

## DETECTION OF DOMESTIC CATTLE GENE INTROGRESSION IN A SMALL POPULATION OF NORTH AMERICAN BISON

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### ABSTRACT

The North American bison (*Bison bison*) in the Great Plains have historically experienced reduced genetic variability due to their near elimination during the 1880's, which led to a population bottleneck and thus inbreeding. Hybridization between North American bison and domestic cattle (*Bos taurus*) has endangered the genetic variability and integrity of the species. Conservation of bison is of great concern due to the cultural importance they have for the inhabitants of the Great Plains. In order to manage and conserve the bison herds, we completed an analysis of the introgression of cattle mitochondrial DNA (mtDNA) in a population of bison from the northern Great Plains using genetic markers. The analysis showed that there was cattle mtDNA introgression in 23.8% of the population among young bulls and females, suggesting that introgression recently occurred. Recommendations to manage and restore the genetic integrity of populations with recent introgression of cattle mtDNA include identification of individuals with genetic markers or the use of breed registries.

### Keywords

North American bison, *Bison bison*, gene introgression, domestic cattle, mitochondrial DNA

### INTRODUCTION

Prior to 1800, an estimated 60 million North American bison (*Bison bison*) freely roamed the plains (Arthur 1984). The decrease in the bison population was a result of numerous historical events over nearly a century. With the exploration of the Great Plains by Meriwether Lewis and William Clark between 1804 and 1806, American Europeans were introduced to the area and this later led to the establishment of Fort Manuel Lisa Raymond, one of the earliest trading posts on the Yellowstone River (Arthur 1984). There was an increase in fur trade in the area, thus the bison were hunted for their hides as commercial leather production started during 1871. Later in the decade, concerned members of

Congress attempted to pass legislation to protect bison populations, but were unsuccessful (Arthur 1984). By 1878 hide hunters completely eliminated bison on the southern and central Great Plains, and by 1884 the northern Great Plain herds were nearly eliminated as well.

The severe decrease in the bison population affected the ecology of the Great Plains as well as the evolutionary characteristics of the remaining population. The near elimination of bison from the Great Plains created an evolutionary bottleneck because only a few remaining individuals were left to repopulate the region. Thus, the population likely underwent reduced genetic variability because a subset of alleles was passed on to progeny. Genetic variability within a species provides an evolutionary mechanism for adaptation, and the reduction in genetic variability can result in reduced fitness, increased mortality, and reduced flexibility of individuals within a population (Alstad 2001).

Since the evolutionary bottleneck of the Great Plains bison population, inbreeding has continued to be a threat to maintaining genetic variability. The small number of individuals that founded the current population has caused inbreeding to occur on a regular basis. Evidence from research with European bison (*B. bonasus*) suggests that individuals with high inbreeding coefficients suffer greater juvenile mortality rates compared to less inbred individuals (Olech 1987, Halbert et al. 2004). To decrease the effects of inbreeding, some bison herds were augmented with bison from different regions (Wilson 2001). Another tactic used to prevent inbreeding within bison populations was to use the process of hybridization, which involved interbreeding of individuals from different species to produce offspring (Futumya 2005).

Several species in the subfamily *Bovinae*, to which bison belong, have historically been cross-bred to improve the overall quality of a domesticated species. Thus, domestic cattle (*Bos taurus*) was used to breed with the North American bison to improve population numbers of bison but also meat quality of cattle. Although hybridization was used in hopes of restoring the Great Plain bison population, it compromises the genetic integrity of the species because of gene swamping of one genome over another and disrupting locally adapted gene complexes (Awise 1994).

The introgression of genes into a population through hybridization is a substantial issue for wildlife conservation, specifically when attempting to maintain the genetic variability and integrity of a species. Maintaining genetic integrity of a species is especially important when the legality of a protected or managed species is challenged (Ward et al. 1999). Currently, the bison populations are not protected, but many agencies work to ensure the conservation of the species. Therefore, it is extremely important that existing bison genome and genetic variability within the species be maintained. The focus of this study is to examine cattle gene introgression in a small bison population within the northern Great Plains using mitochondrial DNA (mtDNA). Mitochondrial DNA is maternally inherited, and can be used to detect cattle gene introgression through female cattle lines. By examining cattle gene introgression, we can better understand the conservation of bison herds in the northern Great Plains.

## METHODS

*Sample Collection*—Muscle tissue samples were collected during the hunting seasons from 2006 to 2008 in a small population found in the central portion of the northern Great Plains. The actual site remains undisclosed to protect the interests of the agency. Samples were taken from three grazing units proximate to each other, named regions A (1600 acres; 50 samples), B (3200 acres; 24 samples), and C (1400 acres; 10 samples). (Fifteen samples were not designated to a grazing unit.) Within each region the samples were identified as either female, young bull, or mature bull based on sex, horn growth, and data provided by the agency. There were a total of 99 samples collected, of which there were 42 females, 45 males (23 young and 22 mature bulls), and 12 with unspecified gender. A semen sample from a bull (domestic cattle) was obtained from the South Dakota State University Beef Breeding Unit, which was used as an additional comparison between cattle and bison.

