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Jonathan A. Jenks
South Dakota State University, jonathan.jenks@sdstate.edu

Christopher N. Jacques
South Dakota State University

Jaret D. Sievers
South Dakota State University

Robert W. Klaver
United States Geological Survey, National Center for Earth Resources Observation and Science, Sioux Falls, South Dakota

R. Terry Bowyer
Idaho State University

See next page for additional authors

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JONATHAN A. JENKS[1], CHRISTOPHER N. JACQUES, JARET D. SIEVERS, ROBERT W. KLAVER, R. TERRY BOWYER, and DANIEL E. RODDY

Department of Wildlife and Fisheries Sciences, South Dakota State University, Brookings, SD 57007 (JAJ, CNJ, JDS)
United States Geological Survey, National Center for Earth Resources Observation and Science, Sioux Falls, SD 57198 (RWK)
Department of Biological Sciences, Idaho State University, Pocatello, ID 83209 (RTB)
Wind Cave National Park, 26611 U.S. Highway 385, Hot Springs, SD 57747 (DER)

ABSTRACT -- The pronghorn (*Antilocapra americana*) was reintroduced into Wind Cave National Park, South Dakota, in 1914 and thus, has inhabited the Park for almost a century. A decline in the population has raised concern for the continued existence of pronghorn inside Wind Cave National Park. Historically, pronghorn numbers reached greater than 300 individuals in the 1960's but declined to about 30 individuals by 2002. The primary objective of our study was to evaluate genetic characteristics of pronghorn to determine if reduced heterozygosity contributed to the decline of pronghorn in Wind Cave National Park. Microsatellite DNA was collected from 75 pronghorn inhabiting Wind Cave National Park in western South Dakota (n = 11), northwestern South Dakota (n = 33), and southwestern South Dakota (n = 31). Pronghorn in Wind Cave National Park had similar levels of observed heterozygosity (0.473 to 0.594) and low inbreeding coefficients (-0.168 to 0.037) when compared with other populations in western South Dakota. Furthermore, indices of population structure indicated no differentiation occurred among pronghorn populations. Results indicated that genetic variability was not a primary factor in the decline of pronghorn in Wind Cave National Park.

[1]Corresponding author. E-mail address: jonathan.jenks@sdstate.edu

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Genetic characteristics of pronghorn (*Antilocapra americana*) populations have been documented across the range of the species (Lee et al. 1989, Lee et al. 1994, Honeycutt 2000, Carling et al. 2003). Pronghorn populations declined throughout North America in the early 1900's with less than 30,000 animals existing by the late 1930's (Cadieux 1987). Extant populations of pronghorn have exhibited reduced variation in mitochondrial DNA when compared with other mammalian populations (Lee et al. 1994). This loss of genetic variation is of great concern in reintroduced and translocated populations where founder populations are often small. Nonetheless, estimates of genetic variation have shown that pronghorn have maintained relatively high levels of heterozygosity following potential bottleneck events of the early 1900's (Honeycutt 2000).

Past classification efforts have recognized five subspecies of pronghorn: American pronghorn (*A. a. americana*, Ord 1815), Oregon pronghorn (*A. a. oregona*, Bailey 1932), Mexican pronghorn (*A. a. mexicana*, Merriam 1901), Sonoran pronghorn (*A .a. sonoriensis*, Goldman 1945), and peninsular pronghorn (*A. a. peninsularis*, Nelson 1912). This knowledge has provided a framework for studying effects of translocations and reintroductions on the preservation of genetic variation. Lee et al. (1989) studied six pronghorn populations in western Texas and recommended that translocations into isolated populations be conducted only if genetic information collected revealed that both populations were similar.

Studies documenting effects of inbreeding, which include decreased fitness, lower resistance to diseases, and lower ability to adapt to changing environmental conditions, have been conducted largely on captive populations (Lacy 1997). Less is known about how inbreeding influences wild pronghorn populations. This information could be especially relevant for the pronghorn population in Wind Cave National Park. In 1914, 13 pronghorn were released into Wind Cave National Park. Pronghorn numbers in Wind Cave National Park increased to greater than 300 individuals in the 1960's but were estimated at about 30 individuals in 2002. The mission of the National Park Service is "...to promote and regulate the use of the ....national parks...which purpose is to conserve the scenery and the natural and historic objects and the wild life therein..." (National Park Service Organic Act 1916:Sec. 1). Thus, an evaluation of the decline of the pronghorn population within Wind Cave National Park was warranted. The objective of our study was to evaluate genetic characteristics of pronghorn to determine if reduced heterozygosity contributed to the population decline in Wind Cave National Park.

