills into other cougar populations (e.g., Wyoming, Montana, Nebraska, and North Dakota; Thompson 2009; Thompson and Jenks 2010), data confirming immigration into the Black Hills remains limited. Our final objective was to compare genetic structure of Black Hills cougars to that of neighboring cougar populations in North Dakota and eastern Wyoming, and conduct population assignment tests to determine evidence of immigration.

Materials and methods

Study area

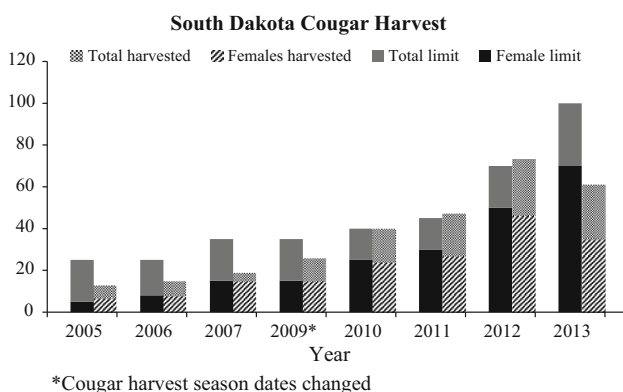We conducted our study in the Black Hills National Forest and surrounding lands (Fig. 1), located in western South Dakota and northeastern Wyoming. The Black Hills represent the eastern most extension of the Rocky Mountains (Froiland 1990), encompassing approximately 8400 km^2 (Fecske et al. 2004). Maximum elevation in the Blacks Hills is 2207 m above mean sea level, with topography consisting of rock outcrops, rolling hills, steep ridges, canyonlands, and gulches (Froiland 1990). The most abundant tree species occurring in the Black Hills is ponderosa pine (*Pinus ponderosa*). White spruce (*Picea glauca*), aspen (*Populus tremuloides*), and birch (*Betula* spp.) trees also are prevalent at higher elevations; burr oak (*Quercus macrocarpa*) draws occur at lower elevations (Larson and Johnson 1999). Prey species available to cougars include: white-tailed deer (*Odocoileus virginianus*), mule deer (*O. hemionus*), elk (*Cervus elaphus*), bighorn sheep (*Ovis canadensis*), mountain goat (*Oreamnos americanus*), porcupine (*Erethizon dorsatum*) as well as a variety of small mammal and domestic livestock species. The cougar is the apex predator in the Black Hills, occurring sympatrically with bobcat (*Lynx rufus*) and coyote (*Canis latrans*). The Black Hills is surrounded by the Northern Great Plains. The closest breeding cougar populations occur in the Pine Ridge region, Nebraska (48 km southeast), Badlands, North Dakota (120 km north/northwest), Laramie Range, Wyoming (160 km southwest), and the Bighorn Mountains, Wyoming (200 km west; Thompson and Jenks 2010).

Capture methods

From 1 January 2003–1 January 2013, we captured cougars primarily with the use of trained hounds; however, we also utilized walk-in live traps, foot-hold traps, and leg-hold traps (Logan et al. 1999). We immobilized cougars with a mixture of telazol (5.0 mg/kg) and xylazine (1.0 mg/kg; Kreeger and Armeno 2007) based on estimated live animal body weight via dart rifle (Dan-Inject, Børkop, Denmark, EU). We weighed, measured, sexed, and estimated age of cougars by tooth wear and pelage characteristics (Anderson and Lindzey 2000). We collected blood samples for genetic analysis from all captured cougars and fitted them with VHF (Telonics MOD-500 (NH), Inc., Mesa, Arizona, USA) or GPS (ATS G2110E, Inc. Isanti, Minnesota, USA; Northstar D-cell, King George, Virginia, USA) radio collars. Immobilized cougars were reversed with 0.125 mg/kg yohimbine, released on site, and observed from a distance to ensure safe recovery. We collected tissue samples from cougars within the Black Hills, South Dakota, from harvest and non-harvest related mortalities. We collaborated with the Wyoming Game and Fish Department to obtain tissue samples collected during 2011–2013 biopsy darting and harvest from cougars in the Wyoming portion of the Black Hills, as well from other eastern regions of Wyoming (i.e.,

