THE UTILIZATION OF GENETIC MARKERS TO RESOLVE MODERN MANAGEMENT ISSUES IN HISTORIC BISON POPULATIONS: IMPLICATIONS FOR SPECIES CONSERVATION

A Dissertation

by

NATALIE DIERSCHKE HALBERT

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

December 2003

Major Subject: Genetics

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ABSTRACT

The Utilization of Genetic Markers to Resolve Modern Management Issues in Historic Bison Populations: Implications for Species Conservation. (December 2003)

Natalie Dierschke Halbert, B. S., Texas A&M University

Chair of Advisory Committee: Dr. James N. Derr

The saga of the American bison (*Bison bison*) is a well-known story of death, destruction, and greed circumvented by early conservationists. The foresight of 5 cattlemen and the Canadian and U.S. governments at the apex of the population bottleneck in the 1880s led to the eventual establishment of several federal bison populations, from which virtually all of the 300,000 extant bison are descended.

A survey of 54 microsatellite loci spanning each autosomal and both sex chromosomes was used to compare levels of genetic variation among 10 of the 11 federal bison populations in the U.S. Although most populations contain moderate levels of genetic variation, the majority of genetic variation is contained within only 4 of the federal populations surveyed. The distribution and partitioning of genetic variation confirm historical records of founding lineages and transfers among populations.

Previously published mitochondrial and nuclear markers were used to survey federal bison populations for evidence of domestic cattle introgression. While only 1 population was found to contain low levels of domestic cattle mitochondrial DNA, 7 of the 10 surveyed populations had detectable introgression of nuclear genes from domestic

cattle. From this, 2 federal bison populations were identified that have both high levels of genetic variation and no evidence of introgression of domestic cattle genes.

The data obtained from this study were used to examine consequences of past and present management practices in closed bison populations. In the case of the Texas State Bison Herd, observed chronic small population size, low levels of genetic variation, low natality rates, and high juvenile mortality rates combined with the results of population modeling indicate a high risk of extinction within the next 50 years unless new genetic variation is introduced into the herd. Alternatively, analysis of population substructure and nonrandom culling reveal the necessity for further investigation into the long-term effects of current management practices in the Yellowstone National Park bison population. This study illustrates that while bison may be considered a conservation success story, long-term survival of protected federal populations requires the development of effective genetic management strategies.

DEDICATION

This dissertation is the culmination of countless hours of research, none of which would have been possible without the encouragement, support, and sacrifices of my husband. From the beginning to the very end, he has been a cheerleader, coach, comedian, and confidant. Thank you, Kyle.

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This work would not have been possible without the managers and biologists of the many parks and refuges involved in this study who provided samples, field data, and historical population accounts, including: Eddie Childers and Brian Kenner from Badlands National Park; Kathleen McPeak from Fort Niobrara National Wildlife Refuge; Steve Cain and Lee Jones from Grand Teton National Park; Lindy Garner and David Wiseman from the National Bison Range; Nancy Gilbertson from Neal Smith National Wildlife Refuge; Michael Oehler and Russell Runge from Theodore Roosevelt National Park; Joe Kimball from Wichita Mountains National Wildlife Refuge; and Barbara Muenchau from Wind Cave National Park. I thank Dr. Tom Roffe for providing samples from radiocollared bison used in a different study from Yellowstone National Park. I am also grateful to Dr. Peter Gogan, USGS-Montana State University, for providing samples and field data from culled and test-release bison from

Yellowstone National Park and for much useful discussion and information regarding the underlying population dynamics of this population presented in Chapter V. Many thanks also go to Danny Swepston and Texas Parks and Wildlife for making the study of the Texas State Bison Herd possible.

For the past 4 years, I have worked and shared bench space with many former and current graduate students, technicians, and student workers who have contributed to the completion of this work and to whom I am grateful. I thank Dr. Robert Schnabel and Christopher Seabury for insightful conversations and friendly debate over many cups of hot coffee. Finally, special thanks go to Claire Kolenda, Stephanie Krenek, Natalia Kuhn, Tim Novak, Sandy Schaffer, and Robyn Schuster for their help in the laboratory.

NOMENCLATURE

Abbreviation	Description
BNP	Badlands National Park
CSP	Custer State Park
EINP	Elk Island National Park
GT	Grand Teton National Park
MBS	Mackenzie Bison Sanctuary
MSGR	Maxwell State Game Refuge
NBR	National Bison Range
NS	Neal Smith National Wildlife Refuge
NPS	National Park Service (U.S.)
TR	Theodore Roosevelt National Park
TRN	Theodore Roosevelt National Park – north unit
TRS	Theodore Roosevelt National Park – south unit
TSBH	Texas State Bison Herd
USDA	United States Department of Agriculture
USFWS	U.S. Fish and Wildlife Service
WBNP	Wood Buffalo National Park
WC	Wind Cave National Park
WM	Wichita Mountains National Wildlife Refuge
YNP	Yellowstone National Park

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CHAPTER I

INTRODUCTION

"I feel real and great interest in the work being done by the American Bison Society to preserve the buffalo - the biggest of the American big game, probably on the whole the most distinctive game animal of this Continent, and certainly the animal which played the greatest part in the lives of the Indians, and which most deeply impressed the imagination of all the old hunters and early settlers. It would be a real misfortune to permit the species to become extinct, and I hope that all good citizens will aid the Society in its efforts for its preservation."
— President Theodore Roosevelt, 1907 (as quoted in Garretson 1938)

Evolution of the *Bison* genus

During the Illinoian glacial period of the Pleistocene epoch approximately 500,000 – 250,000 years before present (BP), bison entered into North America via the Bering land bridge from northern Eurasia (Guthrie 1970; McDonald 1981). Two species, *Bison latifrons* and *B. antiquus*, appear in North American fossil records during this time. The larger of the two, *B. latifrons*, inhabited the wooded environments in the northern portions of the continent and became extinct during the late Wisconsin glacial period (75,000 – 10,000 BP; McDonald 1981; Dary 1989). In the southwestern United States and Mexico, however, *B. antiquus* survived into the Holocene. There is some debate as to whether *B. antiquus* or a new species, *B. occidentalis*, persisted after the large-scale species reduction triggered by environmental changes and increased human

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This dissertation follows the style and format of the journal Genetics.

hunting pressures around 11,000 – 9,500 BP (McDonald 1981; Dary 1989). Regardless of the taxonomic status of bison fossils dating to this period, modern North American bison apparently evolved from *B. antiquus*, *B. occidentalis*, or a mix of the two species around 5,000 – 4,000 BP (McDonald 1981; Dary 1989; Geist 1991; Wyckoff and Dalquest 1997). Modern bison are considerably smaller in horn and body size than their progenitors (Guthrie 1970; McDonald 1981).

The genus *Bison* is represented by two extant species: *B. bison* (North American bison) and *B. bonasus* (European bison; McDonald 1981; Corbet and Hill 1986; Wilson and Reeder 1993; Burzyńska *et al.* 1999). The genus is most closely related to the genus *Bos*, which includes domestic cattle (*B. taurus*), yak (*B. grunniens*), gaur (*B. gaurus*), kouprey (*B. sauveli*), and banteng (*B. javanicus*; Miyamoto *et al.* 1989; Geraads 1992; Wall *et al.* 1992; Janecek *et al.* 1996; Ritz *et al.* 2000). The *Bison-Bos* genera split occurred between 0.5 – 1.5 million years ago in Eurasia (McDonald 1981; Hartl *et al.* 1988; Loftus *et al.* 1994; Bradley *et al.* 1996; Ritz *et al.* 2000). Both bison species are capable of producing fertile offspring through hybridization with domestic cattle and other members of the genus *Bos* (Boyd 1908; Goodnight 1914; Steklenev and Yasinetskaya 1982; Meagher 1986). Consequently, Simpson (1961) and van Gelder (1977) supported the inclusion of the genus *Bison* in the genus *Bos*, the latter pointing out that although "virtually all mammalogists agree verbally that '*Bison*' and '*Bos*' are congeneric," tradition has precluded the everyday use of the inclusion. Further support for the generic inclusion of *Bison* into *Bos* comes from morphological data (Groves 1981), blood protein analysis (Stormont *et al.* 1961), and phylogenetic analyses of

mitochondrial (Burzyńska *et al.* 1999; Miyamoto *et al.* 1989; Janecek *et al.* 1996) and nuclear ribosomal DNA (Wall *et al.* 1992).

The species *Bison bonasus* arose in Europe from *B. schoetensacki* during the late Wisconsin-early Holocene around 10,000 BP (McDonald 1981). *B. bonasus* underwent a continual census decline coincident with habitat destruction, exposure to cattle diseases, and increased hunting pressures between the 1400s and early 1900s and culminating with the elimination of all but 12 captive European bison following World War II (Olech 1987; Pucek 1991; Burzyńska *et al.* 1999). Not only are the descendants of these bison highly inbred (Olech 1987), but the source population used to stock European bison herds contain bison-domestic cattle hybrids as shown through historical (McHugh 1972) and mitochondrial DNA evidence (Ward *et al.* 1999). Furthermore, the 2 bison species are more appropriately considered conspecific, since interspecies hybrids are completely interfertile in both sexes (van Gelder 1977; Corbet 1978; Pucek 1991).

B. bison decline

Based on an estimate by Seton (1937) from observations of Colonel Richard Dodge along the Arkansas River in 1871, a common perception for many years has been that around 60 million bison once roamed the North American continent. More recent examinations, however, have shown this number to be far inflated. Flores (1991) used the 1910 USDA livestock census data and historical rainfall averages to estimate the North American carrying capacity at 28 – 30 million. Similarly, McHugh (1972) estimated the North American carrying capacity at approximately 30 million bison based

on the carrying capacity and acreage of tallgrass versus short-grass prairie and competition with other grazers. Roe (1970) estimated 40 million must have existed in 1830 through analysis of documentation on the number of bison killed between 1830 and 1854. Regardless, it is clear from historical accounts and modern interpretation that bison once roamed the North American continent in the tens of millions.

The apex of the well-known bison slaughter occurred in the mid-1800s and can be mostly attributed to hunting by both Indians and Europeans to supply the profitable bison robe trade (Garretson 1938; Roe 1970). However, there is evidence to suggest that the species was in decline well before this time. Although Native Americans had hunted bison for thousands of years, around 1450 human pressures on bison herds was increased by trade between Indian tribes (Flores 1991). In the late 1600s, the acquisition of horses soon produced entire Indians cultures centered on bison hunting. Natural forces such as fire, snow, and drought, predation by wolves, competition for resources with wild horses, and exotic bovine diseases all played a part in the 1700s and early 1800s in reducing the number of bison in North America (Roe 1970; Flores 1991; Zontek 1995). With the help of horses, Indians eliminated bison west of the Rocky Mountains by the early 1800s (Christman 1971), while European settlers exterminated bison east of the Mississippi River by 1820 (Garretson 1938). By 1800, bison on the southern plains were so scarce that Comanche Indians were literally starving to death (Flores 1991). In the central plains of the U.S. and in Canada, however, it was severe hunting between 1830 and 1880 that caused the nearly complete extinction of the bison species (Garretson 1938; Roe 1970).

Although exact figures are obscure, bison reached a minimum of a few hundred individuals in the late 1800s. Seton (1937) estimated the minimum reached in 1895 of around 800 bison in North America. The famous naturalist William Hornaday (1913) estimated a maximum of 1,300 bison existing in 1888 – 1889, including wild and captive bison in the United States and Canada (Hornaday 1913). Coder (1975) estimated that at the lowest point in 1888, there were only 541 bison in existence in the United States and only around 85 alive in the wild in Montana, the Dakota Territory, Wyoming, Colorado, and Texas. Of these 85 wild bison, all except a small herd in Wyoming were completely hunted out of existence. When it became evident that bison were on their way to extinction in the 1880s, a small number of individuals effectively served to save the species through the recovery efforts summarized below.

The recovery of North American bison

McKay-Alloway Herd

James McKay and Charles Alloway were business partners, who actively participated in annual bison hunts with the Metis Indians in Saskatchewan. By the 1872 Red River hunt, bison were quite scarce, and the pair decided to establish a small herd with some of the few remaining wild bison. In 1873 - 1874, a total of 4 female and 1 male calves were captured and used to establish the McKay-Alloway herd (Coder 1975). Following the death of McKay in 1880, Colonel Samuel L. Bedson purchased 8 of the bison and added 3 wild calves to the small herd. Of the remaining McKay-Alloway herd, 13 were purchased and given to the Canadian (Dominion) government. The 13

bison joined 3 existing bison in the establishment of Banff Park in Alberta, and 2 females and 1 male from the Goodnight herd in Texas were later added (Coder 1975).

Goodnight Herd

At the behest of his wife, Charles Goodnight began his famous bison venture in the panhandle of Texas with the capture of 1 male and 1 female calf in 1878 (Haley 1949). Five additional calves were later obtained, but of the 7 total, one was killed and one sold, thus establishing the Goodnight herd with 5 wild bison (Haley 1949; Coder 1975). The herd grew and prospered; by 1887 there were 13 bison and by 1910, the number had increased to 125 (Dary 1989). The Goodnight herd increased to 200 – 250 bison for several years in the 1920s (Haley 1949). Following Goodnight's death in 1929, the ownership of the herd changed several times and reliable estimates of population size are unavailable. By the 1970s the population was estimated at 40 – 100 bison (Danny Swepston, pers. comm.). In 1997, the remaining 36 bison were donated to Texas Parks and Wildlife and moved to Caprock Canyons State Park in the Texas panhandle (Texas State Bison Herd: TSBH). Over the last 120 years this population has remained reproductively isolated, therefore representing the only extant bison population directly descended from the original Charles Goodnight herd.

Dupree-Philip Herd

Frederick Dupree, a cattle rancher from South Dakota, captured bison in Montana in 1882 after observing the rapid disappearance of bison on the plains. About 9 calves were captured, though 2 or 3 died shortly after arriving at the Dupree ranch (Coder 1975). Dupree died in 1898 and his entire operation of 85 bison was purchased by James "Scotty" Philip in 1901 (Coder 1975; Zontek 1995). In 1914, 36 bison from the Philip herd were used to found the Custer State Park (CSP) population in South Dakota (Garretson 1938).

Jones Herd

Charles "Buffalo" Jones profited enormously on the Great Plains during the mid-1800s from hunting bison to supply the demand for hides. Once moderately wealthy, he used his influence to help save the species from extermination. In 1886 he traveled to Palo Duro Canyon and captured 14 calves from a remnant population of southern plains bison, only 10 of which survived the trip back to Garden City, Kansas. From 1887 – 1889, a total of 46 bison were added to the herd in the same fashion (Coder 1975). Later, purchases of the entire Bedson herd of 86 bison from Canada (McKay-Alloway origin) and 10 adult bison from various owners in Kansas and Nebraska were used to supplement the Jones herd (Garretson 1938; Coder 1975).

Pablo-Allard Herd

After creating trouble for himself by taking two wives from two different Indian tribes at the same time, Samuel "Walking Coyote" Wells captured 3 male and 4 female calves in 1879 in Montana to present to his Pend d'Oreille tribe as a peace offering (Coder 1975). Wells arrived at Flathead Valley with 2 male and 2 female calves, which were used to start a small herd. Charles Allard, Sr. and his interpreter Michael Pablo bought the entire herd of 12 bison from Wells in 1883, thus forming the Pablo-Allard bison herd (Coder 1975; Zontek 1995). In 1893, Pablo and Allard purchased 44 bison from Charles Jones to supplement their herd (Seton 1937). By 1906, the herd was estimated at 350 bison.

National Zoological Park Herd

In the late 1800s another important captive bison herd was founded at the National Zoological Park in Washington, DC as follows: 1888 – 1 male, 1 female from Nebraska; 1889 – 3 males, 1 female from South Dakota; 1897 – 1 male, 2 females from Pablo-Allard herd; 1904 – 4 females, 3 of which were from the Austin Corbin herd in New Hampshire which originated from a mixture of bison from Wyoming, Manitoba, and Charles Jones' herd (Coder 1975). William Hornaday personally saw to the collection of the bison and establishment of the National Zoological Park herd, which would later serve to help create the Wichita Mountains National Wildlife Refuge and Wind Cave National Park populations (see below).

Bison bison in the 20th century

Although several hundred bison existed at the population bottleneck apex in the late 1800s, nearly all bison that exist today are descendants of the 76 – 84 bison used to found the aforementioned 5 private herds in the late 1800s and a remnant wild population in Yellowstone National Park of no more than 30 bison (Garretson 1938; Meagher 1973; Coder 1975). From these few bison, and under the protection of both the U.S. and Canadian governments, the worldwide bison census quickly grew as follows: 1910 – over 2,000 bison; 1920 – over 8,000 bison; 1933 – over 21,000 bison (Hornaday 1913; Seton 1937; Garretson 1938; Coder 1975). As the established federal bison populations grew, excess bison were killed, used to establish new federal populations, or sold in public auctions. The bison story has now come full-circle, as the majority of the 300,000 North American bison in existence today are privately owned and have histories tracing back to a few public bison populations, founded from a limited number of bison secured primarily by 5 private ranchers in the late 1800s.

Taxonomic status of *B. bison*

The species *B. bison* is currently represented by two subspecies which presumably differ in physical size and coat characteristics: wood bison (*B. b. athabascae* Rhoads) and plains bison (*B. b. bison* L; Hall 1981; McDonald 1981; Meagher 1986). Limited support for the division of these two races comes from differences in phenotypic variation (van Zyll de Jong *et al.* 1995). Following exploitation in a manner similar to that experienced by the plains bison, in 1891 an estimated 300 wood bison representing

the only remnant of the subspecies were in the area now belonging to Wood Buffalo National Park (WBNP) in Canada (Banfield and Novakowski 1960). Under protection by the Canadian government, the population had increased to 1,500 – 2,000 bison by 1922. Despite this rapid population growth and over the protests of Canadian scientists (Seton 1937; McHugh 1972), over 6,600 plains bison of Pablo-Allard herd origin were moved into WBNP from 1925 - 1928 (Banfield and Novakowski 1960; Roe 1970). Reports indicate that the plains bison freely roamed and bred with the native wood bison (van Camp 1989; Geist 1991). A presumed pure sub-population of wood bison (Banfield and Novakowski 1960) was used to establish populations at Mackenzie Bison Sanctuary (18 bison; MBS) and Elk Island National Park (24 bison; EINP) in Canada in 1963 and 1965, respectively (Geist 1991).

The subspecific status of wood bison has been a contentious issue. Under the currently accepted taxonomic definition, the wood bison populations receive protection and funding from the Canadian government. However, Burton (1962), Corbet (1978), van Gelder (1977), and Wilson and Reeder (1993) all considered the subspecific status of the wood bison invalid. Geist (1991) noted that the generally accepted phenotypic differences between wood and plains bison are the effects of environmental and not genetic influence. Wood and plains bison have identical chromosome numbers (2n) with the same G-banding patterns (Ying and Peden 1977). Blood group typing does not support subspecific status, indicating more variation within plains bison than between plains and wood bison populations and high similarity between presumably pure wood bison from EINP and both hybrid wood-plains bison from WBNP and pure plains bison

from EINP (Peden and Kraay 1979). Analysis of nuclear restriction fragment length polymorphisms indicated significantly different allele frequencies between wood and plains bison from EINP, although unique wood bison alleles were not found (Bork *et al.* 1991). The significance of the noted differences between wood and plains bison, however, is unknown since differences in allele frequencies were not established among plains bison populations.

More recently, microsatellite DNA analysis has been used to compare allelic distribution and genetic distance between wood bison from EINP, MBS, and WBNP with plains bison populations (Wilson and Strobeck 1999). After finding no alleles private to the wood bison populations and observing limited genetic differences between the presumed subspecies, Wilson and Strobeck (1999) concluded that all wood bison populations have some admixture of plains bison germplasm. Polziehn *et al.* (1996) and Ward *et al.* (1999) showed through phylogenetic analysis of mitochondrial DNA that that each of the Canadian wood bison populations (EINP, MBS, WBNP) contain mitochondrial haplotypes shared with plains bison populations and that the haplotypes unique to wood bison populations are not phylogenetically distinct from other bison haplotypes. Given these lines of evidence, it appears that wood and plains bison are not distinct enough to be considered subspecies. However, the history of the northern wood bison during and after the species bottleneck in the late 1800s makes the Canadian populations a potentially important source of bison genetic diversity.

Krumbiegel and Sehm (1989) used evidence of phenotypic variation and former range to further split the plains bison into two subspecies: southern plains bison (*Bison*

bison bison Linnaeus) and northern plains bison (*Bison bison montanae* Krumbiegel). The division, however, is tenuous and based mostly on analysis of pre-1900 illustrations. Charles Goodnight observed phenotypic differences between the northern and southern plains herds, stating "…while they are no doubt the same species, there is enough difference in the two for any judge of animals to observe it at once…" (Haley 1949). Even if such subspecies at one time did exist, they have been undoubtedly crossbred in the past 100 years (McHugh 1972; Coder 1975; Dary 1989), so that the only known true remnant of the southern plains bison is contained in the TSBH.

Previous *B. bison* genetic investigations

Previous *B. bison* genetic work includes blood group serology (Stormont *et al.* 1961; Sartore *et al.* 1969), protein electrophoresis (McClenaghan *et al.* 1990), mitochondrial DNA sequence (Polziehn *et al.* 1995; Ward *et al.* 1999), nuclear restriction fragment length polymorphism (Bork *et al.* 1991), and nuclear microsatellite analyses (Mommens *et al.* 1998; Wilson and Strobeck 1999; Schnabel *et al.* 2000). Only Wilson and Strobeck (1999) attempted to measure the amount of genetic variation within and among populations, with the specific focus of comparing wood and plains bison populations. However, Wilson and Strobeck (1999) included only 4 of the 11 U.S. federal bison populations in their study, which was limited in both the number of loci (11) and number of samples utilized from each population.

Hybridization of bison and domestic cattle

As early as 1873, Charles Goodnight produced hybrids between bison and cattle (Dary 1989). By breeding his bison bulls to Polled Angus cows, he sought to produce a hearty beef breed (Goodnight 1914; Haley 1949). By 1917, Goodnight had become internationally famous for his "cattelo" operations, having produced around 40 head of such hybrids that seemed resistant to disease, required less feed, and could produce calves for more years than pure Angus (Haley 1949). Charles Jones had similar success in producing and utilizing domestic cattle-bison hybrids, and believed that the cattelo should replace domestic cattle beef breeds (Jones 1907; McHugh 1972). In fact, all of the ranchers involved in establishing the 5 foundation herds either experimented with domestic cattle-bison crosses or purchased bison from others who were involved in such experiments (Garretson 1938; Coder 1975). For instance, McKay and Alloway actively crossbred their bison to domestic cattle, as did Samuel Bedson after purchasing some of the McKay-Alloway bison (Coder 1975). Jones later bought the Bedson herd and performed interspecies crosses himself (Garretson 1938).

Both mitochondrial DNA (Polziehn *et al.* 1995; Ward *et al.* 1999) and nuclear DNA (Ward 2000) analyses have revealed evidence of domestic cattle introgression in bison. Domestic cattle introgression has been detected in several public bison populations and all except 1 of the more than 50 private bison herds examined to date (James Derr unpublished data). As such, it has become increasingly important for the long-term preservation of a pure bison species to identify those populations with no evidence of domestic cattle introgression.

Study objectives

A comprehensive survey and analysis of genetic variation within and among U.S. federal bison populations, which are collectively an important resource of bison germplasm, is necessary to provide baseline genetic information from which future management decisions can be established. Although all 11 of the U.S. federal bison populations operate under the guidelines of the Department of the Interior, each herd is managed independently such that decisions ranging from the choice of supplemental feeding to the type of culling employed are ultimately the responsibility of individual park/refuge mangers. Very little theoretical and almost no applied information is known about the genetic impact of various management decisions on long-term levels of genetic variation and survivability in these bison populations.

The objectives of this study were to examine the levels and distribution of nuclear DNA variation within and among federal bison populations, to investigate the effects of various culling practices on genetic variation, and to use previously established technologies to survey detectable levels of introgression of domestic cattle mitochondrial and nuclear genes in these populations. Detailed knowledge of current levels of genetic variation and domestic cattle introgression will serve as the basis, in part, for future management decisions regarding federal bison populations and will likely have a direct impact on the long-term preservation of the species.

Federal bison populations: history, management status, and previous genetic work

Population abbreviations, locations, and summary of founding stock are shown in Table 1.

Badlands National Park

In 1963, 50 bison from TR and 3 from FN were used to found the BNP bison population (McClenaghan *et al.* 1990). Additionally, 20 bison from the Colorado National Monument herd were used to supplement genetic diversity in the BNP population in 1983, all of which were descended from 2 females and 1 male from the Denver area in 1925 (Berger and Cunningham 1994). The annual growth rate is estimated at 15 – 18%, and bison are culled opportunistically and proportionately with respect to age and sex when the population exceeds the conservative carrying capacity of 600 animals (William Supernaugh pers. comm.). McClenaghan *et al.* (1990) reported low levels of genetic variation in the BNP bison population based on protein electrophoresis data, and attributed this finding to inbreeding. This population has not been previously examined for evidence of domestic cattle introgression.

Fort Niobrara National Wildlife Refuge

Originally established as a bird reservation in 1912, the FN bison herd was founded with a gift of 6 bison from a private rancher in Nebraska and 2 bulls from YNP in 1913 (Garretson 1938; Coder 1975). Additional introductions were made to minimize inbreeding and maintain genetic diversity as follows: 4 bulls from CSP in 1935; 4 bulls

TABLE 1

National Park (NP) and National Wildlife Refuge (NWR) bison populations

Herd (Abbreviation)	Location	Founding Stock Year	Number – Source
Badlands NP (BNP)	South Dakota	1963	3 – Fort Niobrara NWR; 50 – Theodore Roosevelt NP (TRS)
		1983	20 – Colorado National Monument (unknown origin)
Fort Niobrara NWR (FN)	Nebraska	1913	6 – private ranch, Nebraska; 2 – Yellowstone NP
		1935	4 – Custer State Park
		1937	4 – Custer State Park
		1952	5 – National Bison Range
Grand Teton NP (GT)	Wyoming	1948	20 – Yellowstone NP
		1964	12 – Theodore Roosevelt NP
National Bison Range (NBR)	Montana	1908	1 – Goodnight herd; 3 – Corbin (McKay-Alloway); 34 – Conrad (Pablo-Allard)
		1939	2 – 7-Up Ranch (unknown origin)
		1952	4 – Fort Niobrara NWR
		1953	2 – Yellowstone NP
		1984	4 – Maxwell State Game Refuge (Jones)
Neal Smith NWR (NS)	Iowa	1996	8 – Fort Niobrara NWR; 8 – Wichita Mountains NWR
		1997	6 – Fort Niobrara NWR; 8 – National Bison Range
		1998	3 – Fort Niobrara NWR
Sully's Hill National Game Preserve (SH)	North Dakota	1919	6 – Portland City Park, Oregon (unknown origin)
Theodore Roosevelt NP (TR)	North Dakota	1956	29 – Fort Niobrara NWR to found TRS (south unit)
		[1962]	[20 – TR-S bison to found TRN (North unit)]
Wichita Mountains NWR (WM)	Oklahoma	1907	15 – New York Zoological Park
		1940	2 – Fort Niobrara NWR
Wind Cave NP (WC)	South Dakota	1913	14 – New York Zoological Park
		1916	6 – Yellowstone NP
Yellowstone NP (YNP)	Wyoming, Idaho, Montana	1902	< 30 wild; 18 – Pablo-Allard herd; 3 – Goodnight herd

from CSP in 1937; 5 bulls from NBR in 1952. The herd is managed in a controlled grazing program by rotation through fenced units periodically throughout the year. The population has grown from less than 200 from 1940 – 1964 to around 200 – 300 from 1965 – 1985 to current estimates of approximately 350 bison, with recent natural mortality rates averaging < 2.0%/year. The sex ratio is approximately 1:1 in the younger age classes and the average calving rate for 3 year-old and older females is 83%. Bison are culled through public auctions and donations to remove surplus and maintain the carrying capacity of around 350 bison. From the 1970s through the mid-1990s, weight, appearance, and health factors were used to make culling decisions in the calf and yearling age classes, which were reduced by approximately 50% each year (Royce Huber pers. comm.). Criteria for culling older age classes included general health, condition, and reproductive success in females based on calf production. The culling strategy has become more randomized in the past few years. FN calves receive vaccinations for hemorrhagic septicemia, blackleg, and malignant edema and the entire population has been disease-free for over 30 years.

