

**AN ISSUE OF GENETIC INTEGRITY AND DIVERSITY:
ASSESSING THE CONSERVATION VALUE OF A PRIVATE
AMERICAN BISON HERD**

A Senior Scholars Thesis

by

ASHLEY SUZANNE MARSHALL

Submitted to the Office of Undergraduate Research
Texas A&M University
in partial fulfillment of the requirements for the designation as

UNDERGRADUATE RESEARCH SCHOLAR

April 2010

Major: Genetics
Biochemistry

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Approved by:

Research Advisor:

Associate Dean for Undergraduate Research:

James Derr

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ABSTRACT

An Issue of Genetic Integrity and Diversity: Assessing the Conservation Value of a Private American Bison Herd. (April 2010)

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There are 600,000 American bison (*Bison bison*) that exist today, all of which can be traced to the less than 500 bison that were present after a severe bottleneck in the late 1800s. To save the species from extinction and increase the robustness of domestic cattle (*Bos taurus*), ranchers interbred the two species, creating a hybrid animal. The genetic introgression caused by this hybridization can still be seen in the current bison population. Only two bison herds evaluated to date have shown no domestic cattle genetic introgression: Yellowstone National Park (YNP) and Wind Cave National Park (WC). As such, these herds are very important to the conservation of the bison species. A private herd that was reportedly derived from YNP has shown no introgression during initial testing. Using microsatellites randomly dispersed throughout the genome, the genetic integrity of this herd is evaluated. It was found that this herd has an average number of alleles per loci, but that the unbiased heterozygosity values are low when compared to 11 known Department of Interior (DOI) herds. The low heterozygosity

values could be due to a biased sex ratio that is present in the herd. This biased sex ratio would produce inbreeding within the herd, resulting in the low unbiased heterozygosity values. The presence of an average number of alleles per loci suggests that the low heterozygosity value is reversible by removing this biased sex ratio. The relationship of this herd to the reported foundation herd, YNP, is also evaluated. Though this herd is reportedly founded from YNP only, genetic analysis shows this to be untrue. The presence of alleles that are unique to the private herd when compared to YNP supports this claim. A Structure analysis comparing the germplasm of the 11 DOI herds to this private herd shows that the private herd was not founded solely from YNP, shown by the fact that the private herd did not cluster with YNP in this analysis. This information is important for the management of this herd and the overall conservation of the germplasm of this species.

ACKNOWLEDGMENTS

I would like to thank Dr. James Derr and Dr. Natalie Halbert for being incredibly patient with me through this project, for being amazing teachers, mentors, and friends, and for helping foster a love for research.

I would like to thank Laura Englehart for keeping me sane during days in the lab when nothing seemed to work properly and talking through the problems I encountered over countless dinners.

I would also like to thank my family for giving me encouragement through this process and Matt for letting me bounce a million ideas off him while I searched for solutions to problems.

NOMENCLATURE

BNP	Badlands National Park
DOI	Department of Interior
FN	Fort Niobrara National Wildlife Refuge
GT	Grand Teton National Park
LOD	Log of Odds
NBR	National Bison Range
NS	Neal Smith National Wildlife Refuge
PCR	Polymerase Chain Reaction
SUH	Sully's Hill National Wildlife Preserve
TRN	Theodore Roosevelt National Park – North Unit
TRS	Theodore Roosevelt National Park – South Unit
WC	Wind Cave National Park
WM	Wichita Mountains National Wildlife Refuge
YNP	Yellowstone National Park

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CHAPTER I

INTRODUCTION

Over 600,000 American bison (*Bison bison*) exist today. All of these animals can be traced to the less than 500 bison that were present after a severe bottleneck in the late 1800s, with all animals being traced to five populations (Coder 1975). This reduction in population was due to overhunting and habitat loss as human populations increased. Currently bison populations inhabit less than 1% of the historic range (Sanderson *et al.* 2008) with most of the animals now on private ranches (Schnabel *et al.* 2000). During the last 100 years, captive bison were hybridized with domestic cattle (*Bos taurus*) in an effort to increase the robustness of domestic cattle (Ward *et al.* 1999) as well as to save the bison species. This hybridization does not occur naturally and is a result of human influence. The resultant introgression of domestic cattle DNA into the bison germplasm is still detectable in most modern populations (Ward *et al.* 1999; Halbert *et al.* 2005; Halbert and Derr 2007).