South Dakota Cougar Harvest

Legend: ▨ Total harvested　▨ Females harvested　▩ Total limit　■ Female limit

*Cougar harvest season dates changed

Fig. 2 South Dakota cougar harvest from 2005–2013

Casper, Sheridan, and Laramie). Additionally, we collaborated with the North Dakota Game and Fish Department to obtain tissue samples collected from 2003–2012 from harvest, non–harvest related mortalities, and live captures. All animal capture procedures were approved by the South Dakota State University Animal Care and Use Committee (Approval number 11-078A) and followed recommendations of the American Society of Mammalogists (Sikes and Gannon 2011).

Genetic analysis

DNA extraction and genetic analysis was conducted at the National Genomics Center for Wildlife and Fish Conservation, United States Forest Service, Rocky Mountain Research Station (Missoula, Montana, USA). Samples were analyzed at 20 microsatellite loci used in previous studies on cougars (Menotti-Raymond and O'Brien 1995; Menotti-Raymond et al. 1999): Fca43, Fca57, Fca77, Fca90, Fca96, Fac132, Fca559, Fca176, Fca35, Lc109, Fca391, Fca08, Fca30, Fca82, Fca149, PcoA208, PcoB10, PcoC112, PcoB210, PcoC108. Genomic DNA from blood and tissue was extracted with the Dneasy Tissue Kit (Qiagen, Inc., Valencia, CA, USA). Polymerase chain reaction (PCR) volume (10 μl) contained 1.0 ml DNA, 1× reaction buffer (Applied Biosystems, Foster City, CA, USA), 2.0 mM $MgCl_2$, 200 mM of each dNTP, 1 mM reverse primer, 1 mM dye-labeled forward primer, 1.5 mg/ml BSA, and 1U Taq polymerase (Applied Biosystems). The PCR profile was 94 °C/5 min, ([94 °C/1 min, 55 °C/1 min, 72 °C/30 s] × 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).

We grouped individuals into cohorts based on birth year, which were subsequently categorized under the appropriate harvest regime (pre, moderate, and heavy). Cougars can breed as early as 2 years of age; thus, we used a 2 year sliding window approach to avoid the inclusion of parents and offspring in the same cohort. We calculated observed (H_O) and expected (H_E) heterozygosity, effective alleles (A_e), and tested for deviations from Hardy–Weinberg equilibrium (HWE) using GenAlEx 6.5 (Peakall and Smouse 2006, 2012) and Genepop (Raymond and Rousset 1995; Rousset 2008). To account for variation in sample size we used HPRARE 1.0 (Kalinowski 2005), which uses rarefied measures to calculate allelic richness (Ar). Most methods used to estimate effective population size (N_e) assume discrete generations, which can cause severe bias for species with overlapping generations (Luikart et al. 2010), such as cougars. However, if a single cohort is sampled, effective population size can be estimated as

effective number of breeders (Schwartz et al. 1998; Waples 2005). Therefore, we estimated effective population size as the effective number of breeding individuals (from here on referred to as N_B) genetically contributing to the population (Waples and Teel 1990; Waples 2005). We calculated N_B for each cohort using a bias correction method based on linkage disequilibrium (Hill 1981; Waples 2006; Waples and Do 2010), as implemented by NeEstimator V2 (Do et al. 2014). Statistical analysis among cohorts and harvest regimes was determined using an ANOVA in the R statistical program (R Core Team 2015).

We examined population structure between Black Hills, eastern Wyoming, and North Dakota cougar populations from 2003–2013 using STRUCTURE (Pritchard et al. 2000). We used Geneclass2 (Piry et al. 2004) and STRUCTURE (Pritchard et al. 2000) to conduct population assignment tests to determine immigration between the Black Hills and North Dakota cougar populations using samples from 2001–2013. Finally, we assessed overall genetic variation and population structure for cougars in the Black Hills, eastern Wyoming (i.e., Casper, Sheridan, and Laramie) and North Dakota using individuals from the same birth period (2011–2013) to alleviate temporal effects. We used the R statistical program (R Core Team 2015) diveRsity to determine F_{ST} values between the three populations, and performed Fisher's exact test between the Black Hills and eastern Wyoming populations to test for panmixia (Keenan et al. 2013).

