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Genetic analysis of a *Bison bison* herd derived from the Yellowstone National Park population

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The objective of this study is to inventory the current genetic diversity of the bison quarantine feasibility study (BQFS) herd originating from Yellowstone National Park (YNP) using previously described microsatellite, mitochondrial and nuclear DNA markers with the aim to determine the degree, if any, of cattle DNA introgression in this herd. This work can provide an important tool in monitoring and managing bison genetic diversity as brucellosis-free reintroduced herds are re-established throughout the US for conservation purposes. The BQFS composed of 89 *Bison bison* from YNP that were quarantined and tested to qualify as free of brucellosis in 2006–2007. Understanding genetic diversity of the herd is important to determine if any genetic characteristics such as cattle DNA introgression or low genetic diversity may threaten the herd's protected status. We evaluated genetic diversity at 42 microsatellite loci representing each of the nuclear chromosomes in the bison genome. We found no detectable evidence of cattle DNA introgression in this herd through nuclear markers and mitochondrial DNA analysis. Parentage analysis of the BQFS herd indicated that the majority of mature adults were actively breeding and contributing offspring. Genetic diversity levels in the quarantined herd were high and comparable to the YNP parent herd, suggesting a low risk of genetic loss in the near future. Based on these findings, the genetic diversity currently available within the BQFS herd will provide a strong foundation for bison reintroduced herds and for the preservation of the species.

The American bison *Bison bison* is a prime example of conservation success in North America. Until the 1870s, tens of millions of bison roamed the Great Plains (Freese et al. 2007, Halbert and Derr 2008, Hedrick 2009). This population was decimated to near extinction by the mid-1880s through massive slaughters aimed at collecting hides and meat. Extensive genetic analyses of American bison have shown a remarkable conservation of genetic diversity despite the severe population decline over a century ago (Mommens et al. 1998, Wilson and Strobeck 1999, Boyd 2003, Halbert 2003, Halbert and Derr 2008). Stringent genetic monitoring of bison populations and translocation of animals have enabled the preservation of the remaining bison genetic diversity.

Re-establishing new herds of bison in areas of their historic range has been recommended as part of the effort to preserve the wild bison genetic diversity (Freese et al. 2007, Sanderson et al. 2008). Conserving this genetic diversity, however, is compromised by various factors such as small herd size, introgression of cattle genes, intensive management and culling practices, and infectious diseases (Freese et al. 2007, Sanderson et al. 2008). Due to small herd sizes, re-established herds can suffer from inbreeding depression and risk extinction, such as with the Texas State bison herd that has been wrought with problems including a small founder population and evidence of genetic drift and low heterozygosity due to extremely low levels of genetic diversity (Halbert et al. 2004). Smaller populations are also at higher risk for losing genetic diversity more rapidly than herds with more numerous individuals. Introgression of cattle DNA into bison herds has been detected in some bison herds for multiple generations and different management strategies are needed when dealing with these hybridized populations (O'Brien and Mayr 1991, Hill 1993). Finally, indiscriminate culling of animals from bison herds due to proximity to livestock or fear of spreading an infectious pathogen potentially threatens to reduce genetic diversity via mortality, which could act as a population bottleneck and threaten the evolutionary potential of a herd (Halbert 2003).

The Yellowstone National Park (YNP) bison herd retains a portion of genetic diversity from pre-settlement herds (Halbert 2003). This free-ranging herd is the only herd in the US and, along with the herd from Wood Buffalo National Park, is one of two herds in North America that have descended from a continuously free-ranging wild herd. Free-ranging, in this case, is defined as the population being

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maintained without fences and without supplemental feed since 1967. YNP's bison herd was protected as a federal herd around the turn of the 20th century with 46 animals (Meyer and Meagher 1995). Today, after years of monitoring and protection, the summer population contains over 3500 animals. Presently, the bison population in YNP is believed to consist of two genetically distinct breeding groups or subpopulations: the central and northern subpopulations (Halbert 2003, Halbert et al. 2012). Recent evidence suggests potential genetic interchange between the two groups (Gates et al. 2005), but the extent of genetic interchange has yet to be determined. Genetic analyses have determined that despite their population reduction the YNP genetic contribution (i.e. genetic diversity) to the overall US bison population is higher relative to other federal herds (Halbert 2003, Halbert and Derr 2008, Hedrick 2009) such that the YNP herd has been proposed for propagating new herds. The presence of cattle DNA introgression in many bison herds restricts the use of those herds in future conservation planning. Out of ~500 000 plains bison currently in the US and Canada which includes an estimated 400 000 in private herds, fewer than 1.5% are likely free of domestic cattle genes (Freese et al. 2007). The YNP population has been found to contain both high levels of genetic variation and no evidence of domestic cattle. At present there are no current conservation herds sourced by YNP stock and such herds are needed for long-term genetic conservation of this cattle free bison genome (Halbert 2003, Halbert et al. 2005, Freese et al. 2007, Halbert and Derr 2007).

