IDENTIFYING AND PRESERVING BISON LINEAGES: A CASE STUDY OF THE NATIONAL BISON RANGE LINEAGE

A Senior Scholars Thesis

by

JENNIFER CHURCHILL

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 2008

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Associate Dean for Undergraduate Research: Robert C. Webb

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ABSTRACT

Identifying and preserving bison lineages: A case study of the National Bison Range lineage (April 2008)

Jennifer Churchill
Department of Biochemistry
Texas A&M University

Research Advisor: Dr. James Derr
Department of Veterinary Pathobiology

While over 500,000 bison exist today, the long-term conservation of this species is not assured because only five percent of all bison are maintained in conservation herds and genetic evidence of domestic cattle introgression has been detected in nearly all herds evaluated to date. Prior to the introduction of domestic cattle DNA into the National Bison Range (Montana, USA) herd in the 1980s, historical records indicate that four separate herds were formed in Alaska (USA) using bison from the National Bison Range lineage. Bison from these Alaskan herds were examined for the presence of domestic cattle introgression. In addition, 46 nuclear microsatellite markers and mitochondrial sequence data were used to analyze the patterns of genetic diversity and evaluate the relationship of the Alaskan and National Bison Range herds. Neither nuclear nor mitochondrial domestic cattle introgression was detected in the NBR-Alaska bison examined in this study. The bison mitochondrial haplotypes found in the NBR-Alaska herds are consistent with previously identified NBR bison haplotypes. In addition, all of the alleles from the diversity marker panel except for three have been identified in the

NBR herd. The identification of sources of bison germplasm without evidence of domestic cattle DNA introgression is crucial for the conservation of the National Bison Range lineage and preservation of the bison species.

DEDICATION

This thesis is the result of many hard days and long nights. I dedicate this thesis to Jeremy Olguin for helping me through each and every one of them. Thank you for being there to listen to my thoughts, my ideas, and my complaints. Thank you for being there to congratulate me when things went well. Most importantly, thank you for putting a smile on my face each and every time things didn't go as planned.

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Special thanks goes to Dr. Jim Derr and Dr. Natalie Halbert. I am grateful for their encouragement to give this project a try and for their support and guidance through every step along the way. I thank Dr. Derr for giving me the opportunity to grow as a scientist and make the most of my time at Texas A&M. I thank Dr. Halbert for all that she has taught me and for all of the late nights she has dedicated to helping me in the lab. I thank my family for their support and encouragement at home. I also thank Amy Boedecker, Judy King, Manju Mohan, Kavila Krishnan, and Kelly Orr for their laboratory help.

NOMENCLATURE

ADFG	Alaska Department of Fish and Game
CHI	Chitina River
COR	Copper River
DJ	Delta Junction
FWL	Farewell Lake
NBR	National Bison Range
PCR	Polymerase chain reactions

TABLE OF CONTENTS

Page

LIST OF FIGURES

LIST OF TABLES

CHAPTER I

INTRODUCTION

Management of the *Bison bison* species

Near the apex of the North American bison population bottleneck, a small number of individuals independently served to save the bison species from near-extinction by capturing and raising wild bison on 5 private ranches (Coder 1975). Almost all of the bison that exist today descend from the bison used to establish these 5 private herds and one wild population in Yellowstone National Park (Garretson 1938; Meagher 1973; Coder 1975). Of the more than 500,000 bison that exist today, 95 percent of them are maintained in private herds many of which are selectively bred for phenotypic traits such as size, docility, and production. The remaining 5 percent of bison are maintained in private and public conservation herds (Boyd 2003), and the management of these resources is critical for the conservation of the *Bison bison* species. Additionally, evidence of hybridization with domestic cattle (*Bos taurus*) has been detected in both the mitochondrial (Polziehn et al. 1995; Ward et al. 1999) and nuclear (Halbert et al. 2005; Halbert and Derr 2007) genomes of almost all the bison herds examined to date which indicates the extent, origin, and relative level of introgression in specific bison populations. Out of the 11 U.S. federal bison herds, there are only two herds that have neither historical nor genetic evidence of domestic cattle introgression: Yellowstone

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This thesis follows the style of the *Journal of Heredity*.

National Park and Wind Cave National Park (Halbert and Derr 2007). This hybridization comes from direct efforts of cattle ranchers to create more robust breeds by hybridizing the *Bos taurus* and *Bison bison* species. Since bison and domestic cattle are two species that do not naturally interbreed, bison herds with low levels of or no domestic cattle DNA introgression are important to the conservation of bison genetics.