The presence of domestic cattle DNA introgression threatens the integrity of the bison germplasm and, therefore, the identification of this hybridization is necessary for the management of bison populations. Of more than 150 public and private herds evaluated

This thesis follows the style of Genetics.

to date (Halbert and Derr, unpublished data), only two harbor no evidence of domestic cattle DNA introgression: Yellowstone National Park (YNP) and Wind Cave National Park (WC) (Halbert and Derr 2007). Therefore, the identification of bison herds without detectable introgression is critical to the long-term conservation of the species. A private herd of more than 600 individuals has been identified that was reportedly established between the 1920s and 1940s from 5-8 bison that were transplanted from YNP. This herd could be critical to the conservation of bison.

Modern genetic technologies were used to determine the genetic diversity and variation in this herd. Twenty-six polymorphic microsatellites randomly distributed throughout the genome were used to evaluate this diversity (Schnabel *et al.* 2000; Halbert *et al.* 2004). These microsatellite markers represent 26 of the 29 autosomal chromosomes present in the bison genome. The uniqueness of alleles and the establishment of Hardy-Weinberg equilibrium were evaluated from these markers as well. Unique alleles, those not shared by any other herds tested, will determine if this herd is unique among all herds that have been tested. The relationship between the private herd to its reported foundation herd, YNP, was then evaluated, to confirm the origination of this private herd.

The herd was also evaluated for evidence of domestic cattle DNA introgression using 14 microsatellite markers (Halbert *et al.* 2005). Mitochondrial DNA was also examined for introgression (Ward *et al.* 1999). A single animal was found to have a single domestic

cattle allele at one locus. This finding was confirmed with a closely linked microsatellite marker (Halbert *et al.* 2005). This study will examine the specific origin and relatedness of this animal, as well as the overall herd. The historical lineage of this animal is important because it threatens the genetic integrity of this herd. The results of this project will inform management of the conservation value of this herd and therefore contribute to the overall protection of the species.

CHAPTER II

METHODS

DNA extraction

Blood samples were collected from the private herd on Whatman™ FTA cards. The samples, 695 total, were sent to the laboratory at Texas A&M University where they were logged into the local database. Each sample was given a unique identification number in this database for future reference. Whatman™ FTA cards lyse the erythrocytes in the samples and trap the DNA, both nuclear and mitochondrial, in the cellulose fibers. A 1.2 mm micro-punch was made from each sample. The samples were then washed with 150 µL Whatman™ FTA Purification Solution three separate times to thoroughly remove protein debris from the punch and then buffered with 150 µL 1/10x Tris/EDTA Buffer Solution pH 7.4 ± 0.1 (FisherBiotech). Once all liquid was removed from each sample, the punches were used in polymerase chain reactions (PCRs) immediately or stored for no more than one week at 4° C.

Mitochondrial introgression analysis

Once the FTA cards were completely washed a polymerase chain reaction (PCR) was performed on each sample. For the mitochondrial haplotype analysis, a 357 base pair fragment of the noncoding D-loop region was amplified using primers developed in Ward *et al.* 1999. During this analysis, the 16S DNA fragment was used as an internal control for each sample. This amplification was performed using 1 µL of template