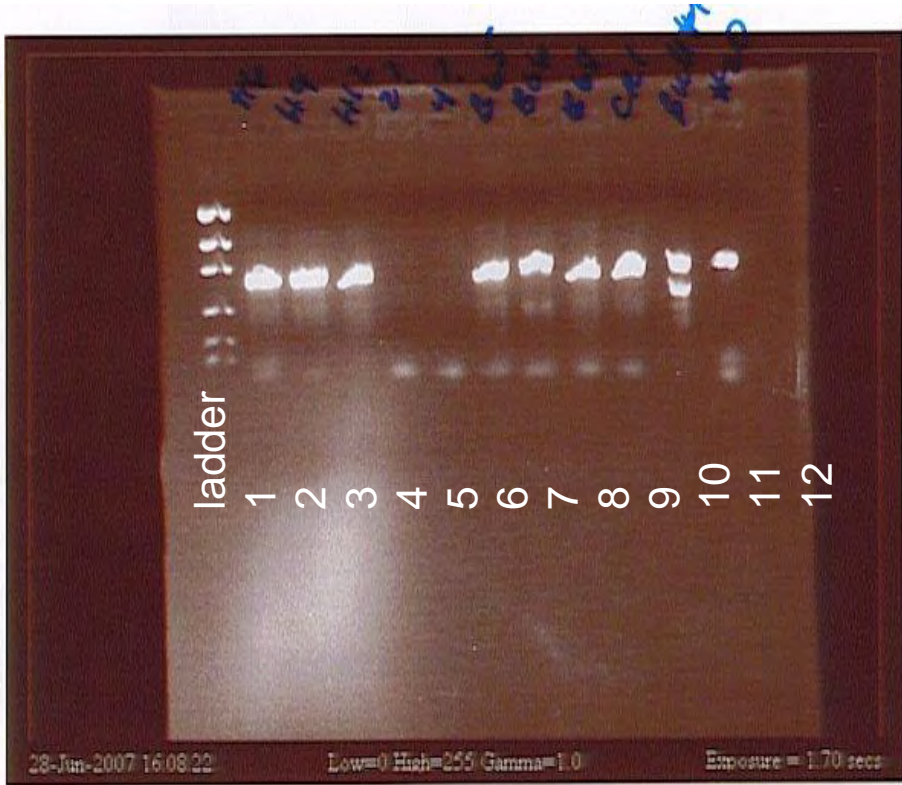
*DNA Extraction and Amplification*—DNA was extracted using a phenol/chloroform method (Chomczynski and Sacchi 1987). Polymerase chain reaction (PCR) was performed to amplify the mitochondrial DNA control regions. PCR was performed using 50 ng genomic DNA, primers specific for the mtDNA region (5'-AGCTAACATAACACGCCCATAC-3'; 15907 and 5'-CCTGAAGAAAGAACCAGATGC-3'; 16264), and primers specific for the 16S rRNA region (5'-CCCGCCTGTTTATCAAAAACAT-3'; 2284 and 5'-CCCTCCGGTTTGAAGTCAAGATC-3'; 2878) (Ward et al. 1999). PCR amplification followed protocol established by Ward et al. (1999). Samples which amplified the mtDNA region were considered to have mtDNA cattle haplotypes, and the 16S rRNA gene was used as an internal control for each reaction. Gel electrophoresis with a 1.5% agarose gel confirmed presence of an internal control at 590 bp and presence of a domestic cattle haplotypes at 357 bp (if gene introgression occurred).

*Statistics*—ANOVA using JMP-IN® software was performed comparing all regions to determine if there were differences in genetic introgression in the herds and if this related to herd management.

## RESULTS

Only 67 samples (region A, 37; region B, 13; region C, 10; and unknown, 7) were amplified with success. All of the samples amplified at the 16S rRNA (or internal control) and 23.9% of the samples amplified at the mtDNA region (or cattle haplotypes) showing the same amplification pattern as the domestic cattle sample. Figure 1 shows a subset of samples of cattle haplotype and internal control regions.

Cattle mtDNA haplotypes were present in 16 samples, most of which came from regions A and B. Ten of these samples were from region A, three were from region B, and three were unassigned. Twenty-seven percent of the samples from



**Figure 1. Results of PCR amplification of DNA from bison (1-9, 11) and domestic cattle (10). Thick, bright bands indicate amplification. Samples 4 and 5 did not amplify due to low DNA concentration. Samples 10 and 12 served as positive and negative controls, respectively. Samples that produced an amplification band at 357 bp, like sample 10, contain a cattle gene.**

region A had the presence of cattle haplotypes, and 23% of the samples from region B had the presence of cattle haplotypes.