The National Bison Range herd

Domestic cattle DNA has been detected in both the mitochondrial and nuclear genomes of the NBR herd (Ward et al. 1999; Halbert and Derr 2007). This introduction of domestic cattle DNA was traced to the addition of 4 females from the Maxwell State Game Refuge in 1984 (Ward et al. 1999; Halbert and Derr 2007). The subsequent mixing of these lineages prohibits recovery of the NBR bison germplasm prior to the 1984 introduction.

The National Bison Range-Alaska herds

The start of the NBR-Alaska herds began in 1928 when 23 bison from the NBR herd were shipped to Alaska to found the Delta Junction bison herd (DJ) (Garretson 1938; Coder 1975). Bison from the DJ herd were then used to start other plains bison herds in Alaska such as the Farwell Lake (FWL), Copper River (COR), and Chitina River (CHI) herds (Figure 1). These NBR-derived herds are now maintained by the Alaska Department of Fish and Game (ADFG). The importance of these NBR-Alaska herds stems from their potential to provide a critical source of germplasm that predates the

introduction of domestic cattle DNA into the NBR lineage and thereby likely represents one of the few sources of bison free from domestic cattle introgression.

Figure 1. - History of the National Bison Range lineage

Research objectives

The main objective of this project is to provide evidence for the direct genetic relationship of the NBR-Alaska herds to the NBR bison and establish their importance in the conservation and management of these herds. This study will determine if there is any evidence of domestic cattle introgression in the bison from the NBR-Alaska herds. The genetic diversity of the NBR-Alaska bison herds will also be examined. This study is looking for a relationship in the genetic diversity with other conservation herds, and most importantly, a direct relationship in the genetic diversity of the NBR-Alaska bison and the NBR bison.

CHAPTER II

MATERIALS AND METHODS

DNA sampling and extraction

Samples from the four Alaskan bison herds were received from archival collections and recent hunter-killed bison through ADFG veterinarians and biologists. The number of samples received from each herd can be found in Table 1 below. These samples were cataloged and archived at Texas A&M University for future reference.

Table 1

Sampling information for each NBR-Alaska herd

Herd	Abbreviation	Location	Year established	Origin	Number of samples
National Bison Range	NBR	Montana	1908*	various	179
Delta Junction	DJ	Alaska	1928	NBR	28
Farwell	FWL	Alaska	1950	DJ	5
Copper River	COR	Alaska	1962	DJ	2
Chitina	CHI	Alaska	1965	DJ	1

*additional bison added in 1939 (private ranch), 1952 (Fort Niobrara National Wildlife Refuge), 1953 (Yellowstone National Park), and 1984 (Maxwell State Game Refuge)

DNA was extracted from hair as follows: cut 7-8 hair follicles from the shafts and placed in 50 µl of 200mM Tris/HCl Buffer; incubate at 96°C for 20 minutes; centrifuge briefly, add 50 µl of 200mM NaOH; centrifuge for 2 minutes at 10,000 rpm; take 50 µl from the top layer of the extraction and place in a new tube; dispose of rest of extraction. For the samples on FTA cards, a 1.2 mm punch was taken from the card. This punch was

washed with FTA solution to remove protein debris and rinsed with 1/10x TE following manufacturer recommendations. FTA punches were allowed to dry for 2 hours at room temperature and then left a 4°C for a maximum of 48 hours before use. When used in reactions, these FTA punches and addition of water were substituted for 1 µl of DNA in the reaction mix.

Mitochondrial haplotype analysis

All polymerase chain reactions (PCR) were performed on GeneAmp PCR System 9700 thermal cyclers. Ward et al. (1999) was followed to detect the mitochondrial DNA (mtDNA) types. PCR products were resolved on 1.2% agarose gels. After determination of the mtDNA types, a 441-bp fragment of the mitochondrial D-loop was PCR-amplifed and sequenced with the primers XXXXX (5'-AAACCAGCAACCCGCTAG-3') and XXXXX (5'-GACTCATCTAGGCATTTTCAGT-3'). Amplification was performed under the following conditions (per 25 µl reaction): 1 µl of template DNA, 20 µM each primer, 10 mM dNTP's, 2.5 mM $MgCl_2$, 10x ABI Buffer, GoTaq® Flexi DNA Polymerase. Amplification was performed using the following PCR method: 1 cycle at 96°C for 10 minutes; 4 cycles at 96°C for 20 seconds, 57°C for 30 seconds (lowered 1 degree every cycle), 72°C for 1 minute; 35 cycles at 96°C for 20 seconds, 54°C for 30 seconds, 72°C for 1 minute; 1 cycle at 72°C for 10 minutes. The amplification products were then resolved on a 1.2% agarose gel and purified with QIAquick gel extraction columns (Qiagen). Sequence reactions were performed using the BigDye® terminator cycle