DNA, 20 μ M each primer, 10 mM dNTPs, 2.5 mM $MgCl_2$, 10x ABI Buffer and GoTaq® Flexi DNA Polymerase for each 25 μ L reaction. The mix was then placed on a GeneAmp PCR System 9700 (Applied Biosystems) which performed the following profile: 1 cycle of 96° C for 3 minutes; 4 cycles of 96° C for 20 seconds followed by 58° C for 30 seconds and 65° C for 1:30 minutes; 26 cycles of 96° C for 20 seconds, 54° C for 30 seconds, and 65° C for 1:30 minutes; 1 cycle of 96° C for 1 minute; 1 cycle of 54° C for 1 minutes; and, finally, 1 cycle of 65° C for 20 minutes. To analyze the PCR product an internal size standard (Mapmarker 1000 ROX; Bioventures) was added and this mixture was then sequenced using an ABI3130xl Genetic Analyzer (Applied Biosystems). This sequence was then analyzed using GeneMapper v3.7 (Applied Biosystems) and compared to those found in Ward *et al.* (1999).

Nuclear introgression analysis

Fourteen nuclear microsatellite markers were used to analyze domestic cattle introgression into the nuclear genome. To analyze these markers, the following PCR mixture was used on each sample for a 25 μ L reaction: 1 μ L of template DNA, 20 μ M each primer, 10 mM dNTPs, 2.5 mM $MgCl_2$, 10x ABI Buffer and GoTaq® Flexi DNA Polymerase. The primer sequences used were those found in Schnabel *et al.* 2000 and Halbert *et al.* 2005. Each markers position in the genome is listed on Table 1. The PCR thermo profile was identical to that used in the mitochondrial introgression analysis. An internal standard (Mapmarker 400 ROX; Bioventures) was added and the mixture was then sequenced using the ABI3130xl Genetic Analyzer (Applied Biosystems).

GeneMapper v3.7 (Applied Biosystems) was again used to analyze the sequence and determine the alleles present for each sample. These alleles were compared to the known American bison and domestic cattle alleles (Halbert *et al.* 2005).

Table 1
Nuclear introgression analysis markers

Marker	Chromosome (Position)	Bison Alleles¹	Domestic cattle alleles¹
AGLA17	1 (0.0)	215	214 – 219
PIT17b7*	1 (30.0)	143 - 159	128 - 143
BM4307	1 (34.8)	185 – 187	183 – 199
BM7145	1 (69.2)	108 – 110	116 – 118
BMS4040	1 (98.8)	75	85 – 99
CSSM42	2 (34.4)	167 – 171	173 – 217
AGLA293	5 (32.0)	218	218 – 239
RM500	5 (55.6)	123	125 -135
SPS113	10 (29.2)	128 – 132	135 – 154
BM4513	14 (62.5)	132 – 134	139 – 166
TGLA227	18 (84.7)	73	79 – 106
RM185	23 (45.1)	92	90 – 108
BMS2270	24 (21.2)	66 – 70	80 – 98
BM1314	26 (24.8)	137	143 – 167
CSSM36	27 (39.8)	158	162 – 185

* - linked microsatellite marker used to confirm domestic cattle allele at marker BM4307

1 – Halbert *et al.* 2005

One closely linked microsatellite marker, PIT17b7 (Table 1), was used to confirm the domestic cattle introgression allele (Halbert *et al.* 2005). The same protocol was used to analyze this marker.

Nuclear polymorphic analysis

Twenty-six polymorphic microsatellite markers were sequenced to be used for evaluation of herd genetic variation and diversity. These markers were analyzed using the same protocol as the nuclear introgression markers. The chromosome and position of each marker in the genome is listed in Table 2, as well as the possible bison alleles (Schnabel *et al.* 2000; Halbert *et al.* 2004).

Genetic diversity analysis

FSTAT 2.9.3.2 (Goudet 2001) was used to determine if the herd was in Hardy-Weinberg equilibrium. The genotypes from all 695 animals at the 26 polymorphic markers were used in this analysis.

Genetic diversity, including unbiased heterozygosity, average number of alleles per marker and unique alleles were analyzed using the Excel Microsatellite Toolkit (Park 2001). This information was then compared with previously published results from the 11 Department of Interior (DOI) public herds (Halbert and Derr 2008).

