DETECTION OF BISON/CATTLE HYBRIDIZATION IN CUSTER STATE PARK BREEDING BULLS USING MICROSATELLITE AND MITOCHONDRIAL DNA MARKERS: TOOLS FOR CONSERVATION MANAGEMENT

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ABSTRACT

Previous studies have shown the presence of cattle genes in the Custer State Park (CSP) bison herd. In this study the degree of hybridity in breeding bulls, was assayed in extant breeding bison bulls as well as samples of archived blood from Custer State Park bulls dating from 1994-2001. Hybridity was determined using both mitochondrial and microsatellite markers. The mitochondrial assay utilized a highly conserved region as a positive control and a cattle specific region in multiplexed PCR reactions. Presence of the cattle specific PCR product and the control PCR product was diagnostic for the presence of maternal cattle DNA. Microsatellite markers were diagnostic for cattle and bison and were also used to assess hybridity. Using seven previously developed microsatellite markers, 90 bison bulls were genotyped on an automated genetic analyzer. The extent of hybridity detected with the mitochondrial test was 3.3% and hybridity detected using microsatellite markers was 10%. The extended goal of the present project is to utilize DNA-based assessments of hybridity in the selection of breeding bulls in the CSP bison herd.

Keywords

Bison, Custer State Park, hybrid, introgression

INTRODUCTION

Historically, the population of American Bison (*Bison bison*) on the Great Plains of North America had been estimated in excess of 50 million. During the expansion westward, bison were slaughtered for their meat, and valuable hides. By the late 1800's, bison were nearly extinct, numbering less than 1000 individuals. During the late 1800s, a small number of private ranchers attempted to rescue the bison from extinction by harboring them on their ranches. These individuals are responsible for saving the bison from extinction and for creating small foundation herds. It is known that many of these ranchers hybridized bison

to domestic cattle (*Bos taurus*) experimentally. These small, private herds were then used to stock federal and state bison populations.

Pete Dupree, a South Dakota rancher, captured five bison calves in the Dakota's to found his own bison herd in 1881. James "Scotty" Philip purchased the entire Dupree herd in 1901 which had grown to about 83 bison. In 1914, 36 bison from the estate of Scotty Philip were sold to the state of South Dakota and immediately brought onto Custer State Forest lands. These 36 bison became the founders of what is now the Custer State Park herd (Dary, 1974). It is well documented that there was a history of hybridization between cattle and bison in the Dupree herd (Coder, 1975), but the status of the 36 bison used to found the CSP herd is not known. There is no record of experimental hybridization following the foundation of the Custer State Park herd, however, recent studies have indicated the existence of cattle genes in the current herd (Polziehn et al, 1995; Ward et al, 1999; Halbert et al, 2005). These cattle genes are thought to trace back to the original 36 animals used to found the CSP herd.

Hybridity can be assessed in bison using microsatellite and mitochondrial DNA (mtDNA) markers. Mitochondrial DNA (mtDNA) is haploid, inherited maternally, lacks recombination and provides a relatively simple approach to DNA testing. Mitochondrial DNA will reveal hybidity in maternal pedigrees, but the absence of cattle mtDNA is not proof of bison purity because of this maternal mode of transmission. Nuclear microsatellite markers have become the preferred molecular marker in many genetic studies because of their high mutation rate, abundance in the genome and the simple codominant phenotypes. In addition, microsatellite markers can be used to determine the extent of nuclear introgression that is not revealed with mitochondrial analysis.

The present study was undertaken at the request of park biologists to investigate the genetic status of the breeding bulls in CSP to evaluate the possible inclusion of DNA testing in the future selection of breeding bulls. Seven microsatellite markers diagnostic for cattle and bison alleles were used to screen each of the 90 breeding bulls in addition to a mitochondrial assay for the presence of cattle mtDNA.

METHODS

DNA extraction

Archived whole blood samples for 90 breeding bulls were obtained from Custer State Park. These individuals represented the animals used for breeding over a period of eight years from 1994-2001. Total genomic DNA from whole blood was extracted using a Qiagen DNeasy Tissue Extraction Kit as per manufacturer's instructions (Qiagen Inc, Valencia, CA).

Mitochondrial Assay

A mitochondrial assay was used to detect the presence of cattle mitochondrial DNA in bison. PCR was performed for each individual blood sample with 2 mitochondrial markers. One primer pair (Bov16s2878/Bov16s2284, table 1)

Table 1. Primers and amplification protocols used for amplification of mitochondrial sequences and for nuclear microsatellite loci. * Expected size range for C-spec and Bov 16S are as presented in Ward, 2000; the size range for the microsatellite loci are as presented in Halbert et al, 2005.