sequencing kit version 1.1 and an ABI3130xl Genetic Analyzer (Applied Biosystems). Sequences obtained from this study were compared with those from Ward et al. (1999) using Sequencher 4.8 (GeneCodes Corp.) and CLUSTALX (Higgins and Sharp).

Nuclear introgression analysis

A set of 14 nuclear microsatellite markers developed specifically to detect domestic cattle DNA fragments in bison were used to evaluate the presence of domestic cattle nuclear DNA introgression (Halbert and Derr 2007). The forward primers for each marker were fluorescently labeled. All microsatellite reactions were performed using the thermal parameters as above. All PCR products were separated on an ABI 3130*xl* Genetic Analyzer (Applied Biosystems) using an internal size standard (Mapmarker LOW). GeneMapper v3.7 was used for allele identification and comparison.

Nuclear diversity assessment

To examine the patterns of genetic diversity within the NBR-Alaska herds, 46 nuclear microsatellite markers distributed throughout the nuclear chromosomes (Halbert 2003) were used. The forward primers for each marker were fluorescently labeled and amplified in multiplexed PCRs. PCR products were separated on an ABI 3130*xl* Genetic Analyzer (Applied Biosystems) using an internal size standard (Mapmarker 400 ROX; Bioventures). GeneMapper v3.7 (Applied Biosystems) was used for allele identification and comparison. These alleles were compared with 179 bison samples from National Bison Range collected in 2000-2001 (Halbert 2003).

CHAPTER III

RESULTS

Mitochondrial haplotype analysis

Since the mtDNA genome is maternally inherited, the presence of domestic cattle mtDNA indicates a domestic cow in the bison lineage. All 33 samples in this study were found to contain bison mtDNA (Figure 2).

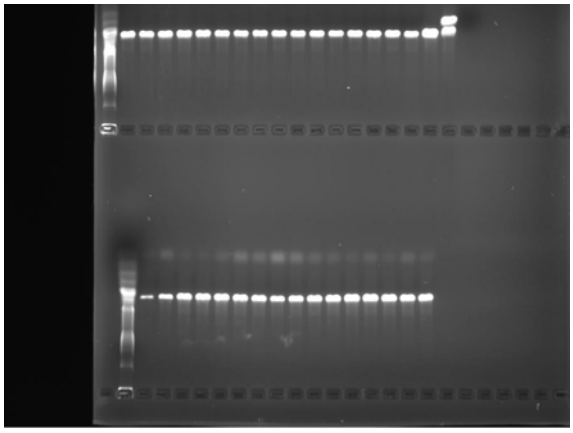

Figure 2. – Results of duplex PCR amplification to determine mtDNA types

To investigate the diversity of the bison mitochondrial haplotypes present in the NBR-Alaska bison, a 441-bp fragment of the non-coding D-loop region of the mitochondrial genome was PCR-amplified and directly sequenced. The sequences were compared to 8 haplotypes previously identified in bison (Ward et al. 1999). A single base pair difference (Figure 3) was identified among the 19 NBR-Alaska bison sequenced for this

region. These two haplotypes are identical to two of the three bison sequences previously identified in the NBR herd (haplotypes 6 and 7; Ward et al. 1999).