Parentage analysis

Twenty-six polymorphic microsatellite nuclear markers were used to determine the parentage structure of the herd. The program CERVUS (Kalinowski *et al.* 2007) was then used to determine the breeding structure of the herd using the calves born in 2007.

Table 2
Nuclear polymorphic analysis markers

Marker	Chromosome (Position)	Bison Alleles	Reference
BMS527	1 (55.9)	163 - 177	Schnabel <i>et al.</i> 2000
BM4440	2 (55.0)	123 - 133	Schnabel <i>et al.</i> 2000
BM2113	2 (106.2)	127 - 153	Schnabel <i>et al.</i> 2000
HUJ246	3 (67.9)	242 - 264	Halbert <i>et al.</i> 2004
BMS1074	4 (74.9)	154 - 160	Halbert <i>et al.</i> 2004
BMS1315	5 (31.8)	135 – 149	Halbert <i>et al.</i> 2004
BM4311	6 (89.7)	90 – 104	Halbert <i>et al.</i> 2004
RM372	8 (19.1)	118 - 136	Schnabel <i>et al.</i> 2000
BM711	8 (83.6)	161 – 175	Halbert <i>et al.</i> 2004
BMS1716	11 (47.7)	189 - 195	Halbert <i>et al.</i> 2004
BMS410	12 (0.0)	79 - 97	Schnabel <i>et al.</i> 2000
BM720	13 (38.6)	203 – 235	Schnabel <i>et al.</i> 2000
BL1036	14 (78.7)	177 – 193	Halbert <i>et al.</i> 2004
BM1706	16 (80.6)	232 – 254	Schnabel <i>et al.</i> 2000
BM17132	19 (58.6)	85 – 95	Schnabel <i>et al.</i> 2000
BM1225	20 (8.0)	239 – 271	Schnabel <i>et al.</i> 2000
BM4107	20 (52.4)	165 – 185	Halbert <i>et al.</i> 2004
TGLA122	21 (67.3)	136 - 150	Halbert <i>et al.</i> 2004
BM47	23 (9.1)	103 – 107	Halbert <i>et al.</i> 2004
BM1905	23 (64.3)	172 – 184	Schnabel <i>et al.</i> 2000
BMS1862	24 (32.8)	178 – 198	Schnabel <i>et al.</i> 2000
ILSTS102	25 (6.5)	113 – 147	Halbert <i>et al.</i> 2004
BMS1001	27 (5.1)	107 – 115	Halbert <i>et al.</i> 2004
BMS1675	27 (64.1)	85 – 91	Halbert <i>et al.</i> 2004
BMS510	28 (22.1)	91 - 95	Schnabel <i>et al.</i> 2000
BMS1857	29 (0.9)	142 - 168	Halbert <i>et al.</i> 2004

The single animal with the hybrid allele was analyzed to determine if it had any offspring in the herd. CERVUS was again used for this evaluation. Any animal born after 2003 was considered a potential offspring in this analysis.

Determination of relationship to YNP

Allelic variation at the 14 microsatellite genotyped for introgression and the 26 microsatellite markers genotyped for parentage analysis were used to compare this private herd to the bison at YNP. This comparison was done using the information obtained in the genetic diversity analysis by the Excel Microsatellite Toolkit.

Structure 2.1 (Pritchard *et al.* 2000) was also used to analyze the relationship of this private herd to YNP. The private herd was compared to all 11 DOI herds reported by Halbert *et al.* 2005. There are eight known clusters when these 11 herds are evaluated in this program (Halbert and Derr 2008). The private herd would be expected to cluster with YNP in this evaluation if it was solely derived from Yellowstone NP founders. Ten independent iterations were averaged to obtain these clusters with the number of clusters defined as eight. Cluster assignments were aligned using Clumpp 1.0 (Jakobsson and Rosenberg 2007) and visualized using Distruct 1.1 (Rosenberg 2004).