LOCUS	PRIMER SEQUENCE	AMPLIFICATION PROTOCOL	SIZE RANGE CATTLE* (bp)	SIZE RANGE BISON* (bp)
Mitochondrial markers				
C-spec	F: 5'-AGCTAACATAACACGCCCATAC-3'	d	357	no product
	R: 5'-CCTGAAGAAAGAACCAGATGC-3'			
Bov 16SrDNA	F: 5'-CCCGCCTGTTTATCAAAAACAT-3'	d	594	594
	R: 5'-CCCTCCGGTTTGAACTCAGATC-3'			
Nuclear microsatellite markers				
BM1314	F: 5'-HEX/TTCCTCCTCTTCTCTCCAAAC-3'	b	143-167	137
	R: 5'-ATCTCAAACGCCAGTGTGG-3'			
BM4513	F: 5'-HEX/GCGCAAGTTTCCTCATGC-3'	a	139-166	132-134
	R: 5'-TCAGCAATTCAGTACATCACCC-3'			
BMS2270	F: 5'-HEX/CTGCGTTAACACCCCACC-3'	c	80-98	66-70
	R: 5'-GCAGGAAGGCTGATGCAC-3'			
BMS4040	F: 5'-HEX/GTCCATAGGGTCACACAGAGTC-3'	a	85-99	75
	R: 5'-CCAAATCTTACCATAGCAAAGG-3'			
CSSM042	F: 5'-6-FAM/GGGAAGGTCCTAACTATGGTTGAG-3'	c	173-217	167-171
	R: 5'-ACCCTCACTTCTAACTGCATTGGA-3'			
CSSM36	F: 5'-6-FAM/GGATAACTCAACCACACGTCTCTG-3'	a	162-185	158
	R: 5'-AAGAAGTACTGGTTGCCAATCGTG-3'			
TGLA227	F: 5'HEX/CGAATTCCAAATCTGTTAATTTGCT-3'	b	79-106	73
	R: 5'-ACAGACAGAAACTCAATGAAAGCA-3'			

a) 94°C 2 min; 35 cycles of 94°C 15 sec, 54.4°C 15 sec +1sec/cycle, 72°C 30 sec; 72°C 2 min. b) 94°C 2 min: 35 cycles of 94°C 15 sec, 54.4°C 15 sec, 72°C 30 sec; 72°C 2 min. c) 94°C 2 min; 35 cycles of 94°C 15 sec, 60.1°C 15 sec, 72°C 30 sec; 72°C 2 min. d) 94°C 2 min; 35 cycles of 94°C 30 sec, 55°C 30 sec, 74°C 1 min.

amplified a segment of the mitochondrial genome of any animal in the Bovidae subfamily (Derr et al, 1992). This marker was used as a control since it amplified segments of both the domestic cattle, and the bison mitochondrial genome. The second primer pair (c-spec16264/c-spec15907, table 1) specifically amplifies a portion of the control region of the mitochondrial genome of cattle (*Bos taurus*) and does not amplify bison DNA (Ward, 2000). Therefore, the presence of this second PCR product in a bison sample indicates the presence of cattle mitochondrial DNA. Each amplification was carried out in 20 ul reactions containing 0.5 U Taq Polymerase, 200 uM each dNTPs, 1.5 mM MgCl2, Tris buffer (Promega, Madison, WI), and 0.5 uM of each primer (Integrated DNA Technologies, Coralville, IA). Thermal profiles for these two primer sets are specified in table

1. Amplification of cattle DNA was performed with each group of reactions as a positive control. The presence or absence of PCR products from the above reactions was determined using 1.5 % agarose gel electrophoresis. The gels were then visualized and photographed. Figure 1 depicts the expected banding pattern for each of the marker sets when used to detect cattle, bison or cattle/bison hybrids.

Microsatellite marker amplification

Seven microsatellite markers were used to detect hybridity in the breeding bull samples from CSP. The forward primers for each of the seven loci were 5' end-labeled with a fluorophore (HEX or 6-FAM) for genotype analysis. Amplification was performed in 20 ul reactions using an Eppendorf Gradient thermocycler (Eppendorf, Westbury, NY). Marker names, primer sequences and thermal profiles are outlined in Table 1. Each 20 ul reaction contained 0.5 U Taq Polymerase, 200 uM each dNTPs, 1.5 mM MgCl2, Tris buffer (Promega), and 0.5 uM of each primer (Integrated DNA Technologies).