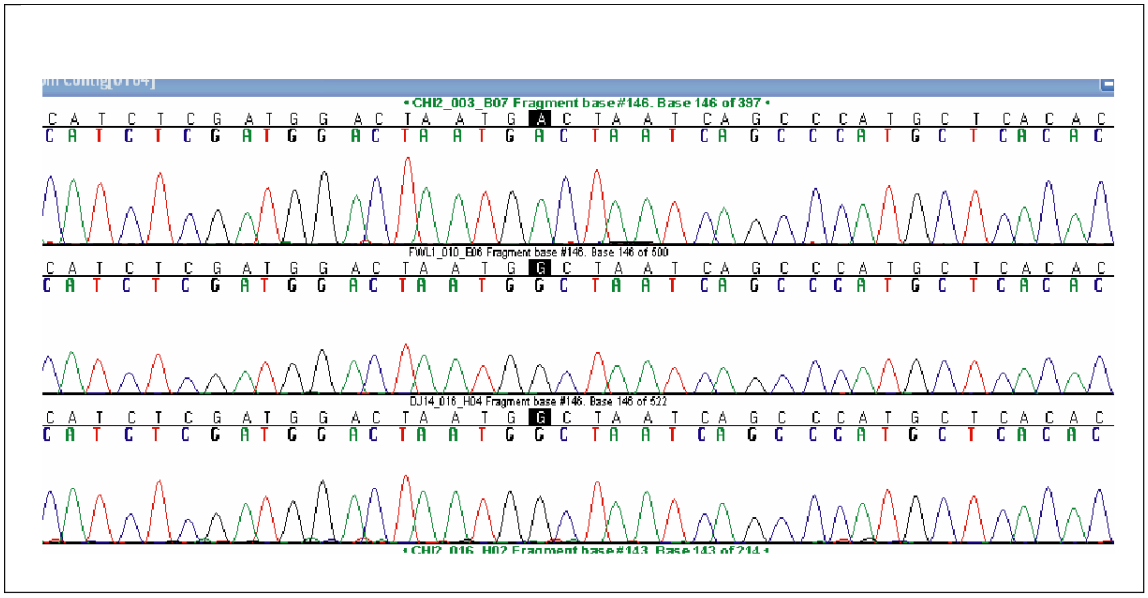

Figure 3. – Sequence variant identified in NBR-Alaska bison

Nuclear introgression analysis

Evaluation of nuclear introgression was performed by analyzing 14 unlinked nuclear markers developed to detect domestic cattle DNA fragments in bison (Halbert and Derr 2007). An average genotyping success of 97.19 ± 3.84% was found at the completion of the analysis. As can be seen in Table 2 below, there were no domestic cattle alleles detected in the NBR-Alaska herds. In addition, the 128-bp allele at marker SPS113 which was previously found to be unique to the NBR lineage among US federal bison herds (Halbert and Derr 2007) was identified in the NBR-Alaska herds.

Table 2

Nuclear markers utilized to detect domestic cattle introgression

Locus	Chromosome(Position)[a]	Domestic cattle alleles	Bison Alleles	NBR-Alaska alleles
AGLA17	1 (0)	214-219	215	215
BM4307	1 (35.2)	183-199	185-187	185, 187
BM7145	1 (69.2)	116-118	108-110	108, 110
BMS4040	1 (98.8)	85-99	75, 95	75
CSSM42	2 (34.4)	173-217	167-171	167, 169, 171
AGLA293	5 (32)	218-239	218-220	218, 220
RM500	5 (55.6)	125-135	123	123
SPS113	10 (29.2)	135-154	128-132	128*, 130, 132
BM4513	14 (62.5)	139-166	132-134	132
TGLA227	18 (84.7)	79-106	73	73
RM185	23 (45.1)	90-108	92	92
BMS2270	24 (21.2)	80-98	66-70	66, 68
BM1314	26 (24.8)	143-167	137	137
CSSM36	27 (39.8)	162-185	158	158

[1] Domestic cattle and bison allele size ranges derived from previously published reports (Halbert et al. 2005; Halbert and Derr 2007).
[2] Among US federal bison herds, the 128-bp allele at marker SPS113 is unique to the NBR lineage, and was also identified in this study within the NBR-Alaska herds

Nuclear diversity assessment

Patterns of genetic diversity within the NBR-Alaska herds were examined by analyzing 46 nuclear microsatellite markers (Table 3) distributed throughout the nuclear chromosomes (Halbert 2003). These markers are spaced throughout the bison genome on 27 of the 29 autosomes and the X chromosome, and they were previously developed and utilized to study patterns of genetic variation in US federal bison herds, including the National Bison Range herd (Halbert 2003). The average genotyping success of these markers was 88.4 ± 7.5%. Genotypes of the NBR-Alaska bison were compared with previously collected genotypes from 179 bison from the NBR herd (Halbert 2003). A

total of 230 alleles were identified in this comparison. Approximately 78% of these alleles were shared between the NBR and NBR-Alaska herds, 21% of these alleles were identified in the NBR herd only, and 1% of these alleles were found only within the NBR-Alaska herds. Among US federal bison herds, 7 alleles from these 46 markers were previously identified exclusively from the NBR herd (Halbert 2003), and 1 of these alleles was also identified in the NBR-Alaska herds.