Relatedness analysis

Rel-A-Tree (Frasier, unpublished) was used to test the relatedness of the hybrid animal with the rest of the herd. This program was used to test for the presence of close relatives in the herd. It was also used to confirm the presence of one offspring in the

herd. For this analysis, the 26 polymorphic microsatellite markers were used for each of the 695 animals.

Three YNP animals selected at random were entered into an analysis to confirm the results for animals that were completely unrelated to this herd. The same 26 polymorphic markers were used in this analysis.

Three individuals known to be born the same year as the hybrid animal were used in a cohort analysis to determine the expected relatedness of animals that were related to the herd. The 26 polymorphic microsatellite markers were again used for this analysis.

CHAPTER III

RESULTS

Mitochondrial introgression analysis

All 695 animals tested have bison mitochondrial DNA. However, mitochondrial analysis is limited and can only detect maternal domestic cattle lineages. Therefore, nuclear analyses were required to investigate further for evidence of domestic cattle introgression.

Nuclear introgression analysis

In the nuclear introgression analysis, 695 samples were analyzed using the 14 nuclear markers (Table 3). One sample was found to have domestic cattle introgression, allele size 197, at a single microsatellite marker, BM4307. Using the closely linked microsatellite marker PIT17B7, it was confirmed that the suspect allele was domestic cattle. This confirmation marker was positive for domestic cattle introgression, shown by the presence of the 139 allele.

Table 3
Alleles present at the nuclear introgression markers

Marker	Chromosome (Position)	Bison Alleles ¹	Alleles found
AGLA17	1 (0.0)	215	215
PIT17b7*	1 (30.0)	143 - 159	139 [†] , 145
BM4307	1 (34.8)	185 – 187	185, 187, 197 [†]
BM7145	1 (69.2)	108 – 110	108, 110
BMS4040	1 (98.8)	75	75
CSSM42	2 (34.4)	167 – 171	167, 169, 171
AGLA293	5 (32.0)	218 – 220	218, 220
RM500	5 (55.6)	123	123
SPS113	10 (29.2)	128 – 132	130, 132
BM4513	14 (62.5)	132 – 134	132, 134
TGLA227	18 (84.7)	73	73
RM185	23 (45.1)	92	92
BMS2270	24 (21.2)	66 – 70	66, 68, 70
BM1314	26 (24.8)	137	137
CSSM36	27 (39.8)	158	158

* - microsatellite marker used to confirm domestic cattle allele present at BM4307

† - domestic cattle allele

1 – Halbert *et al.* 2005

Nuclear polymorphic analysis

The 26 polymorphic markers were analyzed in all 695 individuals, achieving a 98.14% overall genotyping success. Table 4 shows the alleles found in this private herd and their frequencies within the herd.

