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A Comprehensive Evaluation of Cattle Introgression into US Federal Bison Herds

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Abstract

Genetic introgression, especially from interspecies hybridization, is a significant threat to species conservation worldwide. In this study, 11 US federal bison populations were comprehensively examined for evidence of both mitochondrial and nuclear domestic cattle (*Bos taurus*) introgression. Mitochondrial introgression was examined using established polymerase chain reaction methods and confirmed through analysis of D-loop sequences. Nuclear introgression was assessed in 14 chromosomal regions through examination of microsatellite electromorph and sequence differences between bison and domestic cattle. Only one population was identified with domestic cattle mitochondrial DNA introgression. In contrast, evidence of nuclear introgression was found in 7 (63.6%) of the examined populations. Historic accounts of bison transfers among populations were corroborated with evidence of introgressed DNA transmission. While neither nuclear nor mitochondrial domestic cattle introgression was detected in bison from Grand Teton National Park, Sully's Hill National Game Preserve, Wind Cave National Park, or Yellowstone National Park, adequate sample sizes were available only from the last 2 populations to allow for statistical confidence (>90%) in nuclear introgression detection limits. The identification of genetically unique and undisturbed populations is critical to species conservation efforts, and this study serves as a model for the genetic evaluation of interspecies introgression.

Near the apex of the decline of North American bison (*Bison bison*) in the late 1800s, a small number of individuals independently and effectively served to save the species from near-extinction by capturing and raising wild bison on 5 private ranches (Coder 1975). Nearly all bison that exist today are descendants of the less than 100 bison used to found these 5 private herds and a remnant wild population in Yellowstone National Park (YNP) of approximately 30 bison (Garretson 1938; Meagher 1973; Coder 1975). Bison produced in the private herds were used to establish public populations in the United States of America and Canada (Table 1), to which the lineages of the more than 500 000 North American bison in existence today can be traced. Therefore, federal and state bison populations in North America are a critical resource for long-term species conservation.

Hybrids are known to form among nearly all combinations of species from the *Bos* genus (van Gelder 1977), and molecular techniques have been used to assess the extent of nuclear introgression due to hybridization among some members of the genus (Davis et al. 1988; Nijman et al. 1999). Although generally considered to be from different but closely related genera, bison and domestic cattle (*Bos taurus*) can produce fertile offspring from human-controlled crosses (Jones 1907; Boyd 1908, 1914; Goodnight 1914). The 2 species are not known to produce hybrids naturally, and even carefully controlled crosses result in a low birth rate of viable first-generation hybrid offspring (Boyd 1908; Steklenev and Yasinetskaya 1982). Each of the ranchers involved in establishing the 5 bison foundation herds in the late 1800s either experimented with domestic cattle–bison crosses or purchased bison from others who were involved in such experiments (Garretson 1938; Coder 1975). Consequently, both mitochondrial (Polziehn et al. 1995; Ward et al. 1999) and nuclear (Halbert et al. 2005) evidence of domestic cattle introgression has been identified in both public and private bison populations. In a previous study, 14 unlinked microsatellite markers with nonoverlapping allele size ranges between bison and domestic cattle were used to identify bison populations with evidence of nuclear domestic cattle introgression; regions of introgression were subsequently confirmed through analysis of microsatellites closely linked to the original diagnostic loci (Halbert et al. 2005). To date, evidence of mitochondrial or nuclear domestic cattle introgression has been identified in all except 6 of 14 US and Canadian public bison populations (Ward et al. 1999; Halbert et al. 2005) and all except 1 of the more than 50 private bison herds examined to date (Derr JN, unpublished data).

The apparent success of the bison recovery efforts over the past 150 years is threatened by domestic cattle introgression. Hybrid species do not have taxonomic status and are not protected by the Endangered Species Act (O'Brien and Mayr

Table 1. History of establishment for 11 US federal bison populations, derived from Halbert (2003)

Herd	Location	Year	Founding stock (number, source)[a]
BNP	South Dakota	1963	3, FN; 50, TR (TRS)
		1983	20, Colorado National Monument (unknown origin)
FN	Nebraska	1913	6, private ranch, Nebraska; 2, YNP
		1935	4, CSP, South Dakota
		1937	4, CSP, South Dakota
		1952	5, NBR
GT	Wyoming	1948	20, YNP
		1964	12, TR
NBR	Montana	1908	1, Goodnight herd; 3, Corbin (McKay–Alloway); 34, Conrad (Pablo–Allard)
		1939	2, 7-Up Ranch (unknown origin)
		1952	4, FN
		1953	2, YNP
		1984	4, Maxwell State Game Refuge, Kansas (Jones)
NS	Iowa	1996	8, FN; 8, WM
		1997	6, FN; 8, NBR
		1998	3, FN
SUH[b]	North Dakota	1919	6, Portland City Park, Oregon (unknown origin)
		1932	1, WC
		1941–1979	7, FN
		1987	3, NBR
		1994–1997	2, TR
TR	North Dakota	1956 (1962)	29, FN to found south unit (TRS) [20, TRS bison to found north unit (TRN)]
WM	Oklahoma	1907	15, New York Zoological Park[c]
		1940	2, FN
WC	South Dakota	1913	14, New York Zoological Park[c]
		1916	6, YNP
YNP	Wyoming, Idaho, Montana	1902	Approximately 30 indigenous; 18, Pablo–Allard herd; 3, Goodnight herd

BNP, Badlands National Park; FN, Fort Niobrara National Wildlife Reserve; GT, Grand Teton National Park; NBR, National Bison Range; NS, Neal Smith National Wildlife Reserve; SUH, Sully's Hill National Game Reserve; TR, Theodore Roosevelt National Park; WM, Wichita Mountains National Wildlife Reserve; WC, Wind Cave National Park; YNP, Yellowstone National Park.