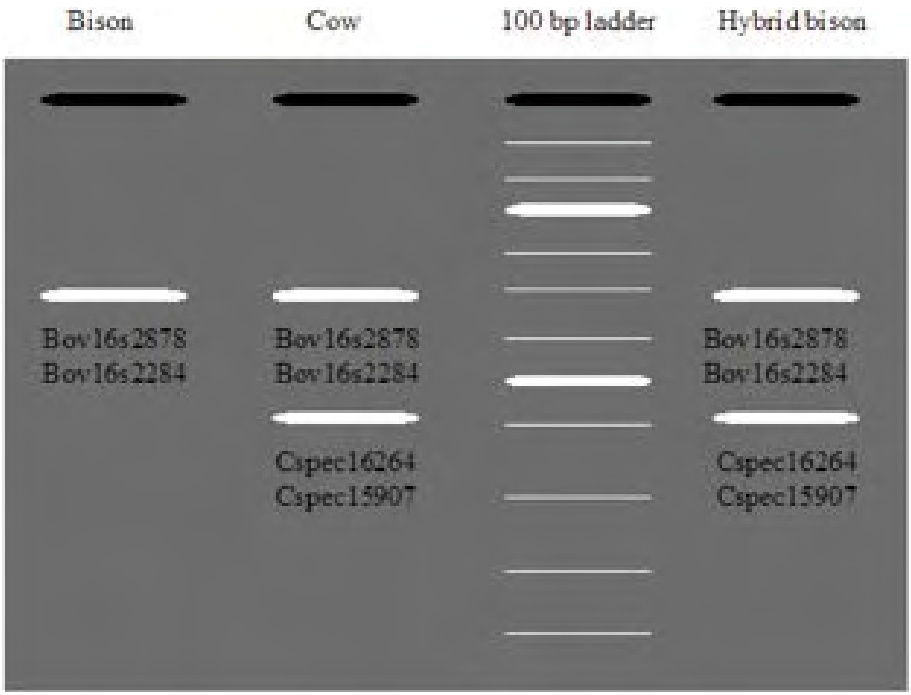

Figure 1. Expected results of the mitochondrial assay. This figure depicts the band patterns with bovine primers Bov16s2878/Bov16s2284 and cattle specific primers c-spec16264/c-spec15907 for bison, cattle and bison/cattle hybrid. The Bov primers amplify a fragment approximately 700 bp in length of DNA from any animal of the bovidae subfamily. The c-spec primers amplify a fragment approximately 400 bp in length from cattle DNA only, and are therefore diagnostic for the presence of cow mitochondrial DNA.

Genotyping

Fragments amplified for each individual bison bull were separated, sized, and genotyped on an ABI 3100 Avant Automated Genetic Analyzer (Applied Biosystems, Foster City, SD). ROX 350 (Applied Biosystems) was used as an internal size standard. Data collection was done using Genescan software version 3.7 (Applied Biosystems). Data analysis for allele determination was done using Genotyper v3.7 (Applied Biosystems). Genotyping was performed by Western South Dakota DNA Core Facility (WestCore), Black Hills State University, Spearfish, SD.

RESULTS

Mitochondrial Assay

The mitochondrial assay was performed on 90 bison bulls. Each of the 90 bulls assayed showed the presence of a band with the Bov16s2878/Bov16s2284 primers, and were therefore positive for the control DNA. Three of the 90 bulls assayed showed the presence of a band amplified with the c-spec16264/c-spec15907 primers. The c-spec primers were specific for cattle mitochondrial DNA and, therefore, indicate that 3.3% of the bison tested using this method are hybrids.

Microsatellite marker amplification

A total of seven microsatellite markers were used to genotype 90 bison and cattle controls. These microsatellite markers are able to amplify both bison and cattle DNA. Hybridity is detected by the presence or absence of alleles that are specific to bison or cattle. Table 2 depicts the genotypes of 11 bison individuals found to be hybrids. In this set of bison bulls, only three of the seven microsatellite markers used detected hybridity in nine animals, a hybrid frequency of 10%. At locus BM4513, two bulls had a cattle allele. At locus BM1314, three bulls had a cattle allele. At locus BMS2270, four bulls had a cattle allele. There were no bison that had more than one cattle allele, and only one bison with a cattle allele at locus BM4513 also tested positive for cattle mtDNA. The total amount of hybridity, mtDNA and microsatellite data combined, detected among the 90 bulls tested was 12.2% (Table 3).