STUDY AREAS

Wind Cave National Park encompassed an area of 115 km^2, with an average elevation of 1,257 m above mean sea level and was situated in Custer County, South Dakota, in the southeastern region of the Black Hills. Wind Cave National Park was enclosed by a 2.5-m woven-wire fence, with cattle guards present at all road entrances to prevent movement by ungulates out of Wind Cave National Park. Wind Cave National Park was characterized by a mozaic of mixed-grass prairie interspersed with a ponderosa pine (*Pinus ponderosa*) dominated forest. Plant species occurring in the mixed grass prairie within Wind Cave National Park included Kentucky bluegrass (*Poa pratensis*), blue grama (*Bouteloua gracilis*), western wheatgrass (*Pascopyrum smithii*), western snowberry (*Symphoricarpos occidentalis*), common juniper (*Juniperus communis*), and northern bedstraw (*Galium boreale*). Plant nomenclature followed Larson and Johnson (1999) and Johnson and Larson (1999).

Fall River County encompassed 507,084 ha, of which 12,545 ha were federal lands administered by the USDA Forest Service (Kalvels 1982). The county was located in the southwestern corner of South Dakota and was bordered by Custer County to the north, Shannon County to the east, Nebraska to the south, and Wyoming to the west. Elevation ranged between 914 and 1,478 m above mean sea level (Kalvels 1982). Approximately 83% of the farm and ranch land in Fall River County was grazed by livestock and 17% was used for cultivated crops, tame pasture, or hay (Kalvels 1982). Fall River County lies within the mixed grass prairie region of western South Dakota and dominant grasses included western wheatgrass, buffalograss (*Buchloe dactyloides*), green needlegrass (*Nassella viridula*), needle-and-thread (*Hesperostipa comata*), sideoats grama (*Bouteloua curtipendula*), blue grama, and prairie Junegrass (*Koeleria pyramidata*). Dominant overstory woody vegetation in Fall River County consisted of ponderosa pine (*Pinus ponderosa*) interspersed with small stands of quaking aspen (*Populus tremuloides*) and paper birch (*Betula papyrifera*) (Kalvels 1982).

Harding County encompassed 693,968 ha; most of the land area was treeless, semi-arid rolling plains (Johnson 1988). The county was located in the northwestern corner of South Dakota and was bordered by Butte County to the south, Perkins County to the east, North Dakota to the north, and Montana to the west. Land elevation ranged between 817 and 1,224 m above mean sea level (Johnson 1988). Most farm or ranch land (88%) in Harding County was used for grazing and 12% was used for cultivated crops, tame pasture, or hay. Dominant grasses on the landscape included western wheatgrass, prairie Junegrass, buffalo grass, green needlegrass, and blue grama. Silver sagebrush (*Artemisia cana*) and big sagebrush (*A. tridentata*) were the dominant shrubs (Johnson 1988).

Pre-hunt estimates of pronghorn populations for Harding and Fall River counties during 2002 were 12,500 and 2,600 individuals, respectively. The primary predator affecting pronghorn in western South Dakota was the coyote (*Canis latrans*). Intensive coyote management (aerial shooting) was practiced in Harding County with moderate management (state trappers responded to landowner complaints) occurring in Fall River County.