[a] Five private foundation herds established in the late 1800s (Coder 1975): McKay–Alloway (Canada), Goodnight (Texas), Dupree–Philip (South Dakota), Jones (Kansas), and Pablo–Allard (Montana).

[b] History of introductions provided by Dixon C (personal communication).

[c] As described by Coder (1975); founded as composite of bison from Nebraska (1888), South Dakota (1889), the Pablo–Allard herd (1897), and the Corbin herd (1904), which originated from bison from Wyoming, Manitoba, and the Jones herd.

1991). Widespread hybridization in other mammalian species has lead to proposals to delist such icons as the red wolf and Florida panther as endangered species (Rhymer and Simberloff 1996). However, bison have at least 2 advantages to successful long-term conservation over other partially introgressed species: a large total population size (>500 000 bison in existence today) and many moderately sized, isolated, and protected public populations (census sizes >200). As such, the purpose of this study was to comprehensively examine bison from US federal populations for evidence of both mitochondrial and nuclear domestic cattle introgression to identify potentially important sources of germplasm for long-term species conservation efforts.

Materials and Methods

Sample Collection and DNA Isolation

Bison blood, hair, or tissue samples were collected from 11 US federal populations (Table 2) by park personnel. DNA was extracted from whole blood following the Super Quik-Gene protocol (Analytical Genetic Testing Center, Denver, CO) and standard phenol–chloroform–isoamyl alcohol extraction (Sambrook et al. 1989) or isolated through application to FTA cards and processing following the manufacturer's recommendations (Whatman, Newton Center, MA). DNA was extracted from hair follicles and tissues following the protocols by Schnabel et al. (2000) and Halbert et al. (2004), respectively, and archived at Texas A&M University for future reference.

Mitochondrial DNA Introgression Assay

All polymerase chain reaction (PCR) and sequence reactions were performed on GeneAmp PCR System 9700 thermal cyclers (PE Biosystems, Foster City, CA). The mitochondrial DNA (mtDNA) assay was as described by Ward et al. (1999) with minimal exceptions as follows (per 25 μl reaction): 50 ng template DNA or 1 FTA punch, 0.2 μM each primer, 1× MasterAmp PCR Enhancer (Epicentre, Madison, WI), 400

Table 2. Total number of bison examined among 11 federal populations for mitochondrial and nuclear domestic cattle introgression

Population	Collection year	Census[a]	Total sampled
BNP	2002	875	492
FN	2001–2002	380	367
GT	1999–2000	600	39
NBR	1999–2002	350	616[b]
NS	2001	63	63
SUH	2004	35	31
TRN	2000	312	294
TRS	2001	371	355
WM	1999, 2002	600	172
WC	1999–2001	350	352[b]
YNP	1997, 1999–2002	3000	520
Sum		6936	3301

[a] Current approximate census population size, as estimated by individual herd managers. When possible, estimates are given of total census population size at the time of collection for this study (or average across collection years).

[b] Total sampled greater than given census size due to sampling of all adults in a population and calves over multiple years. Duplicate samples from the same individual were eliminated from analysis through comparisons of unique collection identifiers (ear tags, microchips) or polymorphic microsatellite analysis (Halbert 2003).

μM deoxynucleoside triphosphate (dNTPs), 2.0 mM $MgCl_2$, 1× reaction buffer, and 1.0 units *Taq* DNA polymerase (Promega, Madison, WI). The thermal parameters for the mtDNA assay were as follows: 96 °C for 3 min; 4 cycles of 96 °C for 20 s, 58 °C for 30 s (−1 °C per cycle), and 65 °C for 90 s; 26 cycles of 96 °C for 20 s, 54 °C for 30 s, and 65 °C for 90 s; and 1 cycle of 96 °C for 60 s, 54 °C for 60 s, and 65 °C for 20 min.

Sequencing of the mtDNA D-loop was performed for bison with suspect domestic cattle fragments. An 1100-bp fragment was amplified using the primers 12S (5′-AACAGGAAGGCTGGGACC-3′) and THR (5′-AGAGAAGGAGAACAACTAACCTCC-3′) located in the 12S rRNA and threonine tRNA genes, respectively, flanking either side of the bovine D-loop. Amplification was performed under the following conditions (per 50 μl reaction): 100 ng template DNA, 0.12 μM each primer, 400 μM dNTPs, 3.5 mM $MgCl_2$, 1× reaction buffer, and 1.25 units AmpliTaq Gold® DNA polymerase (PE Biosystems). PCR products were cleaned using the QIAquick PCR Purification Kit (Qiagen Inc., Valencia, CA). Sequence reactions were performed using the BigDye® terminator cycle sequencing kit version 2.0 (PE Biosystems) and an ABI377 automated sequencer (PE Biosystems) with the THR and internal D811-R (770 bp from THR, 5′GGGGGAATTTTTATGGAGG-3′) primers.

Sequences obtained in this study were compared with those produced by Ward et al. (1999) using CLUSTALX (Higgins and Sharp 1988) with the following alignment parameters: gap opening of 15, gap extension of 6.66, and transition weight of 0.5. Phylogenetic Analysis Using Parsimony (PAUP* 4.0b2; Swofford 2003) was employed for parsimony analysis of the sequences through branch-and-bound algorithms with the following options: unrooted starting trees obtained via stepwise addition, tree-bisection-reconnection used as the branch-swapping algorithm, branches collapsed when maximum length equals zero, and bootstrapping on a 50% majority rule consensus tree with 2000 replicates to test the strength of relationships among taxa.