The presence of bovine brucellosis and its causative agent, the bacterium *Brucella abortus,* within the YNP herd raises concerns about using these animals for reintroduced herds. This chronic, untreatable disease affects both livestock and wildlife ungulate species and the pathogen is easily transmitted within and among species. In cattle *Bos taurus* and bison *Bison bison*, the disease is characterized by abortions, birth of weak or nonviable offspring, and placenta retention (Williams et al. 1993, Rhyan et al. 1994, Thorne 2001). Much of the US has been declared bovine brucellosis free, with the exception of a remaining reservoir of brucellosis-infected animals, predominantly bison and elk *Cervus canadensis*, in the Greater Yellowstone Area (GYA; Meyer and Meagher 1995, Roffe et al. 1999) with a few sporadic occurrences elsewhere. Initially reported by Mohler in 1917, *B. abortus* has since been documented in free-ranging bison of YNP (US; Peterson et al. 1991, Meagher and Meyer 1994, Dobson and Meagher 1996, Cheville et al. 1998, Rhyan et al. 2009). *Brucella abortus* has the potential to be transmitted to domestic cattle which would result in large economic losses for cattle producers (Meagher and Meyer 1994, Kilpatrick et al. 2009).

To protect cattle from *B. abortus* transmission from infected YNP bison, several management approaches have been implemented, such as vaccinating cattle and bison against brucellosis, hazing wandering bison back into YNP, and permanent removal of bison that have left the park (Stevens et al. 1994, Olsen et al. 2003, Clarke et al. 2005). Animals in this study were part of the bison quarantine feasibility study (BQFS) that was initiated to explore alternative options for bison that leave the protection of the park's perimeters. Specifically, this study sought to determine the feasibility of qualifying YNP bison as free of brucellosis through a quarantine protocol (Zaluski et al. 2010, Clarke et al. 2014). If successful, the BQFS herd would be reintroduced into the wild as a brucellosis-free herd. Management as such a herd could benefit from DNA analyses aimed at identifying paternal and maternal genetic contributions in the herd while in captivity (Blouin et al. 1996, Frankham et al. 2002, Wilson et al. 2005, Bowyer et al. 2007). In small populations, such as reintroduced herds, where the effects of inbreeding depression are amplified, it is not beneficial to have multiple offspring from a few sires since loss of genetic diversity may be expedited. Also, parentage analysis could aid in decisions to translocate animals to the herd if certain lineages are overrepresented and more genetically diverse animals need to be introduced to supplement the herd. If more installments of the quarantine process were to be initiated, these genetic analyses could potentially serve as a basis for selecting animals to enter the quarantine process.

The purpose of our study is to inventory the genetic diversity of the BQFS quarantine herd using microsatellite, mitochondrial, and nuclear DNA markers to: 1) determine the degree, if any, of cattle DNA introgression within the BQFS herd; 2) determine the portion of allelic diversity within the BQFS herd relative to the YNP parent herd; and 3) investigate sire contribution to genetic diversity within the captive herd. From a broader perspective, this work may offer an important tool for future monitoring and managing bison genetic diversity as brucellosis-free reintroduced herds are re-established throughout the US.

Methods and materials

Animal selection and sampling

Animals in this study were part of the bison quarantine feasibility study (BQFS) that was started in spring 2005 by the Interagency bison management plan (IBMP) (Zaluski et al. 2010, Clarke et al. 2014). The BQFS is a cooperative investigative study involving: Montana Dept of Livestock; Montana Dept of Fish, Wildlife and Parks; US Dept of the Interior; National Park Service; USDA Forest Service; and USDA Animal and Plant Health Inspection Service (USDA-APHIS). This novel study would demonstrate whether a quarantine protocol is a feasible tool to managing YNP bison and also explore using excess YNP bison in a broader conservation plan for the species.

Yearling calves were initially captured with other bison migrating out of YNP near West Yellowstone in 2005 and Gardiner, MT, in 2006. Individuals in this study (n = 104) were obtained from bison targeted for slaughter because they moved to the park boundaries near West Yellowstone, MT, according to management actions to protect cattle enacted according to the IBMP. The yearling animals were then entered into the BQFS and transported to state-mandated, privately owned pastures in Montana. To qualify for quarantine, yearling animals tested seronegative on brucellosis tests performed at the trap, including the card test for rapid screening and the fluorescent polarization assay (FPA) for confirmation (Gall et al. 2000). Once included in the study, the animals underwent a monitoring program where blood

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was drawn and tested using a panel of nine serologic tests including the card test and FPA for *Brucella* spp. antibodies throughout the year as determined by the UM&R for Brucellosis Eradication (US Dept of Agriculture 2003). After one year of isolation, about half of the animals (n = 61) were chosen randomly without any prior criteria and sent to slaughter. Blood, urine and tissues of slaughtered animals were subsequently tested and bacterial cultured for any seropositive results for *Brucella* spp. antibodies. Remaining members of the population (n = 43) were bred in late summer of 2007, calved out in spring, and continually serologically tested monthly for antibodies to *Brucella* spp. for the remainder of the study period.