Wilson and Strobeck (1999) found levels of genetic variation (average 4.64 alleles/locus) and heterozygosity (average 57.2%) for 11 microsatellite markers across 30 bison samples from FN, which is comparable to that found in other public bison herds. Overall, FN ranked 6th for the average number of alleles/locus and 10th for the average heterozygosity out of 11 populations studied (rank of 11th being the highest; Wilson and Strobeck 1999). Polziehn *et al.* (1995) and Ward *et al.* (1999) both found a single bison mitochondrial haplotype in FN bison and no evidence of domestic cattle

mitochondrial DNA introgression. Ward (2000) found 11/27 (40.7%) of the FN bison sampled had evidence of domestic cattle introgression at 3 linked markers on chromosome 1.

Grand Teton National Park

In 1948, 20 bison (3 bulls, 12 cows, 5 calves) were moved from YNP to Jackson Hole Wildlife Park near Moran, Wyoming (McHugh 1958; National Park Service (NPS) 1996). A population of 15 – 30 bison was maintained in a large enclosure until 1963 when brucellosis was discovered in the herd, at which time all 13 adults were destroyed and 4 yearlings and 5 calves were vaccinated and retained. In 1964, 12 brucellosis-free adult bison (6 male, 6 female) were added to the population from TR, but by 1968 the population was down to 15 or 16 bison (NPS 1996). After becoming free-ranging in 1969, the population began to expand and migrate between GT in the summer and the adjacent National Elk Refuge in the winter. There are currently approximately 600 bison in the GT population.

GT bison receive supplemental feed in the winter on the National Elk Refuge. Bison that escape the confines of the park onto private lands are usually culled. The only recent large reduction in population size was the removal of 37 animals from 1988 – 1990 when the population census size was just over 100 (around a 30 – 35% reduction; NPS 1996). The herd suffers from brucellosis, and public concerns over the threat of transmission of the disease to livestock on adjoining private lands heavily influence

management of the GT bison herd. No prior studies of genetic variation or introgression of domestic cattle DNA have been performed on GT bison.

National Bison Range

Through the work of the American Bison Society, 12 male and 22 female bison from the Conrad herd in Kalispell, Montana were used to establish the Montana NBR herd in 1908 (Garretson 1938; Coder 1975). These bison originated from 30 bison bought from the Pablo-Allard herd in 1902 (Coder 1975). Additions were made in 1908 of 1 male and 2 females from the Corbin herd of McKay-Alloway origin and 1 male from the Goodnight herd (Garretson 1938). By 1924 the census size had grown to approximately 700 and annual reductions began with the removal of 197 bison (David Wiseman pers. comm.). In 1928, 23 surplus bison were shipped to Alaska, 19 of which were used to found a herd near Fairbanks (Delta Junction Herd; Garretson 1938; Coder 1975). The NBR bison population has been supplemented 4 times since its inception: in 1939 with 2 males from the 7-Up ranch (origin unknown), in 1952 with 4 males from FN, in 1953 with 2 males from YNP, and in 1984 with 4 females from Maxwell State Game Refuge (MSGR) in Kansas (David Wiseman pers. comm.).

Current management policy includes rotational grazing of the entire herd through a cross-fencing system, maintenance of a 60% female adult population as an approximation of natural conditions in large bison herds, and removal of animals to maintain a total census size of 370 – 400 bison (David Wiseman pers. comm.). Bison are removed from NBR through annual round-ups, donation to tribes, and public

auctions through random selection within identified age and sex classes. The bison population has been tested and certified as brucellosis-free since 1952. Johne's disease has recently been indicated through either laboratory testing or necropsy evaluation in 5 bison from NBR (2 confirmed cases; Lindy Garner and Thomas Roffe pers. comm.). The source of the disease is unknown and the incidence is believed to be increasing at this point.

Wilson and Strobeck (1999) included 30 bison from NBR in their microsatellite study, where they found an average of 4.91 alleles/locus and 54.4% heterozygosity, ranking NBR 7th out of the 11 populations studied in both of these measures. Through mitochondrial DNA restriction analysis of 22 bison from NBR, Polziehn *et al.* (1995) found 2 types of bison mitochondrial DNA. A more in-depth mitochondrial sequencing survey and subsequent screening of 113 bison from NBR revealed 3 bison haplotypes and 1 domestic cattle haplotype (Ward *et al.* 1999). The most likely source of the domestic cattle mitochondrial introgression was determined to be MSGR (from the 1984 introduction of 4 females), as the domestic cattle haplotype was shared with another bison population with origins from MSGR (Ward *et al.* 1999). Ward (2000) also found 3 linked markers on the telomeric end of chromosome 1 that each demonstrated possible nuclear domestic cattle introgression in 6/38 (15.8%) bison sampled from NBR.

Neal Smith National Wildlife Refuge

The NS bison population is the most recently established of the federal herds, formed as mixture of stock from various herds as follows: 1996 – 8 from FN, 8 from

WM; 1997 – 6 from FN, 8 from NBR; 1998 – 3 from FN. The population was reduced from a maximum of 70 to 35 bison in 2001 and has since been maintained at the current carrying capacity of 35 based on the existing 700-acre enclosure (Nancy Gilbertson pers. comm.). Brucellosis has not been detected in the NS bison population. No documented studies of genetic variation or domestic cattle introgression have been conducted in the NS bison population, although some deductions can be made from previous studies on the source populations.

Sully's Hill National Game Preserve

Sully's Hill National Park was established in 1904 and in 1931 was transferred to the National Wildlife Refuge System as a national game preserve. In 1919, the bison herd at SH was established with 6 bison from the Portland City Zoo in Oregon (Coder 1975). The origin of these bison is unknown. The herd is maintained at 30 – 40 bison and used mostly for exhibition purposes. No previous genetic work is known from the SH population.

Theodore Roosevelt National Park

Theodore Roosevelt National Monument was established in North Dakota in 1947 and became an official national park in 1978. In 1956, 29 bison from FN were used as founding stock for the south unit bison population (TRS). The north unit (TRN) population, located approximately 40 miles from the south unit, was subsequently founded from 20 TRS bison in 1962 (Michael Oehler pers. comm.). The census

population sizes are approximately 320 (TRS) and 310 (TRN) bison based on surveys and round-ups in 2002 and 2001, respectively. Extensive roundups are conducted within each unit every 2 – 3 years, and excess animals are transferred to tribal groups. Culling decisions are based on targeted overall herd size, a 2 female:1 male adult sex ratio, and maintenance of age class proportions with an upper cull limit of 60% for any given age class (Michael Oehler pers. comm.). Based on extensive testing since their foundation, the populations are thought to be free of brucellosis and other known bovid diseases, and studies of genetic variation and examination of domestic cattle introgression have not been previously undertaken on these populations.

Wichita Mountains National Wildlife Refuge

The second of the federal bison herds to be established was in Oklahoma on the Wichita Forest and Game Preserve, founded in 1905 (later to become Wichita Mountains National Wildlife Refuge). In 1907, William Hornaday selected 6 males and 9 females from the New York Zoological Park used to establish the WM bison population (Garretson 1938; Mitchell 1993). The 15 animals were believed by Hornaday to represent 5 bison blood lines. Several of the females had origins from a private herd in Jackson, Wyoming, some came from a private herd in Maine, and one bull was from the Jones herd (Coder 1975). By 1917 there were 92 bison and by 1923 the herd had grown to 147 bison (Mitchell 1993). In 1940, 2 bulls from FN were used to supplement the herd (Joe Kimball pers. comm.).

The WM bison population is maintained in 3 subherds on large fenced pastures. Yearlings are moved between pastures annually to maintain overall genetic diversity. The current WM census population size is approximately 575, with excess bison culled annually through round-ups, donations to tribal organizations, and public auctions (Joe Kimball pers. comm.). Wilson and Strobeck (1999) included 21 bison from WM in their microsatellite study, where they found an average of 3.91 alleles/locus and 47.4% heterozygosity, ranking WM 3rd out of the 11 populations studied in both of these measures (the lowest ranking of the 4 U.S. federal populations examined). Polziehn *et al.* (1995) examined 20 WM bison and found 2 bison mitochondrial haplotypes and no evidence of domestic cattle mitochondrial introgression.

Wind Cave National Park

The WC bison population originated in 1913 with 6 male and 8 female bison donated by the New York Zoological Society through the efforts of the American Bison Society (Coder 1975; Mitchell 1993). In 1916, 6 bison were added to the small herd from YNP. The culling practices to remove excess WC bison have changed dramatically over the history of the herd. In the 1930s and 1940s, old and/or sickly bison were selectively removed from the population while in the 1950s, excess bison were purposely baited into neighboring CSP. When the incidence of brucellosis became widespread (60 – 75%) in the 1950s and 1960s, the management practices were shifted to focus on brucellosis eradication. In 1964, 220 of the 440 bison at WC were shot in the field (50% reduction), and in 1979 the herd was again reduced through round-up and

slaughter from approximately 547 to 353 bison (35% reduction). In 1982, the park was placed under quarantine by the State of South Dakota, due to brucellosis in the herd. Brucellosis continued to drive the culling of the herd until 1985 when the disease was eliminated. In 1986, the WC bison population was released from quarantine by South Dakota and no animals have tested positive for brucellosis antibodies since (Barbara Muenchau pers. comm.).

In an attempt to maintain a 50:50 sex ratio in the younger age classes, annual roundups are conducted and yearling bison are culled from the WC population. Ten yearling bison of each sex are withheld in the park, producing a herd representative of all age classes (Barbara Muenchau pers. comm.). Excess bison are typically transferred to various American Indian tribes. Ward *et al.* (1999) examined 37 WC bison and found a single bison mitochondrial haplotype and no evidence of domestic cattle mitochondrial introgression.

Yellowstone National Park

The first federal park in the world, YNP was founded in 1872. From 1872 – 1886, wild bison in YNP were poached rigorously and without consequence due to inadequate management and funding for law enforcement. At the lowest point in 1902, there were no more than 30 bison remaining in the wild in YNP (Garretson 1938; Meagher 1973). In the same year, Charles "Buffalo" Jones was appointed by President Roosevelt to act as game warden in the preservation of bison in YNP and played an integral role in the building of corrals and supervising the purchase of additional plains

bison to supplement the YNP population (Coder 1975). Introductions of 18 female bison from the Pablo-Allard herd in Montana and 3 bulls from the Goodnight herd in Texas were made in 1902 (Garretson 1938; Coder 1975). These 21 bison were originally fenced and treated as captive. One of the Goodnight bulls died in the first winter and thus made very little, if any, genetic contribution to the captive herd (Garretson 1938). After the first year, the herd had increased by 12 head (Coder 1975). A few additions of some "wild" YNP bison were made to the captive herd (Garretson 1938), which continued to increase in numbers until 1915 when the herd was released into the park, eventually intermingling with the growing wild bison population (Meagher 1973).

The YNP bison population is the most thoroughly studied and arguably the most well-known of all public bison herds in North America; it is also at the center of a significant political controversy. In the late 1800s and early 1900s, the management priority for YNP bison was the protection from hunters and near-extinction. As the population increased, ungulate carrying capacity became the new management priority. For instance, in the northeast section of the park in the Lamar Valley, the bison subpopulation numbered over 1,000 from 1929 – 1932 but was reduced to 143 bison by 1952 through frequent reductions (Meagher 1973). In 1954 there were around 1,477 bison total in YNP, and further reductions for purposes of meeting the carrying capacity of the land were made until in 1967 there were only around 397 bison in the entire park (Meagher 1973).

After 1967, the park adopted a policy of noninterference whereby bison populations were mostly left to their own devices without supplemental feed or direct management. By the 1980s bison began migrating beyond YNP boundaries in significant numbers due to competition for natural resources, especially during severe winters when the overall bison population exceeds 3,000 (Cheville *et al.* 1998). The possibility of transmission of brucellosis to livestock on adjacent private lands has confounded this issue and led to intense political and scientific deliberations concerning the management of this historically valuable bison population.

Wilson and Strobeck (1999) included 33 bison from YNP in their microsatellite study, where they found an average of 5.36 alleles/locus and 54.2% heterozygosity, ranking YNP 9th and 6th, respectively, out of the 11 populations studied. With a combined analysis of 47 bison, both Polziehn *et al.* (1995) and Ward *et al.* (1999) found 2 bison mitochondrial haplotypes and no evidence of domestic cattle mitochondrial introgression. Ward (2000) did not find evidence of domestic cattle nuclear introgression in 28 YNP bison with his study of 100 microsatellites.

CHAPTER II

AN EXAMINATION OF GENETIC VARIATION IN U. S. FEDERAL BISON POPULATIONS

"The efforts of man to atone for the great bison slaughter by preserving the species from extinction have been crowned with success."
— William T. Hornaday 1913

Introduction

In the field of conservation genetics, one rarely has the opportunity to examine the recovery of a species from near extinction. North American bison not only sustained a well-documented population bottleneck, but also recovered seemingly well through a series of founder events. The lineage of nearly all extant bison in both public and private herds can be traced back to less than 100 bison maintained in 5 private herds in the late 1800s (Coder 1975).

Today there are approximately 300,000 bison in North America, most of which are privately owned. The U.S. National Park Service maintains less than 7,000 bison in five herds: BNP ~700 bison, GT ~600 bison, TR ~630 bison in 2 units, WC ~350 bison, and YNP ~2,500 – 3,000 bison. There are less than 1,600 additional bison maintained in five U.S. Fish and Wildlife Service herds: FN ~ 350 bison, NBR ~350 bison, NS ~60 bison, SH ~30 bison, and WM ~600 bison. The vast majority of other public and most private bison herds are derived from these federal bison populations (with the noted exception of Canadian public bison herds, see Chapter I). Additional publicly

maintained bison are found in various state herds, several public zoos, and a few bison sanctuaries. Public and private bison populations are highly fragmented with artificially regulated gene flow. Unlike public populations, private bison herds are often managed specifically for such traits as growth rate and meat production and many have known bison-domestic cattle hybrids. As such, the long-term maintenance of bison genetic variation depends on the practices invoked by managers of federal bison herds.

There is a limited amount of blood group (McClenaghan *et al.* 1990), mitochondrial sequence (Polziehn *et al.* 1995; Ward *et al.* 1999), and microsatellite DNA (Wilson and Strobeck 1999; Schnabel *et al.* 2000) information on some of the federal bison herds. The aforementioned studies neither include information from all of these herds nor address management issues within each herd. A comparative study of genetic variation within and among federal bison populations will be central in examining effects of various management strategies on the long-term survivability and genetic variation of these herds.

Neutral, unlinked genetic markers are the preferred choice for analyses of population-level genetic variation. The neutrality (or near-neutrality) of such markers is vital, since selection on non-neutral genetic loci acts to skew allelic distributions and therefore severely complicate analyses and validity of conclusions. It is furthermore necessary to choose genetic loci not closely linked so as to simplify analyses and provide the most thorough representation of the genome under investigation. Several types of genetic loci meet these qualifications, but the two most popular are single nucleotide polymorphisms (SNPs) and microsatellites. Although SNPs have the advantage of being

more frequent across genomes (approximately 1/1,000 bp in humans), they have an upper limit of only 2 alleles/locus (A/T or G/C). Microsatellites, although less frequent (approximately 1/20,000 – 1/40,000 bp in humans), have the advantage of many more possible alleles for any given locus.

Microsatellites are known to cross-amplify in closely related species due to conservation of unique sequence regions flanking the repeat (Moore *et al.* 1991). The bovine linkage map (Fries *et al.* 1993; Bishop *et al.* 1994; Kappes *et al.* 1997) has provided a multitude of microsatellite markers for cattle, many of which have proven to co-amplify in bison (Mommens *et al.* 1998; Wilson and Strobeck 1999; Schnabel *et al.* 2000). Fluorescent dye labeled microsatellite loci can be co-amplified and genotyped simultaneously through multiplexed polymerase chain reaction (PCR) and high-throughput fluorescent fragment detection systems. Development of a panel of multiplexed polymorphic microsatellite markers across the entire bison genome will not only give a better understanding of the genetic structure of extant federal bison herds, but will also serve as a tool for evaluating the consequences of current management techniques.

Materials and methods

Sample collection and DNA extraction

Liver, whole blood, and/or tail hair samples were collected from 10 of the 11 federal bison herds from the period of 1997 – 2002, the only exception being the small exhibition herd maintained at SH. Additionally, the entire population of 40 bison from

the TSBH was sampled in December 2001 and included in this analysis. Collections were made and kindly provided by herd managers, biologists, and veterinarians.

For the extraction of DNA from liver samples, approximately 0.5 g frozen liver was pulverized in liquid nitrogen with a mortar and pestle. Tissue lysis buffer consisting of 1× STE (100 mM NaCl, 10 mM Tris, 1 mM EDTA), 2% SDS, and 4 mg/ml Proteinase K was added to the powdered tissue and incubated overnight at 55°C in a water bath. The tissue was treated with 10 µg RNAse-A and standard Phenol-Chloroform-Isoamyl Alcohol (PCI) extraction was performed (Sambrook *et al.* 1989).

DNA from whole blood samples was isolated following the Super Quik-Gene protocol (AGTC, Denver, Colorado) and standard PCI as above or through the application of 1 – 2 ml whole blood onto FTA cards (Whatman, Newton Center, Massachusetts). DNA was extracted from hair samples as follows: cut 15 – 20 hair follicles from the hair shafts; place in 200 µl of buffer (400 mM KCl; 100 mM Tris; 0.45% TritonX; 0.45% Tween-20; 0.5 mg/ml proteinase K); incubate in 55°C water bath overnight, vortexing intermittently; centrifuge at 13,000 g for 5 minutes; remove liquid and perform standard PCI extraction as above. With the exception of those stored on FTA cards, all samples were resuspended in 1× TE and concentrations determined through spectrophotometry.

For isolation of DNA from the FTA cards, 1.2 mm punches were washed with FTA solution to remove protein debris and rinsed with 1× TE following manufacturer recommendations. FTA punches were either allowed to dry for 1 hour at room temperature or left at 4°C for a maximum of 24 hours before use. When FTA punches

were used in reactions, they were substituted for 50 ng DNA by addition of water to the reaction mix.

Marker choice and multiplexing

Fifty-four bovine microsatellite markers were selected from the USDA gene mapping database (www.sol.marc.usda.gov). Markers with high PIC values in bison and no known null alleles were preferentially selected (Schnabel 2001), such that there was a minimum of one marker per nuclear chromosome and at least 40 cM between syntenic markers, as mapped in the cattle genome. Fifteen of the markers were utilized following the protocols from Schnabel *et al.* (2000) as designed for bison parentage testing, with minor changes in the fluorescent dyes and PCR protocols utilized. The forward primer for each marker was labeled with 1 of 4 fluorescent dyes (NED, 6-FAM, HEX, VIC; PE Biosystems, Foster City, California). Markers were multiplexed based on non-overlapping allele size ranges and dye types (Table 2).

PCR conditions for multiplexes 3, 80 – 83, 85, 86, and URB011 were as follows (5 µl total volume): 50 ng template DNA or 1 FTA punch; 0.05 – 0.4 µM each primer; 1× MasterAmp PCR Enhancer (Epicentre, Madison, WI); 500 µM dNTPs; 3.0 mM $MgCl_2$; 1× reaction buffer; 0.375 units *Taq* DNA polymerase (Promega, Madison, WI). Conditions for multiplexes 1 and 84 were as above with the exception of 1.5× reaction buffer and 3.5 mM $MgCl_2$. Conditions for multiplex 2 were as above with the exception of 1.2× reaction buffer and 3.25 mM $MgCl_2$. All reactions were run on a GeneAmp PCR System 9700 thermal-cycler (PE Biosystems) under the following parameters (except

TABLE 2

Summary information for 54 nuclear microsatellite loci used in this study

Locus	Label[a]	Multiplex	Chromosome (Position)[b]	R_A	N_A	PIC
AGLA232	NED	83	13 (79.5)	155-173	7	0.586
BL1036	NED	85	14 (78.7)	177-193	5	0.681
BM1225	NED	2	20 (8.0)	239-273	10	0.723
BM1706	6-FAM	2	16 (80.6)	232-254	6	0.400
BM17132	6-FAM	1	19 (58.6)	85-95	5	0.669
BM1824	6-FAM	84	1 (108.6)	178-198	7	0.703
BM1862	6-FAM	80	17 (86.3)	201-215	6	0.746
BM188	HEX	84	26 (40.4)	99-123	9	0.529
BM1905	NED	2	23 (64.3)	172-184	4	0.463
BM2113	6-FAM	2	2 (106.2)	127-153	9	0.688
BM2830	NED	86	5 (120.2)	142-164	10	0.800
BM4028	6-FAM	86	12 (79.7)	108-126	7	0.641
BM4107	HEX	85	20 (52.4)	159-185	8	0.726
BM4311	6-FAM	82	6 (89.7)	90-104	6	0.745
BM4440	NED	2	2 (55.0)	123-143	7	0.719
BM47	6-FAM	85	23 (9.1)	103-111	4	0.303
BM6017	HEX	82	X (4.7)	104-122	6	-
BM711	6-FAM	82	8 (83.6)	161-177	6	0.442
BM720	VIC	2	13 (38.6)	203-235	9	0.711
BM757	HEX	83	9 (0.6)	186-202	9	0.439
BMC4214	HEX	84	3 (123.0)	175-191	6	0.700
BMS1001	NED	80	27 (5.1)	107-115	5	0.432
BMS1074	NED	80	4 (74.9)	152-160	5	0.660
BMS1117	HEX	3	21 (9.9)	89-99	4	0.610
BMS1172	6-FAM	3	4 (27.3)	86-104	7	0.586
BMS1315	HEX	85	5 (31.8)	135-149	5	0.587
BMS1355	NED	81	18 (2.8)	146-154	4	0.544
BMS1675	6-FAM	80	27 (64.1)	85-91	4	0.553
BMS1716	HEX	80	11 (47.7)	185-197	6	0.613
BMS1747	6-FAM	83	14 (4.2)	89-103	5	0.569
BMS1857	6-FAM	85	29 (0.9)	142-168	10	0.820
BMS1862	VIC	1	24 (32.8)	142-170	11	0.714
BMS2258	HEX	81	7 (75.0)	127-152	11	0.816
BMS2639	6-FAM	3	18 (57.0)	168-186	7	0.781
BMS410	NED	1	12 (0.0)	83-97	6	0.555
BMS510	VIC	1	28 (22.1)	91-95	4	0.622
BMS527	6-FAM	1	1 (55.9)	159-177	8	0.692
BMS528	6-FAM	83	10 (19.0)	140-152	5	0.694
BMS601	6-FAM	81	19 (99.5)	172-180	5	0.682
BMS812	NED	86	15 (68.8)	90-122	7	0.697

TABLE 2 CONTINUED

Locus	Label[a]	Multiplex	Chromosome (Position)[b]	R_A	N_A	PIC
BMS911	HEX	81	X (136.2)	100-114	6	-
BMS941	NED	83	17 (30.1)	81-85	3	0.370
HUJ246	NED	80	3 (67.9)	242-264	7	0.637
IL4	6-FAM	84	7 (30.5)	83-105	10	0.804
ILSTS102	NED	85	25 (6.5)	113-153	6	0.645
INRA037	6-FAM	81	10 (69.9)	118-132	6	0.642
INRA133	HEX	82	6 (8.2)	223-240	6	0.444
INRA189	NED	82	Y[c]	96-100	2	-
INRA194	HEX	86	22 (21.8)	144-160	5	0.493
RM372	VIC	1	8 (19.1)	114-138	8	0.770
TGLA122	NED	82	21 (67.3)	136-150	6	0.635
TGLA44	NED	84	2 (0.8)	149-159	6	0.689
TGLA53	6-FAM	86	16 (40.3)	132-142	6	0.608
URB011	6-FAM	URB011	29 (55.6)	139-155	8	0.714
				Range	**2-11**	**0.303-0.820**
				Average	**6.48**	**0.629**
				Std Dev	**2.03**	**0.122**

R_A, called allele size range; N_A, total number of alleles found in this study; PIC, polymorphic information content (Botstein *et al.* 1980); std dev, standard deviation; [a], fluorescent dye label for forward primer; [b], chromosomal positions (cM) as reported in the USDA cattle gene mapping database; [c], in non-pseudoautosomal region (Liu *et al.* 2002)

multiplex 84): 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. The following cycling parameters were used for multiplex 84: 96°C 3 minutes; 2 cycles of 96°C 20 s, 58°C 30 s (-1°C/cycle), 65°C 90 s; 28 cycles of 96°C 20 s, 56°C 30 s, 65°C 90 s; 1 cycle of 96°C 60 s, 56°C 60 s, 65°C 20 min. Multiplex 84 and URB011 were co-loaded into a single injection, as were multiplexes 1 and 2.

Fragment analysis

PCR products were separated on an ABI 310, 377, or 3100 Genetic Analyzer (PE Biosystems) using an internal size standard (Mapmarker LOW, Bioventures, Inc., Murfreesboro, Tennessee). Multiple samples we analyzed on all 3 systems to standardize allele calling, and approximate fragment sizes and called allele sizes are shown in Appendix A. In general, the ABI 310 sized fragments 1 – 1.5 bases below the ABI 377, while the ABI 3100 sized fragments about 0.5 bases below the ABI 310. The reverse primers for BMS1172, BMS410, and BMS527 were 5'-tailed with a viral DNA sequence (GTGTCTT; Brownstein *et al.* 1996) partway through this project to either provide cleaner fragments or prevent problematic overlapping with multiplexed markers, as indicated in Appendix A.

Genotyper 3.6 (PE Biosystems) was used for allele identification and comparison. Fragment sizes and called allele sizes, along with other pertinent information on individual samples, were maintained using an Access 2002 database (Microsoft®).

Data analysis

Polymorphic information content values (PIC; Botstein *et al.* 1980) were calculated over the entire data set using the program Cervus 2.0 (Marshall *et al.* 1998). Tests of Hardy-Weinberg equilibrium (HWE) using an unbiased estimate of the exact probability and the Markov chain method (Guo and Thompson 1992) were performed using the program GENEPOP 3.1d (Raymond and Rousset 1995) for each autosomal

locus in each population. When the null hypothesis of HWE was rejected ($p < 0.01$), score tests (U-tests; Rousset and Raymond 1995) of heterozygote excess and deficiency were performed in GENEPOP to determine the direction of deviation. For all HWE tests, the Markov chain procedures were as follows: 10,000 step dememorization, 150 batches, and 50,000 iterations per batch. Allele frequencies, number of alleles, allelic richness (El Mousadik and Petit 1996), observed heterozygosity, and expected heterozygosity (Nei 1987) were calculated for each locus in each population using the program FSTAT 2.9.3.2 (Goudet 1995, 2001). Allelic richness is a measure of the total number of alleles at a locus in a population independent of sample size, allowing for more valid comparisons between populations. Expected heterozygosity, or unbiased gene diversity, is based only on allele frequencies and is therefore a more robust statistic for comparisons between populations than observed heterozygosity, which is dependent on effective population size.

Overall and pairwise population differences in allelic richness and expected heterozygosity were performed using ANOVA ($p < 0.01$ considered statistically significant) and the Bonferroni multiple comparison procedure (using a 99% confidence interval), respectively, in the statistical package Analyse-it 1.68 (Analyse-it Software, Ltd., Leeds, England). Correlations were assessed using the Pearson correlation test within the same software package.

The contribution of each population to overall allelic richness and gene diversity (heterozygosity) was measured following the equations outlined in Petit *et al.* (1998). This method uses both diversity within and differentiation among populations to

establish the contribution of a particular population to overall genetic diversity. In calculating the contribution of a particular population to overall allelic richness, this method weights rare and private alleles and is not dependent on sample size. All necessary calculations were performed using FSTAT. Overall gene diversity was estimated using h_T', which is corrected for sample size. Gene diversity per population was measured using the unbiased estimator of Nei (1987), and corresponds to expected heterozygosity as discussed above.