Nuclear Introgression Assay

One marker from each of the regions examined by Halbert et al. (2005) was chosen for examination in this study based on the presence of introgression in other bison populations screened (Halbert et al. 2005) and allele size ranges for multiplexing. The forward primer for each marker was fluorescently labeled and multiplexed according to nonoverlapping allele size ranges and dye types (Table 3). All microsatellite amplification reactions were performed using the thermal parameters described above for the mtDNA assay. PCR conditions for multiplexes A and C and all confirming markers were as follows (5 μl reactions): 50 ng template DNA or 1 FTA punch, 0.05–0.4 μM each primer, 1× MasterAmp PCR Enhancer (Epicentre), 400 μM dNTPs, 3.0 mM $MgCl_2$, 1× reaction buffer, and 0.375 units *Taq* DNA polymerase (Promega). PCR conditions for multiplex B were as above with the exception of 1.6× reaction buffer.

All 14 nuclear diagnostic microsatellites were screened across all samples. Markers were rerun as singletons in individuals with suspect domestic cattle–like alleles using essentially the same PCR protocols as above, with water substituted for the extra primer volume. For those populations with suspect domestic cattle–like alleles at a diagnostic locus, bison were divided into 2 classes: those with domestic cattle–like alleles and those with exclusively bison alleles. At least one linked confirming microsatellite (Table 3) was amplified in a singleplex PCR on a subset of each class, with all bison in the former class screened when possible. All PCR products were separated on an ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA) using an internal size standard (Mapmarker LOW; Bioventures, Inc., Murfreesboro, TN). GENOTYPER 3.6 (Applied Biosystems) was used for allele identification and comparison.

Sequence Confirmation of Introgression

For each diagnostic microsatellite marker in which domestic cattle introgression was detected in one or more bison populations, representative alleles were sequenced from bison and cattle through a second PCR, TOPO TA Cloning® (Invitrogen, Carlsbad, CA) and subsequent sequencing following the manufacturer's recommendations. Briefly, isolated white colonies were grown in Luria–Bertani broth containing 50 μg/ml ampicillin. A 1-μl aliquot of the broth was added to 9 μl sterile water and denatured at 100 °C for 10 min. Immediately after denaturation, the samples were placed on ice and used as template for standard microsatellite PCR amplification and allele detection as described above. After determining the alleles represented in each clone, plasmid preparations were obtained and inserts sequenced for clones representing each of the following allele classes: bison allele, domestic cattle–like allele identified in bison, and domestic cattle allele of the same size (electromorphs) from

Table 3. Primary diagnostic and closely linked confirming microsatellite markers (in italics) for 14 chromosomal regions used to detect nuclear domestic cattle introgression in bison

Locus	Label[a]	Multiplex	Chromosome	Position[b]	*Bison bison* allele range[c]	*Bos taurus* allele range[d]
AGLA17	VIC	A	1	0	215	214–219
BM4307	6-FAM	C	1	35.2	185–187	183–199
BMS4017	HEX		1	34.8	145–165	148–158
BM7145	NED	A	1	69.2	108–110	116–118
INRA119	HEX		1	68.7	122–128	130–138
BMS4008	6-FAM		1	71.7	158–164	152–179
BMS4040	NED	B	1	98.8	75, 95[e]	85–99
CSSM42	NED	B	2	34.4	167–171	173–217
AGLA293	HEX	C	5	32	218–220	218–239
RM500	6-FAM	A	5	55.6	123	125–135
SPS113	VIC	A	10	29.2	128–132	135–154
BM4513	NED	A	14	62.5	132–134	139–166
TGLA227	VIC	B	18	84.7	73	79–106
RM185	HEX	C	23	45.1	92	90–108
BMS2270	6-FAM	A	24	21.2	66–70	80–98
ILSTS065	HEX		24	25.2	Null[f]	131–143
BM1314	6-FAM	B	26	24.8	137	143–167
HEL11	6-FAM		26	20.7	142–175	179–203
CSSM36	VIC	A	27	39.8	158	162–185

[a] Fluorescent dye label for forward primer (Applied Biosystems).

[b] Chromosomal position (cM) as reported in the USDA cattle gene mapping database (www.marc.usda.gov).

[c] Based on the YNP and WC populations in this study and the results of Halbert et al. (2005).

[d] Based on the results of Halbert et al. (2005) from 64 domestic cattle (10 Angus, 16 Hereford, 13 Holstein, 12 Shorthorn, 13 Texas Longhorn).

[e] The 95-bp BMS4040 allele was found by Halbert et al. (2005) only in the CSP population and presumed to be of bison origin based on the exclusive presence of bison-like alleles at a nearby locus.

[f] ILSTS065 does not amplify in bison due to the presence of a fixed null allele.

domestic cattle. Multiple clones containing the same allele were sequenced to resolve any suspect sequence anomalies. Preliminary alignments were established using the program CLUSTALX (Higgins and Sharp 1988) as described above, which were then checked manually and adjusted as necessary around the repeat regions. Repeat region length differences and single nucleotide polymorphisms were recoded in a 1–0 matrix containing all the alleles sequenced. PAUP* 4.0b2 (Swofford 1999) was used to establish relationships among alleles as described above.