Tissue samples were collected for DNA extraction from animals that remained after the random slaughter selection. In the fall of 2007, females were divided according to pregnancy status while males were randomly chosen for two groups. The first group (group A) consisted of heifers (n = 21) that were confirmed pregnant plus six bulls ($n_{GroupA} = 27$). Females not confirmed pregnant in the fall of 2007 (n = 14) were placed in a separate quarantine pen with two bull bison (group B; $n_{groupB} = 16$). Both groups were exposed to bulls in a second breeding season in fall of 2008 during the quarantine period. Offspring born from the two calving seasons in 2008 and 2009 were also included in the genetic analyses of the current study ($n_{2008} = 16$; $n_{2009} = 30$; Table 1).

Sample collection and DNA extraction

Tissue samples from ear punches including hair, skin, and muscle were collected in the field under the guidance of USDA-APHIS Veterinary Services personnel by collecting tissue from an ear of each animal. Tissues were placed in bags and kept frozen until DNA extraction could be completed at the USDA-APHIS Wildlife Genetics Laboratory at the National Wildlife Research Center (Fort Collins, CO). Genomic DNA from 107 tissue samples was extracted using the DNeasy Tissue Extraction Kit following the manufacturer's protocol.

Mitochondrial DNA sequencing

A mitochondrial DNA (mtDNA) screen was used to detect evidence of domestic cattle introgression in the bison studied (Ward et al. 1999). The mitochondrial genome contains a D-loop segment from the highly variable control region from which domestic cattle haplotypes can be sequenced if they are present. This screen was performed with minimal exceptions as in Halbert and Derr (2007) by laboratory personnel at the DNA Technologies Core Laboratory (Texas A&M Univ., Bryan, TX). Positive and DNA-free negative controls were used in every polymerase chain reaction (PCR) run. Genotyper ver. 3.6 software was used for allele size identification and comparison and for the presence of domestic cattle haplotypes.

Table 1. Sex distribution of live offspring born to bison quarantine feasibility study groups in 2008 and 2009. Stillbirths were not counted or used in the analyses. Group B included females that were not pregnant in 2007 and then divided into a separate pasture until palpable pregnancy occurred.

	2008		2009	
	Female	Male	Female	Male
Group A	11	5	10	7
Group B	0	0	5	8
Total	11	5	15	15

Microsatellite amplification

Fourteen nuclear markers were used as a second screen to determine whether domestic cattle DNA introgression existed in this study population. The nuclear markers included were: AGLA17, AGLA293, BM1314, BM4307, BM4513, BM7145, BMS2270, BMS4040, CSSM36, CSSM42, RM185, RM500, SPS113 and TGLA227 (Halbert and Derr 2007). The specific nuclear markers were chosen because alleles at this locus are not shared between domestic cattle and bison. These markers were amplified by laboratory personnel at the DNA Technologies Core Laboratory (Texas A&M Univ., Bryan, TX) using the same parameters as Halbert and Derr (2007).

Breeding system analyses in the BQFS herd was performed using 11 previously developed microsatellite markers described by Schnabel et al. (2000) that were validated for parentage testing in bison. These parentage markers included: BM720, BM1225, BM1706, BM17132, BM1905, BM2113, BM4440, BMS410, BMS510, BMS527 and RM372 (Schnabel et al. 2000). These were divided into two multiplex reactions based on non-overlapping allele size ranges and dye type: PRTG 1 and 2 (Table 2). These markers were also used to assess genetic diversity along with 17 additional highly polymorphic bovine microsatellite markers found throughout the cattle genome described by Halbert (2003) which were run as multiplexed into three mixes: 80, 82 and 85 (Table 2). The remaining microsatellite markers included: BL1036, BM47, BM711, BM1862, BM4107, BM4311, BM6017, BMS1001, BMS1074, BMS1315, BMS1675, BMS1716, BMS1857, HUJ246, ILSTS102, INRA189, TGLA122 (Halbert 2003). All multiplexes and PCR conditions were followed as described by Halbert (2003). All PCR products were analyzed using an ABI 3600 DNA Analyzer with a Mapmarker 400 internal size standard.