Genetic differentiation and distance values between populations were calculated to help elucidate genetic relationships among populations. Pairwise F_{ST} values as a measure of genetic differentiation (Weir and Cockerham 1984) between populations were calculated in FSTAT. Two measures of genetic distance, D_S (Nei 1972) and $(\delta\mu)^2$ (Goldstein *et al.* 1995) were calculated using POPDIST 1.1.1 (available online at http://www.biology.ualberta.ca/jbrzusto/GeneDist.html). D_S is widely used for many types of genetic data and is based on the infinite alleles model of evolution, while $(\delta\mu)^2$ was designed specifically for use on microsatellite loci, is based on the stepwise mutation model of evolution, and is not sensitive to fluctuations in population size (Goldstein *et al.* 1995; Goldstein and Schlötterer 1999; Nei and Kumar 2000). The latter, however, has the notable disadvantage of high sampling variances (Nei and Kumar 2000). From the genetic distance data, unrooted trees were created using the neighbor-joining method (Saitou and Nei 1987) in PHYLIP 3.7 (Felsenstein 1993) with randomized input order of taxa. The neighbor-joining method is commonly employed

for creating trees from distance matrix data, does not assume equal rates of evolution across loci (molecular clock), and minimizes overall tree length (Weir 1996).

Results

Sampling

A total of 2,169 samples were genotyped to > 90% completion such that 5 or fewer loci were missing genotypes for any given individual, not including the Y-chromosome marker INRA189. Each individual marker was genotyped to > 90% completion in each population. The total number of samples genotyped for each population and approximate population census sizes are shown in Table 3.

It was not possible to meet the initial sampling goal of a 20% minimum of the census population size in every case. Samples were not obtained from SH. Furthermore, a sub par sampling of approximately 6.5%, 5.8%, and 16.3% of the census population size was obtained from GT, WM, YNP, respectively. In the case of GT and YNP, the absence of working facilities greatly inhibited necessary sampling. The GT samples were all from bison radiocollared, trapped, and tested for other scientific studies, while approximately 92% of the YNP samples were from winter collections of bison leaving park boundaries (all others from radiocollared bison used for other studies). Approximately 30% of the total number of bison from the 10 sampled federal herds were genotyped (2169/6940; Table 3).

TABLE 3

Total number of bison sampled for 54 polymorphic loci by population and sex

Population	Census[a]	Total	Males	Females
BNP	875	312	119	193
FN	379	167	83	84
GT	600	39[b]	10	29
NBR	350	152[b]	83	69
NS	63	49	20	29
TRN	312	270	115	155
TRS	371	324	120	204
TSBH	40	40	19	21
WC	350	293	117	176
WM	600	35[b]	0	35
YNP	3000	488[b]	214	274
Sum	**6940**	**2169**	**900**	**1269**

See Table 1 for population abbreviations. [a], current approximate census population size, as estimated by individual herd managers; when possible, estimates are given of total census population size at time of collection for this study; [b], sex unknown at time of collection and determined by X- and Y-chromosome microsatellite markers for the following samples: GT (39 total), NBR (46 total), WM (35 total), and YNP (11 total)

General comparisons of genetic diversity

The number of alleles per locus across all 54 nuclear markers ranged from 2 (INRA189) to 11 (BMS1862 and BMS2258), with an average of 6.48 ± 2.03 *SD* alleles/locus (Table 2). For the 51 autosomal markers, PIC values ranged from 0.303 (BM47) to 0.820 (BMS1857) with an average of 0.629 ± 0.122 (Table 2).

A total of 350 alleles were detected in the 54 polymorphic nuclear microsatellites utilized in this study. The total number of alleles in each population was used to

calculate the percentage of total allelic diversity present within each population, which ranged from 39.1% in the TSBH to 76.9% in the NBR population (Table 4).

Appendix B details allelic frequencies for all 54 loci. A total of 36 private alleles from 24 loci were distributed as follows: WC 10 alleles; NBR 9 alleles; YNP, TSBH, and WM each with 4 alleles; BNP 3 alleles; TRS and FN each with 1 allele. BMS2258 had the most number of private alleles of any locus with 4 total (1 BNP, 2 WC, 1 WM). A total of 10 fixed loci, not including INRA189 on the Y-chromosome, were distributed as follows: GT 1 locus; TRN 2 loci; TSBH 7 loci. Total number of alleles (N_A), allelic richness (A_R), observed heterozygosity (H_O), and expected heterozygosity (H_E) for each locus in each population are detailed in Appendix C. A_R, H_O, and H_E were calculated from females only for the X-chromosome markers BM6017 and BMS911. Table 4 summarizes the average values for these statistics and total numbers of private alleles and fixed loci in each population.

The correlation between A_R and H_E was tested for all autosomal and X-chromosome loci (53), excluding population-locus pairs with fixed alleles (10), for 573 total population-locus pairs. Figure 1 illustrates the correlation between these two statistics ($r = 0.73 \pm 0.05$ *SD*). The null hypothesis of no correlation was rejected using a two-tailed t-test ($p < 0.0001$). H_E tends to have more intrinsic variation with lower A_R values in this data set, as indicated by the flared shape of the observations in Figure 1. These results compare well with published results on the utility of allelic richness for isozyme data, where a correlation of $r = 0.77$ was observed between allelic richness and heterozygosity (Petit *et al.* 1998).

TABLE 4

Summary statistics for 54 nuclear polymorphic loci across 11 bison populations

	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
N_A	4.50	4.37	4.06	4.98	4.78	3.52	4.24	2.54	4.80	4.25	4.83
% total alleles	69.4	67.4	62.6	76.9	73.7	54.3	65.4	39.1	74.0	64.3	74.6
A_R	4.11	4.06	3.96	4.60	4.60	3.26	3.99	2.52	4.49	4.21	4.44
H_O	0.565	0.576	0.531	0.639	0.605	0.517	0.564	0.371	0.654	0.580	0.615
H_E	0.574	0.590	0.560	0.647	0.631	0.513	0.574	0.373	0.653	0.599	0.627
Private Alleles	3	1	0	9	0	0	1	4	10	4	4
Fixed Loci	0	0	1	0	0	2	0	7	0	0	0

See Table 1 for population abbreviations. N_A, average number of alleles per locus; % total alleles based on 350 total across 54 loci; A_R, average allelic richness; H_O, average observed heterozygosity; H_E, average expected heterozygosity; number of fixed loci excludes INRA189 (Y-chromosome)

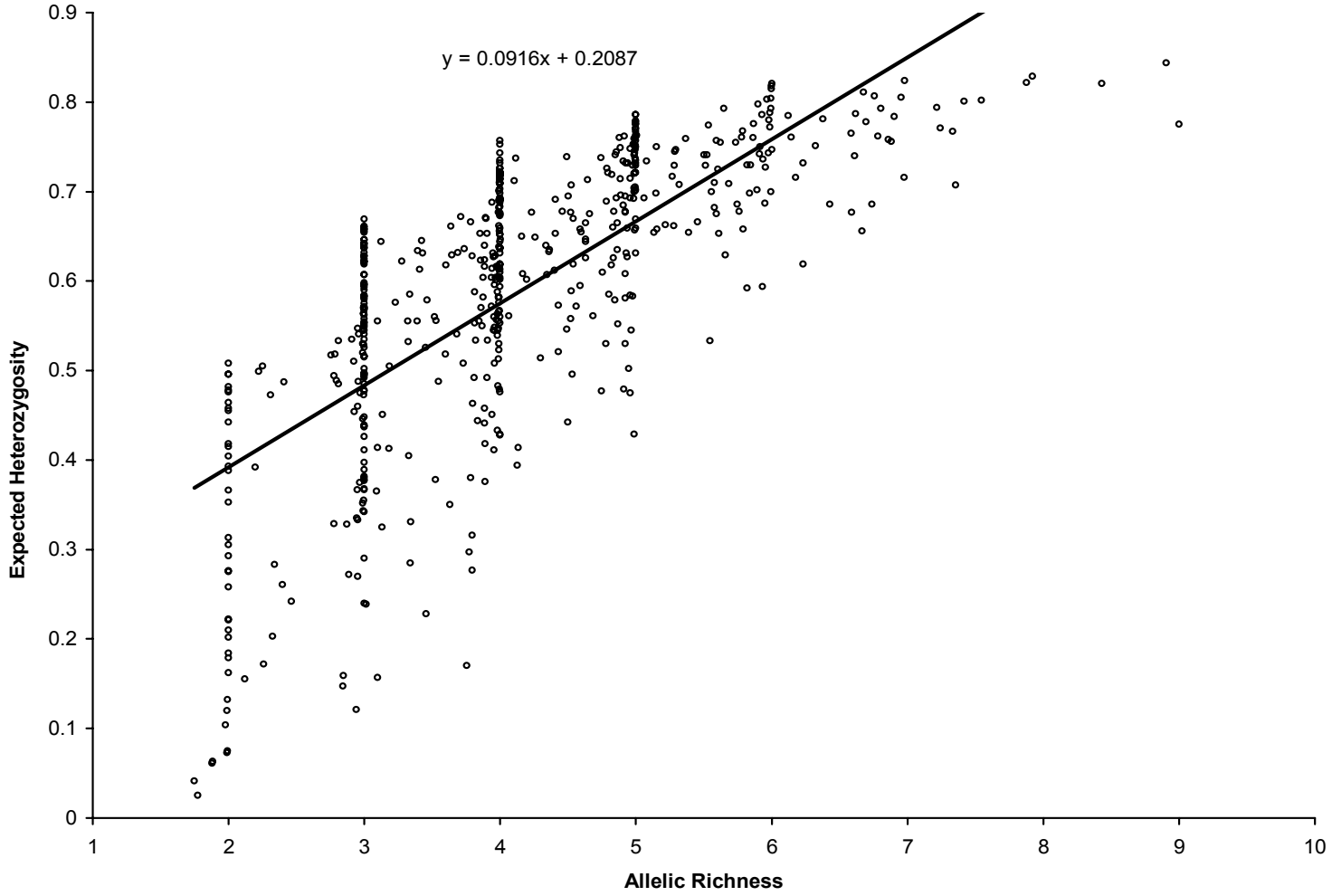

Figure 1. —Correlation between allelic richness and expected heterozygosity.

Average A_R and H_E were used to compare populations due to the relative insensitivity of these statistics to sample sizes (Figure 2). As expected, the two measures were positively correlated among populations. NBR and NS had the highest average A_R (4.60), while WC had the highest average H_E (65.3%). TSBH scored the lowest in both categories (2.52 and 37.3%, respectively). Overall differences in A_R and H_E were significant, with significant differences noted between the following population pairs for A_R: TSBH vs. all populations except TRN; TRN/NBR; TRN/NS; TRN/WC; TRN/WM; TRN/YNP. Similarly, significant differences were indicated between the following populations for H_E: TSBH vs. all populations; TRN/NBR; TRN/NS; TRN/WC. After excluding the TSBH from ANOVA testing, overall differences among the 10 federal bison populations for both A_R and H_E were still significant.

HWE testing

Of the 561 (51 autosomal loci, 11 populations) possible tests of HWE, 552 were performed (excluding 9 monomorphic population-locus pairs). A total of 37 tests (6.7%) rejected the null hypothesis of HWE ($p < 0.01$). BMS601 and INRA133 consistently failed the HWE test and heterozygote deficiency score test over multiple populations (10 and 7, respectively), indicating the presence of null alleles. These 2 loci were omitted from further analysis to prevent bias, bringing the total number of nuclear autosomal markers to 49 (52 total including X- and Y-chromosome markers). The overall rate of rejection when excluding BMS601 and INRA133 of 3.8% ([37 rejected tests – 17 rejected tests of BMS601 and INRA133]/[552 tests – 22 tests of BMS601 and

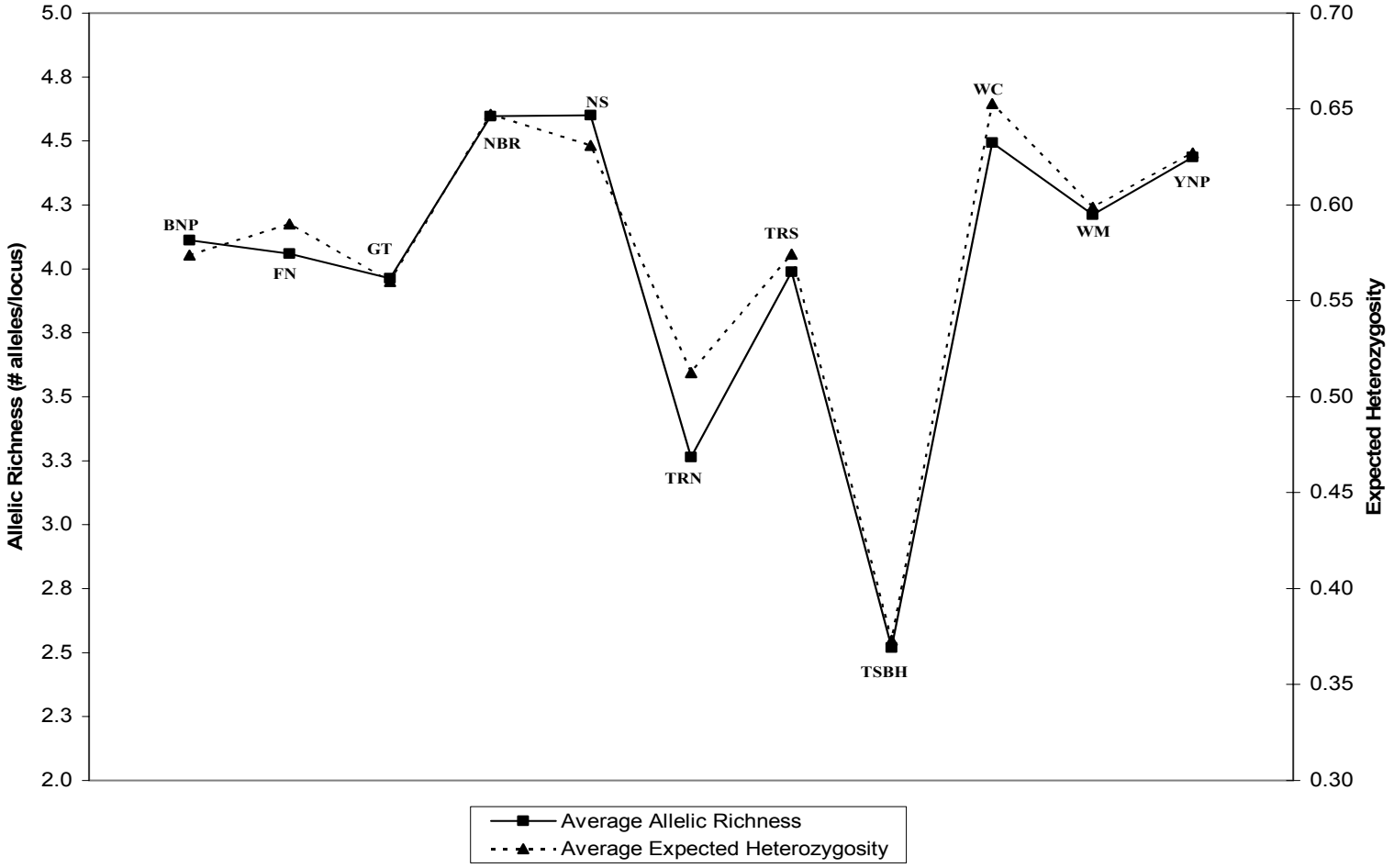

Figure 2. —Comparison of average allelic richness and expected heterozygosity among 11 bison populations. See Table 1 for population abbreviations.

INRA133]) is expected given the large number of tests performed and is comparable to reported HWE deviations in other bison microsatellite studies (Wilson and Strobeck 1999; Schnabel *et al.* 2000).

BNP had the highest rate of heterozygote deficiency of any population, not including BMS601 and INRA133, at 12.2 % (6/49 loci). TRS had the next highest heterozygote deficiency rate at 6.1% (3/49 loci), while all remaining populations had rejection ranges for these tests of 0.0 – 4.1%.

Relative contribution of each population to genetic diversity

The contribution of individual populations to overall genetic diversity was examined across all 10 federal bison populations using 49 autosomal microsatellites. The relative contributions of each population for both allelic richness (C^r_T) and gene diversity (C_T) are shown in Table 5 (individual scores multiplied by 100 for simplification). Each measure is further divided into diversity and differentiation components (C^r_S, C^r_D and C_S, C_D, respectively). The diversity component measures the effect of within-population diversity while the differentiation component measures the level of differentiation of a particular population (*k*) versus all other populations. Negative values indicate a below-average contribution to genetic variation. The additive effects of the 2 subcomponents produce the observed contributions for each measure.

Figure 3 illustrates the relative contribution of each of the tested federal bison populations to overall gene diversity. Only 4 populations made positive contributions to overall gene diversity: NBR, WC, YNP, and WM (in descending order of C_T). NBR,

WC, and YNP contributed positively to overall gene diversity due to high within-population diversity, while WM was actually below average for within-population diversity but highly differentiated from other populations. The remaining 6 populations did not contribute to overall gene diversity. Measurement of population contribution to total allelic richness produced similar results (Figure 4). The same 4 populations had positive contributions to overall gene diversity in a slightly different order: YNP, WC, NBR, and WM (in descending order of C^r_T). NBR, WC, and YNP contributed positively to overall allelic richness due to strong divergence from other populations, while WM showed positive divergence but below average within-population diversity.

Genetic relationships among populations

Pairwise F_{ST} values between populations, as calculated on 49 autosomal loci, are shown in Table 6. Using this measure, the most genetically similar population pairs were FN/NS (F_{ST} = 0.0242) and TRS/BNP (F_{ST} = 0.0397), while those most distinct were TRN/TSBH (F_{ST} = 0.3513) followed by GT/TSBH (F_{ST} = 0.3472). D_S and $(\delta\mu)^2$ genetic distance measures between populations, also calculated on 49 autosomal loci, are shown in Table 7. Based on genetic distance measures, the most closely related populations are FN/NS (D_S = 0.05; $(\delta\mu)^2$ = 0.11), while the most distantly related are TRN/TSBH. (D_S = 0.683; $(\delta\mu)^2$ = 2.297). Figure 5 illustrates neighbor-joining trees based on each of the genetic distance measures.

TABLE 5

Contributions of federal bison populations to overall gene diversity and allelic richness

Population	h_k	C_T	C_S	C_D	A_R	C^r_T	C^r_S	C^r_D
BNP	0.5808	-0.7958	-0.3239	-0.4719	4.2335	-0.9703	-0.0801	-0.8902
FN	0.5976	-0.6229	-0.0523	-0.5706	4.1908	-0.4861	-0.1676	-0.3184
GT	0.5652	-0.3398	-0.5779	0.2381	4.0704	-0.0739	-0.4144	0.3405
NBR	0.6443	1.0850	0.7057	0.3792	4.6747	2.2993	0.8241	1.4752
NS	0.6323	-0.3964	0.5120	-0.9084	4.7291	-0.3933	0.9356	-1.3289
TRN	0.5205	-0.9508	-1.3025	0.3517	3.3540	-1.2657	-1.8828	0.6171
TRS	0.5840	-0.6557	-0.2716	-0.3841	4.1311	-0.9324	-0.2900	-0.6425
WC	0.6564	1.0790	0.9035	0.1756	4.5925	3.9047	0.6557	3.2491
WM	0.5930	0.7750	-0.1262	0.9012	4.2319	0.5373	-0.0835	0.6208
YNP	0.6342	0.7958	0.5418	0.2540	4.5180	4.5547	0.5030	4.0517

See Table 1 for population abbreviations. h_k, gene diversity; C_T, relative contribution to overall gene diversity; C_S, component of C_T due to within-population diversity; C_D, component of C_T due to differentiation between population *k* and all other populations; A_R, allelic richness; C^r_T, relative contribution to overall allelic richness; C^r_S, component of C^r_T due to within-population diversity; C^r_D, component of C^r_T due to differentiation between population *k* and all other populations

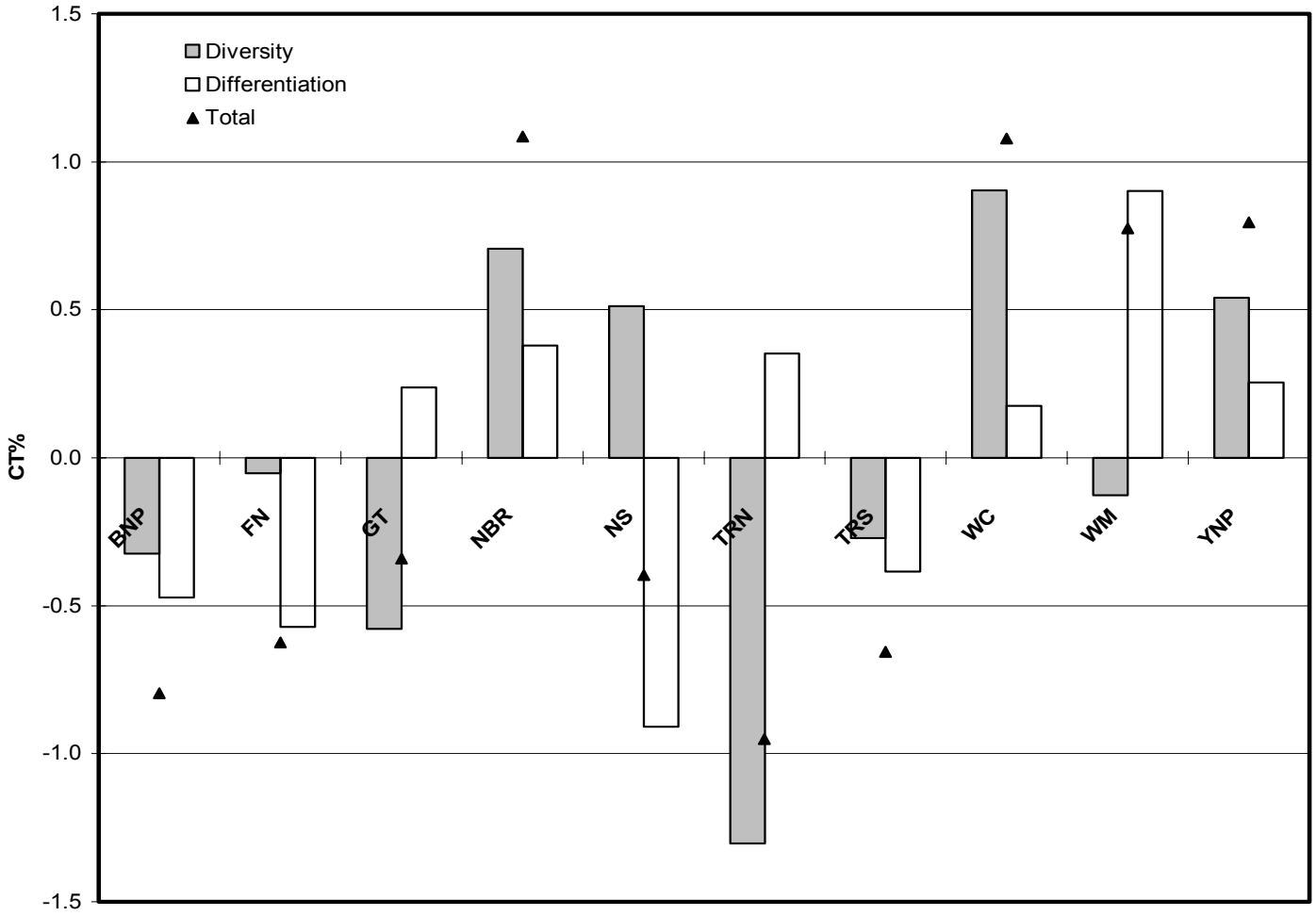

Figure 3. —Relative contribution of individual bison populations to overall gene diversity (heterozygosity), subdivided into diversity and differentiation components. Overall contribution to gene diversity (CT%) is indicated by solid triangles.

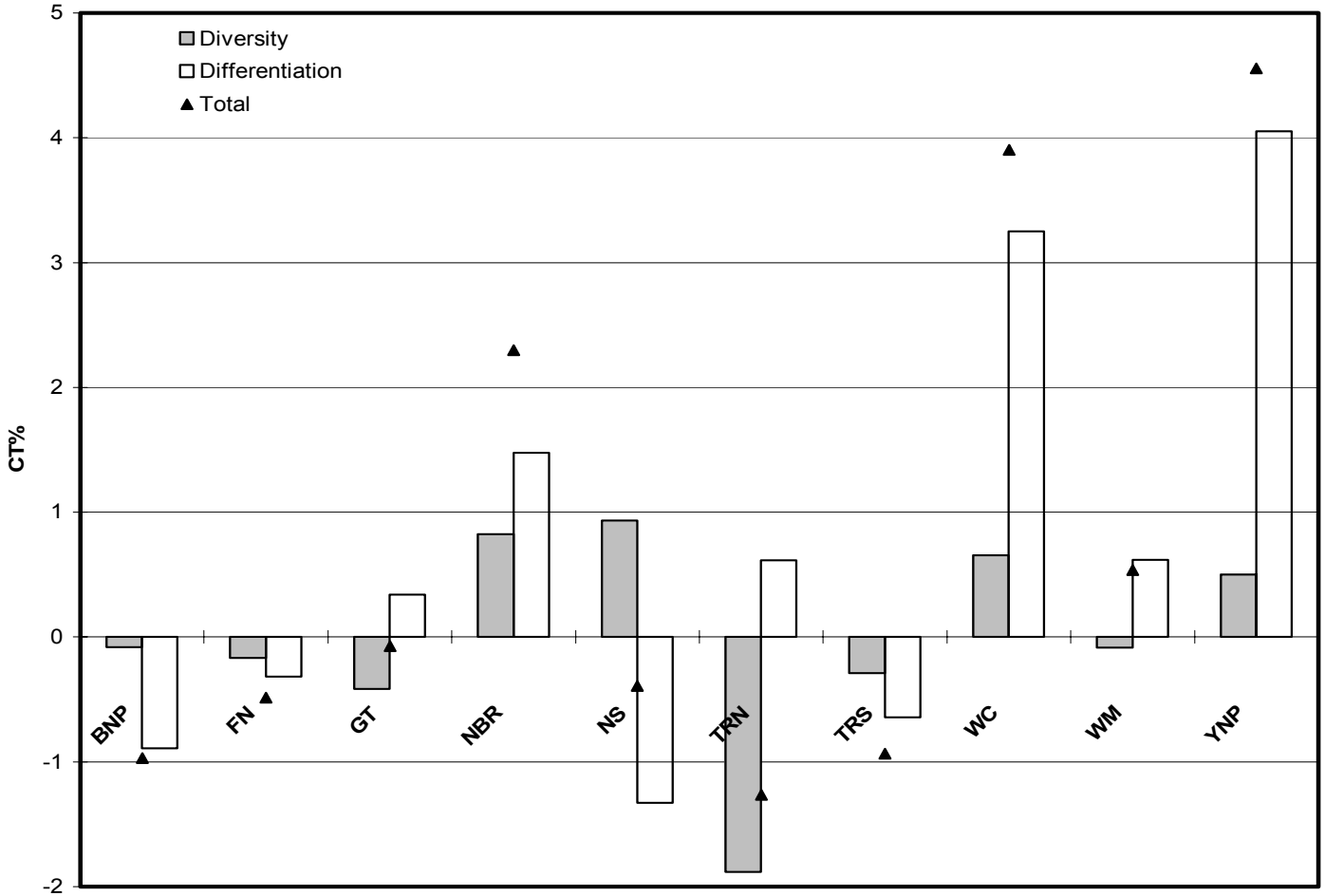

Figure 4. —Relative contribution of individual bison populations to overall allelic richness, subdivided into diversity and differentiation components. Overall contribution to allelic richness (CT%) is indicated by solid triangles.