Statistical Analysis

The statistical model outlined by Halbert et al. (2005) was used to estimate the probability of detecting domestic cattle introgression in the bison populations examined in this study as follows: assume 2 categories of founders for a given bison population, hybrid founders and purebred founders, and let p be the expected proportion of haploid domestic cattle genome represented in the hybrid founders such that an F_1 (first-generation cross) hybrid as a founder would represent the entire domestic cattle genome ($p = 1$) and a BC_1 (first-generation backcross) hybrid as a founder would represent half the domestic cattle genome ($p = 0.5$). Assume then that the hybrid founders are merged with a group of purebred bison and allowed to randomly mate for a sufficient number of generations such that each bison within the population has some proportion, m, of nuclear domestic cattle introgression. In a random sample of n individuals and using t independent, selectively neutral, unlinked diagnostic markers to detect introgression, a marker is considered informative for detecting introgression if it falls into the region of the genome for which domestic cattle DNA was present in the hybrid founders. Therefore, the probability of detecting introgression within a population is represented by

$$P(p, m, n, t) = 1 - \left[p\left(1 - \frac{m}{p}\right)^n + (1 - p) \right]^t, \quad \text{for } m \leq p. \qquad (1)$$

Results

A total of 3301 bison from 11 federal populations were surveyed for evidence of domestic cattle introgression using both mitochondrial and nuclear loci (Table 2). Sampling of all or nearly all bison from individual populations was performed when possible but was not achieved for the Badlands National Park (BNP) (56.2%), Grand Teton National Park (GT) (6.5%), Wichita Mountains National Wildlife Refuge (WM) (28.7%), and YNP (17.3%) populations.

Of the 11 federal populations examined, evidence of domestic cattle mtDNA introgression was found only in bison from National Bison Range (NBR), where suspect cattle D-loop fragments amplified in 11 of 616 tested bison (1.8%). Of these, 2 were females (born in 1984 and 1989) and 9 were males (1 each born in 1989, 1994, 1998, 1999, and 2000 and 3 born in 2002; 1 of unknown age). D-loop sequencing was performed for 8 of the suspect bison, excluding the 3 males born in 2002. Sequence alignments revealed

Table 4. Summary of testing and results for confirming loci by population

			DL suspect			DL nonsuspect		
Population	DL	CL	Domestic cattle allele (CL)	CL tested	CL cattle allele	Bison alleles (CL)	CL tested	CL bison allele
WM	BM1314	HEL11	187	7	7	155, 159, 161	7	7
BNP	BM4307	BMS4017	154	123	121	155, 159, 161, 163	366	366
FN	BM4307	BMS4017	154	73	69	155, 159, 161, 163	211	211
NS	BM4307	BMS4017	154	16	15	153, 155, 159, 161, 163	46	46
TRN	BM4307	BMS4017	154	91	90	155, 159, 161, 163, 165	210	210
TRS	BM4307	BMS4017	154	76	76	153, 155, 159, 161, 163	289	289
NBR	BM7145	INRA119	132	45	45	124, 126, 128	12	12
		BMS4008	166	45	44	160, 162	11	10
NS	BM7145	INRA119	132	2	2	124, 128	3	3
		BMS4008	166	2	2	160, 162	3	3
BNP	BMS2270	ILSTS065	131	30	14	Null[a]	12	12

Results presented only for those populations with suspect cattle-like alleles at DLs. Bison from each population were divided into 2 groups based on their DL genotypes: suspect (possessing cattle-like allele) or nonsuspect (possessing only bison-like alleles). Bison from each group were genotyped for the appropriate linked CL. The domestic cattle and bison called allele sizes for each CL in each population are indicated (following Halbert et al. 2005). DL, diagnostic locus; CL, confirming locus; CL tested, total number of bison tested in each class for the appropriate CL; CL cattle allele, the total number of tested bison with at least one cattle-like allele at the confirming locus; CL bison allele, the total number of tested bison with exclusively bison-like alleles at the confirming locus.

[a] ILSTS065 does not amplify in bison due to the presence of a fixed null allele.

complete identity to the domestic cattle mtDNA haplotype (9*) found in NBR bison by Ward et al. (1999). Likewise, parsimony analysis produced a consensus tree similar to that detailed by Ward et al. (1999), with the domestic cattle haplotypes from NBR bison sharing a node with domestic cattle of various breeds and other haplotypes identified as resulting from bison–domestic cattle introgression.

Allele frequencies for each of the 14 diagnostic microsatellites utilized for the detection of domestic cattle introgression are shown in Table A1 by population, with comparative frequencies for 5 domestic cattle breeds ($n = 64$ total) also shown (Halbert et al. 2005). Suspect domestic cattle–like alleles were detected at 4 of the 14 diagnostic microsatellites as follows: WM—9.01% frequency of BM1314 157-bp allele; BNP—13.55%, Fort Niobrara National Wildlife Refuge (FN)—13.48%, Neal Smith National Wildlife Refuge (NS)—13.49%, Theodore Roosevelt National Park north unit (TRN)—16.26%, and Theodore Roosevelt National Park south unit (TRS)—11.51% frequency of BM4307 197-bp allele; NBR—3.83% and NS—1.59% frequency of BM7145 116-bp allele; and BNP—3.15% frequency of BMS2270 94-bp allele (Table A1). In each population where potential domestic cattle introgression was detected at a diagnostic locus, confirmation of domestic cattle introgression was obtained through the detection of domestic cattle alleles at one or more linked loci (Table 4). In some cases, a small number of bison had a cattle-like allele at one locus but not at the other, indicating recombination (e.g., NBR BM7145/BMS4008; Table 4). One notable exception was the BMS2270/ILSTS065 markers in the BNP population, where less than 50% (14 of 30) of the tested bison were confirmed to have cattle-like alleles at both loci. This discrepancy is most likely due to recombination between these markers or genotyping error at the ILSTS065 locus, where the absence of a PCR product was interpreted as evidence of a bison-like allele when in fact amplification failure would produce the same result (Halbert et al. 2005). The ILSTS065 locus was coamplified with BMS2270 in the secondary screen in an attempt to eliminate genotyping error.