Geneclass2 assigns/excludes individuals using predefined population subdivision based on different genetic assignment criteria (Piry et al. 2004). We employed the frequency based method (Paetkau et al. 1995) using the Monte Carlo resampling method with 1000 simulated individuals and an alpha of 0.01 (Paetkau et al. 2004). STRUCTURE uses both allele frequency and a Bayesian model-based clustering method to infer population structure on the basis of genotypes (Pritchard et al. 2000). To infer population structure, we excluded available geographic information, and assumed individuals had mixed ancestry (admixture model) and correlated allele frequencies. We set both burn-in periods to 10,000 and evaluated 1–10 possible genetic clusters (K), with three iterations. To select the appropriate number of genetic clusters (K), we used Structure Harvester, which collates STRUCTURE results (Earl and vonHoldt 2012) to determine the most suitable K using the Evanno method (Evanno et al. 2005).

Results

We analyzed 675 unique genotypes from Black Hills cougars (pre-harvest: n = 288; moderate harvest: n = 289; and heavy harvest: n = 98), 113 unique genotypes from

Table 1 Estimates (at 20 loci) of observed (H_O) and expected (H_E) heterozygosity, allelic richness (Ar), and number of effective alleles (A_e) of Black Hills cougars by cohort year under different harvest regimes: pre (2001–2006), moderate (2007–2010), and heavy (2011–2013)

Genetic variation of Black Hills cougars by cohort							
Harvest regime	Cohort year	N		H_O	H_E	Ar	A_e
Pre-harvest	2001–2002	56	Mean	0.56	0.55	4.07	2.49
			SE	0.04	0.04	0.35	0.17
	2002–2003	76	Mean	0.57	0.56	4.02	2.57
			SE	0.04	0.04	0.34	0.18
	2003–2004	96	Mean	0.56	0.56	4.01	2.56
			SE	0.04	0.04	0.32	0.17
	2004–2005	117	Mean	0.57	0.55	3.95	2.51
			SE	0.04	0.04	0.31	0.16
	2005–2006	132	Mean	0.56	0.55	3.93	2.53
			SE	0.04	0.04	0.33	0.17
Moderate harvest	2006–2007	149	Mean	0.57	0.56	4.01	2.60
			SE	0.05	0.04	0.31	0.18
	2007–2008	146	Mean	0.56	0.56	3.94	2.57
			SE	0.05	0.04	0.31	0.18
	2008–2009	142	Mean	0.54	0.55	3.86	2.52
			SE	0.04	0.04	0.32	0.18
	2009–2010	138	Mean	0.55	0.55	3.97	2.51
			SE	0.04	0.04	0.30	0.17
Heavy harvest	2010–2011	110	Mean	0.55	0.55	3.98	2.51
			SE	0.04	0.04	0.30	0.17
	2011–2012	79	Mean	0.55	0.56	3.90	2.57
			SE	0.04	0.04	0.31	0.18
	2012–2013	52	Mean	0.54	0.55	3.99	2.50
			SE	0.04	0.04	0.33	0.17

N indicates sample size

North Dakota cougars, and 62 unique genotypes from eastern Wyoming cougars at 20 microsatellite loci. We observed no significant difference in observed ($P = 0.473$) or expected ($P = 0.886$) heterozygosity levels, allelic richness ($P = 0.764$), or effective alleles ($P = 0.745$) in cougars in the Black Hills under different harvest regimes (Table 1). Significant deviations from HWE occurred at PcoB10 ($P < 0.05$) during the pre-harvest regime, Fca90 ($P < 0.05$), Fca149 ($P < 0.05$), and Pco108 ($P < 0.05$) during the moderate harvest regime, and at Bco210 ($P < 0.05$) during the heavy harvest regime. We found effective population (N_B) size ranged from a low of 38 (28–55; 95 % CI) pre-harvest and a high of 98 (70–150; 95 % CI) moderate harvest, before declining to 62 (42–106; 95 % CI) heavy harvest (Table 2).