METHODS

A helicopter capture service (Helicopter Capture Service, Marysville, Utah, Hawkins and Powers, Greybull, Wyoming) equipped with a modified 0.308 caliber net gun captured adult female pronghorn in Wind Cave National Park and Harding County, South Dakota, in January 2002, and Fall River County, South Dakota in February 2003. Whole blood samples were collected in Vacutainers (Becton Dickinson, Rutherford, New Jersey) that contained EDTA(K_3) from captured pronghorn. Samples were placed on ice and refrigerated until the extraction of DNA could be completed. Genetic analysis was conducted by an independent laboratory (Biogenetic Services Inc., Brookings, South Dakota), which identified alleles at 7 microsatellite loci within samples. Microsatellite DNA contained genetic material from both parents and thus, was considered a better index of heterozygosity than mitochondrial DNA (Ramey et al. 1999). Microsatellite DNA was purified from samples containing 100 μl of whole blood by using a Puregene DNA isolation kit. The protocol identified by Gentra Systems, Inc. (Minneapolis, Minnesota) was followed for the purification and analysis of microsatellite DNA. Primer sequences used to identify alleles and genotypes were obtained from a study of pronghorn on the National Bison Range, Montana, where 14 microsatellite markers were identified (Carling et al. 2003). The Genes in Populations computer program (May et al. 1992) was used to determine allele frequency, heterozygosity, the coefficient of inbreeding (F_{IS}), and population structure (F_{ST}). Methods used in our study were approved (Approval Number 02-A002) by the Institutional Animal Care and Use Committee at South Dakota State University.

RESULTS

A total of 75 blood samples was collected from captured pronghorn in Wind Cave National Park (n = 11) and Harding County, South Dakota (n = 33) during 2002, and in Fall River County, South Dakota (n = 31) during 2003. Five polymorphic loci (Aam2, Aam3, Aam8, T268, and T108) and two monomorphic loci (T26, T156) were identified across all 3 populations (Table 1). To maintain consistency with other studies of genetic variation in wildlife populations, only polymorphic loci were included in further analyses.

Table 1. Summary of microsatellite DNA data for pronghorn from three locations in South Dakota: number of alleles (A), observed heterozygosity (H_O), expected heterozygosity (H_E), and inbreeding coefficient (F_{IS}). A blank cell represents "no data."

			Locus							
Population	Parameters	n	Aam2	Aam3	Aam8	T268	T108	T26	T156	Mean
Wind Cave National Park		11								
	A		6	7	5	1	2	1	1	
	H_O		0.727	0.455	0.182[a]	0.000	0.500	0.000	0.000	0.466
	H_E		0.698	0.818	0.504	0.000	0.500	0.000	0.000	0.630
	F_{IS}		-0.041	0.444	0.639		-1.000			0.011
Harding County		33								
	A		6	9	4	2	2	1	1	
	H_O		0.636[a]	0.424[a]	0.364[a]	0.061	0.970[a]	0.000	0.000	0.491
	H_E		0.777	0.819	0.719	0.059	0.500	0.000	0.000	0.575
	F_{IS}		0.181	0.482	0.495	-0.031	-0.941			0.037
Fall River County		31								
	A		6	7	5	1	2	1	1	
	H_O		0.581	0.581[a]	0.871[a]	0.000	0.935[a]	0.000	0.000	0.742
	H_E		0.703	0.764	0.721	0.000	0.498	0.000	0.000	0.672
	F_{IS}		0.175	0.240	-0.209		-0.879			-0.168
Pooled										
	Total A		6	10	6	2	2	1	1	
	Mean H_O		0.648	0.486	0.472	0.020	0.802	0.000	0.000	0.485
	Mean H_E		0.726	0.800	0.648	0.020	0.499	0.000	0.000	0.539

[a]Chi-square analysis showed significant differences between observed and expected allele frequencies at $P < 0.05$.

Pronghorn in Wind Cave National Park and Fall River County had 21 different alleles ($\bar{x}$ = 4.2 alleles per locus, SE = 0.993), whereas pronghorn in Harding County had 23 different alleles ($\bar{x}$ = 4.6 alleles per locus, SE = 1.131) (Fig. 1). Observed heterozygosity ranged from 0.473 for pronghorn in Wind Cave National Park to 0.594 for pronghorn in Fall River County and expected heterozygosity ranged from 0.504 for pronghorn in the Park to 0.575 for pronghorn in Harding County (Table 1).