Microsatellite data analysis

To assess genetic diversity in the BQFS herd, expected (unbiased genetic diversity, H_E; Nei 1987) and observed heterozygosities (H_O), as well as mean number of alleles per locus (N_A), were obtained by using the Microsatellite Toolkit for Microsoft Excel to analyze the parentage and genetic variation microsatellite markers (Park 2001). Unbiased genetic diversity was assessed as a measure of expected heterozygosity in the populations based on allele frequencies, which reduces effects due to sample size variations compared to observed heterozygosities. The genetic diversity estimate from the BQFS population was compared to previously collected genetic data from its parent population (YNP; Halbert and Derr 2007).

Genetic differentiation was investigated through examination of pairwise F_{ST} (Weir and Cockerham 1984) values

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Table 2. Summary information for 28 nuclear microsatellite loci used in this study: range of alleles in base pairs (R_A), number of alleles observed (N_A), and observed heterozygosity (H_O) in bison quarantine feasibility study (BQFS) and Yellowstone National Park (YNP). Adapted from Halbert et al. 2004.

Marker	Chromosome (position)‡	Label*	Multiplex†	R_A	$N_{A\text{-}BQFS}$	$N_{A\text{-}YNP}$	$H_{O\text{-}BQFS}$	$H_{O\text{-}YNP}$
BL1036	14 (78.7)	NED	85	177–193	4	4	0.73	0.60
BM1225	20 (8.0)	NED	PRTG 2	239–273	5	5	0.72	0.69
BM1706	16 (80.6)	6-FAM	PRTG 2	232–254	4	5	0.58	0.42
BM17132	19 (58.6)	6-FAM	PRTG 1	85–95	5	5	0.71	0.71
BM1862	17 (86.3)	6-FAM	80	201–215	5	5	0.74	0.71
BM1905	23 (64.3)	NED	PRTG 2	172–184	3	3	0.33	0.36
BM2113	2 (106.2)	6-FAM	PRTG 2	127–153	4	4	0.54	0.57
BM4107	20 (52.4)	HEX	85	159–185	5	5	0.69	0.63
BM4311	6 (89.7)	6-FAM	82	90–104	6	6	0.76	0.73
BM4440	2 (55.0)	NED	PRTG 2	123–143	4	5	0.62	0.61
BM47	23 (9.1)	6-FAM	85	103–111	2	3	0.20	0.20
BM6017	X (4.7)	HEX	82	104–122	5	5	0.44§	0.45§
BM711	8 (83.6)	6-FAM	82	161–177	4	4	0.51	0.54
BM720	13 (38.6)	VIC	PRTG 2	203–235	6	7	0.91	0.78
BMS1001	27 (5.1)	NED	80	107–115	5	5	0.65	0.64
BMS1074	4 (74.9)	NED	80	152–160	4	4	0.61	0.57
BMS1315	5 (31.8)	HEX	85	135–149	4	4	0.70	0.64
BMS1675	27 (64.1)	6-FAM	80	85–91	3	3	0.33	0.41
BMS1716	11 (47.7)	HEX	80	185–197	3	4	0.39	0.40
BMS1857	29 (0.9)	6-FAM	85	142–168	6	6	0.76	0.78
BMS410	12 (0.0)	NED	PRTG 1	83–97	4	4	0.65	0.63
BMS510	28 (22.1)	VIC	PRTG 1	91–95	4	4	0.79	0.68
BMS527	1 (55.9)	6-FAM	PRTG 1	159–177	5	6	0.70	0.68
HUJ246	3 (67.9)	NED	80	242–264	4	4	0.61	0.58
ILSTS102	25 (6.5)	NED	85	113–153	3	3	0.61	0.61
INRA189	Y	NED	82	96	1	1	—	—
RM372	8 (19.1)	VIC	PRTG 1	114–138	5	6	0.70	0.70
TGLA122	21 (67.3)	NED	82	136–150	4	6	0.66	0.79
Average					4.18	4.50	0.62	0.60
Standard deviation					1.16	1.26	0.16	0.14

*flourescent label used with forward primer.
†thermocycler parameters: 96°C 3 min; 4 cycles of 96°C 20 s, 58°C 30 s (−1°C/cycle), 65°C 90 s; 26 cycles of 96°C 20 s, 54°C 30 s, 65°C 90 s; 1 cycle of 96°C 60 s, 54°C 60 s, 65°C 20 min.
‡as reported in the USDA cattle gene mapping database.
§calculated on female population only.

to assess the degree of genetic differentiation (if any) occurring between the BQFS herd and its parent YNP population. If genetic differentiation was detected then it would suggest that the BQFS herd did not sample the YNP bison genetic diversity sufficiently. F_{ST} was estimated using the program FSTAT 2.9.3 (Goudet 2002) which was also used to test for Hardy–Weinberg equilibrium. Deviations from Hardy–Weinberg equilibrium can indicate inbreeding or population fragmentation in a population, sampling bias, or problems with the microsatellite markers used (Frankham et al. 2002, Templeton 2006). We assessed departures from Hardy–Weinberg equilibrium using FSTAT 2.9.3 and GenePop 4.1 (Rousset 2008).