STRUCTURE analysis for Black Hills, eastern Wyoming, and North Dakota resulted in a K = 3 (mean Ln P(K) = −37,526.6; Fig. 3), when excluding prior population information. Plotting these results geographically indicated there was more genetic similarity between Black Hills and eastern Wyoming cougars, in comparison to North Dakota cougars. Hierarchical analysis using samples

Table 2 Effective number of breeders (N_B) by cohort year under different harvest regimes: pre (2001–2006), moderate (2007–2010), and heavy (2011–2013)

| Harvest regime	Cohort year	N	N_B	95 % CI
Pre-harvest	2001–2002	57	38	28–55
	2002–2003	77	41	32–55
	2003–2004	97	41	34–51
	2004–2005	119	70	56–91
	2005–2006	134	80	66–100
Moderate harvest	2006–2007	151	78	64–97
	2007–2008	149	78	62–94
	2008–2009	145	106	82–145
	2009–2010	140	112	81–163
Heavy harvest	2010–2011	111	93	68–135
	2011–2012	80	80	57–121
	2012–2013	53	76	52–132

N indicates sample size

only from the Black Hills and eastern Wyoming resulted in a K = 2 (mean Ln P(K) = −31,844.8; Fig. 4) with no obvious geographic structuring. STRUCTURE analysis for
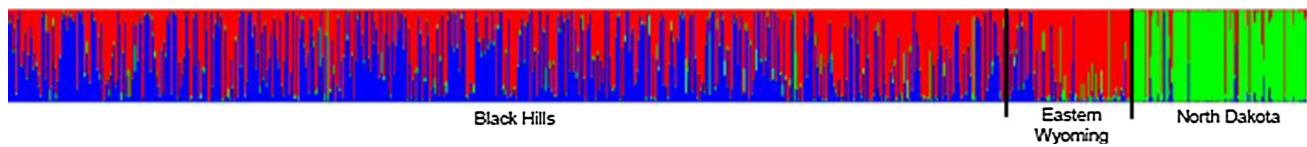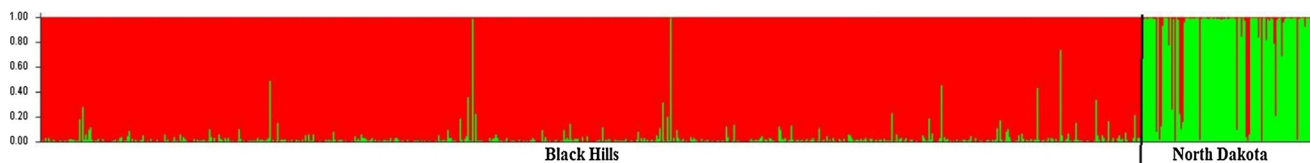
Fig. 3 Population structure of Black Hills (*red* and *blue*), eastern Wyoming (*red* and *blue*), and North Dakota (*green*) cougars inferred by STRUCTURE, K = 3. Populations are divided by a *vertical black line*. Each individual is represented by a single column, where the color(s) of the column represent degree of similarity to each population
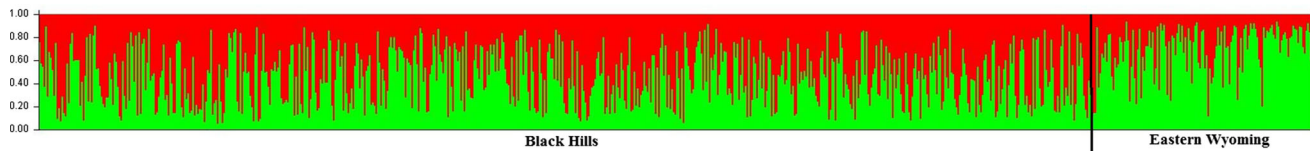
Fig. 4 Population structure of Black Hills (*red* and *green*) and eastern Wyoming (*red* and *green*), cougars inferred by STRUCTURE, K = 2. Populations are divided by a *vertical black line*. Each individual is represented by a single column, where the color(s) of the column represent degree of similarity to each population