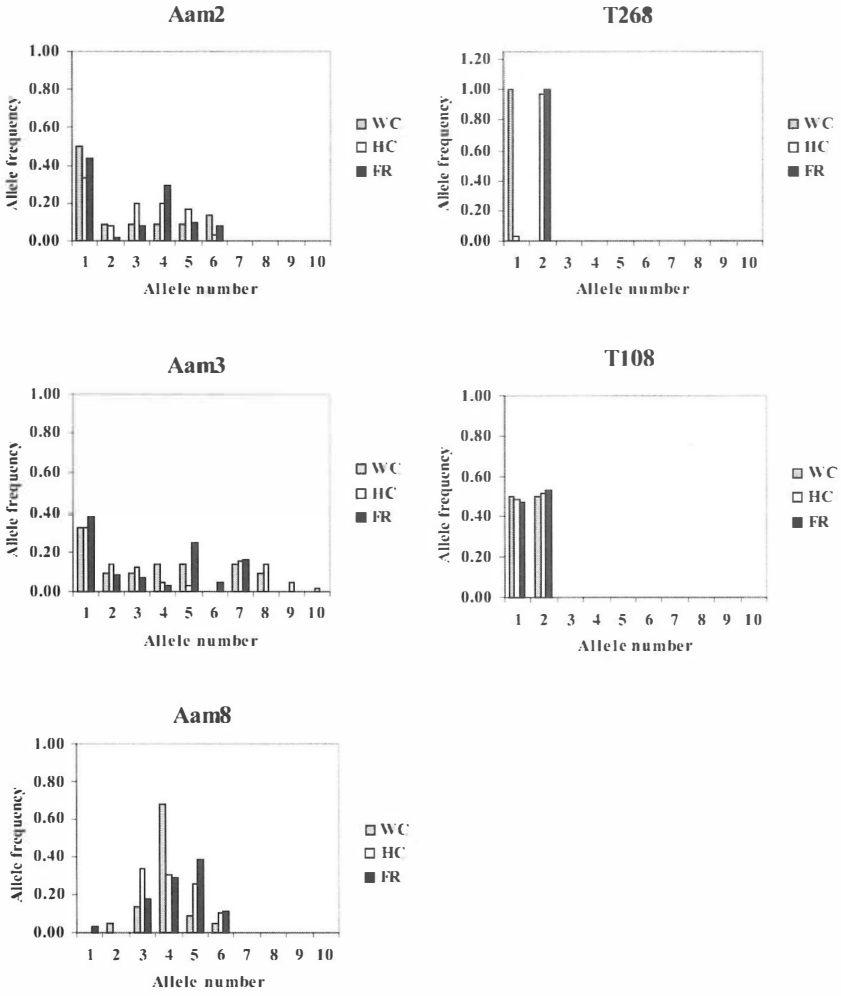

Figure 1. Allele frequencies at five polymorphic microsatellite loci typed in DNA samples (n = 75) of pronghorn in Wind Cave National Park (WC), Harding County (HC), and Fall River County (FR), South Dakota, 2002-2003.

Inbreeding coefficients (F_{IS}), which can range from -1.0 when all individuals are heterozygous to 1.0 when no individuals are heterozygous, were less than or equal to 0.037 for pronghorn populations in western South Dakota (Table 1). Also, pairwise measures of population structure (F_{ST}), which range from 0.0 when no differentiation occurs to 1.0 when complete differentiation occurs, for pronghorn were low between Wind Cave National Park and Harding County (F_{ST} = 0.028), Wind Cave National Park and Fall River County (F_{ST} = 0.032), and Harding County and Fall River County (F_{ST} = 0.015) populations.

DISCUSSION

Genetic variation can be influenced by a small number of founders, significant declines in population size, isolation by geographic features (e.g., mountain ridges) or human induced barriers (e.g., fences, fragmented landscapes). Although many of these factors have existed at some time for pronghorn in Wind Cave National Park, results of our genetic analysis reflected only slight effects on genetic variability. For example, multi-loci heterozygosity and inbreeding coefficients for pronghorn in Wind Cave National Park were similar to free-ranging pronghorn populations in western South Dakota.