Genetic characteristics of the BQFS herd were compared to previously tested bison from seven established North American bison populations (Halbert and Derr 2007; Table 3; geographical distribution in Fig. 1). In addition, STRUCTURE 2.3.3 (Pritchard et al. 2000) was used to assess the relationship of BQFS herd individuals to these known bison populations by grouping individuals into genetic clusters with a priori population information using a Bayesian approach. The goal of this analysis was to confirm whether the BQFS herd clustered entirely into the YNP herd or if potential admixture with any other herd was detected. Though admixture from a non-YNP herd has been previously undocumented, if admixture was observed then concern for genetic contribution from mixed lineages or potential cattle DNA introgression would prompt further genetic testing and could compromise conservation management decisions for the BQFS herd.

In STRUCTURE, we used an admixture model to assess the fraction of a BQFS herd individual's genome that was attributable to the known populations. The correlated alleles option was used in all tests along with 20 000 burn in replicates and 40 000 Markov chain iterations. Individuals in the BQFS herd needing assignment were given a value of zero in the Popflag column, and all individuals from defined herds were given a value of 1, which allowed repeated updating of allele frequencies of all groups except the individuals in the targeted herd. Because seven clusters (BNP, FN, NBR, TRN, TRS, WC and YNP) were expected based on previous work by Halbert and Derr (2007), only six to eight ($K = 6–8$) inferred clusters were assessed. The data was analyzed using the LOCPRIOR model within STRUCTURE, which uses the sampling locations of individuals to assist the clustering process. Six tests for each value of K were performed.

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Table 3. Seven established North American bison populations used to compare genetic characteristics with the bison quarantine feasibility study (BQFS) herd (Halbert and Derr 2008). All herds were used to compare to genomes of BQFS individuals and establish relationships based on genetic clusters. Heterozygosity and alleles present in YNP samples were also used to compare with BQFS herd individuals. These samples are archived at Texas A&M University.*

Herd name	Abbreviation	Location	Sample size
Badlands National Park	BNP	South Dakota	328
Fort Niabrara National Wildlife Reserve	FN	Nebraska	178
National Bison Range	NBR	Montana	179
Theodore Roosevelt National Park - North Unit	TRN	North Dakota	309
Theodore Roosevelt National Park - South Unit	TRS	North Dakota	368
Wind Cave National Park	WC	South Dakota	345
Yellowstone National Park	YNP	Wyoming, Idaho, Montana	505

*Note: genetic data was used with permission

All other settings used default parameters (Pritchard et al. 2010). Results from all runs were summarized and inspected using STRUCTURE Harvester ver. 0.6.7 (Earl and von Holdt 2011) where the best estimates of *K* were inferred by examining averages and standard deviations of the log of the probability of the data (Ln *P*(*D*)). The clusters were aligned using the program CLUMPP 1.1.2 (Jakobsson and Rosenberg 2007) using the *Greedy* option and 1000 repeats of randomized input order. Consequent cluster designations were visualized using the program Distruct 1.1 (Rosenberg 2004).

Parentage analyses were completed for each breeding season. In the first breeding season (2007), all males (n = 8) were considered potential sires for the offspring born in 2008. We conducted separate analyses completed for both groups (group A and group B) for the second breeding season (2008) as males were not intermixed between groups. Parentage was determined using birthdates of each animal and multilocus microsatellite genotype matches to assign candidate parents to juveniles using the program Parente (Cercueil et al. 2002) to assess paternity. Approximate ages and genotypes from the offspring, known dams, and potential sires were used in the program. Known dam and calf pairings were based on daily observations and monitoring by field researchers (Rebecca Frey pers. comm.). We were able to assume nearly 100% sampling as all animals within the herd were sampled and included in the analyses with the exception of one calf that died without a tissue sample being collected; therefore no genetic analyses were completed on this individual (field tag 86-82). Because all animals were sampled, the probability of excluding the sire from the analysis is low as compared to a wild population where all adults may not be sampled. A scoring error rate of 1% was assumed, based on recommendations of previous parentage