Table 4
Alleles present at the nuclear polymorphic markers

Marker	Chromosome (Position)	Bison Alleles	Alleles present (Frequencies)	R
BMS527	1 (55.9)	163 - 177	167 (13.48), 173 (41.49), 175 (10.99), 177 (34.04)	a
BM4440	2 (55.0)	123 - 133	123 (7.27), 125 (29.26), 127 (49.82), 129 (13.65)	a
BM2113	2 (106.2)	127 - 153	129 (21.28), 133 (15.96), 143 (56.74), 145 (6.03)	a
HUJ246	3 (67.9)	242 - 264	256 (17.03), 258 (8.70), 260 (25.18), 262 (49.09)	b
BMS1074	4 (74.9)	154 - 160	154 (11.70), 156 (5.85), 158 (61.88), 160 (20.57)	b
BMS1315	5 (31.8)	135 – 149	135 (90.07), 137 (7.45), 141 (1.24), 147 (1.24)	b
BM4311	6 (89.7)	90 – 104	90 (0.89), 92 (2.13), 96 (16.68), 98 (0.89), 104 (76.42)	b
RM372	8 (19.1)	118 - 136	114 (0.18), 118 (10.71), 128 (0.36), 130 (84.82), 134 (3.39), 136 (0.18), 138 (0.36)	a
BM711	8 (83.6)	161 – 175	161 (72.87), 167 (27.13)	b
BMS1716	11 (47.7)	189 - 195	189 (13.12), 191 (51.77), 193 (1.60), 195 (33.51)	b
BMS410	12 (0.0)	79 - 97	83 (65.96), 85 (20.39), 89 (13.48), 93 (0.18)	a
BM720	13 (38.6)	203 – 235	203 (3.30), 213 (8.87), 225 (33.51), 227 (0.18), 229 (0.53), 231 (53.55)	a
BL1036	14 (78.7)	177 – 193	177 (0.18), 181 (84.40), 191 (1.60), 193 (13.83)	b
BM1706	16 (80.6)	232 – 254	232 (14.89), 238 (67.20), 250 (16.84), 252 (1.06)	a
BM17132	19 (58.6)	85 – 95	85 (65.25), 87 (15.07), 89 (6.21), 91 (13.48)	a
BM1225	20 (8.0)	239 – 271	241 (84.57), 245 (0.53), 249 (12.77), 253 (0.71), 265 (0.35), 269 (0.71), 271 (0.35)	a
BM4107	20 (52.4)	165 – 185	159 (7.62), 165 (55.32), 173 (22.34), 179 (1.77), 181 (11.17), 183 (1.77)	b

Table 4 continued

Marker	Chromosome (Position)	Bison Alleles	Alleles present (Frequencies)	R
TGLA122	21 (67.3)	136 - 150	142 (59.59), 148 (32.47), 150 (7.93)	b
BM47	23 (9.1)	103 – 107	103 (73.67), 105 (17.79), 107 (8.54)	b
BM1905	23 (64.3)	172 – 184	172 (72.16), 176 (17.73), 184 (10.11)	a
BMS1862	24 (32.8)	178 – 198	201 (12.06), 202 (0.18), 205 (43.09), 207 (28.01), 211 (16.31), 215 (0.35)	a
ILSTS102	25 (6.5)	113 – 147	113 (0.18), 143 (90.39), 145 (9.07), 147 (0.36)	b
BMS1001	27 (5.1)	107 – 115	107 (0.18), 109 (1.77), 111 (34.57), 113 (18.79), 115 (44.68)	b
BMS1675	27 (64.1)	85 – 91	87 (84.40), 89 (10.99), 91 (4.61)	b
BMS510	28 (22.1)	91 - 95	91 (77.05), 92 (5.87), 94 (17.08)	a
BMS1857	29 (0.9)	142 - 168	142 (18.79), 148 (46.45), 150 (0.18), 156 (24.82), 158 (1.60), 160 (8.16)	b

R – Reference

a – Schnabel *et al* 2000

b – Halbert *et al* 2004

Genetic diversity analysis

The program FSTAT was used to evaluate the potential for population subdivision in the herd. It was found that the herd as a whole is in Hardy-Weinberg Equilibrium across all microsatellite loci. The p-value for this evaluation ranged from 0.62433 to 0.62567.

The Excel Microsatellite Toolkit analysis determined the unbiased heterozygosity of the private herd in comparison to 11 Department of Interior (DOI) herds (Halbert and Derr 2008). These values are present in Table 5. This table shows that the current unbiased heterozygosity of this private herd is relatively low at 0.4887, with the DOI herds ranging from 0.5576 to 0.6404. The average number of alleles per marker, however, is within the range of values of the known herds (3.58 – 4.81) with a value of 4.42.