DISCUSSION

In the past decade, with the advance of molecular genetics techniques, the impact of hybridization between wild and domestic or nonindigenous flora and fauna on the wild species populations has raised new concerns for wildlife conservation. It has been recognized that hybridization events can threaten a rare species existence (Rhymer and Simberloff, 1996). While hybridization in

Table 2. Genotypes of the eleven hybrid bison. * = the Cattle microsatellite alleles that were amplified; NP = no PCR product was amplified; af = amplification failed.

SAMPLE NUMBER	BM1314	BM4513	BMS2270	C-SPEC
BR950156	(132/132)	(127/143*)	(059/063)	NP
BR950131	(132/154*)	(127/129)	(061/061)	NP
BR960163	af	(127/129)	(061/079*)	NP
BR970301	(132/132)	(127/129)	(059/061)	400bp
BR970308	(132/132)	(127/127)	(061/081*)	NP
BR970327	(132/132)	(127/129)	(059/061)	400bp
BR980201	(132/132)	(127/143*)	(063/063)	400bp
BI990055	(132/154*)	(127/129)	(059/063)	NP
BI990052	(132/154*)	(127/129)	(063/063)	NP
BI990076	af	(127/127)	(059/081*)	NP
BI210057	(132/132)	(129/129)	(059/081*)	NP

Table 3: Comparison of the frequency of hybrids detected in bison from this study with previous studies.

LOCUS	NUMBER OF INDIVIDUALS	HYBRID FREQUENCY DETECTED	PUBLISHED FREQUENCY
mtDNA and nuclear loci	11	12.22%	na
mtDNA	3	3.33%	20.6%[1]
Nuclear loci	9	10%	30.8%[2]
BM1314	3	3.4%	4.05%[2]
BM4513	2	2.22%	0.00%[2]
BMS2270	4	4.44%	2.56%[2]

[1]Ward et al., 1999; [2]Halbert et al., 2005

naturally occurring populations of species can provide a source for novel genetic variation that is sometimes beneficial to species evolution (Lewontin and Birch, 1966; Dowling and Secor, 1997), anthropogenic hybridization poses a threat. The most extreme example is the complete admixture of the two parental species that leaves no pure stock to conserve (Allendorf and Leary, 1988; Allendorf et al., 2001; Rhymer et al., 1994). In the case of bison, we know that a bottleneck occurred in the North American population as the species was decimated and population numbers plummeted from more than 50 million animals to 1000 animals. The subsequent hybridization of some of the remaining animals with domestic cattle and the ensuing introgression has left its mark in some of the remaining wild and captive herds. However, none of the remaining populations

that contain remnant hybrid animals, including the Custer State Park population, have undergone introgression to this extent. For example, Halbert et al., 2005 tested 100 microsatellite markers covering 29 of the 30 bison autosomes and the X chromosome for their ability to differentiate cattle and bison genomic regions. They found only 14 diagnostic regions, of which only seven regions over five autosomes showed evidence of cattle introgression. The Halbert study included 14 bison populations, five of which were found to have some small degree of cattle introgression. While overall CSP bison harbored cattle alleles on four different autosomes, only five individuals in the Halbert study were found to harbor two introgressed genomic regions, and only seven were found to harbor a single introgressed region. A Y chromosome marker BYM-1 has also been developed and when tested revealed that there has been no Y chromosome introgression in any of the herds where mtDNA haplotypes were found (Ward et al., 2001). We argue that implementation of a management plan for natural herds that includes testing individuals for hybridity would be a valuable tool to work toward ridding herds containing hybrid animals of remnant cattle DNA. This approach would be especially useful for herds such as CSP that are known to harbor a low frequency of hybrid animals and which are an important source for supplementing and founding new herds.

The results presented here are consistent with previous findings that reported evidence of bison-cattle hybridization that occurred over 100 years ago (Polziehn et al, 1995; Ward et al, 1999; Halbert et al, 2005). The hybridization event(s) most likely occurred in bison owned by Peter Dupree in the late 1800's. Bison from this herd were subsequently sold to James "Scotty" Philip and are known to be the founders of the CSP herd. The hybridity seen today represents the remnant introgressed cattle DNA. An estimated 30-35 generations have passed since the CSP herd was established. While it is not known how many individuals of the initial herd were hybrids, previous studies and historical accounts provide ample evidence that at least some of the original 36 individuals were second generation hybrids (Garretson, 1938; Coder 1975; Dary 1989; Polziehn, 1995; Ward, 1999; Halbert, 2005). With the passage of 30 or more generations, and many of the animals backcrossing to wild-type bison, the amount of genetic introgression would decrease and become more fragmented throughout the genome. These results show that using seven microsatellite loci in combination with the mtDNA detected about ½ the amount of hybrid animals as previous studies using a few more loci. To increase the power of detecting hybrid individuals, we could include the use of other diagnostic microsatellite markers.