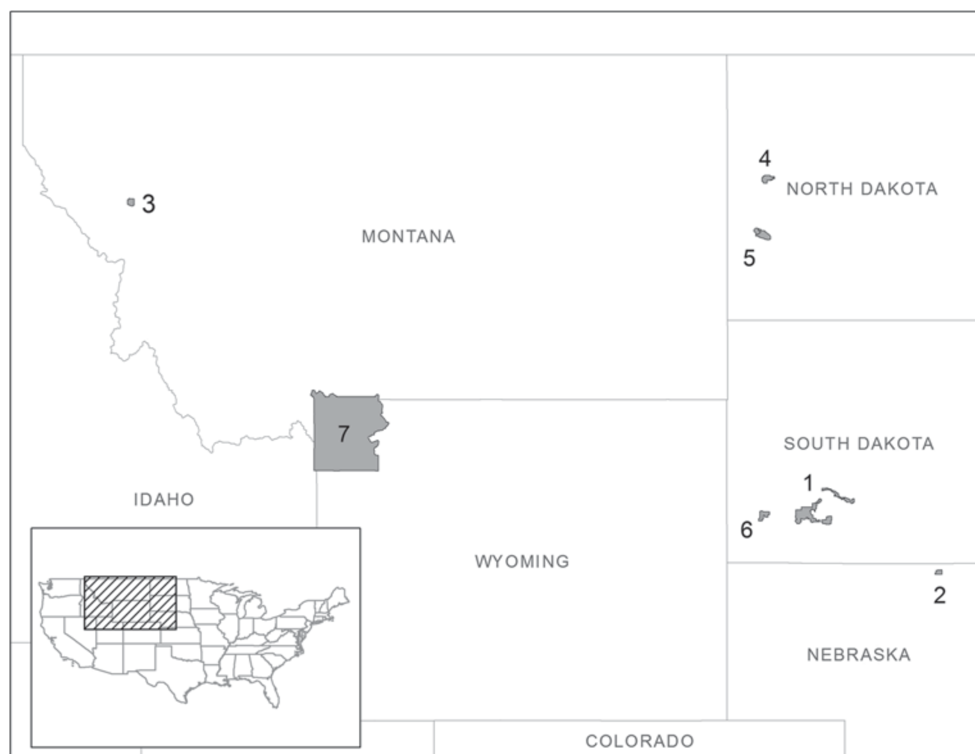

Figure 1. Geographical distribution of seven known North American bison populations represented in our study (1 – Badlands National Park; 2 – Fort Niabrara National Wildlife Refuge; 3 – National Bison Range; 4 – Theodore Roosevelt National Park North Unit; 5 – Theodore Roosevelt National Park South Unit; 6 – Wind Cave National Park; and 7 – Yellowstone National Park).

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studies to account for genotyping error, mutations, or null alleles (Marshall et al. 1998, Schnabel et al. 2000, Cercueil et al. 2002, Halbert et al. 2004). A minimum parentage probability of 80% was considered acceptable for the correct identification of parental assignment. Though sexual maturity is most commonly reached between two and four years for bison, animals in our study herd did not successfully breed until the three-year threshold (Meagher 1986). Thus, we considered all individuals aged three years or older were as potential parents.

Results

Using mtDNA screening, no domestic cattle haplotypes were present in any of the BQFS bison samples. In addition, the nuclear marker screen showed no domestic cattle alleles were present in our study herd. These results demonstrated that there was no detectable cattle DNA introgression in the BQFS herd using these markers.

When analyzing the parentage and genetic variation microsatellite markers (n = 28), a single monomorphic microsatellite marker was detected in the BQFS herd as in the YNP herd (Y-chromosome marker INRA189; Table 2). Values for number of alleles per locus, size ranges, and heterozygosity were comparable between the BQFS population and its parent population (YNP; Table 2). The number of alleles averaged 4.17per locus in the BQFS herd, which is less than the averaged 4.5 alleles per locus reported for the YNP population. The number of alleles in the BQFS study herd represents a 7.11% loss of alleles from the YNP parent population. Average heterozygosity ranged from 0.20 to 0.91 in the BQFS herd as compared with 0.20 to 0.85 in the total YNP population (not significantly different, p = 0.52).

Pairwise F_{ST}-values averaged 0.008 across both the BQFS and YNP populations demonstrating low differentiation between the herds. None of the loci within the BQFS herd violated Hardy–Weinberg equilibrium ($p > 0.05$, all $p \geq 0.11$). Linkage disequilibrium was observed in 4.3% of the pairwise marker combinations in the BQFS population on 11 different chromosomes (nominal p = 0.01); no significant deviation from linkage equilibrium was noted in the YNP population (Halbert and Derr 2007).