Fig. 5 Population structure of Black Hills (*red*) and North Dakota (*green*) cougars inferred by STRUCTURE, K = 2. Each individual is represented by a single column, where the color(s) of the column represent degree of similarity to each population

Black Hills and North Dakota populations resulted in a K = 2 (mean Ln P(K) = −36,200.6; Fig. 5), but suggested geographic structuring into a Black Hills and a North Dakota group. We used these results to confirm immigration of cougars (n = 2) into the Black Hills from North Dakota, as well as emigration out of the Black Hills (n = 6) into North Dakota, based on 98 % ancestry in the Black Hills cluster (Table 3). Results from Geneclass2 allowed assignment of two cougars to North Dakota that were originally sampled from the Black Hills cougar population; these individuals were the same as those identified by STRUCTURE. However, we were only able to assign four cougars (of the six identified by STRUCTURE) to the Black Hills population that were originally sampled from North Dakota, based on a >85 % probability (Table 3).

Genetic variation between the three populations was comparable (Table 4), and we observed no significant

difference in observed (P = 0.605) or expected (P = 0.617) heterozygosity, allelic richness (P = 0.261), or effective alleles (P = 0.296). While we found no significant deviations from HWE in the Wyoming cougar population, we found deviations from HWE at Fca132 (P < 0.05), Fca391 (P < 0.05), and PcoA208 (P < 0.05) in the North Dakota cougar population. There were 96 alleles in Black Hills, 64 alleles in North Dakota, and 86 alleles in the eastern Wyoming cougar population, as well as alleles unique to the Black Hills (n = 2), North Dakota (n = 3), and eastern Wyoming population (n = 6). Overall, F_{ST} between Black Hills and North Dakota was 0.10 (0.07–0.13; 95 % CI), 0.07 (0.04–0.09; 95 % CI) between North Dakota and eastern Wyoming, and 0.03 (0.02–0.04; 95 % CI) between Black Hills and eastern Wyoming (Table 5); thus, indicating population differentiation, as confidence intervals do not incorporate zero. Fisher's exact

Table 3 Population assignment of Black Hills and North Dakota cougars using STRUCTURE and Geneclass2 during 2001–2013

Population sampled	# Sampled	STRUCTURE		Geneclass2	
		Black Hills	North Dakota	Black Hills	North Dakota
Black Hills	675	673	2	673	2
North Dakota	113	6	107	4	109

Table 4 Mean and standard error of genetic variability of Black Hills, North Dakota, and eastern Wyoming cougars during 2011–2013

	Black Hills		North Dakota		Eastern Wyoming	
N	98		25		37	
	Mean	SE	Mean	SE	Mean	SE
H_O	0.54	0.04	0.52	0.04	0.56	0.04
H_E	0.55	0.04	0.52	0.04	0.57	0.04
Ar	3.74	0.30	3.40	0.24	4.06	0.31
# Alleles	96	0.41	64	0.13	86	0.50
Effective alleles/locus	2.52	0.18	2.25	0.13	2.62	0.19

Table 5 Estimation of population subdivision (F_{ST}) between Black Hills (n = 98), North Dakota (n = 25), and eastern Wyoming (n = 45) cougar populations during 2011–2013

Overall F_{ST}

Black Hills	North Dakota	Eastern Wyoming	
0.10*	–	–	North Dakota
0.03	0.07*	–	Eastern Wyoming

Significant F_{ST} values indicated by * (P < 0.05)

test between Black Hills and eastern Wyoming revealed a significant difference (P < 0.05) at all but 6 loci.