TABLE 6

Pairwise F_{ST} values among 11 bison populations

	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM
FN	0.0459	-								
GT	0.1235	0.1161	-							
NBR	0.1505	0.1398	0.1361	-						
NS	0.0667	0.0242	0.1114	0.0982	-					
TRN	0.0702	0.0991	0.1457	0.1909	0.1130	-				
TRS	0.0397	0.0631	0.1123	0.1487	0.0767	0.0703	-			
TSBH	0.3103	0.3018	0.3472	0.2859	0.2949	0.3513	0.3070	-		
WC	0.1375	0.1290	0.1418	0.0960	0.0961	0.1645	0.1401	0.2602	-	
WM	0.1609	0.1495	0.1924	0.1339	0.1053	0.2266	0.1676	0.3285	0.1119	-
YNP	0.1464	0.1360	0.1022	0.0976	0.0990	0.1851	0.1501	0.2347	0.0855	0.1372

See Table 1 for population abbreviations. F_{ST} values based on 49 autosomal loci.

TABLE 7

D_S and $(\delta\mu)^2$ genetic distances among 11 bison populations

	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
BNP	-	0.074	0.222	0.322	0.120	0.097	0.062	0.651	0.296	0.326	0.312
FN	0.163	-	0.216	0.312	0.050	0.147	0.105	0.622	0.292	0.313	0.298
GT	0.588	0.496	-	0.296	0.223	0.226	0.200	0.643	0.325	0.415	0.204
NBR	0.570	0.560	0.597	-	0.227	0.377	0.320	0.634	0.225	0.310	0.216
NS	0.259	0.110	0.569	0.419	-	0.176	0.141	0.557	0.228	0.226	0.221
TRN	0.406	0.514	0.631	0.758	0.505	-	0.098	0.683	0.321	0.437	0.378
TRS	0.147	0.211	0.481	0.559	0.295	0.272	-	0.645	0.307	0.348	0.325
TSBH	1.673	1.534	1.319	1.658	1.498	2.297	1.650	-	0.576	0.601	0.458
WC	0.556	0.569	0.664	0.594	0.498	0.64	0.588	1.886	-	0.256	0.188
WM	0.604	0.597	0.829	0.69	0.471	0.927	0.682	1.335	0.411	-	0.312
YNP	0.601	0.588	0.383	0.396	0.481	0.881	0.650	1.131	0.375	0.513	-

See Table 1 for population abbreviations. D_S above diagonal; $(\delta\mu)^2$ below diagonal

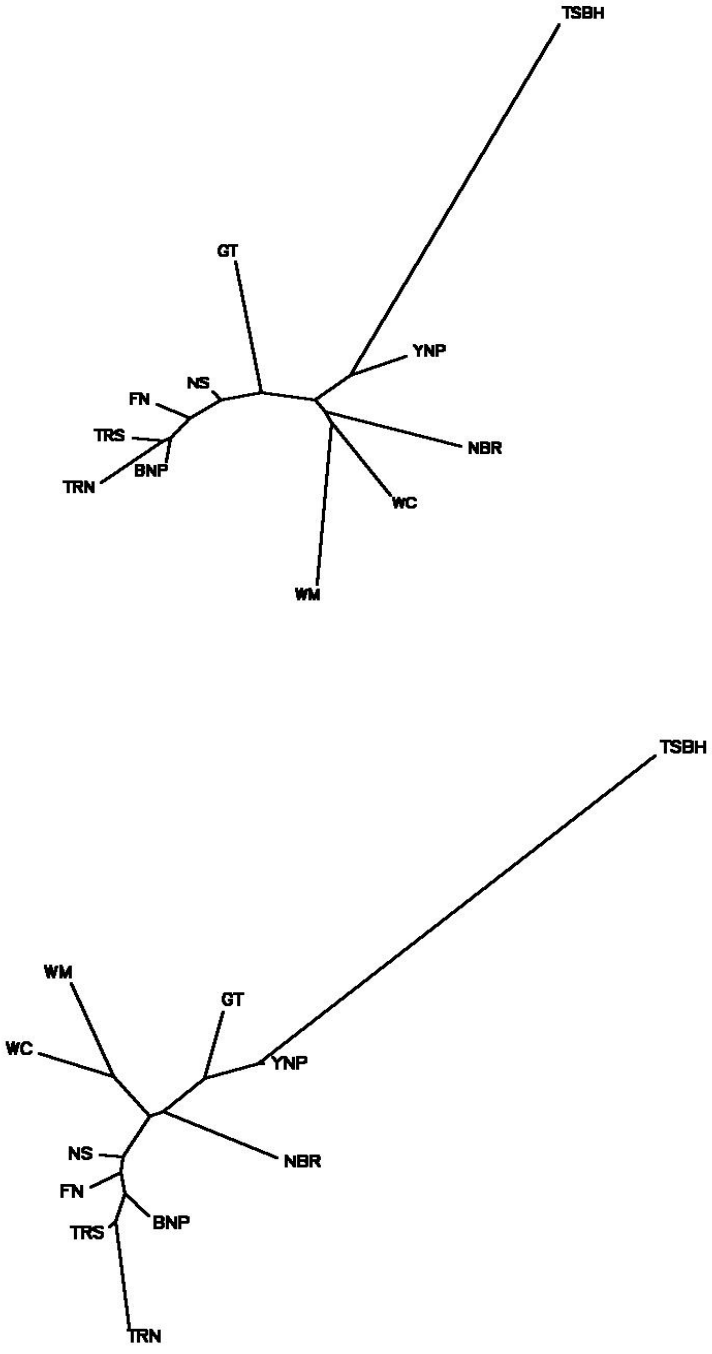

Figure 5. —Neighbor-joining tree diagrams for D_S (top) and $(\delta\mu)^2$ (bottom) distance measures

Discussion

Levels of overall bison genetic variation and heterozygosity

The levels of bison genetic variation and heterozygosity described here are similar to those reported previously in bison using microsatellite markers. Not including the TSBH, which clearly has significantly lower levels of genetic variation and heterozygosity compared to the other tested populations (see below), allelic richness ranged from 3.26 – 4.60 while expected heterozygosity ranged from 51.3 – 65.3% in the federal bison populations. Similarly, Wilson and Strobeck (1999) reported 3.18 – 6.55 alleles/locus (uncorrected for sample size) and average observed heterozygosity ranging from 29.5 – 66.9% in a microsatellite survey of 11 loci across 11 public bison populations. Schnabel *et al.* (2000) also reported average expected heterozygosity across 15 of the same markers utilized in this study in 16 public and private bison populations of 41.6 – 69.8%.

Compared with domestic cattle, bison tend to have somewhat less genetic variation and lower average heterozygosity (Ritz *et al.* 1996; Mommens *et al.* 1998; Schnabel *et al.* 2000), most likely due to differences in management techniques between the two species, the dramatic bison census size bottleneck around 120 years ago, and the maintenance of extant bison in relatively small, fragmented populations. However, the bison populations examined in this study have substantially greater nuclear genetic variation than reported in other post-bottleneck mammalian species (e.g. O'Brien *et al.* 1983; Hoelzel *et al.* 1993; Wisely *et al.* 2002).

Intrinsic population growth rates and the duration and severity of bottleneck events are known to affect the predicted rate of loss of rare alleles (Nei *et al.* 1975). In bison, a combination of these factors likely led to a moderate loss of genetic variation compared with other post-bottleneck species. For instance, the duration of the population bottleneck apex was relatively short, lasting only 20 – 30 years. Following the census bottleneck, the total number of bison increased rapidly from a conservative estimate of 800 bison in 1888 to more than 8,000 bison by 1920 (see Chapter I). Furthermore, the selection of bison used in the 5 foundation herds (Chapter I) from Texas to Canada and across the Great Plains region at the apex of the bottleneck – effectively sampling a large portion of the historic range – may have inadvertently captured a large portion of pre-bottleneck genetic variation. Direct support of this hypothesis would be possible through genetic analysis of bison remains dating to pre-1880 in a manner similar to that used in this study.

Evidence of non-random mating

Although most population-locus combinations did not indicate a significant departure from HWE, heterozygote deficiency was observed in 12.2% of the tested loci at BNP. This level of rejection is at least twice that observed in the other tested bison populations, and is somewhat unexpected given the relatively large census population size (~875 bison). McClenaghan *et al.* (1990) found similar results in BNP bison with a survey of 24 blood protein loci. Only 1 of the 24 loci was polymorphic, and when the population was subdivided by origin (see Chapter I), the sampled portion from TR

indicated a deficiency of heterozygotes. Furthermore, at least 2 of the other tested monomorphic loci were known to be polymorphic in other federal bison populations (NBR/WM; McClenaghan *et al.* 1990).

These results indicate that for at least some loci, significant deviations from HWE due to an overabundance of homozygotes exist in the BNP bison population. Both population subdivision and inbreeding could account for these results, and further investigation is necessary to reveal the source of the observed heterozygote deficiencies.

Comparisons of genetic diversity among federal bison populations

Clear differences exist among federal bison populations in overall A_R and H_E. Among the federal populations, TRN has significantly lower levels of genetic variation than other tested populations. Comparisons of the total number of alleles present in individual populations indicates NBR, YNP, NS, and WC (in descending order) have the highest overall allelic diversity, while TRN has the lowest.

Analyses of the contribution of individual populations to overall allelic richness and gene diversity reveal 4 of the 10 federal populations contribute the most to these measures: NBR, WC, WM, and YNP (Table 5). Of these, WM had below-average within-population diversity components for both gene diversity and allelic richness (Figure 3, 4). The WM bison population, while genetically divergent from other tested populations, does not have appreciable levels of within-population diversity that contribute to the overall genetic diversity of federal bison populations. The notable high contribution of the divergence subcomponent to overall allelic richness (Figure 4) in

NBR, WC, WM, and YNP correlates with these populations having the most number of private alleles (Table 4). These results are consistent with the design of the tests to weight rare and private alleles in calculating overall contribution of individual populations to allelic richness (Petit *et al.* 1998).

Although the NS population has high levels of overall allelic diversity, the recent origin of the NS population from other federal populations prohibits a positive contribution of NS to overall allelic richness and gene diversity. This is clearly illustrated in Figures 3 and 4, where NS displays positive within-population diversity subcomponents but below-average differentiation subcomponents for both measures. This is an important point, since average allelic richness and expected heterozygosity (Table 4, 5) alone would have placed NS within the top 2 or 3 populations for these measures. When making decisions on primary targets for conservation efforts, preference should obviously be given to source populations of genetic diversity.

Each of the 6 populations that did not contribute positively to overall gene diversity and allelic richness are at least partially from the same founding stock (BNP, FN, GT, NS, TRN, TRS). FN bison were used as founding stock for the TRS and NS populations, while TRS bison were used as founders for the TRN and BNP populations and as supplements to the GT bison population (Table 1). The genetic similarity of these populations is clear with the exception of GT as a possible outlier of the group (Figure 5; see discussion below). The similarity of these 6 populations has a direct influence on the genetic contribution of each to the overall genetic diversity of federal bison populations.

The insignificant contribution of the BNP bison population to overall genetic diversity is somewhat unexpected, since BNP was the only federal population supplemented with bison from the Colorado National Monument (Table 1). It is possible that the bison used to supplement BNP were not significantly different from other bison in federal populations, such that the addition of these bison may have changed the genetic constitution of the BNP population but not the contribution of this population to overall genetic diversity. Furthermore, these supplemental bison may have produced few offspring, thereby contributing minimally to the germplasm of the BNP population. Lastly, non-random sampling may have precluded the detection of diversity due to the introduced bison, especially if the "introduced" group acts as a subpopulation with little interaction with the "resident" group. The 2002 roundup, from which the tested samples were obtained, included only 496 of the estimated 875 bison (~56%) in the entire BNP population. Of these, 312 were used in this study. Although the goal of testing 20% of the census population size was clearly exceeded (~35% genotyped), population substructure may have prohibited a truly random sampling from this population during roundup. The genetic similarity between the tested bison from BNP and TRS, as indicated by relatively low F_{ST}, D_S, and $(\delta\mu)^2$ estimates (Table 6, 7) is further evidence that one or more of the above-mentioned scenarios has probably occurred. In fact, the F_{ST} estimate between BNP and TRS (0.0397) is lower than that between the two TR populations (0.0703). Further testing of additional BNP bison and analysis of potential population substructure is necessary to resolve this issue.

The TRN population was founded exclusively from TRS in 1962, just 6 years after the TRS population was created from FN bison (Table 1). Although genetically similar based on F_{ST} and distance measures (Table 6, 7), there are several notable difference between the two populations. A single private allele (BM2113, allele 151) is found in the TRS population that is not found in any other tested population. This private allele is most likely due to a new mutation, as it occurs at a low frequency and is only 2 base pairs different from a fairly frequent allele (allele 153) shared among all 6 populations related to FN and NBR (Appendix B). Further, 2 loci are fixed in the TRN population, but polymorphic in the TRS population (BMS1001 and BMS941; Appendix B). In both cases, the most frequent allele in the TRS population is that which is fixed in the TRN population. Furthermore, the TRN population has lower average allelic richness and expected heterozygosity compared with the TRS population (Figure 2). Although the TR populations have been managed similarly, they have been maintained at different census numbers. Until 1998, the south unit consistently maintained a 2 – 3 fold larger population (~250 – 400) than the north unit (~100 – 250). The most likely source of the noted differences in fixed loci, allelic richness, and heterozygosity between the populations is random genetic drift, which tends to eliminate rare alleles and decrease overall heterozygosity, especially in small populations. Because no other introductions have been made into either TR population since their inception, and since both are under the same management plan, one logical management alternative would be the movement of bison from the TRS to the TRN population to increase genetic diversity and heterozygosity in the latter.

Genetic relationships among bison populations

The genetic relationships among the tested bison populations as determined through F_{ST}, D_S, and $(\delta\mu)^2$ measures were, in general, consistent with the known histories of the populations. The neighbor-joining trees shown in Figure 5 for D_S and $(\delta\mu)^2$ are mostly identical in the placement and grouping of populations. Small differences exist in the placement of GT and NBR (see below).

The nearest neighbor of the TSBH is YNP, as might be predicted due to the introduction of 3 bison from the original Goodnight herd into YNP in 1902. One Goodnight bull was also sent to the NBR in 1908, but either the contribution of this single bull was insignificant in the overall genetic constitution of the NBR bison population, or the admixture of many other sources of bison into the NBR has obscured the contribution (Table 1). The TSBH is located on the longest branch of either tree, an effect of the amount of genetic differentiation between the TSBH and even the most closely related federal bison populations. The last 120 years of chronic small population size have driven genetic drift – and likely inbreeding (see Chapter IV) – in the TSBH. Consequently, the TSBH has significantly lower levels of heterozygosity and allelic variation compared with other tested populations and the highest number of fixed loci (7/53 = 13.2%; Table 4). If true genetic differences once existed between the southern and northern plains bison herds, and the only pure remnant of the southern plains bison is contained in the TSBH, the observed long branch may also be the effect of differences in initial genetic composition of the TSBH compared with the federal populations (see

Chapter I). Some support for this suggestion comes from the 4 private alleles found in the TSBH population. However, the TSBH is known to contain domestic cattle introgression dating back to the experiments of Charles Goodnight in the late 1800s and early 1900s (see Chapter IV) and the observed private alleles may also due to this historic introgression.

The closest genetic neighbor of YNP is WC ($D_S = 0.188$, $(\delta\mu)^2 = 0.375$) followed by GT ($D_S = 0.204$, $(\delta\mu)^2 = 0.383$). The YNP bison used to supplement the WC herd (Table 1) markedly influenced the genetic constitution of the WC population. Although WC and WM share a common branch in both distance trees, the raw distance measures indicate that YNP has the closest genetic similarity with WC followed by WM ($D_S = 0.256$, $(\delta\mu)^2 = 0.411$). The close genetic relationship of the WC and WM bison populations is consistent with the history of these populations since both were founded with bison from the New York Zoological Park herd. The GT population was founded with bison from YNP, and migration from YNP to GT is known to occur at low rates (Table 1; Steve Cain pers. comm.). The neighbor-joining tree based on $(\delta\mu)^2$ distances is most consistent with recent migration, since the GT population is nearer to YNP than the NBR/WC/WM group.

The placement of the NBR population on the trees is inconsistent, and may result from the multiple unique sources of bison used to supplement the population (Table 1). Again, the $(\delta\mu)^2$ distance tree is most consistent with known history, since the D_S tree actually places NBR on a branch with WC and WM. The only historic ties between NBR and WC are that both have been supplemented with YNP bison (only 2 added to

NBR in 1953), and no known relationship exists between WM and NBR. The $(\delta\mu)^2$ distance tree places the NBR population alone on a branch between the WC/WM and GT/YNP nodes.

The close genetic relationship between NS and FN is corroborated with F_{ST}, D_S, and $(\delta\mu)^2$ estimates. The relationship is consistent with the fact that more FN bison were used as stock for NS than from any other population (17 FN, 8 NBR, 6 WM; Table 1). As such, FN represents approximately 51% of the founders of the NS population, and assuming all introduced bison have contributed equally to breeding, has the most representation in the genetic constitution of the current NS bison population.

The overall topology of the FN/BNP/TRN/TRS group is also consistent with known population histories. As discussed above, FN was used both directly and indirectly as founding stock for all 3 of the other populations. Of the group, TRN has a notable long branch in both trees, most likely due to genetic drift as discussed above.

Although few differences exist between the trees presented in Figure 5, the tree based on $(\delta\mu)^2$ estimates is generally more consistent with historic records from individual populations than the tree produced from D_S estimates. The two distance measures are fundamentally different in their treatment of genetic data. While $(\delta\mu)^2$ is calculated using average squared differences in allele sizes between populations, D_S is dependent on the frequency distributions of shared alleles between populations (Goldstein and Pollock 1997). The theoretical advantage of $(\delta\mu)^2$ for use on microsatellite data is the basis of this measure on the stepwise mutation model, which is

a better fit to the known patterns of microsatellite evolution than the infinite alleles model on which D_S is based (Goldstein *et al.* 1995; Goldstein and Schlötterer 1999).

Concluding remarks

In general, the bison populations represented in this study display a moderately high level of overall genetic variation, especially considering the severe bottleneck sustained only 120 years ago and small number of founders used for each population. There are, however, notable differences in overall allelic variation and heterozygosity and the contribution of each population to overall genetic variation among federal bison populations. The genetic relationships among the populations analyzed in this study are mostly supported by known historic records of genetic sources for individual populations.

This study represents the first in-depth examination of the genetic constitution and relationships of U.S. federal bison populations. As such, the results and conclusions of these data are expected to have a lasting impact on future management decisions of these populations. These data also provide background genetic information for the investigation and analysis of the effect of various management strategies employed within bison populations, as will be further discussed in Chapters IV and V.

CHAPTER III

AN ANALYSIS OF DOMESTIC CATTLE INTROGRESSION IN U. S. FEDERAL BISON POPULATIONS

"When I started to breed cattelo, people ridiculed me; they thought I was crazy. I guess if I had told them what I hoped to do they would have sent me to the insane asylum … Here, in the cattelo is an animal that has not only got the beef which is the finest in the world, but an animal that will furnish furs that you can wrap yourselves in and be comfortable."

— Charles "Buffalo" Jones 1907

Introduction

Natural interspecies hybridization, with or without introgression of genetic material, is known within all biological kingdoms and from the highest orders including plants, fishes, birds, and mammals. From an evolutionary view, species with the ability to naturally hybridize are by necessity closely related. Those species which produce fertile hybrids in either direction of the interspecies cross are more correctly considered conspecific, although the demotion of separate species to subspecies status is often avoided for historical and/or political reasons. An example of this is found in the case of North American bison (*Bison bison*) and European bison (*B. bonasus*), as discussed in Chapter I. Natural hybridization is likely an important evolutionary process (Arnold 1992; Dowling and Secor 1997). Some natural hybrids have higher levels of fitness than the parental taxa, and are therefore likely to take over parental taxa distribution(s) and/or invade new ecological niches (Arnold and Hodges 1995). In this manner, natural

hybrids may lead to new adaptive complexes and eventually new species. The influence of natural hybridization on speciation has not, however, been studied with great detail.

As the impact of humans on wildlife species has become better understood and molecular biology techniques have advanced, human-influenced (anthropogenic) interspecies hybridization has become an ecologically and politically important topic. Both natural and anthropogenic introgression between threatened and introduced species are cause for alarm since widespread introgression can lead to population or species extinction (Rhymer and Simberloff 1996). Furthermore, hybridization as a direct result of anthropogenic activity, especially in "wild" species, is generally discouraged (Simberloff 1996) so as to minimize human impact on the evolution of natural species.

Within mammals, molecular biology techniques have detected interspecies hybridization in primates (Painter *et al.* 1993) and deer (Carr *et al.* 1986; Abernethy 1994) and between wild and domestic cats (Randi *et al.* 2001) and dogs (Wayne and Jenks 1991; Gottelli *et al.* 1994), although hybridization has been observed within other mammalian groups (van Gelder 1977; Rhymer and Simberloff 1996). Within bovids, protein electrophoresis has detected hybridization between banteng (*Bos javanicus*) and domestic cattle (*B. taurus*; Davis *et al.* 1988), while RFLP analyses have indicated hybridization between zebu (*B. indicus*) and domestic cattle (Nijman *et al.* 1999). Hybrids are known to form among nearly all combinations of species from the *Bos* genus (van Gelder 1977).

Although bison and domestic cattle can produce fertile offspring, all reports of hybrids are from human-controlled matings (Jones 1907; Boyd 1908, 1914; Goodnight

1914; Steklenev and Yasinetskaya 1982; Steklenev *et al.* 1986). That is, bison and domestic cattle do not produce hybrids naturally and will mate preferentially with their own species if given opportunity. Human-controlled mating of male bison to female domestic cattle has been recorded extensively, although the reciprocal cross is exceedingly difficult to produce (Boyd 1914; Goodnight 1914; Steklenev and Yasinetskaya 1982). Even in the first case, the birth rate of first-generation hybrid offspring is quite low due to a combination of a high death rate of the birthing mothers in conjunction with fetuses too large to pass and accumulations of perifetal fluids in late pregnancy (Boyd 1908; Steklenev and Yasinetskaya 1982). Male first- and second-generation hybrid progeny are sterile in nearly all cases, as predicted by Haldane's (1922) rule since males are the heterogametic sex, and presumably due to differences in Y-chromosome structure and possibly increases in chromosome aberrations and polyploid cells (Steklenev and Yasinetskaya 1982; Steklenev *et al.* 1986). Alternatively, female hybrid progeny can productively produce fertile offspring, especially when backcrossed to bison (Boyd 1908, 1914; Goodnight 1914; Steklenev and Yasinetskaya 1982).

Polziehn *et al.* (1995) and Ward *et al.* (1999) found evidence of domestic cattle mitochondrial DNA (mtDNA) introgression in several public bison populations, including CSP and NBR. Notably, Ward *et al.* (1999) did not find evidence of domestic cattle mtDNA introgression in bison from 4 other U.S. federal populations (FN, WC, WM, YNP) or in wood or plains bison from Canadian federal parks. The mitochondrial screen developed by Ward *et al.* (1999) involves the co-amplification of a 16S mtDNA

fragment as an internal PCR control and a D-loop mtDNA fragment. The D-loop fragment primers are designed in a conserved region of the domestic cattle mtDNA which is highly degenerate in wild bovids, including bison, such that amplification only occurs when domestic cattle mtDNA is present. Verification of suspect domestic cattle mtDNA in bison and wild bovids is achieved through sequencing and phylogenetic analysis (Ward *et al.* 1999). Support for Y-chromosome domestic cattle introgression in North American bison was not found through analysis of a single Y-chromosome marker (Ward *et al.* 2001). The apparent uniparental introgression from female domestic cattle to bison is corroborated by historical and experimental evidence, as discussed above.

A total of 20 autosomal microsatellites distributed on 12 chromosomes with non-overlapping allele size ranges in bison and domestic cattle have been used to detect domestic cattle nuclear introgression in several public bison populations (Ward 2000). When possible, one or more additional diagnostic microsatellites within ~8 cM of the original locus were used as confirming markers to establish regions of chromosomes introgressed from domestic cattle (Ward 2000). This type of screen has the advantage of eliminating most, if not all, confounding results at single loci due to allelic size homoplasy. The probability of cattle-like alleles in two or more linked loci as the result of two mutation events in a single bison population (i.e. not as the result of introgression) is exceptionally small.

The combination of mitochondrial and nuclear analyses have revealed evidence of domestic cattle introgression in all state-managed bison populations examined, but not in some U.S. and Canadian federal bison herds (Ward *et al.* 1999; Ward 2000). Of the

11 U.S. federal bison populations discussed in Chapter I, 5 have previously been examined for genetic evidence of domestic cattle mitochondrial DNA introgression (FN, NBR, WC, WM, YNP; Ward *et al.* 1999) while only 3 have been examined for evidence of domestic cattle nuclear DNA introgression (FN, NBR, YNP; Ward 2000). In both analyses, no more than 38 bison from any given federal population were examined.

Extensive examination of over 50 private bison herds across the U.S. has revealed domestic cattle mitochondrial and/or nuclear introgression in all except 1 herd using 12 of the nuclear microsatellites described by Ward (2000; James Derr unpublished data). In private herds, the introgression event need not trace back to one of the foundations populations, since in the past several decades bison and cattle have been actively hybridized on private ranches for the production of a healthier meat alternative to pure domestic cattle. The issue of bison "purity" has been contentious in both the private and public sectors. For example, CSP has served as the source for many private ranch bison operations through yearly bison auctions. Although CSP was once heralded as most likely formed from pure bison (Morris 1997), it has since been shown to contain around 20% domestic cattle mitochondrial DNA and evidence of nuclear introgression at several microsatellite markers (Ward *et al.* 1999; Ward 2000).

The apparent success story of the recovery of the bison species in the past 150 years is in jeopardy if domestic cattle introgression is widespread in bison populations. Hybrid species do not have taxonomic status and are not protected by the Endangered Species Act. Widespread hybridization in other mammalian species has lead to proposals to delist such icons as the red wolf and Florida panther as endangered species

(Rhymer and Simberloff 1996). One advantage of the bison situation compared with other examples of interspecies hybridization is that of sheer numbers. Several closed bison populations, such as those maintained by the NPS and USFWS, exist that have not been investigated with genetic markers for detection of domestic cattle introgression. As such, the goal of this study was to comprehensively examine bison from U.S. federal populations for evidence of mtDNA and nuclear DNA domestic cattle introgression using the previously developed methods of Ward *et al.* (1999) and Ward (2000). If one or more of these populations do not demonstrate evidence of domestic cattle introgression using current genetic technologies, and do not share a history with those populations which do contain cattle introgression, they will likely become invaluable source(s) of "pure" bison germplasm. As Polziehn *et al.* (1995) asserted, "Given the number of bison available to establish new herds, preference will most likely be given to animals with no evidence of hybridization."

Materials and methods

DNA was isolated from liver, whole blood, and/or tail hair samples of bison from 10 federal populations as described in Chapter II. All PCR and sequence reactions were run on a GeneAmp PCR System 9700 thermal-cycler (PE Biosystems). The mtDNA screen was performed as described by Ward *et al.* (1999) with minimal exceptions as follows (per 25 µl reaction): 50 ng template DNA or 1 FTA punch; 0.2 µM each primer; 1× MasterAmp PCR Enhancer (Epicentre, Madison, WI); 400 µM dNTPs; 2.0 mM $MgCl_2$; 1× reaction buffer; 1.0 units *Taq* DNA polymerase (Promega, Madison, WI).

The thermal parameters for the mtDNA screen were: 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. Sequencing of the mtDNA D-loop was performed for bison with suspect domestic cattle fragments. A 15,238-base pair (bp) fragment was amplified using the primers 12S (5'-AACAGGAAGGCTGGGACC-3') and THR (5'-AGAGAAGGAGAACAACTAACCTCC-3') located in the 12S ribosomal RNA and threonine tRNA genes, respectively, flanking either side of the bovine D-loop. Initial amplification was performed under the following conditions (per 50 μl reaction): 100 ng template DNA; 0.12 μM each primer; 400 μM dNTPs; 3.5 mM $MgCl_2$; 1× reaction buffer; 1.25 units AmpliTaq Gold® DNA polymerase (PE Biosystems, Foster City, California). PCR products were cleaned using the QIAquick PCR Purfication Kit (QIAGEN Inc., Valencia, California). Sequence reactions were performed using the Big-dye® terminator cycle sequencing kit version 2.0 (PE Biosystems) and an ABI377 automated sequencer (PE Biosystems) with the THR and internal D811-R (770-bp from THR; 5'GGGGGAATTTTTATGGAGG-3') primers.