Both bison-like and domestic cattle–like alleles identified in bison were sequenced and compared with domestic cattle electromorphs for the following microsatellite loci: BM1314, BM4307, BM7145, and BMS2270 (GenBank accession numbers DQ887282–DQ887321). Although the BM4307 197-bp domestic cattle–like allele was detected in 5 different bison populations (Table A1), only bison from FN were sequenced for this microsatellite. Bison from FN were used at least in part in the establishment of each of the other 4 populations (Table 1), and therefore, the FN population is presumed to be the source of the 197-bp allele in the other populations. Similarly, the BM7145 116-bp domestic cattle–like allele was identified in the NBR and NS populations (Table A1) but was sequenced only from NBR bison because the NS population was recently derived in part from NBR bison (Table 1). For each locus, at least one domestic cattle allele was found with 100% identity to the sequence of the bison electromorph presumed to be of domestic cattle origin, thereby supporting the hypothesis that the electromorphs shared between bison and domestic cattle are due to introgression of domestic cattle genomic DNA in bison and not symplesiomorphy or convergence. Further supporting this hypothesis was the identification of single-nucleotide fixed changes between bison alleles and domestic cattle–derived alleles outside of the microsatellite repeat region for BM1314 and BM7145. Analysis of character differences through tree-building algorithms confirmed the common origin of domestic cattle alleles and electromorphs found in bison as well as the separation of these alleles from those of bison origin (Figure 1).

Four bison populations without any evidence of mitochondrial or nuclear introgression were identified in this study: GT, Sully's Hill National Game Preserve (SUH), Wind Cave National Park (WC), and YNP. Previous studies using

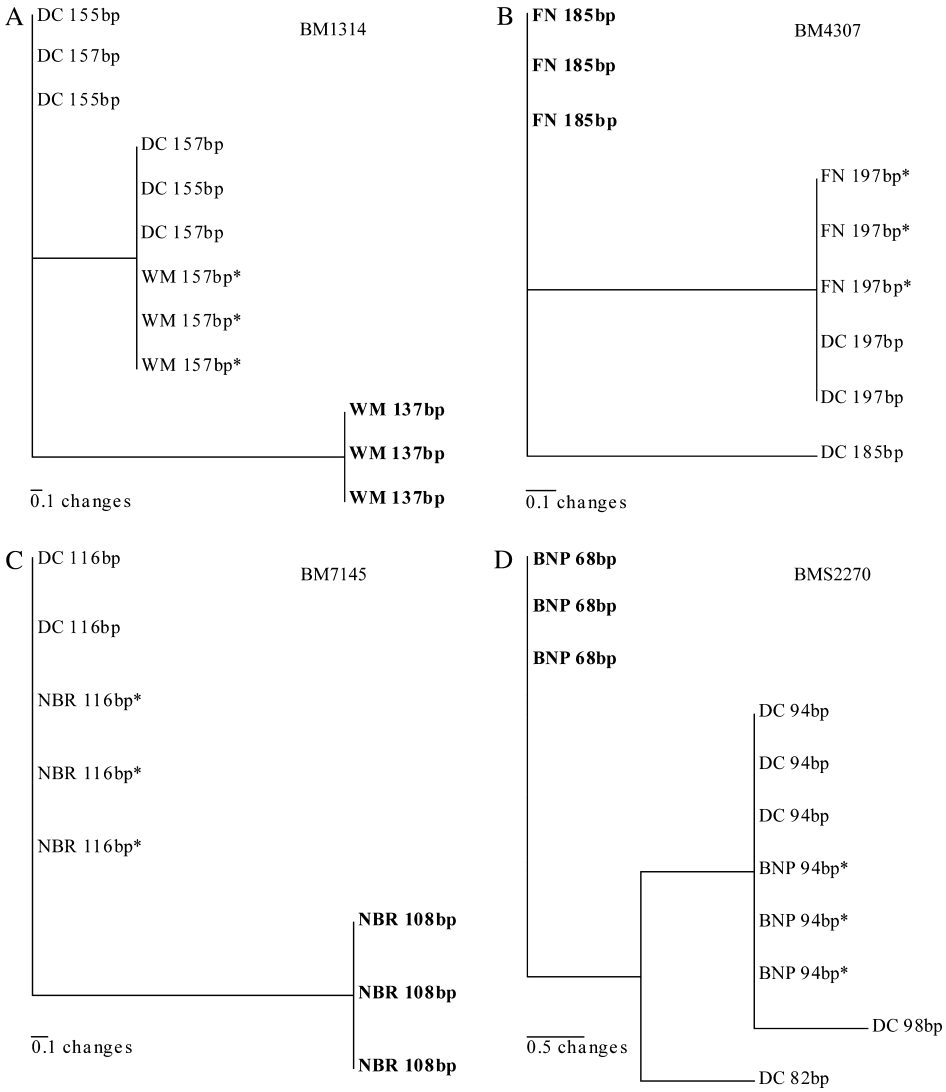

Figure 1. Parsimony analysis depicting relationships among allele sequences for diagnostic markers (**A**) BM1314, (**B**) BM4307, (**C**) BM7145, and (**D**) BMS2270 using branch-and-bound tree-building algorithms. DC-like alleles found in bison are indicated with an asterisk (*), and true bison alleles are shown in boldface type. Each node represents sequence from a different individual or different allele within an individual. DC, domestic cattle.

smaller sample sizes also failed to identify domestic cattle mitochondrial introgression in YNP and WC bison (Polziehn et al. 1995; Ward et al. 1999) and nuclear introgression in YNP bison (Halbert et al. 2005).