Discussion

Our results indicate that cougars in the Black Hills have maintained genetic variability despite the semi-isolated nature of the region, years of increasing harvest, and harvest pressure from both South Dakota and Wyoming. Multiple measures of genetic variation (i.e., heterozygosity and allelic richness), confirm that Black Hills cougars have maintained genetic variability since the pre-harvest period (Thompson 2009), though we acknowledge that simulations would provide further insight on the processes observed. We found no significant difference in heterozygosity and allelic richness in our cohort analysis across the three harvest regimes: pre–harvest, moderate harvest, and heavy harvest (Table 1), despite the populations' recent decrease in size from its highest estimate of approximately 250 individuals (Fig. 6). Observed heterozygosity for Black Hills cougars was similar to that of other cougar populations in the western United States, i.e., Texas, Wyoming, Colorado, Utah, Arizona, New Mexico, and Idaho (H_O 0.52–0.61; Walker et al. 2000; Anderson et al. 2004; Mcrae et al. 2005; Loxterman 2011; Holbrook et al. 2012), and paralleled other studies (Sinclair et al. 2001; Anderson et al. 2004) that found no evidence of population subdivision. Though measures of genetic variation indicate this population has retained genetic viability, we recognize

that unlike demographic effects, which are immediate and more recognizable, genetic effects occur over many generations and therefore, may not be apparent for several generations (Harris et al. 2012).

Loss of genetic diversity has been documented in some wild populations following decreases in population size and/or N_e [African elephant, *Loxodonta africana africana*, Whitehouse and Harley (2001); mountain goat, *Oreamnos americanus*, Ortego et al. (2011); Saimaa ringed seal, *Phoca hispida saimensis*, Valtonen et al. (2012); Far eastern leopard, *Panthera pardus orientalis*, Sugimoto et al. (2014)]. Other studies of wild populations found either no reduction in genetic diversity [coyote, *Canis latrans*, Williams et al. (2003); Kerguelen mouflon, *Ovis aries*, Kaeuffer et al. (2007)], or occasionally even an increase in diversity that resulted from increased immigration [great reed warblers, *Acrocephalus arundinaceus*, Hansson et al. (2000)]. Williams et al. (2003) found dispersal/immigration preserved genetic variation in a coyote population despite decades of intensive removal. Our cohort estimates of N_B (Table 2; Fig. 6) reflected the changes in estimated population size corresponding to the harvest regimes, with lower estimates during the pre- and heavy harvest regimes, and higher estimates during the moderate harvest regime. Although N_B decreased during the heavy harvest regime, estimates were similar if not higher than pre–harvest N_B estimates. Thus, it is likely that a combination of dispersal and immigration and relatively stable N_B, has resulted in genetic maintenance in Black Hills cougars across the three harvest regimes. However, we believe that additional genetic monitoring is warranted to monitor the recent decline observed in N_B to ensure this is not a continuing trend.

Wright (1978) suggested that an F_{ST} value between 0 and 0.05 indicates little genetic differentiation; based on F_{ST} (Table 5), South Dakota and eastern Wyoming cougar populations were more closely related in comparison to North Dakota cougars. Furthermore, STRUCTURE results (Fig. 3) and the similarity in genetic variability at 20 microsatellite loci (Table 3) between South Dakota and eastern Wyoming cougars would suggest that these

Cougar population estimates, N$_B$ & H$_o$

Population estimate
NB
Ho

Year
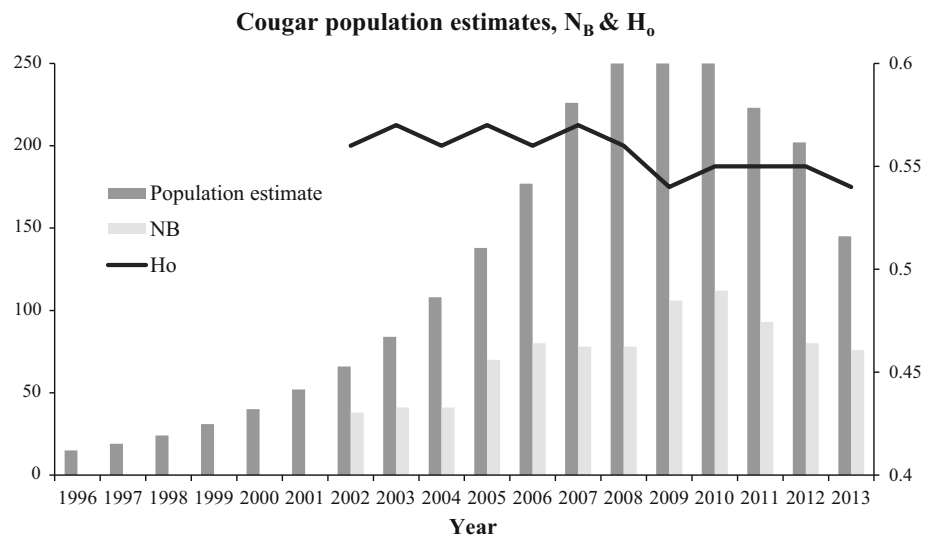
*Population estimates derived here are for the South Dakota portion of the Black Hills only