Sequences obtained in this study were compared with those produced by Ward *et al.* (1999) using ClustalX (Higgins and Sharp 1988) with the following alignment parameters: gap opening = 15; gap extension = 6.66; transition weight = 0.5. Phylogenetic Analysis Using Parsimony (PAUP* 4.0b2, Swofford 1999) was employed for parsimony analysis of the sequences through heuristic searches with the following options: unrooted starting trees obtained via stepwise addition; tree-bisection-reconnection used as branch-swapping algorithm; branches collapsed when maximum

length = zero; bootstrapping on 50% majority rule consensus tree with 2000 replicates to test the strength of relationships among taxa.

From the 20 autosomal microsatellites, 15 were chosen for examination in this study based on the presence of introgression in other bison populations screened by Ward (2000) and allele size ranges for multiplexing. Every chromosomal region where evidence of domestic cattle introgression was found in bison populations by Ward (2000) was included in this study. The forward primer for each marker was labeled with 1 of 4 fluorescent dyes (NED, 6-FAM, HEX, VIC; Applied Biosystems, Foster City, California). Markers were multiplexed based on non-overlapping allele size ranges and dye types (Table 8). Multiplexes A and B were designed by Todd Ward and Robert Schnabel. Table 8 lists the confirming markers utilized in this study, which were run as singletons.

All microsatellite amplification reactions were performed using the thermal parameters described above for the mtDNA screen. PCR conditions for multiplexes A and C and all confirming markers were as follows (5 µl reactions): 50 ng template DNA or 1 FTA punch; 0.05 – 0.4 µM each primer; 1× MasterAmp PCR Enhancer; 400 µM dNTPs; 3.0 mM $MgCl_2$; 1× reaction buffer; 0.375 units *Taq* DNA polymerase (Promega). PCR conditions for multiplex B were as above with the exception of 1.6× reaction buffer.

All 15 nuclear diagnostic microsatellites were screened across all samples. Markers were rerun as singletons in individuals with suspect cattle-like alleles, with the same PCR protocols same as above with replacement of water for extra primer volume.

TABLE 8

Primary diagnostic and confirming microsatellite markers used to detect nuclear domestic cattle introgression in bison

Locus	Label[a]	Multiplex	Chromosome	Position[b]	$R_{A\text{-}BISON}$	$R_{A\text{-}DC}$
AGLA17	VIC	A	1	0.0	215	214-219
AGLA293	HEX	C	5	32.0	218-220	218-239
BM1314	6-FAM	B	26	24.8	137	143-167
HEL11	6-FAM			20.7	142-175	179-203
BM4307	6-FAM	C	1	35.2	185-187	183-199
BMS4017	HEX			34.8	145-165	148-158
BM4513	NED	A	14	62.5	132-134	139-166
BM7145	NED	A	1	69.2	108-110	116-118
INRA119	HEX			68.7	119-130	132-138
BMS4008	6-FAM			71.7	158-164	152-179
BMC3224	6-FAM	B	29	43.6	176	182-190
BMS2270	6-FAM	A	24	21.2	66-70	80-98
ILSTS065	HEX			25.2	null[c]	131-143
BMS4040	NED	B	1	98.8	75, 95[d]	85-99
CSSM36	VIC	A	27	39.8	158	162-185
CSSM42	NED	B	2	34.4	167-171	173-217
RM185	HEX	C	23	45.1	92	90-108
RM500	6-FAM	A	5	55.6	123	125-135
SPS113	VIC	A	10	29.2	128-132	135-154
TGLA227	VIC	B	18	84.7	72-73	79-106

Primary diagnostic and closely-linked confirming loci (in italics) are presented for 15 chromosomal regions. Only those confirming loci used in this study are shown. $R_{A\text{-}BISON}$, called allele size range in bison based on the YNP and WC populations in this study and the results of Ward (2000); $R_{A\text{-}DC}$, called allele size range in domestic cattle based on the results of Ward (2000) except for BMC3224 (see text); [a], fluorescent dye label for forward primer; [b], chromosomal position (cM) as reported in the USDA cattle gene mapping database; [c], ILSTS065 does not amplify in bison due to the presence of a fixed null allele; [d], the 95-bp BMS4040 allele was found by Ward (2000) only in the CSP population and presumed of bison origin based on the presence of bison-like alleles at a nearby locus

Individuals with suspect cattle-like alleles were then screened with the appropriate linked confirming marker (Table 8). In populations where suspect domestic cattle alleles were identified, a small number of additional bison without the cattle-like allele at the diagnostic locus were screened for the same linked confirming marker.

Results

A total of 3,378 bison from 10 federal populations were surveyed for evidence of domestic cattle introgression using both mitochondrial and nuclear loci (Table 9). No samples were collected from SH, as discussed in Chapter II. This survey represents approximately 49% (3378/6900; Table 9) of the total pool of bison from the 10 sampled populations. Testing of all or nearly all bison from individual populations was performed when possible. Complete sampling was not possible for the BNP (56.6%), GT (6.5%), WM (29.3%), and YNP (17.7%) populations.

Of the 10 federal populations examined, evidence of domestic cattle mtDNA introgression was found only in those bison from NBR. Of the 636 bison tested from the NBR population, suspect cattle D-loop fragments amplified in 11 bison (1.7%). Of these, 2 were females (born in 1984, 1989) and 9 were males (1 each born in 1989, 1994, 1998, 1999, 2000 and 3 born in 2002; 1 of unknown age). The sex of one of the males was unknown at the time of collection and determined through amplification of the 96-bp allele at the Y-chromosome locus INRA189 (see Chapter II, Appendix B). D-loop sequencing was performed for 8 of the suspect bison, excluding the 3 males born in 2002. Sequence alignments revealed complete identity to the domestic cattle mtDNA haplotype (9*) found in NBR bison by Ward *et al.* (1999). Likewise, parsimony analysis

TABLE 9

Total number of bison sampled among 10 federal populations for mitochondrial and nuclear screen for domestic cattle introgression

Population	Census[a]	Total
BNP	875	495
FN	379	379
GT	600	39
NBR	350	636[b]
NS	63	63
TRN	312	312
TRS	371	371
WC	350	375[b]
WM	600	176
YNP	3000	532
Sum	**6900**	**3378**

See Table 1 for population abbreviations. [a], current approximate census population size, as estimated by individual herd managers. When possible, estimates are given of total census population size at time of collection for this study. [b], collection taken over multiple years such that total collection is greater than given census size.

produced a consensus tree similar to that detailed by Ward *et al.* (1999), with the domestic cattle haplotypes from NBR bison sharing a node with domestic cattle of various breeds and other haplotypes identified as resulting from bison-domestic cattle introgression.

Allele frequencies for each of the 15 diagnostic microsatellites utilized for the detection of domestic cattle introgression are shown in Table 10 by population, with comparative results presented by Ward (2000) for CSP (n = 39) and 5 domestic cattle breeds (n = 64 total) also shown. One exception was BMC3224, where 96 independent

cattle samples representing more than 30 breeds were genotyped and used to estimate the domestic cattle allele frequencies reported in Table 10 (samples kindly provided by Christopher Seabury). Suspect cattle-like alleles were detected in 4 of the 15 diagnostic microsatellites within the 10 tested federal bison populations as follows: WM - 8.86% frequency of BM1314 157-bp allele; BNP - 13.67%, FN - 13.51%, NS - 13.49%, TRN – 16.34%, and TRS - 11.28% frequency of BM4307 197-bp allele; NBR - 3.78% and NS - 1.59% frequency of BM7145 116-bp allele; BNP - 3.13% frequency of BMS2270 94-bp allele.

In every population where potential domestic cattle introgression was detected at a diagnostic locus, confirmation was obtained through the detection of domestic cattle alleles at a linked locus (Table 11). For those populations with suspect cattle-like alleles at one or more diagnostic loci, the bison were divided into two classes: those with cattle-like alleles and those with exclusively bison alleles. Screening of the confirming locus was then performed on a subset of each class, with all bison in the former class screened when possible. For instance, in the WM bison population, where the 157-bp suspect BM1314 cattle-like allele was found, the liked locus HEL11 was used to screen 7 bison with the cattle-like allele and 7 bison with exclusively bison alleles at the BM1314 locus. In the first class, all 7 bison were confirmed to have a cattle-like allele at the HEL11 locus (187-bp). In the second class, all 7 bison were confirmed to have bison alleles at the HEL11 locus (155-, 159-, and/or 161-bp). That is, the presence of cattle-like alleles was confirmed with 2 linked loci in 7 bison from WM. Similar results were found with other loci in all other screened populations.

TABLE 10

Allele frequencies for 15 diagnostic microsatellites

AGLA17	BNP	FN	GT	NBR	NS	TRN	TRS	WC	WM	YNP	CSP	AN	HE	HO	SH	TLH	DC
214												10.00	31.25	30.77	12.50	7.69	19.53
215	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	98.70						
216																7.69	1.56
219											**1.30**	90.00	68.75	69.23	87.50	84.62	78.91

AGLA293	BNP	FN	GT	NBR	NS	TRN	TRS	WC	WM	YNP	CSP	AN	HE	HO	SH	TLH	DC
218	100.00	100.00	100.00	99.27	100.00	100.00	100.00	96.47	100.00	100.00	96.20		40.63				10.83
220				0.73				3.53			3.80			3.85			0.83
222												25.00		3.85	4.17		5.83
225																33.33	5.00
226																11.11	1.67
228												75.00	56.25	84.62	45.83	16.67	57.50
230															29.17	16.67	8.33
232															12.50	5.56	3.33
236																11.11	1.67
239													3.13	7.69	8.33	5.56	5.00

TABLE 10 CONTINUED

BM1314	BNP	FN	GT	NBR	NS	TRN	TRS	WC	WM	YNP	CSP	AN	HE	HO	SH	TLH	DC
137	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	91.14	100.00	95.90						
143																3.85	0.79
145																3.85	0.79
147												5.56	25.00				7.14
153												5.56	31.25	3.85			9.52
155												66.67	34.38	61.54	33.33	23.08	42.06
157									**8.86**		**4.10**	16.67		34.62	58.33	42.31	29.37
159												5.56				19.23	4.76
163													6.25			3.85	2.38
165															8.33		1.59
167													3.13			3.85	1.59

BM4307	BNP	FN	GT	NBR	NS	TRN	TRS	WC	WM	YNP	CSP	AN	HE	HO	SH	TLH	DC
183												11.11					1.64
185	59.49	82.11	94.87	89.26	77.78	74.26	71.74	90.22	65.52	100.00	75.60	22.22	12.50	16.67	9.09		11.48
187	26.84	4.39	5.13	10.74	8.73	9.41	16.98	9.78	34.48		21.80		3.13				0.82
189											**2.60**	11.11	37.50	54.17	54.55	19.23	36.07
191													6.25	4.17	9.09	15.38	7.38
197	**13.67**	**13.51**			**13.49**	**16.34**	**11.28**					55.56	40.63	25.00	22.73	38.46	36.07
199															4.55	26.92	6.56

TABLE 10 CONTINUED

BM4513	BNP	FN	GT	NBR	NS	TRN	TRS	WC	WM	YNP	CSP	AN	HE	HO	SH	TLH	DC
132	93.78	94.20	96.15	99.59	90.48	84.18	100.00	74.51	95.35	82.88	67.90						
134	6.22	5.80	3.85	0.41	9.52	15.82		25.49	4.65	17.12	32.10						
139													3.13	7.69		3.85	3.13
141																3.85	0.78
143												30.00	31.25	3.85	16.67	23.08	21.09
145												15.00	9.38	3.85		30.77	11.72
147												5.00	15.63	38.46	37.50	7.69	21.09
149												40.00	15.63	15.38	12.50	23.08	20.31
151												5.00		11.54	25.00	3.85	8.59
154													15.63		4.17		4.69
160												5.00		15.38			3.91
162													9.38			3.85	3.13
164														3.85			0.78
166															4.17		0.78

BM7145	BNP	FN	GT	NBR	NS	TRN	TRS	WC	WM	YNP	CSP	AN	HE	HO	SH	TLH	DC
108	76.27	87.00	98.72	91.00	88.89	87.10	67.08	66.12	100.00	82.26	71.80						
110	23.73	13.00	1.28	5.23	9.52	12.90	32.92	33.88		17.74	26.90						
116				**3.78**	**1.59**						**1.30**	90.00	96.88	65.38	95.83	88.46	87.50
118												10.00	3.13	34.62	4.17	11.54	12.50

BMC3224	BNP	FN	GT	NBR	NS	TRN	TRS	WC	WM	YNP	CSP	AN	HE	HO	SH	TLH	DC
176	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00							
182																	4.17
184																	53.65
186																	27.08
188																	14.58
190																	0.52

TABLE 10 CONTINUED

BMS2270	BNP	FN	GT	NBR	NS	TRN	TRS	WC	WM	YNP	CSP	AN	HE	HO	SH	TLH	DC
66	12.12	34.34	30.77	76.60	40.48	7.41	26.11	43.77	64.20	31.85	47.40						
68	84.75	65.66	69.23	23.40	53.97	92.59	72.22	37.54	16.05	59.27	34.60						
70					5.56		1.67	18.70	19.75	8.88	15.40						
80													3.33	3.85			1.59
82													23.33		33.33	11.54	14.29
84													10.00	3.85	12.50	34.62	12.70
86																7.69	1.59
88												10.00		30.77			7.94
90											**2.60**	10.00	26.67	11.54	12.50	26.92	18.25
92												20.00		19.23	20.83	3.85	11.90
94	**3.13**											5.00			4.17	7.69	3.17
96													30.00				7.14
98												55.00	6.67	30.77	16.67	7.69	21.43

BMS4040	BNP	FN	GT	NBR	NS	TRN	TRS	WC	WM	YNP	CSP	AN	HE	HO	SH	TLH	DC
75	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	98.70						
85													18.75	3.85	8.33	15.38	10.16
87												5.00				3.85	1.56
95											1.30	90.00	65.63	96.15	91.67	80.77	83.59
97												5.00	3.13				1.56
99													12.50				3.13

TABLE 10 CONTINUED

CSSM36	BNP	FN	GT	NBR	NS	TRN	TRS	WC	WM	YNP	CSP	AN	HE	HO	SH	TLH	DC
158	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00						
162												10.00	9.38	16.67	29.17	34.62	19.84
167														8.33		3.85	2.38
169													3.13	4.17			1.59
171												5.00	12.50	4.17	12.50		7.14
173												5.00	9.38	29.17		42.31	17.46
175												20.00			8.33	7.69	6.35
177																7.69	1.59
179												55.00	43.75	33.33	16.67	3.85	30.16
181												5.00	21.88		33.33		12.70
185														4.17			0.79

CSSM42	BNP	FN	GT	NBR	NS	TRN	TRS	WC	WM	YNP	CSP	AN	HE	HO	SH	TLH	DC
167	69.22	77.95	55.13	62.25	62.26	54.38	63.78	67.39	35.34	58.50	63.5						
169	2.25		2.56	6.59			0.28	8.12	22.41	6.92	8.1						
171	28.53	22.05	42.31	31.17	37.74	45.62	35.94	24.49	42.24	34.58	28.4						
173													9.38		33.33		8.59
175												15.00			4.17		3.13
177														3.85	4.17	3.85	2.34
179												30.00	12.50	23.08	37.50	34.62	26.56
181													3.13		4.17		1.56
193														7.69			1.56
205													3.13	7.69	4.17	3.85	3.91
207																3.85	0.78
209															4.17		0.78
211													3.13			3.85	1.56
213												55.00	68.75	15.38	8.33	46.15	39.84
217														42.31		3.85	9.38

TABLE 10 CONTINUED

RM185	BNP	FN	GT	NBR	NS	TRN	TRS	WC	WM	YNP	CSP	AN	HE	HO	SH	TLH	DC
90																7.69	1.61
92	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	96.20	5.00					0.81
94													3.57	19.23	8.33	3.85	7.26
96												20.00	3.57		33.33		10.48
98												10.00		11.54			4.03
100											**3.80**	15.00	21.43	11.54	4.17	11.54	12.90
102												30.00	60.71	34.62	20.83	30.77	36.29
104												10.00	3.57	19.23	12.50	3.85	9.68
106												10.00	7.14	3.85	16.67	42.31	16.13
108															4.17		0.81

RM500	BNP	FN	GT	NBR	NS	TRN	TRS	WC	WM	YNP	CSP	AN	HE	HO	SH	TLH	DC
123	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00						
125																11.11	1.67
127												10.00	34.38	15.38	12.50	38.89	22.50
129														3.85			0.83
131												20.00	6.25	19.23	12.50	50.00	19.17
133												65.00	59.38	57.69	70.83		53.33
135												5.00		3.85	4.17		2.50

TABLE 10 CONTINUED

SPS113	BNP	FN	GT	NBR	NS	TRN	TRS	WC	WM	YNP	CSP	AN	HE	HO	SH	TLH	DC
128				13.03	4.76												
130	85.34	65.24	38.46	56.35	61.11	53.92	62.22	54.27	67.54	44.83	70.50						
132	14.66	34.76	61.54	30.62	34.13	46.08	37.78	45.73	32.46	55.17	29.50						
135												5.00				11.54	3.17
137												10.00	33.33		16.67		12.70
139												20.00		15.38	12.50	19.23	12.70
141													3.33		8.33		2.38
143																3.85	0.79
145														19.23		19.23	7.94
147												10.00				19.23	5.56
149												5.00	10.00	57.69	25.00		19.84
151												50.00	53.33	7.69	29.17	26.92	33.33
154															8.33		1.59

TGLA227	BNP	FN	GT	NBR	NS	TRN	TRS	WC	WM	YNP	CSP	AN	HE	HO	SH	TLH	DC
72	5.86	3.11	34.62	13.80	3.97	0.68	3.49	40.25	21.13	26.92							
73	94.14	96.89	65.38	86.20	96.03	99.32	96.51	59.75	78.87	73.08	100.00						
79																16.67	2.50
83												15.00		3.85	16.67	22.22	10.00
85													31.25		4.17		9.17
90												5.00	3.13			5.56	2.50
92												40.00	25.00	19.23	66.67	11.11	32.50
94												15.00	28.13	19.23	4.17	5.56	15.83
96												10.00	9.38	3.85	8.33	11.11	8.33
98														3.85			0.83
101												15.00	3.13	42.31		27.78	16.67
106														7.69			1.67

Frequencies given as percentages. Suspect domestic cattle allele frequencies in bison populations are indicated in bold. See Table 1 for population abbreviations. All CSP, AN, HE, HO, SH, TLH, DC allele frequencies derived from Ward's (2000) data with the exception of BMC3224. CSP, Custer State Park bison; AN, Angus; HE, Hereford; HO, Holstein; SH, Shorthorn; TLH, Texas Longhorn; DC, overall domestic cattle frequencies (based on AN, HE, HO, SH, TLH except for BMC3224)

TABLE 11

Summary of testing and results for confirming loci by population

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

See Table 1 for population abbreviations. Results presented only for those populations with suspect cattle-like alleles at diagnostic loci (DL). Bison from each population were divided into groups based on their DL genotypes: suspect (possessing cattle-like allele(s)) or non-suspect (bison-like alleles). Bison from each group were genotyped for the appropriate linked confirming locus (CL). The domestic cattle and bison called allele sizes for each CL in each population are indicated (following Ward 2000). CL tested, total number of bison tested in each DL class for the appropriate CL; CL cattle allele, the total number of tested DL suspect bison with a cattle-like allele at the CL; CL bison allele(s), the total number of tested DL non-suspect bison with exclusively bison-like alleles at the CL; [a], BMS2270 does not amplify in bison due to the presence of a fixed null allele

In some cases, a small number of bison had a cattle-like allele at one locus but not at the other, indicating recombination (e.g. NBR BMS4008; Table 11). One notable exception is with the BMS2270/ILSTS065 screening pair for the BNP population, where less than 50% (14/30) of the tested bison were confirmed to have cattle-like alleles at both loci. These results may be the product of recombination or genotyping error for the ILSTS065 locus, where the absence of a PCR product was interpreted as evidence of a bison-like (null) allele when in fact amplification failure would produce the same result. The ILSTS065 locus was co-amplified with BMS2270 in the secondary screen in an attempt to eliminate such genotyping error.

Discussion

This study has identified domestic cattle introgression in some, but not all, tested federal bison populations. Those populations included in previous studies (Polziehn *et al.* 1995; Ward *et al.* 1999; Ward 2000) have been examined in much greater detail for both nuclear and mitochondrial loci, and the detection of domestic cattle introgression has been investigated for the first time in several federal bison populations through this study.

Sources of domestic cattle introgression in U.S. federal bison populations

Of the 4 sources of bison stock used to found or supplement FN (Table 1), the most likely to have contributed the domestic cattle introgression observed today is either the private ranch in Nebraska or CSP, since the domestic cattle alleles found at BM4307

and BMS4017 are not found in either NBR or YNP bison (Table 10, 11; BMS4017 allele distribution for NBR and YNP based on data not presented here). Bison from CSP also have domestic cattle introgression in the BM4307/BMS4017 region, although the alleles are of different sizes (Table 10; Ward 2000). Furthermore, CSP and FN bison have demonstrated evidence of domestic cattle introgression at the linked PIT1-7B7 locus (Chromosome 1, 34.0 cM), with 139-bp alleles in both populations (Ward 2000). These findings indicate CSP as the likely source of FN domestic cattle introgression.

Although the allele frequency observed for the BM4307 197-bp allele (0.135) was substantially lower than that observed by Ward (2000) of 0.204, it is still much greater than the frequency of the BM4307 189-bp allele in CSP bison (0.026). The higher frequency in the FN population observed by Ward (2000) was most likely due to small sample size error (n = 27, < 8% of the population census size of approximately 350 – 400). Drift and/or selection might also explain the observed differences in the FN samples collected in 1995 used by Ward (2000) and those from 2002 used in this study. However, neither of these natural forces would be expected to act in the significant manner necessary to explain these differences in such a relatively short time (< 2 generations with a 4-year generation time in bison) with a population of consistently greater than 350 bison during the interim.

As Ward (2000) pointed out, the presumed source of CSP for the observed domestic cattle introgression in FN bison is confounded by a lack of similar sized alleles between the two populations, but may be explained by the small sample size from the CSP population (n = 39). That is, the BM4307/BMS4017 domestic cattle alleles found

in the FN population might actually be present, but as of yet undetected, in the CSP population. Alternatively, these alleles might have been eliminated from the CSP population by random genetic drift since the introduction of CSP bison into FN in the 1930s. The bison from a private source in Nebraska used to found the FN bison population (Table 1) cannot be eliminated as a source of the observed domestic cattle introgression, since the history of this source is unknown.

The observation of the same BM4307/BMS4017 domestic cattle alleles in the TR bison populations as found in the FN population is expected based on the history of these populations (Table 1, 10, 11). Notably, the TRN population has an approximately 44% higher frequency of the BM4307 compared to the TRS population (Table 10), consistent with differences observed using other microsatellite loci and the hypothesis of genetic drift acting more strongly in the TRN population based on a historically smaller census size than the TRS population (Chapter II).

Two separate regions of domestic cattle introgression are found in the BNP population (Table 8, 10, 11): one on chromosome 1 (BM4307/BMS4017) and the other on chromosome 24 (BMS2270/ILSTS065). Similarity in allele size and frequency of BM4307 alleles with FN and TRS, the two original source populations for BNP, indicate the source of the chromosome 1 domestic cattle introgression was FN (Table 1, 10). The BMS2270 94-bp domestic cattle allele, however, is not shared with either TRS or FN, and is presumably from the 1984 introduction of bison from Colorado of unknown origin (Table 1). Ward (2000) also showed domestic cattle introgression in CSP bison in the BMS2270/ILSTS065 region. The BMS2270 90-bp allele and ILSTS065 143-bp allele

found in CSP (Table 10, 11; Ward 2000) are different, however, from those found in the BNP population.

Ward *et al.* (1999) found 2.7% (3/113) of the tested bison from NBR had domestic cattle mtDNA, comparable to the 1.7% found in this study. The NBR female with domestic cattle mtDNA born in 1984 was identified as 1 of the 4 females introduced from Maxwell State Game Refuge (MSGR; Table 1; Lindy Garner pers. comm.). The other 3 females from this introduction were included in this study; all had a bison-type mtDNA. However, none of these 4 females had the BM7145 116-bp cattle allele. Furthermore, none of the bison identified as having domestic cattle mtDNA also had the BM1745 116-bp cattle allele. These results indicate 2 independent domestic cattle introgression events in the NBR population. The source of domestic cattle mtDNA introgression in NBR bison is MSGR, as corroborated by the following observations: all 9 bison with domestic cattle mtDNA sequenced in this study were identical to those found in NBR bison by Ward *et al.* (1999), including a single female from MSGR, and this haplotype was found to be identical between the two populations.

Ward (2000) found the same domestic cattle alleles in the MSGR population as found in the NBR population for the BM7145/INR119/BMS4008 region (Table 10, 11), so it is somewhat surprising that MSGR could not be confirmed as the source of the domestic cattle introgression in this region. Barring sampling error, the only explanation for this finding is a second source of domestic cattle introgression. One possible way to narrow the timing of the introduction of domestic cattle introgression into the NBR population would be to sample bison from the Delta Junction population in Alaska,

which was founded exclusively from NBR bison in 1928 (see Chapter I). The Delta Junction population would be a potentially important source of bison genetic variation if it does not share the domestic cattle introgression in this region with NBR.

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

Excluding the possibility of recent, undocumented introgression, there are only 2 possible sources of the domestic cattle introgression observed in the BM1314/HELL11 region in the WM bison population: the New York Zoological Park or FN (Table 1). Coder (1975) reported that one of the bulls from the New York Zoological Park was from the Jones herd, where hybridization experiments are known to have occurred (Chapter I). Furthermore, the FN population was supplemented with CSP bison in 1935 and 1937, just before the 1940 transfer of 2 bulls to WM. Although domestic cattle introgression was not observed in the BM1314/HELL11 region in the FN population, the same alleles found in the WM population (157-bp and 187-bp, respectively) were found in the CSP population by Ward (2000; Table 10, 11). These findings may be the result of genetic drift over the last 60 years to effectually eliminate the introgressed

BM1314/HELL11 region from the FN population and/or the introduction of a single bull directly from CSP (or an F1 of a CSP bull) to WM – through FN – that did not produce many, if any, offspring while at FN. The later possibility seems likely based on the timing of the movement of bison from CSP to FN and from FN to WM (Table 1).

Although it is not possible to completely exclude the possibility of domestic cattle introgression in any given bison population using the markers and methods in this study (see below), evidence of introgression was not found in the GT, WC, and YNP bison populations. The presence of the BM4307 197-bp domestic cattle allele in the TRS population but not the GT population is surprising given the introduction of bison from TRS into the GT population in 1964 (Table 1). The small sample size from GT may have precluded the detection of domestic cattle introgression at this locus. It is also possible that the contribution of the TRS bison in the 1960s to the GT population was low enough that the allele in question was not maintained (genetic drift). Further sampling from the GT population is necessary to resolve this issue.

Statistical significance of detecting domestic cattle introgression

Including the survey of Ward (2000), a total of 3,748 bison from 21 state and federal populations have been examined for evidence of both domestic cattle mitochondrial and nuclear DNA introgression. Of these, 8 bison populations have been shown to be free of domestic cattle introgression for the markers utilized in this study (plains bison unless otherwise noted): Elk Island National Park (wood bison, $n = 25$), Canada; Mackenzie Bison Sanctuary, Canada (wood bison, $n = 36$); Wood Buffalo

National Park, Canada (wood bison, *n* = 23); Elk Island National Park (plains bison, *n* = 25), Canada; Grand Teton National Park, Wyoming (*n* = 39); Henry Mountains, Utah (*n* = 21); Wind Cave National Park, South Dakota (*n* = 375); Yellowstone National Park, Wyoming (*n* = 560, including those from Ward 2000). The confirmation of potential mtDNA introgression through sequencing and phylogenetic analysis and potential nuclear DNA introgression with linked markers makes the probability of a type I statistical error across a bison population negligible. That is, the probability that a population identified using these techniques as having domestic cattle introgression actually does not is insignificant.