Discussion

In the current study, we identified domestic cattle introgression in some, but not all, tested federal bison populations. A rapid, cost-effective multiplexed PCR assay was developed to facilitate screening of 14 unlinked microsatellite markers on sufficiently large numbers of bison from individual populations. Therefore, we were able to examine populations included in previous studies (Polziehn et al. 1995; Ward et al. 1999; Halbert et al. 2005) in much greater detail for both nuclear and mitochondrial evidence of introgression. Additionally, the prevalence of domestic cattle introgression has been investigated in several federal bison populations for the first time through this study (BNP, GT, NS, SUH, TRN, and TRS). Furthermore, we used both linked confirming microsatellite markers and sequence analysis of diagnostic marker alleles to validate our assay and confirm the origin of domestic cattle–derived alleles in the bison populations examined herein.

Ward et al. (1999) identified domestic cattle mtDNA in 2.7% (3 of 113) of the bison tested bison from NBR, which is comparable to the 1.8% level observed in the current study. A female bison from NBR with domestic cattle mtDNA born in 1984 (see Results) was identified as 1 of the 4 females introduced from Maxwell State Game Refuge (Table 1; Garner L, personal communication). The other 3 females from this introduction were also included in this study; all contained bison mtDNA. The source of domestic cattle mtDNA

introgression in NBR bison is Maxwell State Game Refuge, as corroborated by the following observations: 1) all 9 bison with domestic cattle mtDNA sequenced in this study were identical to those found in NBR bison by Ward et al. (1999), including a single female introduced into the population from Maxwell State Game Refuge, and 2) this haplotype was found to be identical between the 2 populations. Halbert et al. (2005) previously identified domestic cattle–derived alleles of the same size from Maxwell State Game Refuge bison as from NBR bison for the BM7145/INR119/BMS4008 region. In this study, we found approximately 3.8% of the bison examined from NBR contained the same domestic cattle–derived 116-bp allele for BM7145 (Table A1, Figure 1) as that found in Maxwell State Game Refuge (Halbert et al. 2005), which was confirmed with the linked markers INRA119 and BMS4008 (Table 4). However, we did not detect the BM7145 116-bp cattle allele in samples from the 4 females from Maxwell State Game Refuge that were introduced into the NBR population. Furthermore, none of the 11 NBR bison identified as having domestic cattle mtDNA also had the BM1745 116-bp cattle allele. These results indicate 2 independent domestic cattle introgression events in the NBR population, possibly through one or more undocumented bison introductions.

The observation of the same BM4307/BMS4017 domestic cattle alleles in the TR bison populations as found in the FN population is expected based on the history of these populations (Table 1). Two separate regions of domestic cattle introgression were identified in the BNP population (Table A1): one on chromosome 1 (BM4307/BMS4017) and the other on chromosome 24 (BMS2270/ILSTS065). Similarities in allele size and frequency of BM4307 alleles (Table A1) indicate that introductions from FN and TRS were the source of the detected BNP chromosome 1 domestic cattle introgression (Table 1). The BMS2270 94-bp domestic cattle allele, however, is not shared with either TRS or FN and is presumably from the 1984 introduction of bison from Colorado of unknown origin (Table 1). Halbert et al. (2005) also identified domestic cattle introgression in Custer State Park (CSP) bison in the BMS2270/ILSTS065 region. The BMS2270 90-bp allele and ILSTS065 143-bp allele found in CSP (Halbert et al. 2005) are of different sizes, however, from those found in the BNP population (Tables A1 and 4).

The NS bison population shares domestic cattle alleles in the BM4307/BMS4017 region with FN and in the BM7145/INR119/BMS4008 region with NBR, as would be predicted based on the history of this population. The NS population does not share BM1314 domestic cattle alleles with WM, from which 8 bison were used as NS founders (Table 1). Because the frequency of the BM1314 157-bp domestic cattle allele in the WM population is only around 8.9%, it is most likely that this allele was not introduced into the NS population by chance, although drift or unequal contribution of founders might also explain this finding.

Excluding the possibility of recent, undocumented introgression, there are only 2 possible sources of the domestic cattle introgression observed in the BM1314/HEL11 region in the WM bison population: the New York Zoological Park or FN (Table 1). Coder (1975) reported that one of the bulls from the New York Zoological Park was from the Jones herd, where hybridization experiments are known to have occurred. Furthermore, the FN population was supplemented with CSP bison in 1935 and 1937, just before the 1940 transfer of 2 bulls to WM (Table 1). Although domestic cattle introgression was not observed in the BM1314/HEL11 region in the FN population (Table A1), the same alleles found in the WM population (157- and 187-bp, respectively) are found in the CSP population (Halbert et al. 2005). These findings may be the result of genetic drift over the last 60 years to effectively eliminate the introgressed BM1314/HEL11 region from the FN population or the introduction of a single bull directly from CSP through FN to WM that did not produce many, if any, offspring while at FN. The later possibility seems likely based on the timing of the movement of bison among these populations (Table 1).

This study has identified at least 3 federal bison populations with presumed multiple sources of domestic cattle introgression: BNP, NS, and NBR. The importance of utilizing both mtDNA and nuclear loci for the detection of hybridization and introgression was predicted by Rhymer and Simberloff (1996) and is substantiated in this study with results from the NBR population; without both the mtDNA and nuclear loci, the true extent of introgression in this population would have been underestimated. These results also emphasize the importance of the warning given by Simberloff (1996) for extreme caution when purposely mixing individuals from populations, especially when interspecies hybridization is a possible compounding issue. In the case of both the BNP and NBR populations, the observed domestic cattle introgression was in part due to additions made to these populations in the 1980s under the honorable auspices of increasing genetic diversity and limiting inbreeding depression (Berger and Cunningham 1994; Wiseman D, personal communication, respectively).