Table 5
Comparison of heterozygosity and number of alleles across the 11 DOI herds and private herd

Population	Sample size	H _E ¹	Alleles ²
Private herd	695	0.4887	4.42
BNP	328	0.5910	4.46
FN	178	0.6080	4.42
GT	39	0.5610	3.96
NBR	179	0.6290	4.92
NS	62	0.6259	4.81
SUH	29	0.5745	3.58
TRN	309	0.5610	3.62
TRS	368	0.5892	4.35
WC	345	0.6404	4.81
WM	37	0.5576	4.12
YNP	505	0.6084	4.62

The genotype data for the 11 DIO herds was obtained from Halbert and Derr 2008.

1 – unbiased heterozygosity values

2 – average number of alleles per polymorphic microsatellite marker

Parentage analysis

The overall breeding structure of the herd was evaluated using the calves born in 2007.

When run in the program CERVUS, the number of sires to offspring was evaluated.

Table 6 shows the number of offspring produced by each sire. As this table shows, the number of sires that are reproductively successful is relatively small. Of the 59 offspring matched to a sire, 62.71% (39) were produced from only four sires. The net effect of highly unequal male reproductive success is a reduction in the effective population size which could contribute to low heterozygosity values over subsequent generations.

Table 6
Sire parentage success (2007 offspring)

Sire	Number of offspring
1	10
2	10
3	9
4	8
5	6
6	4
7	3
8	3
9	2
10	2
11	1
12	1

Parentage analysis showed that the suspect animal had a single offspring since 2001. This offspring, born in 2007, had a LOD (log of odds) score of 11.1 and 0 mismatching genotypes to the suspect hybrid animal. Analysis before 2001 was impossible due to lack of sampling prior to this date. The markers used in this analysis are shown in Table 4 along with the alleles genotyped and their respective frequencies.

Determination of relationship to YNP

The alleles present at the 14 nuclear introgression markers (Table 3) and the 26 polymorphic microsatellite markers (Table 4) were compared to the alleles found in the YNP herd (Halbert and Derr 2008). It was determined that there are 99 alleles shared between these two herds. YNP, however, has 21 unique alleles and the private herd has 16 unique alleles (Figure 1). If the private herd were solely derived from YNP, it would be expected to have no or very few unique alleles.

In the Structure analysis, the overall genetic diversity of the private herd and the 11 DOI herds was compared. When the results of Structure were visualized in Distruct, it was found that the private herd did not cluster with YNP as expected (Figure 2). This, along with the presence of unique alleles, would suggest that this herd was not solely derived from YNP.

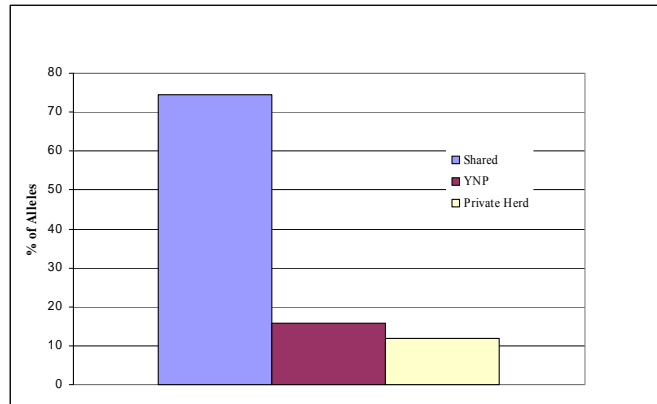


Figure 1. – Allelic comparison between YNP and private herd.

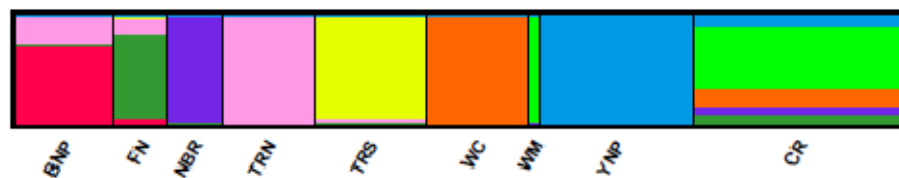


Figure 2. – Structure analysis of private herd (CR) with the 11 DOI herds. The DOI herds group into 8 known clusters (Halbert and Derr 2008) labeled above as BNP, FN, NBR, TRN, TRS, WC, WM, and YNP. This figure shows how the private herd relates to the DOI herds within the 8 clusters. It would be expected to cluster with YNP but, as shown, it groups with many different clusters including WM, WC, FN, and NBR.