Our analyses documented polymorphism in 5 of 7 loci (71%). Pronghorn in the National Bison Range, Montana, showed at least 8 of 14 (57%) loci to be polymorphic (Carling et al. 2003). The reason for fewer polymorphic loci observed in pronghorn populations in western South Dakota, when compared to pronghorn in other western states, is uncertain. Number of polymorphic loci identified was higher in pronghorn from Harding County (n = 5) than in those from Fall River County and Wind Cave National Park (n = 4).

Lower number of polymorphic loci in pronghorn from southwestern than northwestern South Dakota might have occurred because pronghorn were reduced throughout South Dakota to a low of 700 animals in 1924 (Yoakum 1978). These remaining pronghorn occurred in west-central South Dakota, to the north and east of the Black Hills. From 1950 to 1952, a total of 177 pronghorn was trapped in Butte and Meade counties, South Dakota in an effort to restore the species throughout western South Dakota (Berner 1952). To that end, a total of 121 of these animals was released in Harding, Tripp, Custer, and Jackson counties, South Dakota. Those pronghorn released in the southern portion of South Dakota (i.e., Custer and Jackson counties) could have been less diverse genetically (lower polymorphic loci) than those occurring in more northern regions of the state. Furthermore, Jacques and Jenks (2007) documented dispersal of pronghorn from Fall River County to Harding County but not the converse. They also suggested that quality of habitat was higher in northwestern than southwestern South Dakota, which resulted in smaller home ranges and less movement of pronghorn in Harding

County than in Fall River County, South Dakota. Consequently, low founder population size, semi-geographical isolation by the Black Hills, and primary dispersal movements to the north and west could be responsible for the lower number of polymorphic loci in pronghorn in Fall River County and Wind Cave National Park than in pronghorn in Harding County, South Dakota.

Microsatellite analysis has been used to compare heterozygosity between translocated and native populations of pronghorn (Carling et al. 2003), bighorn sheep (*Ovis canadensis*) (Ramey et al. 1999), and grizzly bear (*Ursus arctos*) (Craighead et al. 1995). Radio-collared pronghorn in Wind Cave National Park maintained a relatively high level of genetic diversity, despite a reduced population size. Because radio-collared pronghorn likely were able to move into and out of Wind Cave National Park via openings in the north fence that separates Custer State Park and Wind Cave National Park, we presume that the same opportunities for movement existed for pronghorn inhabiting Custer State Park and potentially those on other grasslands adjacent to Wind Cave National Park. Also, few adult males were identified in Wind Cave National Park during our study. Therefore, we would expect contributions from nonresident pronghorn to reproduction to have a significant influence on the genetic composition of the population.

Multi-loci heterozygosity values and allele frequencies obtained in our study were higher than what has been reported for populations of elk (*Cervus elaphus*) in Pennsylvania and California (mean number of alleles = 1.8, 1.9; observed heterozygosity = 0.222, 0.220; expected heterozygosity = 0.254, 0.219) and moose (*Alces americanus*) (mean number of alleles = 2.6; observed heterozygosity = 0.219; expected heterozygosity = 0.296) that have undergone known genetic bottlenecks (Broders et al. 1999, Williams et al. 2002, Williams et al. 2004). Thus, despite the low population size of pronghorn inhabiting Wind Cave National Park, genetic variability seems to have been conserved.

The index of population structure (F_{ST}) ranges from 0.0 (no differentiation among populations) to 1.0 (complete differentiation via subpopulations). Hundertmark et al. (2006) documented that F_{ST} values for pairwise comparisons of moose in Alaska, southeastern Alaska, and British Columbia, Canada ranged from 0.29 to 0.66, which indicated that significant differentiation occurred among some populations. In our study, F_{ST} values for comparisons between pronghorn populations were less than or equal to 0.032 indicating no differentiation among populations.

Other factors that could be responsible for the decline in the pronghorn population in Wind Cave National Park include poor forage conditions and low survival of adult and neonatal-aged pronghorn. In fact, pronghorn in Wind Cave National Park consumed low levels of shrubs (< 40%) and had low neonate survival (22-42%) (Sievers 2004). The coyote likely contributed to the mortality of at least 50% of radio-collared neonates. However, survival of adults averaged 75%. These results indicated that genetic variation was not a primary factor in the decline of pronghorn in Wind Cave National Park.

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