Bayesian clustering strongly supported seven genetic clusters (Fig. 2). Although the Ln $P(D)$ increased slightly with K = 6 clusters, the smaller standard deviation around K = 7 clusters suggested that animals from the BQFS population were not genetically distinct from the established YNP herd (Fig 2). All replicates were similar as animals in the BQFS cluster were more than 98.97% associated with the same cluster as YNP for all of the simulations. This confirms that the BQFS animals used were representatives of the YNP population and not migrants from another herd. Confirmation of the BQFS individuals originating from the YNP population contributes to the yet unidentified animal emigration from other herds within the YNP herd.

We were able to definitively identify both sire and dam for 15 of 17 calves born in the spring of 2008 and all 17 calves born in spring of 2009 for group A. For one of the two 2008 calves without definitive parent identification (field tag 86-68), the dam was confirmed but paternity was assigned to two equally potential sires (field tag 03-05, 44.82%; field tag 01-05, 45.03%). The second calf (field tag 86-82) died from maternal neglect before a sample could be obtained. In group B, matched parent pairs were identified for 12 of 13 calves. For this calf without definitive parent identification (field tag 9652), genetic mismatches between potential dams and calf led to matches of two potential dams (field tag 52-06, 2 mismatches; field tag 65-06, 3 mismatches), but the sire was unambiguously identified (field tag 58-06). The dam assignment could not be resolved but was decided based on behavioral interactions between one of the dams (field tag 52-06) and the calf. In the total BQFS herd, six of eight adult bulls accounted for 46 live progeny over the two year quarantine period. The bull that produced the most offspring sired 15 calves. The average for the six bulls siring offspring was 7.67 ± 5.19 offspring/adult male. Overall, the cows averaged 1.34 ± 0.59 offspring/adult female.

For group A, four out of six bulls produced progeny in at least one breeding season. In 2008, the three bulls that produced offspring sired one, 5 or 6, and 9 or 10 calves, respectively. The following year, two males produced a single offspring and another bull produced 15 offspring. Only two bulls produced at least one calf in sequential breeding seasons. There were seven cows that raised at least one calf and 13 that raised a calf both years. In group B, there were only two bulls and both sired multiple calves. Notably, one bull dominated the siring by producing 10 calves. Nearly every female (13 of 14 heifers) produced and raised a calf during the 2009 breeding season.

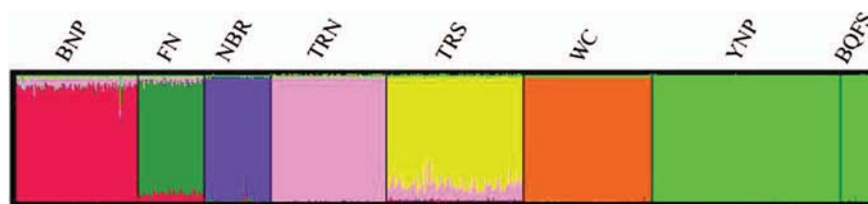

Figure 2. Results of the STRUCTURE analysis which compared individual membership proportions of bison quarantine feasibility study (BQFS) individuals to seven known bison populations (BNP – Badlands National Park, South Dakota; FN – Fort Niabrara National Wildlife Refuge, Nebraska; NBR – National Bison Range, Montana; TRN – Theodore Roosevelt National Park North Unit, North Dakota; TRS – Theodore Roosevelt National Park South Unit, North Dakota; WC – Wind Cave National Park, South Dakota; and YNP – Yellowstone National Park, Wyoming, Idaho, Montana). The colors represent the seven geographically defined populations of bison. Each vertical line represents one individual. The analysis demonstrates the genetic continuity of the BQFS individuals with their parent population (YNP) and also confirms the presence of seven populations of bison.

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Discussion

Cattle DNA introgression into the bison genome is a common occurrence in North American bison populations (Halbert 2003, Halbert et al. 2005, Freese et al. 2007, Halbert and Derr 2007). Markers identifying the presence of cattle DNA in bison have been used to determine the management status and regulatory strategies for conservations herds in the US (Steklenev and Yasinetskaya 1982, Polziehn et al. 1995, Rhymer and Simberloff 1996, Ward et al. 1999, Halbert 2003, Halbert and Derr 2007, Sanderson et al. 2008, Hedrick 2009). Detecting bison with cattle DNA introgression is an important factor in choosing animals for conservation herds and preserving the bison genome. Assays using mitochondrial and nuclear DNA markers in this study population supported previous findings that cattle DNA has not introgressed into the YNP herd at a detectable level (Halbert 2003, Halbert et al. 2005, Freese et al. 2007, Halbert and Derr 2007). The results provide strong evidence that reintroduced herds originating from YNP contain genetically pure animals and future taxonomic status will not be threatened.