Certainly, the choice of genetic markers used in a management program that looks for hybridity in individuals is very important. The mtDNA marker is only able to detect the maternally inherited mitochondrial DNA. While this test can be useful, it is important to reiterate that a positive mtDNA test confirms hybridity, but a negative mtDNA test does not mean the animal tested is not a hybrid. In order to rule out hybridity a number of diagnostic nuclear markers should be used. A management strategy that utilizes DNA testing to detect hybridity in a bison herd could benefit from the utilization of mtDNA markers, nuclear microsatellite markers, and other diagnostic nuclear markers that are currently being developed (R. Schnabel, University of Missouri-Columbia, personal

communication) together in the most efficient way possible. Perhaps the most cost effective strategy would be to run the mtDNA test first to identify quickly and inexpensively hybrids harboring cattle mtDNA since those individuals would very likely harbor cattle genomic DNA. Those animals testing negative for the mtDNA can then be tested for hybridity using nuclear markers. Data from these markers could be used in various aspects of management from determining kinship and paternity, to potentially identifying the presence of disease resistant loci.

While genotype information on all individuals in the herd is valuable information, the implementation of this is problematic. In a herd such as that residing in Custer State Park or in Yellowstone National Park external tags or markings are not consistent with a non-domestic animal and detracts form the aesthetics. Thus the challenge is not in obtaining the genetic information for each individual but rather in the adoption of an efficient method of identifying individuals in the field. Once this is accomplished there is the potential for gathering other useful information such as herd kinship, sire contribution to the herd, herd structure (maternal groupings), inbreeding, and selective culling. The implementation of a management strategy that utilizes genetic technology will greatly benefit the conservation of bison in a natural state, and the preservation of the existing genetic diversity of the species.

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LITERATURE CITED

Allendorf, F.W., R.F. Leary, P. Spruell, and J.K. Wenburg. 2001. The problems with hybrids: setting conservation guidelines. Trends Ecol. Evol. 16: 613-6622

Allendorf, F.W., Leary, R.F. 1988. Conservation and distribution of genetic variation in a polytypic species, the cutthroat trout. Conserv. Biol. 2:170-184

Coder, G.D. 1975. The national movement to preserve the American buffalo in the United States and Canada between 1880 and 1920. PhD Thesis, Ohio State University.

Dary, David A. 1974. The Buffalo Book; The Full Saga Of The American Animal p. 231-232

Dowling, T.E., Secor, T.L. 1997. The role of hybridization and introgression in the diversification of animals. Ann. Rev. Ecol. Syst. 28:593-619

Garretson, M.S. 1938. The American Bison: The Story of its Extermination as a Wild Species and its Restoration Under Federal Protection. New York Zoological Society, New York.

Lewontin R.C., and Birch, L.C. 1966. Hybridization as a source of variation for adaptation to new environments. Evolution 20:315-336

Polziehn, R.O., Strobeck, C.M., Sheraton, J., Beech, R. 1995. Bovine mtDNA discovered in North American Bison Populations. Conserv. Biol. 9:1638-1643

Rhymer, J.M., D. Simberloff. 1996. Extinction by hybridization and introgression. Ann. Rev. Ecol. Syst. 27: 83-109.

Rhymer, J.M., M.J. Williams, and M.J. Braun. 1994. Mitochondrial analysis of gene flow between New Zealand mallards (Anas platyrhynchos) and grey ducks (A. superciliosus). Auk 111: 970-978.

Ward, T.J., Skow, L.C., Gallagher, D.S. Schnabel, R.D., Nall, C.A., Kolenda, C.E., Davis, S.K., Taylor, J.F., Derr, J.N. 2001. Differential introgression of uniparentally inherited markers in bison populations with hybrid ancestries. Animal Genetics 32:89-91

Ward, T.J., Bielawski, J.P., Davis, S.K., Templeton, J.W., Derr, J.N. 1999. Identification of domestic cattle hybrids in wild cattle and bison species: a general approach using mtDNA markers and the parametric bootstrap. Animal Conservation 2:51-57

Wilson, P.J. et al. 2000. DNA profiles of the eastern Canadian wolf and the red wolf provide evidence for a common evolutionary history independent of the gray wolf. Can. J. Zool. 78: 2156-2166.