Hybridization between distinct populations, and in some cases species, is known to increase viability and adaptive response (Spielman and Frankham 1992; Arnold and Hodges 1995), even when the original hybridization is disadvantageous (Lewontin and Birch 1966), as in the case of domestic cattle and bison. Because bison and domestic cattle do not naturally hybridize and there are clear negative fitness consequences in at least the F_1 generation, it seems plausible that the introgression and maintenance of domestic cattle genes into bison germplasm might also be under negative selection. Any potential negative fitness effects are not apparent, however, as the introgressed domestic cattle regions in the populations examined have been maintained for 15–20 generations post-hybridization. However, the location of genes and their respective functions within and near the 14 nuclear regions examined in this study are largely unknown; it is therefore not possible at this point to accurately assess the involvement of natural selection on the maintenance of domestic cattle introgression in these regions.

A total of 3713 bison from 22 US and Canadian populations have been examined for evidence of both domestic cattle mitochondrial and nuclear DNA introgression to date (Ward et al. 1999; Ward 2000; Halbert et al. 2005). Of these,

Table 5. Probability of detection of introgression across a range of individuals sampled (*n*) from a population using 14 nuclear diagnostic markers (*t*) across low, conservative ranges of *m* (level of introgression across population) and *p* (proportion of domestic cattle genome represented in hybrid founders). Appropriate levels of *m* were estimated based on detected levels of introgression in extant bison populations by Halbert et al. (2005)

		n (number of individuals sampled per population)											
m	*p*	25	50	75	100	150	200	250	300	350	400	500	600
0.001	0.250	0.2866	0.4782	0.6092	0.7007	0.8133	0.8746	0.9103	0.9322	0.9463	0.9558	0.9670	0.9730
	0.500	0.2925	0.4950	0.6364	0.7359	0.8570	0.9199	0.9536	0.9722	0.9828	0.9890	0.9951	0.9976
	1.000	0.2954	0.5036	0.6502	0.7536	0.8777	0.9393	0.9699	0.9850	0.9926	0.9963	0.9991	0.9998
0.005	0.250	0.7681	0.9114	0.9521	0.9674	0.9777	0.9807	0.9816	0.9820	0.9821	0.9822	0.9822	0.9822
	0.500	0.8077	0.9541	0.9865	0.9952	0.9990	0.9996	0.9998	0.9999	0.9999	0.9999	0.9999	0.9999
	1.000	0.8270	0.9701	0.9948	0.9991	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
0.010	0.250	0.9128	0.9677	0.9779	0.9807	0.9820	0.9822	0.9822	0.9822	0.9822	0.9822	0.9822	0.9822
	0.500	0.9547	0.9953	0.9990	0.9997	0.9999	0.9999	0.9999	0.9999	0.9999	0.9999	0.9999	0.9999
	1.000	0.9703	0.9991	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
0.015	0.250	0.9535	0.9780	0.9814	0.9820	0.9822	0.9822	0.9822	0.9822	0.9822	0.9822	0.9822	0.9822
	0.500	0.9870	0.9990	0.9998	0.9999	0.9999	0.9999	0.9999	0.9999	0.9999	0.9999	0.9999	0.9999
	1.000	0.9956	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000

9 populations have been identified with no evidence of domestic cattle introgression (plains bison unless otherwise noted): Elk Island National Park, Canada (wood bison, $n = 25$); Mackenzie Bison Sanctuary, Canada (wood bison, $n = 36$); Wood Buffalo National Park, Canada (wood bison, $n = 23$); Elk Island National Park, Canada ($n = 25$); GT ($n = 39$); Henry Mountains, Utah ($n = 21$); SUH ($n = 31$); WC ($n = 352$); and YNP ($n = 548$, including those from the studies of Ward et al. [1999] and Halbert et al. [2005]). As previously discussed, the ability to detect domestic cattle nuclear introgression in hybrid bison populations is dependent on the proportion of domestic cattle genome represented in the original hybrid founders (*p*), the average proportion of domestic cattle introgression in each bison (*m*), the number of individuals sampled (*n*), and the number of independent, selectively neutral, unlinked diagnostic markers (*t*) used to detect introgression (Halbert et al. 2005). Given sufficient sample sizes, a high probability of detection using 14 markers is expected even when *p* and *m* are low, as indicated in Table 5. In fact, the probability of detection when $n = 350$ or more, such as for the WC and YNP populations (Table 2), is greater than 94.6% even when assuming a 0.1% level of introgression (*m*) and only 25% of the domestic cattle genome originally represented in the hybrid founders (*p*). Although it is not possible to prove unequivocally that domestic cattle introgression does not exist in these populations, our analyses suggest that if introgression does exist, it is most likely at exceedingly low levels ($\ll$0.1% per individual).

Conversely, while domestic cattle nuclear introgression was not detected in the GT and SUH populations, small sample sizes preclude similar confidence in our detection limits. When $n < 50$, as is the case for both the GT and SUH populations in this study, the probability of detection is only around 50% based on a 0.1% level of introgression (Table 5). In fact, we would expect to find domestic cattle introgression in each of these populations based on their histories (Table 1). For example, based on the introduction of FN bison into the SUH population (Table 1), we would expect the BM4307 197-bp domestic cattle allele to have been detected in SUH bison (Table A1). As nearly the entire SUH population was examined in this study (Table 2), the BM4307 197-bp domestic cattle allele most likely either was not introduced with the bison from FN or has been lost from the SUH population due to drift. In either case, it is probable that other regions of domestic cattle introgression, as yet unexamined, exist within the nuclear genome of SUH bison based on the history of this population. Similarly, bison were introduced from TRS into the GT populations in 1964, although the BM4307 197-bp domestic cattle allele identified in the TRS population (derived from FN; Table 1) was not found in the GT population in this study (Table A1). The small sample size from GT (Table 2) may have precluded the detection of domestic cattle introgression at this locus. It is also possible that the contribution of the TRS bison in the 1960s to the GT population was such that the allele in question was not maintained (genetic drift). Further sampling from the GT population, which has a current census size exceeding 600 bison, is necessary to resolve this issue.