Table 3

Nuclear markers utilized to assess genetic diversity

Locus	Chromosome(Position)	Size range	Number of alleles
AGLA232	13 (79.5)	155-173	7
BL1036	14 (78.7)	177-193	5
BM1225	20 (8.0)	239-273	10
BM1706	16 (80.6)	232-254	6
BM17132	19 (58.6)	85-95	5
BM1824	1 (108.6)	178-198	7
BM1862	17 (86.3)	201-215	6
BM188	26 (40.4)	99-123	9
BM1905	23 (64.3)	172-184	4
BM2113	2 (106.2)	127-153	9
BM2830	5 (120.2)	140-164	10
BM4311	6 (89.7)	90-104	6
BM4440	2 (55.0)	123-143	7
BM47	23 (9.1)	103-111	4
BM6017	X (4.7)	104-122	6
BM711	8 (83.6)	161-177	6
BM720	13 (38.6)	203-235	9
BM757	9 (0.6)	186-202	9
BMC4214	3 (123.0)	175-191	6
BMS1001	27 (5.1)	107-115	5
BMS1074	4 (74.9)	152-160	5

Table 3 Continued

Locus	Chromosome(Position)	Size range	Number of alleles
BMS1117	21 (9.9)	89-99	4
BMS1172	4 (27.3)	86-104	7
BMS1315	5 (31.8)	135-149	5
BMS1355	18 (2.8)	146-154	4
BMS1675	27 (64.1)	85-91	4
BMS1716	11 (47.7)	185-197	6
BMS1747	14 (4.2)	89-103	5
BMS2258	7 (75.0)	127-152	11
BMS2639	18 (57.0)	168-186	7
BMS410	12 (0.0)	83-97	6
BMS510	28 (22.1)	91-95	4
BMS527	1 (55.9)	159-177	8
BMS528	10 (19.0)	140-152	5
BMS911	X (136.2)	100-114	6
BMS941	17 (30.1)	81-85	3
HUJ246	3 (67.9)	256-264	5
IL4	7 (30.5)	83-105	10
ILSTS102	25 (6.5)	113-153	6
INRA037	10 (69.9)	18-132	6
INRA194	22 (21.8)	144-160	5
RM372	8 (19.1)	114-138	8
TGLA122	21 (67.3)	136-150	6
TGLA44	2 (0.8)	149-159	6
TGLA53	16 (40.3)	132-142	6
URB011	29 (55.6)	139-155	8

CHAPTER IV

CONCLUSIONS

Historical records indicate the 4 Alaska bison herds included in this study are directly descended from bison from the NBR herd prior to the introduction of domestic cattle DNA in 1984. This study provides genetic support for these historical records. Domestic cattle introgression was not detected in the NBR-Alaska bison examined in this study while animals from the NBR herd were previously identified with evidence of both nuclear and mitochondrial introgression (Halbert and Derr 2007). The bison mitochondrial haplotypes found in the NBR-Alaska herds are consistent with previously identified NBR bison haplotypes (Ward et al. 1999). All of the alleles analyzed from the diversity marker panel except for three have been identified in the NBR herd. Two alleles (one introgression marker and one diversity marker) specific and limited to the NBR herd lineage among US federal bison herds were identified in the NBR-Alaska herds.

Three alleles from the diversity marker panel were found to be present only in the NBR-Alaska herds. These alleles have been identified in multiple bison herds besides NBR and might have been lost from the NBR herd after the establishment of the NBR-Alaska herds, or they might have arisen through undocumented introductions into the NBR-Alaska herds, making further investigation necessary to definitively identify the origin of these alleles. Additionally, it is not clear at this point whether the 21% of alleles found

only in the NBR herd were not captured in the initial transfer of bison to the NBR-Alaska herds, were not represented in this study due to small sampling sizes, or were introduced into the NBR herd after 1928 (see Table 1).

Overall, this study supports the direct relationship of the NBR-Alaska herds to the NBR lineage prior the introduction of domestic cattle DNA into the NBR herd. The NBR herd represents a historically and genetically important lineage, and the potential to rescue the NBR lineage through the Alaska satellite herds is significant to the conservation of bison germplasm.

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CONTACT INFORMATION

Name:	Jennifer Churchill
Professional Address:	c/o Dr. James Derr Department of Veterinary Pathobiology Texas A&M University College Station, TX 77843
Email Address:	jenchurchill@tamu.edu
Education:	BS Biochemistry, Texas A&M University, May 2009 Undergraduate Research Scholar