Conversely, there is a chance that one or more of the 8 bison populations identified in this study or by Ward (2000) as being free of cattle DNA actually contain some level of domestic cattle introgression (type II statistical error). This type of error is not difficult to imagine given the small portion of the bison genome under investigation with a 15 marker screen. Many factors unique to each studied population play a role in the calculation of this probability, including the original frequency of introgression in founding stock, the number of generations since the foundation of the population, and the number of bison sampled.

Alternatively, the probability of detecting introgression at a particular significance level (e.g. 0.1%, 1%, 5% population level of introgression) in a sample of size "N" with "n" loci fixed for different alleles in two species can be estimated using the equations set forth by Davis *et al.* (1988). The logic behind these calculations is that although the probability of detecting domestic cattle introgression in a single bison after

several generations past the initial hybridization event is quite small, the probability of detecting of low levels of hybridization across a population is necessarily higher as more individuals are sampled. With the 15 loci used in this study, the presence or absence of alleles in the range of domestic cattle alleles determines the detection of introgression as opposed to allele frequency differences for shared alleles between the two species; as such, these loci can be treated as "fixed" (Davis *et al.* 1988).

Table 12 indicates the associated probabilities of detecting domestic cattle introgression assuming a hybrid ancestry for the population under investigation for various sample sizes and levels of detection using 15 loci and including the exact number of sampled individuals from GT, WC, and YNP in this study. With these loci, the probability of detecting domestic cattle introgression at a 0.01% population level in the WC and YNP populations is > 99.99%. In the GT population, however, the probability of detection at a 0.01% level drops to around 60%. The method used here for estimating error rates is not perfect and makes several assumptions which may be violated in some bison populations, such as random mating and neutral evolution of domestic cattle genes in hybrid bison populations. However, the probabilities presented in Table 12 can be taken as a rough estimate of the degree of confidence that can be placed on the conclusions of this investigation.

Biological significance of detecting domestic cattle introgression

Hybridization between distinct populations, and in some cases species, is known to increase viability and adaptive response (Spielman and Frankham 1992; Arnold and

TABLE 12

Probability of detection of domestic cattle introgression in bison

	SAMPLE SIZE									
% Introgression	**GT 39**	**50**	**100**	**150**	**200**	**300**	**WC 375**	**YNP 532**	**700**	**800**
5%	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
1%	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
0.1%	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
0.01%	0.6078	0.6988	0.9093	0.9727	0.9918	0.9993	0.9999	1.0000	1.0000	1.0000
0.001%	0.0894	0.1131	0.2134	0.3023	0.3812	0.5132	0.5934	0.7211	0.8136	0.8534
0.0001%	0.0093	0.0119	0.0237	0.0354	0.0469	0.0695	0.0861	0.1199	0.1546	0.1747
0.00001%	0.0009	0.0012	0.0024	0.0036	0.0048	0.0072	0.0090	0.0127	0.0167	0.0190

Probabilities of detection are given for various combinations of sample sizes and levels of introgression of domestic cattle nuclear DNA. Exact sample sizes for Wind Cave National Park (WC) and Yellowstone National Park (YNP) are shown. This study did not find evidence of domestic cattle introgression in either the WC or YNP bison populations.

Hodges 1995), even when the original hybridization is disadvantageous (Lewontin and Birch 1966), as in the case of domestic cattle and bison. Since bison and domestic cattle do not naturally hybridize, and there are clear negative fitness consequences in at least the F1 generation, it seems plausible that the introgression and of domestic cattle genes into bison germplasm might also be under negative selection. The maintenance of introgressed domestic cattle regions for 15 – 20 generations post-hybridization in the federal populations examined in this study suggests that any negative fitness effects, at least in these regions, is minimal. However, the location of genes and their respective functions within and near the 15 nuclear regions examined in this study are largely unknown; it is therefore not possible at this point to directly investigate the involvement of natural selection on domestic cattle introgression in these regions.

Of the 3 populations identified in this study as free of domestic cattle introgression (GT, WC, YNP), WC and YNP are among the 4 populations identified in Chapter II as having the most contribution to overall bison genetic variation in federal populations. It is unknown whether relative contribution to overall bison variation in the NBR and WM populations is truly due to bison variation or the addition of domestic cattle alleles into these populations. By the same reasoning, it seems particularly providential that WC and YNP would by far have the highest contribution to allelic richness among the studied populations (Figure 4). Assuming neutral selection on introgressed cattle genomic fragments, those populations with observed domestic cattle introgression should logically have more genetic variation among the federal

populations. The findings of this study oppose this expectation and raise concern as to the possible negative effect of domestic cattle introgression on bison fitness.

The examination of multiple related populations has allowed for resolution of some of the hypotheses previously set forth by Ward (2000). For instance, Ward (2000) suggested a possible association between the frequency of domestic cattle introgression at the PIT1 7B7 locus, a growth factor hormone gene located in the BM4307/BMS4017 region, and nonrandom selection based on size and conformation characteristics in FN bison. Ward (2000) found identical allele frequencies for the PIT1 7B7 139-bp domestic cattle allele and the domestic cattle alleles at linked the BM4307 and BMS4017 loci in the FN population. For at least 20 years, the FN bison population was under artificial selection for physical size during yearly roundups (Chapter I). Similar frequencies of the domestic cattle BM4307 197-bp allele were found in the FN, TRS, and BNP populations (Table 10). Neither of the latter 2 populations are known to have been under any type of direct artificial selection during their history. Since the TRS and BNP populations were founded from FN stock in the 1950s and 1960s before the documented artificial selection in the FN population (1970s – 1990s), the relatively high frequency of the BM4307 197-bp domestic cattle allele and adjacent growth hormone locus in the FN population is most likely due to initial allele frequency and not artificial selection.

Management implications

This study has identified at least 3 federal bison populations with presumed multiple sources of domestic cattle introgression: BNP, NS, and NBR. The importance

of utilizing both mtDNA and nuclear loci for the detection of hybridization and introgression was predicted by Rhymer and Simberloff (1996) and is substantiated in this study with results from the NBR population; without both the mtDNA and nuclear loci, the true extent of introgression in this population would have been underestimated.

These results also emphasize the importance of the warning given by Simberloff (1996) to use extreme caution when purposely mixing individuals from populations, especially when interspecies hybridization is a possible compounding issue. In the case of both the BNP and NBR populations, the observed domestic cattle introgression was in part due to additions made to these populations in the 1980s under the honorable auspices of increasing genetic diversity and limiting inbreeding depression. Although the management decisions were made in good faith and at the time nothing was known about the incidence of domestic cattle introgression in bison populations, no genetic research was actually conducted prior to the introductions to determine if the perceived problems of low variation and inbreeding were real. There are likely other species and populations which have been influenced in similar ways by widespread conservation efforts to mix isolated populations as much as possible for the maintenance of natural variation. The purposeful mixing of populations, especially those that are highly fragmented and isolated through direct human influence, is extremely important in the maintenance of genetic diversity to prevent the potentially negative impacts of forces such as genetic drift; however, management decisions based on these criteria should obviously be made with caution and the best available tools and resources.

In the future, the possibility of domestic cattle introgression should be given high priority in management decisions involving the movement of bison among populations, especially those that are under governmental protection for the long-term conservation of the bison species. At this point, it is virtually impossible to completely rid any of the 7 populations described in this study from all domestic cattle introgression. It is possible, however, to eliminate currently detectable domestic cattle introgression from these populations. This management strategy has at least 3 limitations. First, with the exception of NS, these hybrid populations have been in existence for a minimum of 40 years, representing a maximum of 10 generations, during which time equilibrium between genetic constitution and environment has likely been met. The elimination of detectable domestic cattle introgression may disrupt this equilibrium and cause at least short-term fitness consequences. Second, the culling of these identified bison would be far from random and may eliminate important bison alleles from a given population. Lastly, this would be a very costly endeavor involving years of testing beyond the scope of this study to ensure the detectable cattle introgression is eliminated. Furthermore, only detectable hybridization would be eliminated in populations where undetected regions of domestic cattle introgression are likely based on their hybrid ancestry, but not possible to identify without virtually complete genome sequencing. Some alternatives to the "hybrid reduction" management strategy are discussed in Chapter VI.

For the 3 populations without evidence of domestic cattle introgression, a different variety of management decisions will be important in the future, mostly involving the maintenance of current variation and precautionary measures against

future possibility of domestic cattle introgression. For instance, the WC bison population shares a fence with CSP, where domestic cattle introgression has been detected at both nuclear microsatellite and mtDNA loci (Ward *et al.* 1999; Ward 2000). Occasional escapees have been known to enter the WC property through broken fences, although resourceful herd managers and differences in identification systems between the two populations have thus far allowed for quick and accurate removal of CSP bison from WC property (Barbara Muenchau pers. comm.). The importance of maintaining the WC population in isolation from the neighboring CSP population is underscored by the results of this study.

In conclusion, this study has assessed levels of domestic cattle introgression in 10 federal bison populations and identified at least 2 populations, WC and YNP, which at this time do not have any evidence of domestic cattle introgression and also have high levels of unique genetic variation in relation to other federal populations. As such, these populations should be given conservation priority and be maintained in isolation from those populations identified in this study and by Ward (2000) as containing domestic cattle introgression.

CHAPTER IV

THE LEGACY OF CHARLES GOODNIGHT:

CONSERVATION GENETIC ANALYSIS OF THE TEXAS STATE BISON HERD

"In making contract with you to dispose of my ranch through the Goodnight American Buffalo Ranch Co., I do so for the reason that I am getting too old to give it the necessary attention without overtaxing my energies and that I hope and expect that it will be perpetuated and fill a unique page in the history of a generation yet unborn."
— Charles Goodnight (letter to H. A. Fleming 1910)

Introduction

The Texas State Bison Herd (TSBH) is the only known direct descendent of the bison herd established by Charles Goodnight in the 1880s. The TSBH has remained a small ($n < 100$) closed bison population for the past 120 years since its foundation with 5 wild bison. In 1997 the herd was donated to Texas Parks and Wildlife (see Chapter I for a historical review).

There are likely few bison populations worldwide managed as closely as the TSBH, which receives supplemental feed, yearly vaccinations, and almost daily monitoring by state biologists. The bison are not afflicted with any known ungulate disease (Danny Swepston pers. comm.). Nevertheless, the TSBH is suffering from low natality and high mortality rates compared to other captive bison herds. From 1997 – 2002, the natality rate (number of calves/adult female/year) averaged 39.2% (Swepston 2001; Table 13). In comparison, Berger and Cunningham (1994) reported

TABLE 13

Average age, census population size, mortality rate, and natality rate estimates from the Texas State Bison Herd

Year	Average Age[a]	Census Size[b]	<1 Yr Mortality[c]	>1 Yr Mortality[d]	Natality[e]
1997	3.56	36	3/4 (0.750)	5/36 (0.139)	4/21 (0.190)
1998	4.50	32	2/4 (0.500)	3/32 (0.091)	4/17 (0.235)
1999	5.35	31	7/11 (0.636)	2/31 (0.065)	11/15 (0.733)
2000	5.73	33	1/4 (0.250)	1/33 (0.030)	4/17 (0.235)
2001	6.23	35[f]	3/10 (0.300)	0/35 (0.000)	10/16 (0.625)
2002	6.20	40	4/5 (0.800)	0/40 (0.000)	5/15 (0.333)
TSBH average (small herd)			0.539	0.055	0.392
Captive bison average (large herd)[g]			0.042	0.032-0.042	0.600

[a], Exact ages unknown for animals born before 1997, and figured conservatively as either yearlings or adults (3^{+}). This skews the average age of the herd below actual age, but does not change the average increase in age over 6 years; [b], Census size before death and birth for given year; [c], Ratio given as number of deaths / number of calves born up to 1 year in age; [d], Ratio given as number of deaths / number total bison >1 year in age; [e], Ratio given as number of births / number of total adult females ages 3^{+}; [f], 2 steers donated to Armand Bayou Nature Center (Houston, Texas); [g], Derived as conservative estimates from Berger & Cunningham (1994); mortality rate for age classes 0-2 reported as 0.042 and for ages 3 or more as 0.032 per year

approximately 60% natality at BNP over a 5 year study period and Meagher (1973) estimated a natality rate of 52% in YNP bison. TSBH mortality rates from 1997 – 2002 averaged 53.9% for calves, which is significantly higher than the 4.2% calf mortality rate previously reported, while the average 5.5% mortality rate for TSBH bison older than one year is only slightly higher than the 3.2 – 4.2% previously reported (Berger and Cunningham 1994; Table 13). Consequently, the census population size has only increased from 36 to 40 bison over the past 6 years, and the average age of the population has risen by 2.64 years (Swepston 2001; Table 13).

In 2000, 8 mature (> 3 years old) bulls were fertility tested using electroejaculation (Genetic Resources International, Navasota, Texas). Of these, 4 exhibited normal sperm motility and morphology, while the remainder had abnormalities outside acceptable baseline ranges including low motility, bent tails, and detached heads (Danny Swepston pers. comm.). Although some abnormal readings are expected from a single collection on bulls never before worked for fertility testing, the semen characteristics are certainly suggestive of fertility problems in the TSBH. In December 2001, all 18 adult female bison were pregnancy tested using the pregnancy specific binding protein test (Texas Veterinary Medical Diagnostic Laboratory, College Station, Texas). Results indicated that 15 bison (~83%) were pregnant. From these apparent pregnancies, 5 calves were born and only 1 survived into 2003 (Danny Swepston pers. comm.), confirming the trend of poor recruitment in this herd. As such, it is probable that male infertility and the inability of females to carry pregnancies to term are

negatively affecting the recruitment and population growth rates observed in the TSBH over the past 6 years.

Charles Goodnight was a cattleman and rancher by nature, and was internationally famous for breeding bison to Angus domestic cattle (*Bos taurus*) in an effort to produce a more robust and hardy beef breed (Goodnight 1914; Haley 1949). Evidence of introgression is still present in the descendants of Goodnight's original experiments, as domestic cattle mtDNA was found in 6 of the original 36 TSBH members (population abbreviation JA; Ward *et al.* 1999; Ward 2000). Subsequent genetic testing demonstrated both a unique bison mitochondrial type and distribution of nuclear alleles in the TSBH compared with various wood and plains bison herds (Ward 2000). The unique genetic composition in the TSBH is further indicated by the presence of private alleles and high pairwise F_{ST} values compared with those between tested federal bison populations (Table 4, 6). Furthermore, both genetic distance measures utilized in Chapter II indicate more genetic difference between TSBH and YNP, the closest genetic neighbor, than found in any of the pairwise distance comparisons between the 10 federal bison populations examined (Table 7).

The noted low levels of genetic variation in the TSBH compared with other bison populations (Table 4; Figure 2) combined with chronic small population size, low recruitment, and high juvenile mortality observed in this population indicate the TSBH may be in danger of extinction. As such, the purpose of this study was to investigate past and future genetic consequences of current population trends in the TSBH. Parentage analysis was performed on calves born from 1998 – 2001 to develop an

understanding of the breeding system in the TSBH and directly measure effective population size. Additionally, differences in genetic variation between adult and calf groups were measured. A stochastic model was developed to simulate the effects of current natality and mortality rates on population size, genetic diversity, and heterozygosity in the TSBH over the next 100 years. The model was designed to further simulate the importation of bison males into the TSBH to investigate potential effects of increased fitness and introduction of new allelic variation on long-term census population size, genetic diversity, and heterozygosity.

Methods and results of genetic analysis

Sample collection, DNA extraction, and multiplexed PCR was performed as described in Chapter II for 19 male and 21 female bison from the TSBH. This collection represents the entire population as of December 2001. One additional calf was born in 2002 and several have recently been born in 2003 (Danny Swepston pers. comm.). A total of 54 polymorphic nuclear microsatellite loci were genotyped to 100% completion (Table 2).

Likelihood-based parentage testing was performed using CERVUS 2.0 (Marshall *et al.* 1998) with the following analysis parameters: 10,000 cycles, 1% genotyping error rate, 80% relaxed confidence, and 95% strict confidence. Parentage was established with confidence for offspring born from 1998 – 2001, revealing that 5 bulls and 11 cows produced the 15 tested progeny. The average number of offspring was 3.00 ± 2.12 *SD* for the males and 1.36 ± 0.45 for the females. The bull producing the most progeny

sired 6 offspring (40%) while the most number of offspring per cow was 3 (20%). All 5 males that sired offspring were fertility tested in 2000. Semen samples from 4 of these males displayed normal motility and morphology. The fifth sample had normal motility, but exhibited morphological abnormalities.

Additionally, observed heterozygosity and number of alleles per locus were compared between the TSBH calves born from 1998 – 2001 (n = 15) and extant adults (n = 25) for the 51 autosomal loci. The adult group averaged 38.7% heterozygosity and 2.61 alleles/locus, while the calf group averaged 35.2% heterozygosity and 2.41 alleles/locus, although the differences were not statistically significant (unpaired t-test, $p > 0.001$). There is a 7.6% difference in the total number of alleles present in the current adult population (131 alleles) but absent from the 1998-2001 calf population (121 alleles).

Model description

The stochastic model simulates changes in census population size, heterozygosity, and genetic diversity based on a 1-year time step using Visual Basic® 6.0 (Microsoft®). The initial conditions of the model include sex, age, and genotype at each of 51 unlinked autosomal microsatellites for the 40 extant bison from the TSBH as of December 2001. Females and males in the model are considered potentially reproductive from 3 – 13 years and 4 – 14 years of age, respectively (Berger and Cunningham 1994). Following observed competition and breeding success among males, when a 7 – 12 year-old male exists in the simulated populations, those younger or

older do not mate in a given year (Berger and Cunningham 1994). Each time step (year), the age of each bison is advanced and potential breeders recalculated. Females and males are selected and paired randomly from the potential breeding pool. For each locus, an allele from each parent is randomly chosen and assigned to the offspring. The offspring sex ratio is 1:1, as is generally found in closed bison populations (Berger and Cunningham 1994; Swepston 2001).

"Small herd demography" natality and mortality rates were calculated from TSBH data from the past 6 years (Table 13). "Large herd demography" natality and mortality rates were taken as conservative estimates of those calculated by Berger and Cunningham (1994), with the mortality rate for age classes 0 – 2 years of 4.2% per year and for age classes 3^{+} years of 3.2% per year. In the model, natality rates are applied to potentially breeding females such that under the small herd demography scenario, for instance, 39.2% of the 3 – 13 year-old females are randomly selected to mate and produce offspring. Once bison reach 20 years of age, the mortality rate is assumed to be 50%, based on the rarity of bison in captive populations that survive much past this age. Furthermore, a mortality rate of 100% is applied to any bison that reach the age of 30 during the simulation. When the census population size is greater than 200 bison, excess calves are randomly culled so as to keep the population from exceeding the approximate carrying capacity at CCSP.

Several possible scenarios were evaluated concerning the potential importation and subsequent reproduction of male bison into the TSBH. A random number generator was used to select individuals from 142 male YNP bison. Three distinct sets of 3 bison

(9 total) were chosen and completely genotyped for the same 51 microsatellite loci utilized for the TSBH. All males are imported in the model as 7 year-olds (breeders). Breeding may occur either randomly with replacement, with one male mating all potential females in a given year with replacement the following year, or with 3 imported males breeding all potential females during the year of importation followed by random mating of all potentially breeding males (native and imported) in all subsequent years. Migration occurs with either 3 males imported into the population in year 1 or with 9 males total imported at a rate of 3 males every 5 years (importation of 3 males in years 1, 6, and 11). In general, fitness was assumed to increase following the importation of new bison into the TSBH, as reflected by birth and death rates (Lewontin and Birch 1966; Spielman and Frankham 1992). Unless otherwise noted, 20 replicates were used in each evaluation and averages were taken for each year across all replicates for the parameters of interest.

Model evaluation

Choice of particular males for importation

To examine the sensitivity of the model to the choice of particular imported bison, comparisons were made among simulations of 3 groups of 3 bison each from YNP. In each case, the 3 bison were imported into the population in year 1 and allowed to mate all potentially breeding females preferentially, with random mating of all imported and native males in every subsequent year. No further assumptions were made regarding the

fitness of the imported males versus the original TSBH males, and large herd natality and mortality rates were used (Table 13).

Currently, there are a total of 133 alleles in the TSBH for the 51 nuclear microsatellite loci tested. The addition of the 3 bison from group 1 adds 62 new alleles to the population, while group 2 adds 67 new alleles and group 3 contributes 63 new alleles. Figure 6 illustrates differences in heterozygosity and the total number of alleles for the 51 markers in question over 100 years with each of the 3 sets of imported males.

Differences among average values for heterozygosity and number of alleles at 50 and 100 years between the 3 groups were tested using analysis of variance (ANOVA). Average heterozygosity at 50 and 100 years and number of alleles present at 100 years were not significantly different between the 3 groups ($p > 0.05$). However, the average number of alleles at 50 years between the 3 groups was significantly different ($p < 0.05$). Differences in 50-year average number of alleles were further tested using Tukey's HSD (absolute difference) test, which revealed that the differences between groups 1 and 3 were non-significant ($p > 0.05$) while the other pairwise comparisons showed significant differences ($p < 0.05$). Percent differences between initial and final (100 year) values for total number of alleles are -15.0% (1), -14.3% (2), and -14.5% (3) and for heterozygosity are -12.0% (1), -16.0% (2), and -14.3% (3). Therefore, it appears that heterozygosity and genetic diversity are not substantially influenced after 100 years by our choice of one particular group of bison over another for simulation of importation into the TSBH. In further analyses involving the importation of only 3 bison into the model, group 1 was used since this group contributes the least number of new alleles into the TSBH and is

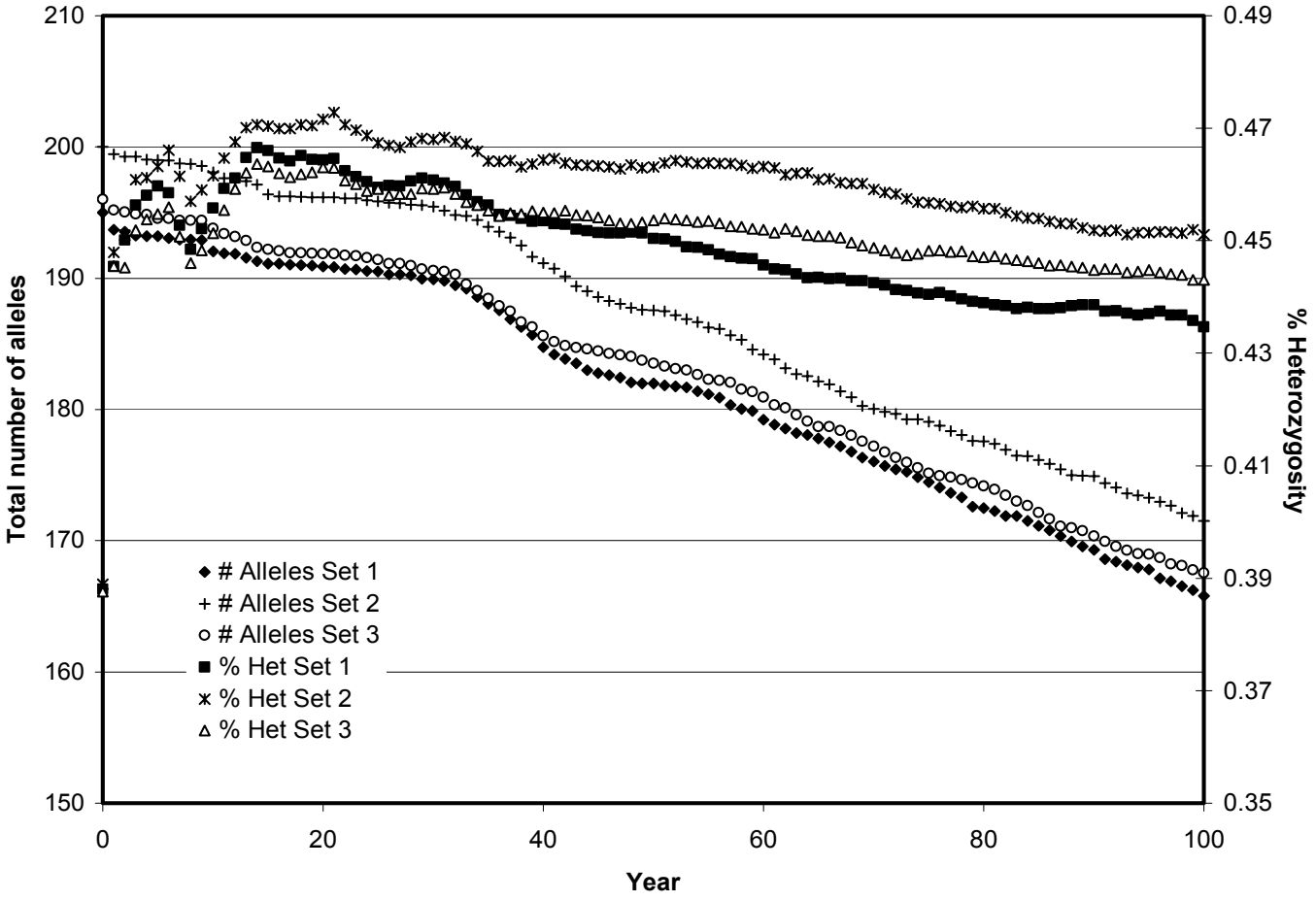

Figure 6. —Evaluation of the effects of male choice for importation on the TSBH genetic simulation model. Total number of alleles and heterozygosity (%Het) are compared each year among 3 groups of 3 imported males each.

therefore likely to provide conservative estimates of predicted changes in heterozygosity and genetic diversity.

Effects of natality and mortality rates

To examine the sensitivity of population size and genetic variation to changing demographic parameters within the model, the potential effects of natality and mortality rates on population size, genetic diversity, and heterozygosity were examined. Using both calculated and previously published rates as a guide, the effects of natality rates ranging from 40 – 60% and calf mortality rates ranging from 5 – 50% were calculated (Series A – F; Table 14). Since mortality rates for those bison > 1 year of age in the TSBH are similar to previously published reports, large herd mortality rates for age classes > 1 year were used in these evaluations. Each evaluation assumed random mating and no migration.

All of the 120 simulated populations (6 series with 20 simulations each) survived to 100 years. Average population size, total number of alleles, and heterozygosity values for each year for each series of simulations are graphed in Figures 7, 8, and 9, respectively. Although the mean population size trajectory of each series eventually reaches $n = 200$, the population growth rates are clearly different among the series. The year that each simulation reached $n = 200$ was calculated and averaged across all 20 iterations for each series, and shown in Table 14 as the average time to reach carrying capacity. The differences among series in average time to carrying capacity are significant (ANOVA, $p < 0.05$).