Genetic markers have been developed to compare genetic diversity and differentiation in bison herds from around North America and also to accommodate population control measures for each herd (Mommens et al. 1998, Wilson and Strobeck 1999, Schnabel et al. 2000, Boyd 2003, Halbert 2003, Halbert et al. 2004, Freese et al. 2007, Sanderson et al. 2008, Hedrick 2009). Levels of genetic variation in bison vary among North American herds. Among the herds with increased levels of genetic diversity compared to other herds, YNP has been highlighted as an important reservoir of bison genetic diversity (Halbert 2003, Halbert and Derr 2008, Hedrick 2009) in spite of its brucellosis disease status. Because of its potential to contribute to overall bison genetic diversity, research on the YNP herd has focused on conservation and decreasing the prevalence of brucellosis in the GYA. We found that heterozygosity in the BQFS population was comparable to that found in its parent YNP population despite having a slight decrease in alleles. The similar genetic composition between the BQFS herd and its YNP parent herd effectively ensures YNP genetic diversity will continue to be present in the herd following reintroduction. In the future, another genetic inventory could be taken to determine whether supplemental genetics through addition of breeding adults or employing advanced reproductive techniques like artificial insemination would be beneficial to the overall genetic composition of the herd (Wilson et al. 2005).

Parentage analyses resulted in information about the breeding system of this captive herd and genetic contribution by males. This information is critical for monitoring potential inbreeding and providing management advice based on minimizing the effects of inbreeding and genetic drift to conserve the diversity of the restored herds (Blouin et al. 1996, Frankham et al. 2002, Halbert et al. 2004). Data showed that nearly all adults were contributing to future generations, albeit unequal genetic contribution was noted by bulls in both groups. In the two quarantined breeding seasons, mating was polygynous as multiple males were confirmed to have sired at least one offspring. It appeared that these bison exhibited a hierarchical mating system where breeding was monopolized by a small number of highly successful males each year as seen in semi-natural bison populations (Taylor et al. 2000, Roden et al. 2005, 2011) and other wild species such as reindeer *Rangifer tarandus*, red deer *Cervus elaphus* and African buffalo *Syncerus caffer* (Wolff 1998, Taylor et al. 2000, Roden et al. 2005, 2011). Successful matings occurred for multiple bulls which helped to promote genetic admixture for the offspring and increase the effective population size of the reintroduced herd which may subdue the effects of genetic drift for the initial generations.

Through parentage analysis and behavioral observations, we recognized maternal neglect and subsequent death of one calf observed by researchers (Rebecca Frey pers. comm.). Our analysis identified a mis-mothering event where two cows abandoned their calves to adopt an unrelated calf (Aitken 2011). Consequently, the second calf died due to neglect from both dams. This behavior has been recorded in other ruminant species including domestic cattle and sheep but has not been previously documented in bison (Gonyou and Stookey 1985, Dwyer and Lawrence 2000, Aitken 2011). Due to the limited area in the quarantine study, the bison may not have had adequate area to separate or adequate bonding opportunity at the time of calving may have increased the chances of mis-mothering. Further, the dams had not previously calved and it has been shown that mis-mothering and offspring rejection is more prevalent in primiparous or inexperienced females (Dwyer and Lawrence 2000).

The results of this study support the efforts of the BQFS conservation method for preserving the genetic diversity of YNP. However, limiting factors in animal selection from the parent YNP herd should be considered. As mentioned before, at least two genetically distinguishable breeding groups or subpopulations comprise the YNP herd (Halbert 2003, Halbert et al. 2012). Due to the specific collection location near west Yellowstone in 2005 and Gardiner, MT, in 2006, only the northern Yellowstone group is believed to be represented in the BQFS herd. The northern group has been shown to have slightly lower levels of genetic diversity (Halbert et al. 2012). However, it is not clear that these differences in genetic diversity are significant compared to the metapopulation. Therefore, it is recommended that the same genetic analyses should be conducted on future herds that are subjected to the quarantine protocol to warrant the animals as a viable herd for reintroduction (free of cattle DNA introgression and similar heterozygosity values compared to the parent YNP herd).

Using DNA analyses to assess the BQFS herd's genetic diversity provided important information for future management and conservation strategies. Together with assessing domestic cattle DNA introgression, these genetic analyses provided a preliminary assessment of genetic diversity and breeding a conservation herd originating from YNP. Further, this study represents a potential model for reintroducing a native species to areas of its historic range under the condition that the animals complete the quarantine protocol for being declared brucellosis-free, though the quarantine protocol may be financially prohibitive (Zaluski et al. 2010, Clarke et al. 2014). By using genetic analyses when choosing animals to translocate from the founding population to a new herd, management teams increase the chances of retaining genetic diversity, minimizing inbreeding, and

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maximizing genetic variability in future generations. Ongoing genetic monitoring of these herds after reintroduction to the wild could facilitate the long-term conservation of genetic diversity and preservation of the American bison species.

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