populations may be acting as a large population as postulated by Anderson et al. (2004). STRUCTURE analysis between Black Hills and eastern Wyoming also depicted little genetic differentiation (Fig. 4). Though Fisher's exact test revealed statistical significance at 14 loci, rejecting panmixia; this is likely a reflection of the number of unique alleles in the Wyoming cougar population (n = 6) than in the Black Hills population (n = 2). However, a more thorough assessment of the population structure of Wyoming cougars using a larger sample size may provide more insight.

Although Black Hills cougars likely colonized the North Dakota Badlands, there seems to be genetic differentiation between the two Dakota populations, as evidenced by genetic variability results (Tables 4 and 5) and population assignment tests (Table 3). This differentiation in the North Dakota population likely resulted from genetic drift within and/or immigration from other cougar populations, such as Montana, which has been documented (Wilkens 2014). In addition, we were able to assign individual cougars to either North Dakota or South Dakota populations with relatively high probabilities (>85 %); confirming both immigration into the Black Hills from neighboring cougar populations, and emigration from the Black Hills into North Dakota. Our results also suggest eastern Wyoming and South Dakota experience a higher frequency of cougar movements (emigration and immigration) compared to the North Dakota population.

Regardless of habitat barriers that may limit dispersal movements among populations (McRae et al. 2005), such constraints are not preventing cougar dispersal movements out of or into the Black Hills (Thompson and Jenks 2010). Several radio marked individuals from this project were documented dispersing from the Black Hills into North

Dakota, Wyoming, and Montana. Additionally, we documented immigration into the South Dakota portion of the Black Hills from an ear-tagged Wyoming cougar. Population immigration likely has sustained the genetic variability of Black Hills cougars, as dispersal facilitates transfer of genetic material, thereby maintaining genetically healthy populations (Logan and Sweanor 2001; Sinclair et al. 2001; Anderson et al. 2004; Biek et al. 2006). Moreover, no clinical signs of inbreeding depression (e.g., crooked tails, cowlicks) have been documented in cougars in the Black Hills (Thompson 2009; Jansen 2011; Juarez 2014).

Conservation and management implications

Genetic assessment of the Black Hills cougar population revealed that the population has maintained genetic viability likely as a result of compensatory emigration/immigration, during years of increased harvest. However, we recommend continued genetic monitoring of cougars in the Black Hills every 3–5 years given that genetic changes occur on an evolutionary time scale (i.e., over many generations; Harris et al. 2012). The cougar populations evaluated in this study may not show genetic effects of harvest pressure and decreasing population size for several generations. As cougars in the Black Hills experience harvest pressure from both South Dakota and Wyoming, continued genetic monitoring is warranted (Schwartz et al. 2007). Furthermore, because cougars in North Dakota and other regions in Wyoming also are experiencing harvest, continued assessment of genetic variation between the three neighboring cougar populations will be important. Dispersal of Black Hills cougars has been documented to

North Dakota, Wyoming, Montana, Nebraska, Oklahoma, and Minnesota (Thompson 2009; Thompson and Jenks 2010), which illustrates the difficulty of managing cougars within agency boundaries. Therefore, because cougars do not adhere to state boundaries we also recommend a large scale cougar database, which could provide an effective means for collaboration among management agencies to continue to successfully maintain healthy populations of the North American cougar.

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