TABLE 14

Summary results for evaluation of the effects of mortality and natality rates on the TSBH model

Series	Natality Rate	Calf Mortality Rate	Average Year $n = 200$	Growth Rate (bison/year)[a]	Average Total Number of Alleles	Average Heterozygosity
A	0.6	0.05	14.8 ± 1.5	10.9	120.0 ± 4.2	34.4 ± 2.2
B	0.5	0.05	18.8 ± 2.9	8.4	120.9 ± 3.0	34.1 ± 2.1
C	0.4	0.05	27.0 ± 5.4	5.7	120.7 ± 3.5	34.2 ± 1.3
D	0.6	0.50	54.6 ± 18.8	1.9	118.1 ± 4.3	33.4 ± 1.3
E	0.6	0.35	27.0 ± 8.6	5.9	119.8 ± 4.2	33.7 ± 1.6
F	0.6	0.20	18.5 ± 2.0	8.5	121.1 ± 4.7	34.2 ± 1.8

Model evaluation conditions include large herd mortality rates for bison > 1 year in age, no migration, and random mating. Summary statistics include average time to reach carrying capacity ($n = 200$), growth rate, genetic diversity (total number of alleles), and heterozygosity after 100 years. [a], slope of regression line of average population size up to average year that population reaches $n = 200$

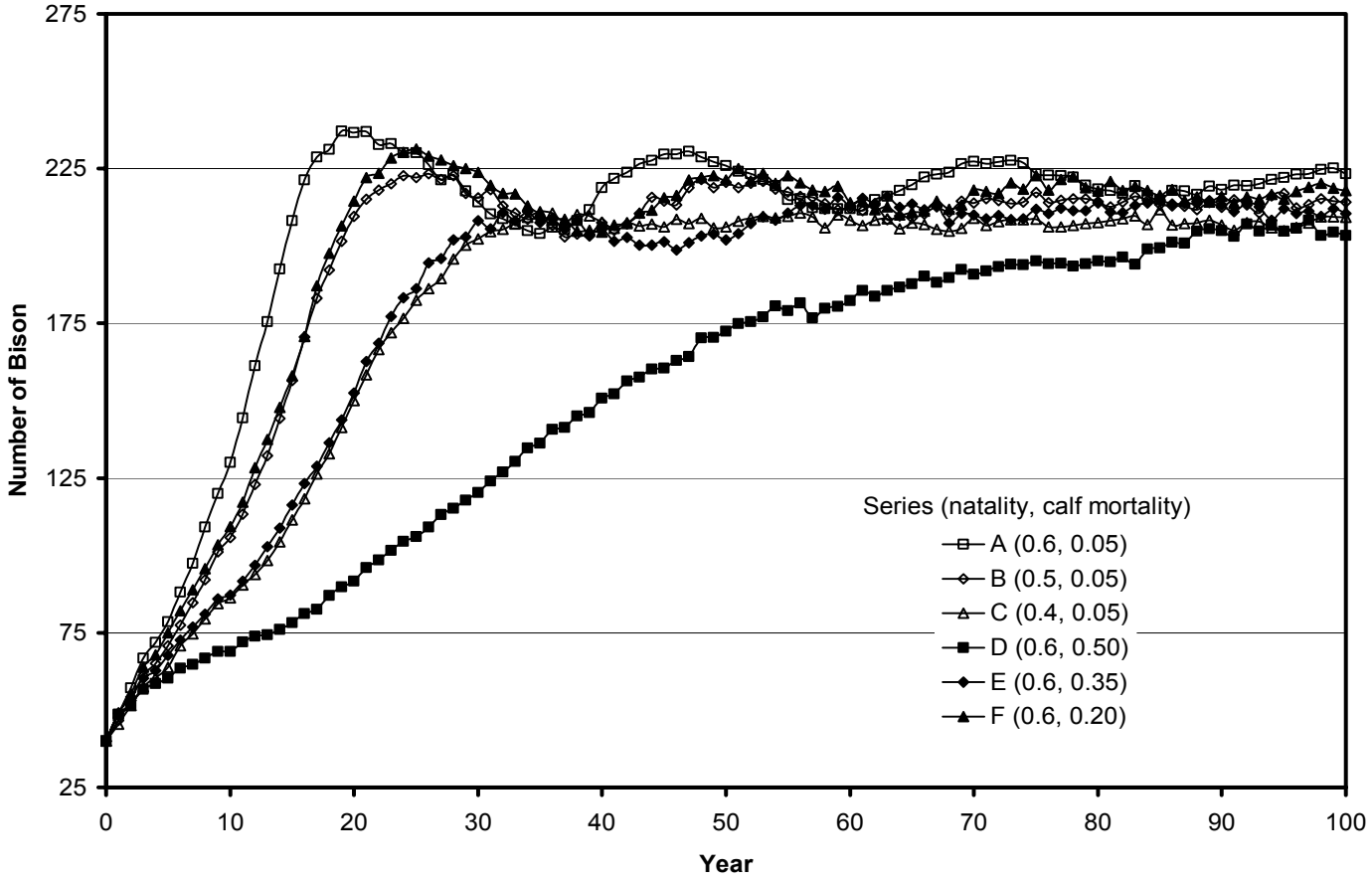

Figure 7. —Evaluation of the effects of mortality and natality rates on population size in the TSBH genetic simulation model. The various series of simulations with different natality and calf mortality rates are compared. Model evaluation conditions include large herd mortality rates for bison > 1 year in age, no migration, and random mating.

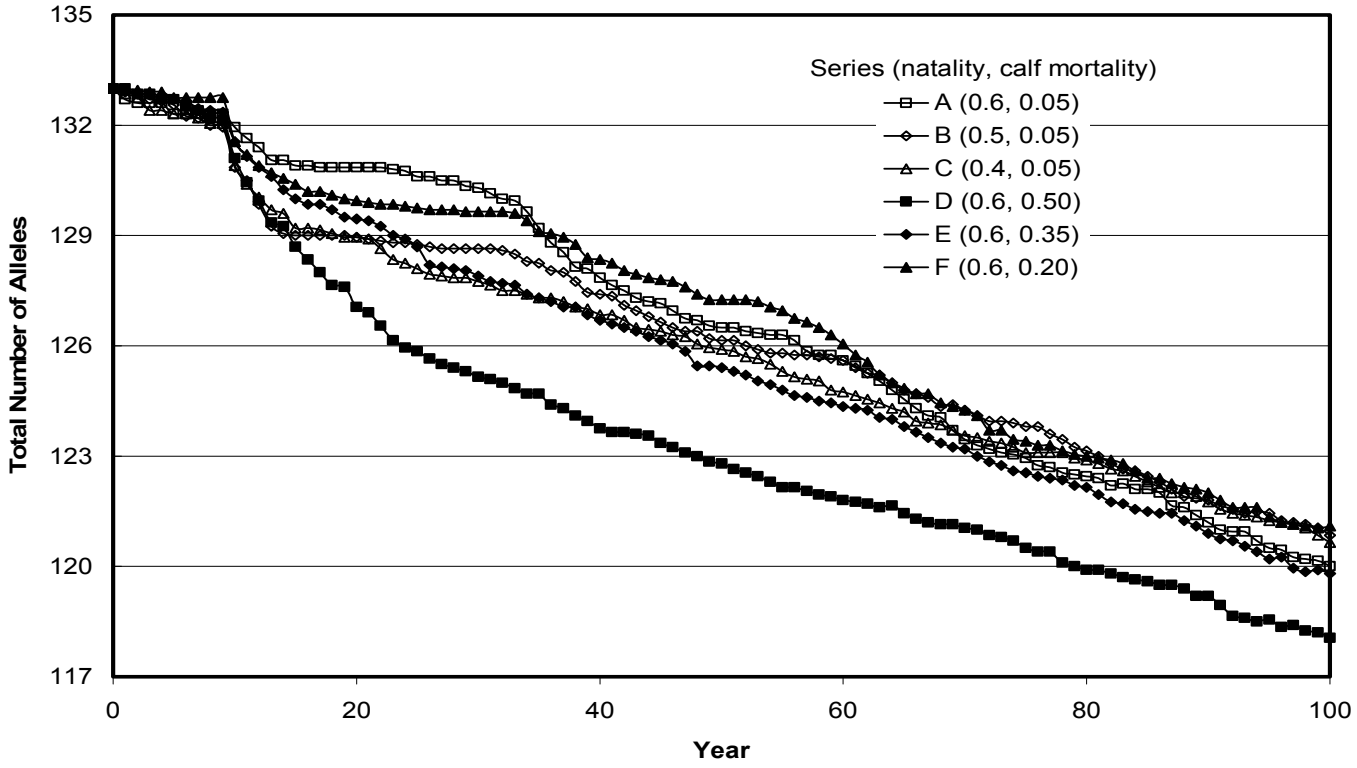

Figure 8. —Evaluation of the effects of mortality and natality rates on total number of alleles in the TSBH genetic simulation model. The various series of simulations with different natality and calf mortality rates are compared. Model evaluation conditions include large herd mortality rates for bison > 1 year in age, no migration, and random mating.

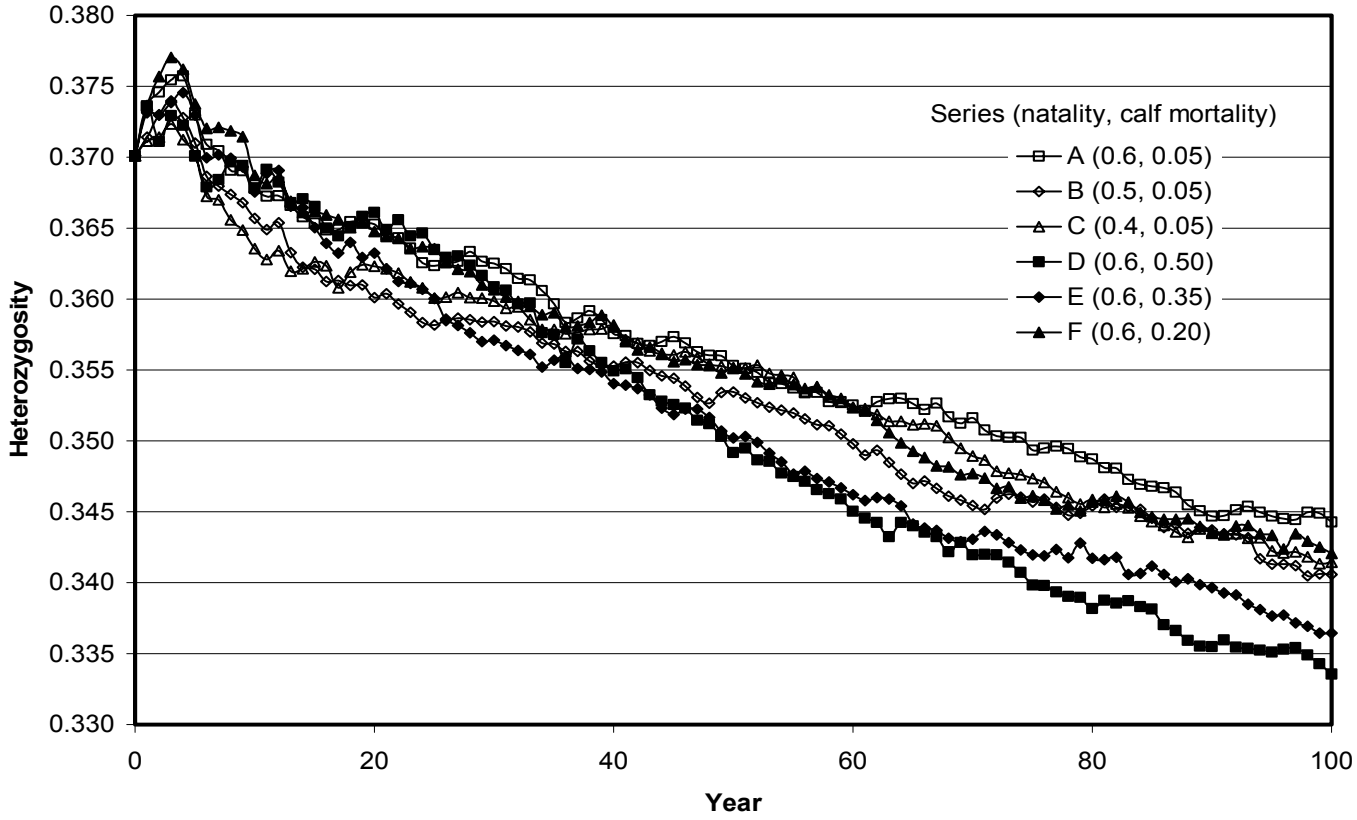

Figure 9. —Evaluation of the effects of mortality and natality rates on heterozygosity in the TSBH genetic simulation model. The various series of simulations with different natality and calf mortality rates are compared. Model evaluation conditions include large herd mortality rates for bison > 1 year in age, no migration, and random mating.

The average growth rate of the population for each series was calculated as the slope of the regression line of the average population size for each treatment from year 0 until the average population reached $n = 200$ individuals. Growth rates, average genetic diversity (total number of alleles), and average heterozygosity in year 100 for the various natality and mortality treatments are shown in Table 14. The finite growth rate of approximately 10.9%/year for series A models that observed in the Badlands National Park bison population of 10.8%/year before systematic yearly culling began in 1972 (Berger and Cunningham 1994).

The results of these analyses indicate that population growth is more sensitive to changes in calf mortality rates than to changes in natality rates. Series C approximates the current natality rate at the TSBH (40%), but includes a considerably reduced calf mortality rate (5% vs. ~50%), while series D approximates the calf mortality rate of 50% in the TSBH, but improves the natality rate (60% vs. ~40%; Table 13). Figure 7 clearly shows a difference in the rate of growth and the average time to reach carrying capacity between these two series (5.7 vs. 1.9 bison/year and 27.0 vs. 54.6 years for series C and D, respectively; Table 14). Furthermore, if only these 2 series are considered, the difference in the average total number of alleles in year 100 is statistically significant (two-tailed *t*-test, $p < 0.05$; difference in average heterozygosity not significant, $p > 0.05$). As such, it seems that genetic diversity and population growth rate are more sensitive to reductions in calf mortality rates than to increases in natality rates in this population. Similar conclusions have been made through modeling Sage Grouse

populations, where individual survival was found to have a larger impact on population persistence than fecundity (Johnson and Braun 1999).

Model Use

The model was used to evaluate 7 different mating and migration scenarios (Table 15). Average population size, genetic diversity, and heterozygosity comparisons are shown in Figures 10, 11, and 12, respectively. Under the first scenario mating was assumed random, natality and mortality rates were as calculated from the TSBH records (small herd demography), and no bison were imported into the population. On average, the population went extinct in 47.9 years ± 12.2 *SD* (rate based on extinction of one or both sexes). Of the 20 iterations, none of the populations survived to 100 years. Consequently, the standard deviations for calculations of population size, total number of alleles, and heterozygosity become quite large after 50 years; therefore, averages for these parameters were calculated based on the surviving populations each year only up to 50 years. Average calculations of census size, genetic diversity (total number of alleles), average heterozygosity, average age, and number of fixed loci from the first scenario are shown in Table 16. This evaluation indicates that within 21 years, 10% of the original genetic diversity within the TSBH will be lost (measured between initial total number of alleles and the total number of alleles remaining in a given year).

The second scenario was similar to the first except that one male was randomly chosen to mate all potentially reproductive females in a given year. This scenario was

TABLE 15

Description of management scenarios evaluated for the TSBH genetic model

Scenario	Description	Mating	Demography	Importation
1	Baseline	Random (7 - 12 year-old male mate preference)	Small herd	None
2	Single male	One 4 - 14 year-old male mates all females in given year with replacement	Small herd	None
3	Artificial insemination	Random (7 - 12 year-old male mate preference)	Large herd natality Small herd mortality	None
4	3 migrants with random mating	Random (7 - 12 year-old male mate preference)	Large herd	3 males in 1st year
5	3 migrants with preferential mating	migrants mate all females in year of importation, random mating otherwise	Large herd	3 males in 1st year
6	9 migrants with preferential mating	migrants mate all females in year of importation, random mating otherwise	Large herd	3 males in years 1, 6, 11
7	9 migrants with average natality & mortality	migrants mate all females in year of importation, random mating otherwise	Half fitness[a]	3 males in years 1, 6, 11

[a], 50% natality rate, 29% calf mortality rate, 4.85% mortality rate for age classes 1 – 2 and 4.35% mortality rate for age classes 3^{+}

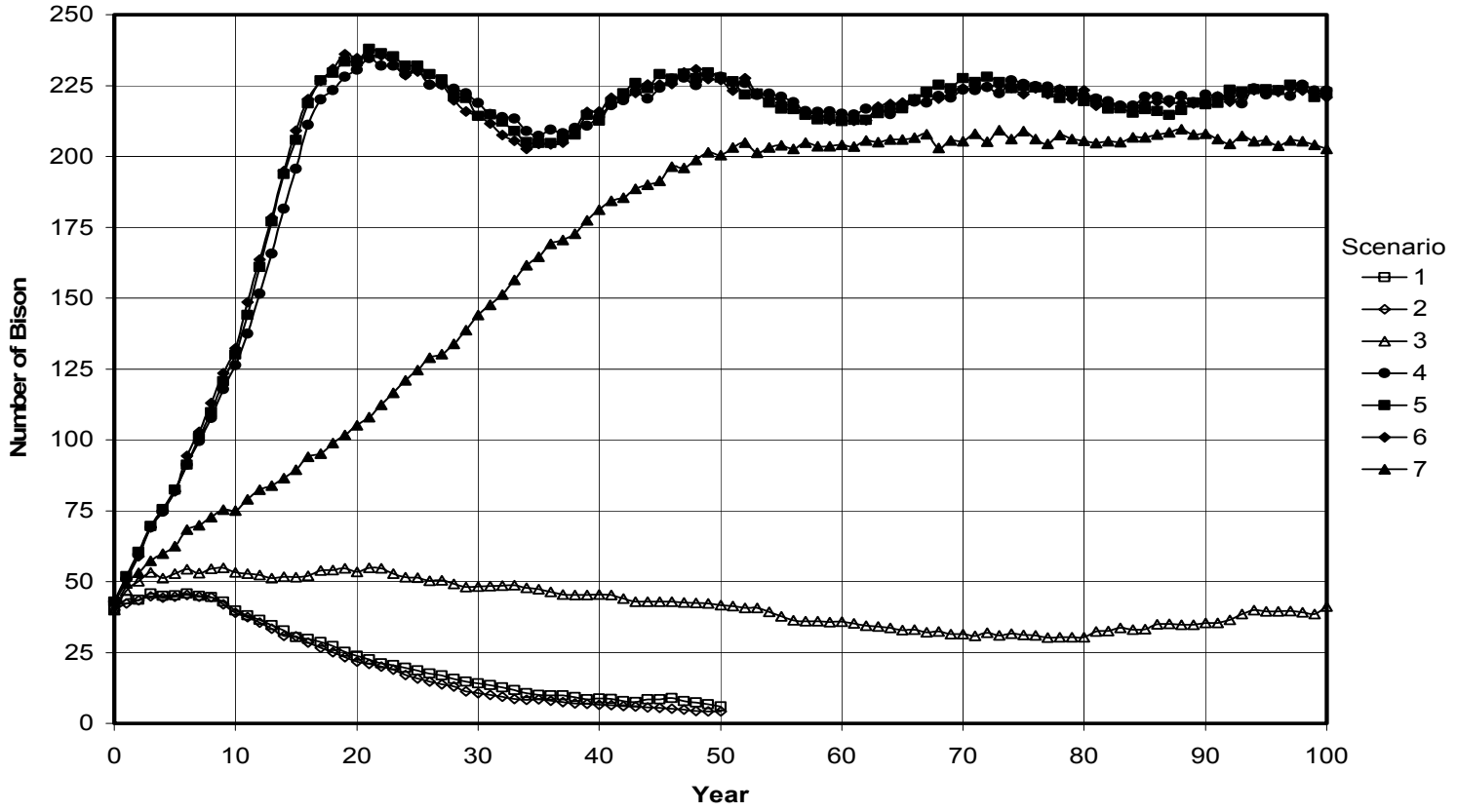

Figure 10. —Comparison of average population size among 7 management scenarios for the TSBH genetic simulation model. See Table 15 for details of each scenario. Averages for scenarios 1 and 2 were only calculated to 50 years, after which the majority of simulated populations were extinct (averages taken each year from only those populations where $n > 0$).

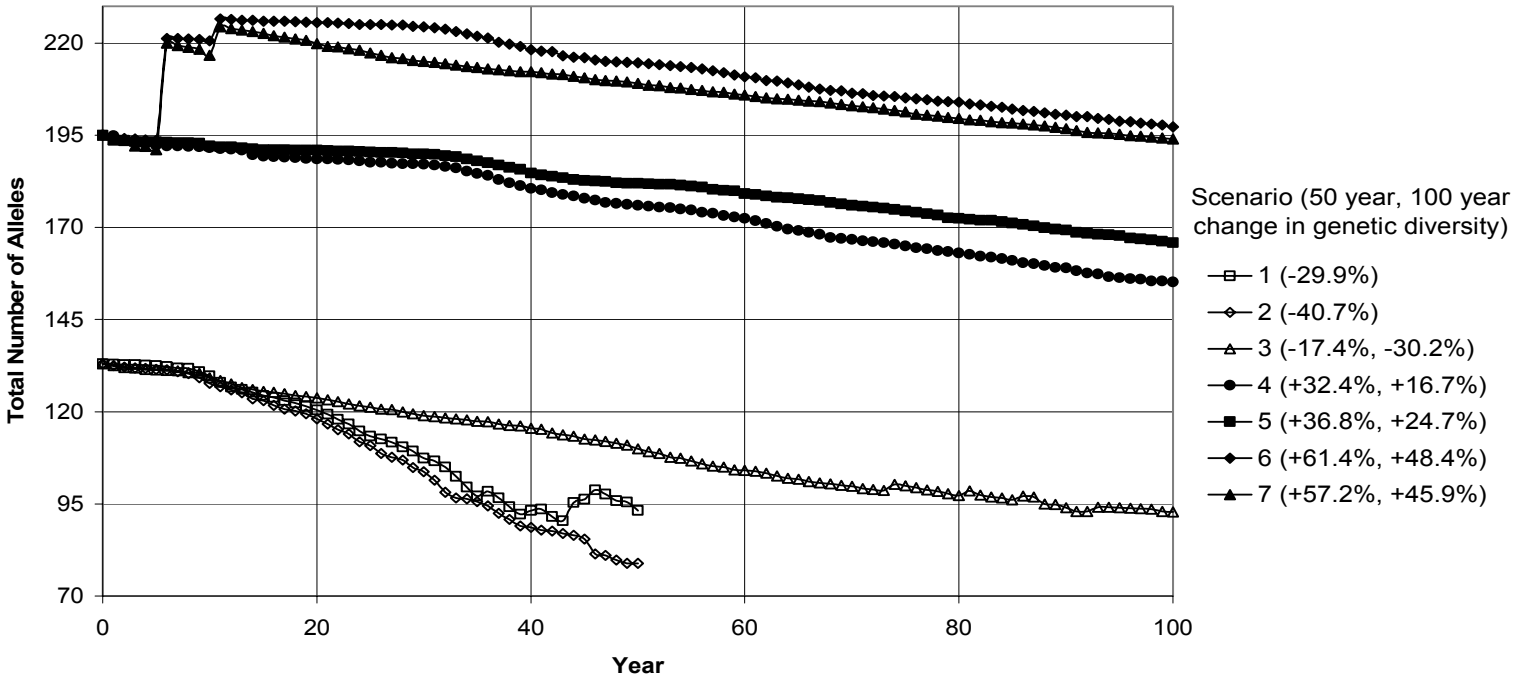

Figure 11. —Comparison of total number of alleles among 7 management scenarios for the TSBH genetic simulation model. See Table 15 for details of each scenario. Averages for scenarios 1 and 2 were only calculated to 50 years, after which the majority of simulated populations were extinct (averages taken each year from only those populations where $n > 0$).

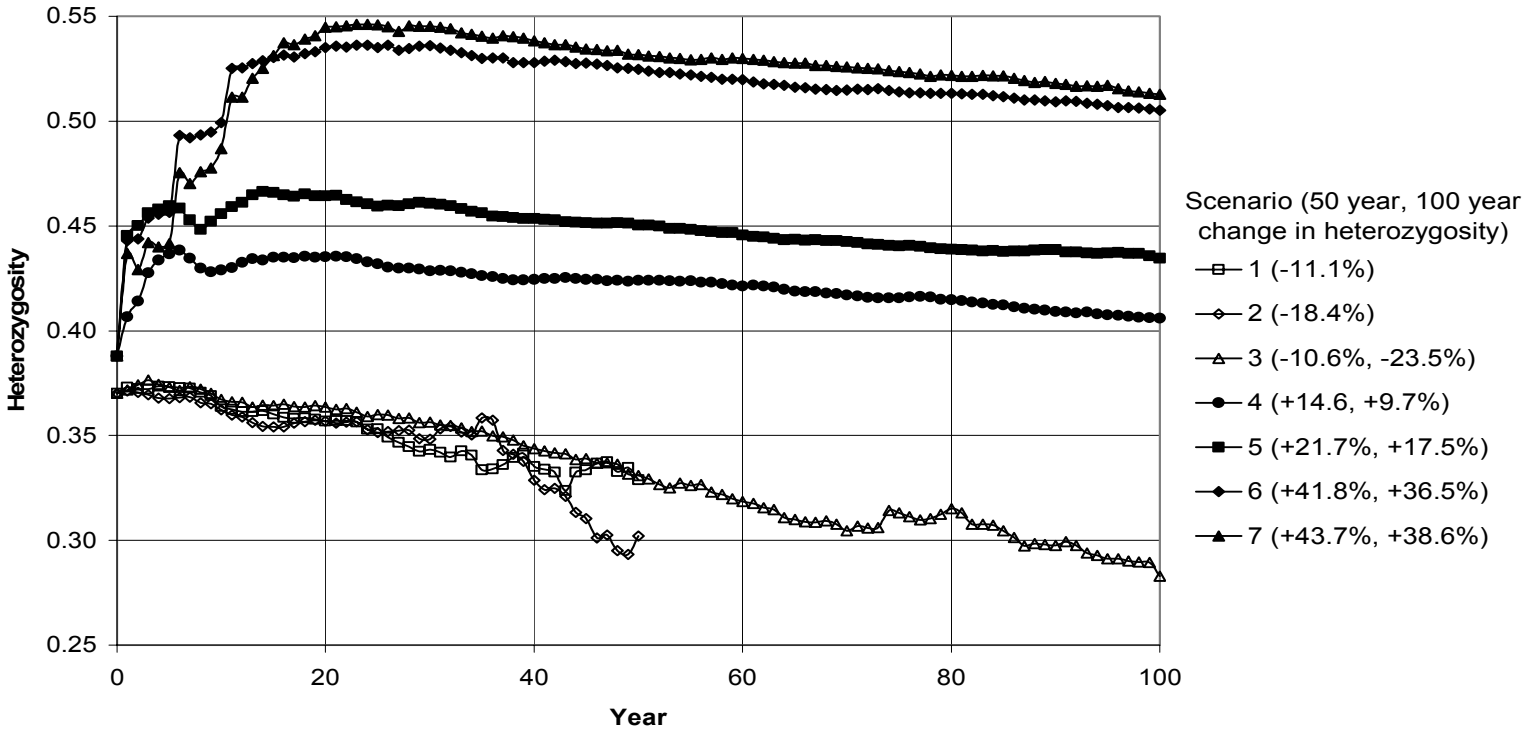

Figure 12. —Comparison of heterozygosity among 7 management scenarios for the TSBH genetic simulation model. See Table 15 for details of each scenario. Averages for scenarios 1 and 2 were only calculated to 50 years, after which the majority of simulated populations were extinct (averages taken each year from only those populations where $n > 0$).

designed to model the effects of one or a few males used preferentially to breed females within a population, as is a common practice in private bison populations and has recently been employed in the TSBH in an effort to improve recruitment rates (Danny Swepston pers. comm.). As with scenario 1, all 20 simulations were extinct by year 100. In scenario 2, 10% of the original genetic diversity within the TSBH is lost within 18 years. The average age of the population at 50 years is 14.1 ± 3.1 years, which is comparable to the average of 14.0 ± 3.8 years from scenario 1 (Table 16; averages not significantly different using two-tailed *t*-test, $p = 0.9278$).

To simulate the possible effects of artificial insemination in the TSBH, the third scenario included an increased natality rate of 60% from the baseline conditions, random mating, and no migration. Under this scenario, mortality rates were assumed to remain the same as those currently found in the TSBH. The high incidence of calf mortality

TABLE 16

Summary of simulated scenario 1 population characteristics after 50 years

Year	Census Size	Total Number of Alleles	Average % Heterozygosity	Average Age	Total Number of Fixed Loci
0	40	133	37.0	6.20	5
10	39.9 ± 6.7	129.7 ± 3.0	36.4 ± 1.1	9.7 ± 0.9	5.7 ± 0.7
20	23.9 ± 8.8	120.5 ± 4.0	35.7 ± 2.3	10.1 ± 1.5	7.9 ± 1.5
30	14.1 ± 8.5	107.4 ± 9.9	34.3 ± 3.2	11.3 ± 2.7	11.8 ± 4.7
40	8.7 ± 7.1	93.2 ± 16.3	33.5 ± 6.1	13.6 ± 3.9	19.1 ± 8.7
50	6.0 ± 4.2	93.2 ± 12.7	32.9 ± 4.1	14.0 ± 3.8	18.9 ± 7.8

despite intense and careful management of this population indicates that the main causative agent of the high calf mortality rates is likely genetically-based and a by-product of low genetic variation. As such, artificial insemination will likely act only to increase birth rates, but will not substantially affect the survival of the calves. Of the original genetic diversity, 10% is lost within 28 years, indicating a slower rate of genetic deterioration compared with scenarios 1 and 2. The average age of the population at 50 years is 8.4 ± 1.5 years, which is significantly lower than that from either of the previous scenarios (two-tailed *t*-test, $p < 0.0001$).