The combined results of this study and those of Ward et al. (1999) and Halbert et al. (2005) indicate that relatively few bison populations exist without evidence of domestic cattle introgression, and even fewer have been examined with appropriately large sample sizes to warrant statistical confidence in the detection limits (WC and YNP only). Therefore, further investigation of bison populations without known historic links to populations harboring domestic cattle nuclear introgression, and from which domestic cattle introgression has not been detected, is necessary (e.g., Henry Mountains and several federal Canadian populations, see above). The identification of key sources of germplasm through this study represents a critical step in the long-term conservation of the bison species. Germplasm integrity should be a principle consideration in the establishment of new conservation herds and movement of bison between established herds. For example, to circumvent further degradation of germplasm integrity, bison should not be transferred from hybridized to non-hybridized herds. This study underscores the importance of thorough genetic evaluation of interspecies introgression for wildlife population management and species conservation.

Appendix

Table A1. Allele frequencies for 14 diagnostic microsatellite markers. See Table 1 for sample sizes. Domestic cattle (DC) allele frequencies derived from Halbert et al. (2005). Alien (domestic cattle) alleles detected in bison populations and verified using confirming microsatellites (Table 3) are indicated in bold

	BNP	FN	GT	NBR	NS	SUH	TRN	TRS	WM	WC	YNP	DC
AGLA17												
214												19.53
215	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	
216												1.56
219												78.91
AGLA293												
218	100.00	100.00	100.00	99.25	100.00	100.00	100.00	100.00	100.00	96.40	100.00	10.83
220				0.75						3.60		0.83
222												5.83
225												5.00
226												1.67
228												57.50
230												8.33
232												3.33
236												1.67
239												5.00
BM1314												
137	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	90.99	100.00	100.00	
143												0.79
145												0.79
147												7.14
153												9.52
155												42.06
157									**9.01**			29.37
159												4.76
163												2.38
165												1.59
167												1.59
BM4307												
183												1.64
185	59.45	82.09	94.87	89.01	77.78	100.00	73.78	72.30	66.18	89.74	100.00	11.48
187	27.00	4.43	5.13	10.99	8.73		9.97	16.19	33.82	10.26		0.82
189												36.07
191												7.38
197	**13.55**	**13.48**			**13.49**		**16.26**	**11.51**				36.07
199												6.56
BM4513												
132	93.78	94.14	96.15	99.59	90.48	100.00	84.25	100.00	95.35	74.57	82.92	
134	6.22	5.86	3.85	0.41	9.52		15.75		4.65	25.43	17.08	
139												3.13
141												0.78
143												21.09
145												11.72
147												21.09
149												20.31
151												8.59
154												4.69
160												3.91
162												3.13
164												0.78
166												0.78
BM7145												
108	76.33	86.89	98.72	90.88	88.89	100.00	87.01	66.90	100.00	65.90	82.02	
110	23.67	13.11	1.28	5.29	9.52		12.99	33.10		34.10	17.98	
116				**3.83**	**1.59**							87.50
118												12.50

Table continues

Table A1. Continued

	BNP	FN	GT	NBR	NS	SUH	TRN	TRS	WM	WC	YNP	DC
BMS2270												
66	12.20	34.31	30.77	76.75	40.48	50.00	7.44	26.06	64.60	43.60	31.88	
68	84.65	65.69	69.23	23.25	53.97	50.00	92.56	72.25	15.53	37.65	59.21	
70					5.56			1.69	19.88	18.75	8.91	
80												1.59
82												14.29
84												12.70
86												1.59
88												7.94
90												18.25
92												11.90
94	**3.15**											3.17
96												7.14
98												21.43
BMS4040												
75	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	
85												10.16
87												1.56
97												83.59
98												0.78
99												3.91
CSSM36												
158	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	
162												19.84
167												2.38
169												1.59
171												7.14
173												17.46
175												6.35
177												1.59
179												30.16
181												12.70
185												0.79
CSSM42												
167	69.24	77.99	55.13	62.46	62.26	67.74	54.80	63.57	34.50	67.21	58.51	
169	2.26		2.56	6.44				0.29	22.81	8.01	6.83	
171	28.50	22.01	42.31	31.10	37.74	32.26	45.20	36.14	42.69	24.78	34.65	
173												8.59
175												3.13
177												2.34
179												26.56
181												1.56
193												1.56
205												3.91
207												0.78
209												0.78
211												1.56
213												39.84
217												9.38
RM185												
90												1.61
92	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	0.81
94												7.26
96												10.48
98												4.03
100												12.90
102												36.29
104												9.68
106												16.13
108												0.81

Table continues

Table A1. Continued

	BNP	FN	GT	NBR	NS	SUH	TRN	TRS	WM	WC	YNP	DC
RM500												
123	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	
125												1.67
127												22.50
129												0.83
131												19.17
133												53.33
135												2.50
SPS113												
128				13.08	4.76							
130	85.34	65.44	38.46	56.17	61.11	54.84	53.61	62.54	67.54	54.43	44.61	
132	14.66	34.56	61.54	30.76	34.13	45.16	46.39	37.46	32.46	45.57	55.39	
135												3.17
137												12.70
139												12.70
141												2.38
143												0.79
145												7.94
147												5.56
149												19.84
151												33.33
154												1.59
TGLA227												
73	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	
79												2.50
83												10.00
85												9.17
90												2.50
92												32.50
94												15.83
96												8.33
98												0.83
101												16.67
106												1.67

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