Relatedness analysis

When three YNP animals were analyzed for relatedness to the private herd using Rel-A-Tree the private herd, average values of -1.24176, -1.125484, and -0.855761 were obtained. This shows that randomly selected individuals that do not belong to this herd give negative average relatedness values.

Using the parentage analysis data, 10 known half-siblings were compared using Rel-A-Tree. An average of 0.249117 was obtained, with a range from 0.142007 to 0.47715, when these 10 individuals were analyzed for relatedness. The expected average relatedness for half-siblings is 0.25. This comparison gives a range of values that can be used in determining the relatedness of the suspect animal to the herd.

Overall, the suspect animal has an average relatedness value of -0.12522. However, 177 animals give positive relatedness values, with 43 individuals having values within what was given when known half-siblings were evaluated.

CHAPTER IV

CONCLUSIONS

A genetic evaluation of this private bison herd shows that allelic diversity is higher than the average value for the 11 US federal bison herds. However, the discovery of relatively low unbiased heterozygosity values is of concern because it is the result of managing for a highly skewed breeding structure in the herd. If left unchecked, this unequal reproductive success between males and females will result in a significantly reduced effective population size and will lead to further reductions in heterozygosity and increased inbreeding. Increasing levels of inbreeding were also documented through our parentage analysis of the 2007 calves. In that year almost 2/3 of the calves were sired by only 4 bulls. These breeding values will result in detrimental and permanent changes to the genetic diversity of the herd if it is not corrected over the next few years. Nevertheless, the good news is with the relative healthy levels of allelic diversity existing in this herd, the low levels of heterozygosity are reversible simply by equalizing the reproductive success between males and females.

Information from this study clearly shows that the founders of this private herd did not all originate from the YNP bison herd. This statement is based on our Structure cluster analyses that compared allelic diversity from this private herd with all 11 US federal bison herds. While this herd and YNP bison herd do share 99 alleles in common, the YNP bison herd has 21 unique alleles not found in this private herd and the private herd has 16 alleles not found in the YNP bison herd. In fact, there is considerable evidence of

genetic links between this private herd and multiple US federal bison herds. In addition, no unique alleles were identified that exist in this private herd when compared to the eleven US federal bison herds.

Based on our analyses of the hybrid microsatellite markers, only one animal was identified in the private herd as having an allele derived from cattle. Extensive analyses of within herd relatedness uncovered various levels of kinship with at least 177 animals that have existed in the herd. In addition, 43 of these animals have values within the range of known half-siblings. These results are only possible if the lineage that includes this animal has been in the herd for many generations. Whether this individual is part of a lineage that dates back to the founding of this herd or this individual more recently joined the herd is not completely known. However, it is clear that she has a large number of first, second and third degree relatives within the herd, that she is not a recent immigrant (within the last few years) into the herd and that her genetic lineage is intertwined throughout this herd. A more detailed genomic analysis of animals in this herd would most likely turn up additional chromosomal regions of hybrid origin. However, there is no question that this private herd has extremely low levels of hybridization compared with most US federal bison herds and all of the commercial private bison herds.

Long-term conservation genetic recommendations for this private bison herd include:

1. Continue to increase the population size of this herd to exceed at least 2000 breeding animals.
2. Strive to better equalize the reproductive success of males and females over time so that as many males as possible sire offspring each generation.
3. Insure that there are no new immigrants are allowed into this herd.
4. Monitor the genetic integrity and heterozygosity status of this herd by sampling the calves each year until the herd size is stabilized.

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May 2010
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