Of the 16 samples that showed cattle mtDNA haplotypes, six were female, seven were male (young bulls), and three were unassigned. Twenty-one percent of the females from region A had the presence of cattle haplotypes, and 67% of the females from region B had the presence of cattle haplotypes. Thirty-eight percent of the young bulls from region A had the presence of cattle haplotypes, and 25% of the young bulls from region B had the presence of cattle haplotypes. There was no significant difference between the regions with the presence of cattle haplotypes ( $P > 0.05$ ), and between all regions with or without the presence of cattle haplotypes ( $P > 0.05$ ).

## DISCUSSION

Hybridization is known to occur in bison, and speciation is incomplete (Verkaar et al. 2003). Introgression of genes through hybridization in wild

animals may compromise genetic integrity (Verkaar et al. 2003), thus it is important to elucidate the genetic structure of small populations to better manage for “pure” bison herds. This is particularly important since bison suffer from large reductions in population size, largely as a consequence of over-harvest and habitat loss (Ward et al. 1999).

In a small population of bison in the northern Great Plains, there appears to be a high percentage of cattle gene introgression. Previous research by Ward et al. (1999) found cattle mtDNA haplotypes present in 6 out of 15 (40%) bison populations, and in 30 out of the 572 (5.2%) individual bison tested. Of the regions included in the study, Custer State Park in South Dakota, found in the northern Great Plains, had one the highest percentages of domestic cattle gene presence, occurring in 7 out of 34 (20.6%) individual bison tested (Ward et al. 1999). Our study showed that two of the three regions (67%) had cattle mtDNA haplotypes and 23.6% of the individuals. This is higher than the overall cattle gene introgression shown in the overall study conducted by Ward et al. (1999) and in Custer State Park (Ward et al. 1999).

The introgression of cattle genes in this population is apparent, but the most informative data from this study reveal that there is cattle mtDNA haplotypes in mature bulls throughout all the regions tested. Young bulls are important for breeding (Wolff 1998), so this may suggest that cattle mtDNA haplotypes were introduced during recent breeding events. It is also possible that these individuals were introduced into these regions from recent events (e.g., bison sales and purchases) introducing bison with cattle haplotypes. Moreover, gene introgressions through male bison are more common than through female bison (Verkaar et al. 2003) largely because of breeding activities. Mitochondrial DNA markers limit our analyses to maternal lines, e.g., females and F1 generation bulls, so there may be mature bulls with cattle introgression left undetected due to the type of markers that were used in this study.

For the population in the northern Great Plains, a very complete conservation method should be used. This includes the use of genetic markers to identify individuals within the population who carry the domestic cattle hybrids and the subsequent removal of these individuals from the breeding process. Conservationists may want to start identifying young bulls in the population with domestic cattle genes first because of the importance of young bulls to breeding. In the future, new individuals brought into the population should be screened using mtDNA markers. When immunization shots are given to these animals, the aforementioned can be conducted. This will help ensure the genetic integrity of these regions and this population.

Other suggestions may include a breed registry or captive breeding and reintroduction. A breed registry with parentage and genetic make-up can be maintained for bison, much like that which zoos maintain in animal conservation programs. During harvests, sales, or purchases, agencies will be able to examine which animals to keep and sell based on parentage and genetic data to maintain the genetic integrity of bison. Purchases should not be made unless the animals are known to be free of cattle haplotypes. When multiple populations exist in an area, captive breeding and reintroduction may be an option. Animals can be shifted from one region to another, where at least one region is maintained as a

“pure” bison herd. The region(s) identified as herds with cattle gene introgression can be managed accordingly. Management could include selling hunts to sportsmen or sportswomen or harvesting animals for hunger programs. Both are viable options to monitor gene flow activity of bison populations.

The evidence suggesting the lack of cattle mtDNA haplotypes in mature bulls within this population provides multiple methods for restoring the genetic integrity of the population. Due to the small sample size and scope of this study, we suggest further testing to verify the lack of domestic cattle genes in the mature bulls. As suggested by Ward et al. (1999), the simplest method for dealing with a population with domestic cattle hybrid introgression is to ignore it. Ignoring the genetic introgression provides an avenue for the depletion of the genetic integrity of the species. It is best to use management and conservation measures as suggested above to restore or maintain the genetic integrity of bison populations, given the historical and cultural significance of this animal (Arthur 1984).

#### ACKNOWLEDGEMENTS

We would like to acknowledge the Department of Biology at the University of South Dakota where the preliminary conceptual and laboratory work was carried out. We would also like to thank the South Dakota State University Beef Breeding Unit for providing a cattle semen sample. Finally, we would like to thank the interested parties and individuals in the collection of samples. All combine to provide an interesting undergraduate research project.

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