To further investigate extinction rates, population size and birth rates were compared between scenarios 1, 2, and 3 for 100 simulations each. Results for these simulations are shown in Table 17. ANOVA indicates that the averages for time to extinction, year of last birth, and proportion of years with no births are not uniform across the 3 scenarios ($p < 0.001$). Tukey's HSD further reveals that for all three measurements, scenarios 1 and 2 were not significantly different ($p > 0.01$), while

TABLE 17

Extinction data summarizing results of 100 simulations under scenarios 1, 2, and 3

	Scenario 1	*Scenario 2*	*Scenario 3*
Proportion Extinct Simulations[a]	0.99	1.0	0.26
Time to Extinction[b]	47.4 ± 13.3	47.1 ± 13.1	77.2 ± 15.7
Year of Last Birth[c]	40.0 ± 15.0	39.0 ± 13.1	91.8 ± 15.7
Proportion Years with No Birth[d]	38.3 ± 9.2%	39.6 ± 8.4%	7.5 ± 11.3%

See Table 15 for a description of the various scenarios presented. [a], proportion of simulations in which one or both sexes becomes extinct at or before 100 years; [b], average time until one or both sexes becomes extinct, taken only for those simulations that become extinct at or before 100 years; [c], average taken for all simulations; [d], total number of years with no births/total number of years with population size > 0

scenario 3 was significantly different from either 1 or 2 ($p < 0.01$). Using the confidence interval for the average time to extinction, there is a 99% chance of extinction given the conditions of scenarios 1, 2, and 3 in approximately 50.8, 50.5, and 81.3 years, respectively.

The last 4 scenarios simulated the effects of bison importation into the TSBH (Table 15). The fourth scenario included random mating, large herd natality and mortality rates, and 3 migrants in the first year of the simulation. Scenario 5 was similar to the fourth except the migrants were allowed to preferentially mate in the year of importation, followed by random mating in all subsequent years. In the 6th scenario, a total of 9 bison were imported into the population at a rate of 3 bison every 5 years with preferential mating in the year of importation (random mating all other years) and assuming large herd natality and mortality rates. Finally, the 7th scenario utilized the best-case importation scenario of 9 bison total, but assuming fitness would not increase to produce natality and mortality rates as those seen in other captive bison populations (i.e. large herd demography). Instead, the average between the small herd and large herd natality and mortality rates was used for this scenario (Table 15). There is a significant difference in the total number of alleles and heterozygosity in year 100 between the 4 scenarios (ANOVA, $p < 0.0001$). Tukey's HSD reveals significant pairwise differences between all scenarios in average heterozygosity and all pairs except 6/7 in total number of alleles in year 100 ($p < 0.01$). Average total number of alleles and heterozygosity in year 100 were further compared between scenarios 6 and 7 and found to not be significantly different using a two-tailed *t*-test ($p = 0.1147$ and 0.2734, respectively). In

scenarios 4, 5, and 6, the average time for the population to reach carrying capacity (n = 200) is approximately 15 –16 years, while that for scenario 7 is 49 years. The average time to incur a 10% loss of genetic diversity for treatments 4, 5, 6, and 7 is 49, 69, 71, and 59 years, respectively, while the average age at 50 years is 9.3, 9.5, 9.5, and 8.2 years, respectively.

Table 18 illustrates the proportion of fixed loci out of 51 total present during the simulation on average. Averages are taken only from those simulations with a population size $n > 0$ in a given year. For scenarios 1 – 3 with no added genetic diversity through importation, the proportion of fixed loci starts at almost 10% and increases steadily thereafter. In the last four scenarios, which include bison importation, a single locus is fixed in year 0 (by chance, the 3 imported males from group 1 are homozygous for the same allele that is fixed in the TSBH population at a single locus). As such, the proportion of fixed loci starts at around 2% and either increases at a substantially slower rate compared with scenarios not including importation (scenarios 4 and 5), or actually decreases with the importation of additional groups of males (scenarios 6 and 7; Table 18).

Discussion

Potential causes of biological and genetic observations in the TSBH

A minimum effective population size (N_e) of 50 individuals is commonly used as a population management goal to minimize inbreeding for short-term population survival (Franklin 1980; Soulé 1980). If all adults from the TSBH are considered (10

TABLE 18

Average proportion fixed loci out of 51 total loci from 20 simulations

Year	Scenario 1	Scenario 2	Scenario 3	Scenario 4	Scenario 5	Scenario 6	Scenario 7
0	9.8%	9.8%	9.8%	2.0%	2.0%	2.0%	2.0%
10	11.2%	12.7%	11.8%	2.1%	2.0%	0.0%	0.0%
20	15.4%	16.7%	14.3%	2.4%	2.0%	0.0%	0.0%
30	23.1%	24.8%	16.8%	2.5%	2.1%	0.0%	0.0%
40	37.5%	40.0%	18.4%	2.7%	2.5%	0.0%	0.0%
50	37.1%	51.0%	22.1%	3.1%	2.6%	0.0%	0.0%
100	—	—	37.1%	5.1%	3.2%	0.4%	0.6%

See Table 15 for a description of the various scenarios presented. Averages taken only from those simulations with a population size > 0 in a given year.

males, 15 females), then $N_e = 24$ (Wright 1931; Caballero 1994). The N_e formula of Lande and Barrowclough (1987) uses N_{em} and N_{ef} as the number of effective males and females (calculated here as 4.0 and 20.1, respectively) based on variance in offspring number, which in this case results in an effective population size of 13.3. This N_e estimate is lower than the previous calculation due to polygynous mating in bison, and indicates that genetic diversity will be lost at a rate equivalent to an idealized population of approximately 13 individuals. Regardless of how N_e is calculated, the effective population size of the TSBH is substantially lower than the recommended minimum of 50 for avoiding inbreeding depression and ensuring short-term population survival.

Genetic drift is expected to decrease genetic diversity at a rate inversely proportional to population size (Lacy 1987). The TSBH calf population for a 4-year period has a demonstrated loss of genetic diversity compared to the current adult

population as represented by a 7.6% difference in the total number of alleles present. This 4-year period represents the approximate generation time in bison, and the rate of erosion of genetic diversity is therefore estimated by this statistic. Clearly, genetic drift is currently causing a reduction in genetic diversity, and will continue to do so as long as the effective population size remains dangerously small.

Closed populations that have survived one or more population bottlenecks, especially when followed by consistently small census population sizes, will generally display an overall loss of genetic diversity (Nei *et al.* 1975). Empirical examples of populations with reduced genetic variability following historic bottleneck events include the Alpine ibex (Maudet *et al.* 2002), black-footed ferret (Wisely *et al.* 2002), cheetah (O'Brien *et al.* 1983), elephant seal (Bonnell and Selander 1974; Hoelzel *et al.* 1993), Florida panther (Roelke *et al.* 1993), and greater prairie chicken (Bouzat *et al.* 1998). The survival of closed populations, however, is likely affected much less by initial population size than by maintenance population size due to the consequences of genetic drift in continuously small populations (Nei *et al.* 1975). In fact, Senner (1980) reports that increasing the initial population size above 5 has little theoretical effect on long-term population survival, but that small increases in the maintenance population size have dramatic effects on the probability of long-term population survival. These theoretical results are congruent with historical data for the TSBH when compared to other extant bison herds.

Inbreeding increases at a rate inversely proportional to population size, thereby resulting in a single common lineage among all individuals of a closed population given

ample generations (Senner 1980). Inbreeding is known to have widespread detrimental effects in naturally outbreeding mammalian species. Examples include low birth weight, decreased litter sizes, increased mortality, and increased sterility in Poland China swine (McPhee *et al.* 1931); cryptorchism, high levels of defective sperm, and heart defects in the Florida panther (Roelke *et al.* 1993); low sperm counts and high juvenile mortality rates in cheetahs (O'Brien *et al.* 1985); increased rates of juvenile mortality in several ungulate species (Ralls *et al.* 1979); and vulnerability to infectious diseases in several mammalian species (O'Brien and Evermann 1988). Several demographic features of the current TSBH population concur with documented examples of inbreeding depression and/or loss of genetic variation through drift, such as low natality rates, probable male infertility, and high calf mortality rates, although inbreeding is not indicated through HWE testing. Three of the tested males that exhibited sperm motility and morphology abnormalities did not sire any offspring in the 4 year test period, which acts to further drive down the effective population size and increase the potential for inbreeding in the TSBH. Drift has likely compounded the issue of reduced fitness through the random loss of potentially important alleles and fixation of deleterious mutations (Lande 1994), possibly explaining the nearly stagnant growth rate of this population compared with other closed bison populations.

Considerations of bison importation into the TSBH

There are very few examples of populations that have recovered in census size following bottleneck events despite apparent lack of genetic variation (e.g. elephant seal;

Bonnell and Selander 1974), and the long-term fitness effects on such populations are unknown. However, the TSBH has not shown any trend towards increased recruitment or decreased calf mortality rates in the past 6 years of intense management and care. Though it is remotely possible this population might survive the current inbreeding depression through purging of deleterious alleles, the end result would almost certainly be a further reduction in genetic variation, increasing the probability of catastrophic demise by disease or natural disaster (Franklin 1980; Soulé 1980). Furthermore, the TSBH already exhibits low heterozygosity, which has been associated with an increased risk of population extinction (Saccheri *et al.* 1998).

Immigration is commonly recommended to alleviate inbreeding depression and improve population fitness in small closed populations. Furthermore, immigration into small populations is likely to increase the probability of population persistence, as indicated by ecological modeling of African wild dog populations (Vucetich and Creel 1999). The number of migrants necessary to counter the effects of drift is commonly taken to be one migrant per generation (OMPG) based on theoretical and experimental evidence (Spieth 1974; Franklin 1980; Spielman and Frankham 1992). However, OMPG is sufficient only for minimizing loss of polymorphism and heterozygosity within subpopulations while allowing for divergence in allele frequencies among subpopulations. OMPG is not sufficient in cases involving small populations, where individual viability will likely decrease and deleterious mutations will increase, collectively leading to an increased probability of extinction (Couvet 2002). Mills and Allendorf (1996) also argue that OMPG is inadequate and suggest a minimum of 1 and

maximum of 10 MPG to prevent erosion of local adaptations and outbreeding depression in cases such as those involving inbreeding depression, when N_e is much less than total population size, when migrants are likely to be at a disadvantage in terms of survival and breeding success, when the receiving population has been isolated for many generations, and/or when demographic or environmental variation indicates high danger of extinction without aggressive supplementations. Notably, the TSBH meets each of these criteria.

The decision to import unrelated bison into the TSBH should not be made hastily or carelessly. Although this population is genetically distinct from other North American bison populations (Table 4, 6, 7; Figure 5; Ward 2000), all evidence to date including natality and mortality rates and measures of genetic diversity and heterozygosity indicates that the TSBH is in a perilous genetic and demographic situation that will most likely lead to extinction. Even Simberloff (1996), while strongly warning of the dangers of hybridization between species and subspecies, recognized that introductions of new genetic variation into small populations should be considered even when the loss of a local gene pool is evident when the risk of population extinction is threatening. That is, if the gene pool would likely be lost without the introduction of new genetic variation, the introduction is justified as a last resort. The potential benefits from the addition of bison into the TSBH of increased genetic diversity, reduced levels of inbreeding, increased fitness, and increased adaptive response are critical to the long-term survival of this population (Lewontin and Birch 1966; Soulé 1980).

Any potential source of bison for importation into the TSBH should be disease-free, have comparatively high levels of genetic diversity, and should have no history of

hybridization with domestic cattle. The choice of males from YNP was based not on the feasibility of actually obtaining and importing these bison, but rather on meeting the last 2 of the aforementioned criteria. It is important to investigate the effects of increased genetic diversity as a product of bison importation into the TSBH regardless of the actual source. A direct historic link does exist between the TSBH and YNP, as Charles Goodnight donated 3 bison bulls to help establish the YNP population in 1902 (Table 1). Although the YNP bison population currently suffers from brucellosis, they are known to have high levels of genetic diversity compared with other closed bison populations, including the TSBH (Table 4; Wilson and Strobeck 1999; Schnabel *et al.* 2000). Furthermore, the YNP bison population has no history of hybridization with domestic cattle, and neither domestic cattle mitochondrial DNA nor nuclear alleles have been detected in YNP bison (Chapter III). The WC bison population also meets the aforementioned criteria and is disease-free, providing an additional potential source for the TSBH.

The effects of importing male bison into the TSBH were examined in this study. However, it is clear that one or both sexes could actually be used in such a scenario. This choice was based on two criteria. First, males were chosen to minimize dilution of the unique bison mitochondrial DNA type found exclusively in the TSBH (Ward *et al.* 1999; Ward 2000; population abbreviation JA). There is currently no evidence that the TSBH has a unique Y-chromosome constitution compared with other bison populations, as this population shares a common bison allele with all other tested populations at the Y-chromosome marker INRA189 (Appendix B). Second, importing a small number of

males is more time- and cost-effective than importing the same number of breeding females, since males can make a larger genetic impact on a population in a shorter amount of time.

Implications of modeling results

Without the addition of novel genetic variation into the TSBH, the model reveals that the population is likely to become extinct. The management practice of breeding a single male to all females in a given year (scenario 2) compared with random mating (scenario 1) has probable genetic consequences of reduced heterozygosity and overall diversity, although the probability of population survival is equivalent (Table 17; Figure 10, 11, 12). Furthermore, the average proportion of fixed loci is notably higher after 50 years in scenario 2 versus 1. Although bison natural mating practices include polygamy (Berger and Cunningham 1994), unnatural exacerbation of this mating regime seems imprudent given the small size of this population and already low genetic variation. Indeed, the least cost- and time-intensive change in the current management strategy that will at least slow the degradation of genetic diversity in the TSBH would be to employ a more random mating scheme. However, the extinction rate under either of these scenarios is convincingly high enough that neither of these management schemes is recommended for the long-term survival of this population.

The third scenario was designed to simulate the potential effects of artificial insemination in the TSBH. The rate of extinction under this scenario is slower than that from scenarios 1 and 2, but the model indicates that if the population does survive under

this scenario, it will remain stagnant in size (Figure 10). Even if this population were to survive the next 100 years through artificial insemination, it would likely have even less genetic variation and heterozygosity than observed today (Figure 11, 12). Furthermore, the model predicts the fixation of about 37% of microsatellite (presumably neutral) loci in the next 100 years, which will place the population at further risk of catastrophic demise due low selective response (Table 18). The application of artificial insemination as a viable management scheme would be prohibitively expensive and time consuming, and the model indicates the genetic and demographic benefits are limited.

With the addition of unrelated bison into the TSBH and under the assumption of increased fitness (scenarios 4 – 7), the model clearly shows increases in population size, a slower rate of genetic deterioration, improvements in heterozygosity, and a substantially slower rate of fixation of neutral loci (Figure 10, 11, 12; Table 18). The model indicates that if bison were imported into the TSBH, the best breeding strategy would be to allow the imports to make a large contribution to the breeding pool through selective breeding of these bison (scenario 5). If the same bison are imported, but not given mating preference (scenario 4), the genetic contribution over time will be less and genetic deterioration, therefore, will occur more quickly (after 100 years: 16.7% gain of alleles, 9.7% gain of heterozygosity, and 5.1% fixed neutral loci in scenario 4 versus 24.7%, 17.5%, and 3.2% in scenario 5, respectively). Even with relatively few bison imported into the population, the long-term effects are substantial. In all of the scenarios that include bison importation, the average proportion of fixed loci after 100 years is

significantly less (0.4% – 5.1%) than the current proportion (9.8%) found in this population (Table 18).

The minimum number of bison immigrants necessary to substantially improve the probability of population survival is unknown. To that end, both a single importation event of 3 bison (scenarios 4 and 5) and an importation event approximately once per generation for the next 3 generations (scenarios 6 and 7) were examined with this model. Clearly, the amount of new genetic variation added into the TSBH with 9 total immigrants is significantly more than that with only 3, leading to higher levels of genetic diversity and heterozygosity over the next 100 years (Figure 11, 12).

The introduction of new variation into small, closed populations tends to increase fitness and adaptive response (Lewontin and Birch 1966; Spielman and Frankham 1992). However, the level of improvement in fitness and probability of population persistence is difficult to measure and likely different for every species and population. In this model the introduction of new bison in scenarios 4 – 6 was assumed to trigger increased fitness such that natality and mortality rates would mimic those observed in larger captive bison populations (Table 13, 15; Berger and Cunningham 1994). Spielman and Frankham (1992) reported reproductive fitness increases in isolated, small, inbred *Drosophila melanogaster* populations with OMPG to approximately half that of the original populations. Scenario 7 simulates the effects of importing 9 bison into the TSBH while increasing natality and decreasing mortality to halfway between the current TSBH and large herd rates (Table 15). Although the differences in natality and mortality rates clearly change the population growth rate (Figure 10), the expected levels of genetic

diversity and heterozygosity after 100 years are not statistically different from the same treatment using the large herd natality and mortality rates (scenario 6, Figure 11, 12). Therefore, even with moderate changes in fitness coupled with immigration of bison into the TSBH, the likely fate if this population is substantially improved.

Concluding remarks

These experiments have shown that without the introduction of new genetic variation, the TSBH will likely continue to suffer both genetically and demographically. Furthermore, without intervention this population faces a 99% chance of extinction in the next 51 years. Artificial insemination is not a reasonable management alternative for the TSBH due to the necessarily high investment of time and resources, diminishing return of low population survivability, and long-term genetic erosion. Although careful consideration should always be given to the potential negative effects of immigration of new individuals into closed populations, especially when the source and recipient population are known to have different genetic backgrounds, sufficient evidence exists concerning the current status and probable fate of the TSBH to justify and necessitate such drastic measures. The likely long-term advantages of importation of new bison into this historically valuable resource include increased genetic variation, improved population fitness, and a significantly higher probability of population survival.

CHAPTER V

AN INVESTIGATION OF SUBPOPULATION STRUCTURE AND NON-RANDOM CULLING PRACTICES IN THE YELLOWSTONE NATIONAL PARK BISON POPULATION

O, give me a home where the buffalo roam,
Where the deer and the antelope play,
Where seldom is heard a discouraging word,
And the skies are not cloudy all day.
— Brewster Higley, "Home on the Range" 1873

Introduction

As the oldest and largest of the federal bison herds, the YNP bison population receives the most national and international public attention and is furthermore valuable from a conservation standpoint for several reasons. First, it is the only *Bison bison* population in the United States descended, in part, from a continuously wild herd. In fact, Canada maintains the only other population in the world which can claim this status. Second, it is one of only a few bison populations in the world considered "free-ranging." In this regard, the YNP population is not maintained by fences, has not received supplemental feed since 1967, and is subjected to population size management through natural forces such as predation, competition for resources, and natural mortality. Finally, the YNP bison population is a valuable genetic resource. As demonstrated in Chapters II and III, the YNP population represents a large source of

unique bison genetic variation apparently unscathed by introgression from domestic cattle.

As discussed in Chapter I, the possibility of transmission of brucellosis to livestock on private lands adjacent to YNP has been the focus of the YNP bison population management in recent years. An agreement between the U.S. Department of the Interior, National Park Service, USDA Forest Service, and USDA Animal and Plant Health Inspection Service was made in 2000 concerning the management of YNP bison as they exit park boundaries (Bison Management Plan for the State of Montana and Yellowstone National Park 2000, Final Environmental Impact Statement available at http://www.nps.gov/yell/technical/planning; hereafter IBMP-2000). In general, the necessary spatial separation between YNP bison and livestock on adjacent private lands has and will be attempted through hazing of bison back onto park property followed by, when necessary, the capture, brucellosis testing, and slaughter or release of the captured bison depending on test results. This plan does not aim to eradicate brucellosis in YNP bison, but only to control the spread of the disease to private livestock. The IBMP-2000 generally assumes that any culling as a result of this plan will be genetically random and therefore have no real impact on the genetic constitution of the YNP bison population. These assumptions, however, are largely untested. As acknowledged in the IBMP-2000, further research into the genetic implications of the current plan are necessary to understand fully both the current genetic constitution and potential impacts of the current management plan on the future of the YNP bison population from a genetic perspective.

For some time, park biologists have documented geographically distinct bison herds within YNP. Meagher (1973) recognized 3 herds based on winter distribution, noting that none of the herds were isolated geographically year-round: Mary Mountain (Firehole and Hayden Valley), Pelican Valley, and Lamar. The known contemporary winter movements of these 3 herds are given in IBMP-2000. In the range of the YNP bison population, the Mary Mountain herd is located to the southwest, the Pelican Valley herd to the southeast, and the Lamar herd to the north. The Mary Mountain herd is known to migrate in the winter primarily in a westward direction towards West Yellowstone, although recent northward movements have been noted (towards Gardiner). The Pelican Valley herd moves eastward and westward during the winter, although these migrations are not far enough from the summer range to reach park boundaries. The Lamar herd moves north and northwest during the winter towards Gardiner. The IBMP-2000 emphasizes the potential impact of bison winter movements into the West Yellowstone and Gardiner areas on the transmission of brucellosis to livestock on adjacent private lands, focusing on overall YNP bison census population size and predicted population growth rates to determine the impact of current management strategies.

Recent radiotelemetry data have indicated little interchange of bison between the "northern" (Lamar) and "central" (Mary Mountain and Pelican) herds (Edward Olexa, USGS unpublished data). Further, analyses of both tooth wear (David Christianson, Montana State University unpublished data) and parturition timing and synchrony (Peter Gogan, USGS unpublished data) have demonstrated differences between these two

herds. The current practice of culling bison without regard to possible subpopulation structure has potentially negative consequences of reduced genetic diversity and alteration of current genetic constitution both within individual subpopulations and the overall YNP bison population.

Unlike the management plan for the YNP bison population, most other federal bison management plans include culling that is purposely randomized with respect to age and/or sex structure to some extent. The details of each plan vary from yearly culling of 1 year-olds at WC to proportionate culling across age classes at TR every 3 years (Chapter I). In contrast, YNP bison are culled based on their misfortunate location at the park boundaries and, when possible, on their brucellosis status. Since bison are known to naturally assemble in matriarchal groups including several generations of related females and the most recent calf crop (Seton 1937; Haines 1995), it is possible that the culling of bison at the YNP boundaries is non-random with respect to family groups, a practice that over sufficient time may lead to systematic loss of genetic variation.

In this study, preliminary genetic analyses were conducted to investigate potential population substructure and non-random culling in YNP bison. Various analyses of population differentiation, including modeling of population substructure, were used to examine the likelihood of at least 2 genetically distinct bison subpopulations within YNP. Parentage analysis was performed to detect parent-offspring relationships and family groups within bison migrating out of the YNP boundaries.

Materials and methods

Sample collection, DNA extraction, and microsatellite analyses were performed as described in Chapter II. All samples used in this study were procured from those bison exiting the YNP boundaries at West Yellowstone or Gardiner in the winters of 1996 – 97, 1998 – 99, and 2001 – 02 (Table 19). Not all bison handled by park personnel were sampled, not all sampled bison were tested in this study due to budget and time restrictions, and the samples included here are a mix of both slaughtered and tested/released bison. Cementum-annuli methods were used to age individual bison. Samples and accompanying field data were kindly provided by Peter Gogan (USGS, Montana State University). All samples were genotyped to 90% completion as described in Chapter II. BMS601 and INRA133 were excluded from all analyses based on their propensity for null alleles such that 49 of the 51 autosomal loci previously described were utilized in this study (Table 2).

Per locus genotypic differentiation was calculated using the exact G-test of Goudet *et al.* (1996) in GENEPOP 3.1d (Raymond and Rousset 1995) with the following Markov chain parameters: 10,000 step dememorization, 150 batches, and 50,000 iterations per batch. Differentiation between groups was considered significant when $p < 0.05$. The effective number of migrants (N_m) between subpopulations was calculated using the private alleles method of Slatkin (1985) and the correction for sample size of Barton and Slaktin (1986) in GENEPOP.

The STRUCTURE program was utilized to test the probability of subpopulation structure through a clustering method for multilocus genotype data (Pritchard *et al.*

2000). The model underlying the program assumes *K* subpopulations and uses allele frequencies to assign samples to subpopulations in a probabilistic manner. In this study, *K* was tested for 1 – 5 subpopulations using a burnin period of 20,000 replicates, 500,000 Markov Chain Monte Carlo replicates, and a new random seed for each run. Appropriate burnin and replications for this dataset were determined as described by Pritchard *et al.* (2000). The assignments of individual bison to various subpopulations were compared *a posteriori* to actual collection sites.

Likelihood-based parentage testing was performed using CERVUS 2.0 (Marshall *et al.* 1998) with the following analysis parameters: 50,000 cycles, 1% genotyping error rate, 80% relaxed confidence, and 95% strict confidence assuming that only 10% of the candidate parents were sampled in each case. Parentage analysis was performed in a stepwise fashion for individual years and locations (4 groups total, see Table 19). First, 0 – 1 year-olds were treated as offspring with all bison 2^+ years of age treated as candidate parents. Next, each age class from 2 – 6 years was treated individually as offspring with all bison in any older age classes treated as candidate parents (for the 2 year-old offspring, all bison 3^+ years old were potential parents, etc). Two samples from the 2001 – 02 West Yellowstone group were of unknown age and were therefore treated first as calves with the 0 – 1 age group and then used as potential parents during each remaining analysis. Seven samples from this same group had field classifications as "adults" and were included with the 3 year-old group as offspring and all other analyses as potential parents.

TABLE 19

Sex and age distribution of YNP bison sampled

Location	Year	Age											Sex		Total
		Fetal	0	1	2	3	4	5	6	7	8	9	10+	Males	Females	
Gardiner	1996 - 97		46	27	29	12	7	11	7	9	6	4	8	63	103	166
West Yellowstone	1996 - 97		6	3	5	23	3	8	4	2	1	2	6	32	31	63
West Yellowstone	1998 - 99	29				4	18	17	9	10	4	2	3	48	48	96
West Yellowstone	2001 - 02		18	6	7	29	6	10	1	2	4	2	9	42	61	103[a]

All bison samples were collected in the winter as indicated in the area surrounding Gardiner or West Yellowstone. Ages are approximate and determined by cementum aging techniques. [a], 9 samples not recorded in age categories are from 2 age unknown bison and 7 with age classification of "adult," assumed to be at least 3 years old for the purposes of this analysis

The 29 fetal samples from the West Yellowstone 1998 – 99 collection included 12 females and 17 males. In all 29 cases, both the cow and fetus were sampled and the true cow matching each fetus is known from the field data. As such, these 29 samples were used as a control group to test the efficacy of the parentage analysis to match offspring with parents, with all 67 remaining samples from this group considered candidate parents.

Preliminary analyses failed to find any parent-offspring matches across location-year groups. Although there is a remote possibility that an offspring and parent were sampled in different locations and/or different years, the probability was considered low enough that these analyses were not exhausted and are not presented here. Therefore, the results presented below are only from parentage analysis within the same location-year group.

Results

Subpopulation structure testing

Genotypic differentiation results are summarized in Table 20, with the number of loci with significant differentiation between sample groups indicated as a percentage of the total number of tested loci. The data were divided and tested in 5 groups, with number of sampled individuals in each group found in Table 19. First, the Gardiner and West Yellowstone samples for 1996 – 97 were compared, with 65.3% of the loci between the two groups significantly different in genotypic distribution. Next, all Gardiner samples were compared with all West Yellowstone samples (77.6% loci significantly

TABLE 20

Summary results for genotypic differentiation among YNP bison groups

Comparison	Loci tested	% Genotypic differentiation
Gardiner 97 vs. WYell 97	49	65.3
Gardiner 97 vs. WYell all	49	77.6
WYell 97 vs. WYell 99	49	8.2
WYell 97 vs. WYell 02	49	8.2
WYell 99 vs. WYell 02	49	12.2

Location-year groups and number of sampled bison are found in Table 19. Indicated years correspond to the late winter (e.g. 97 = winter 1996 – 97). WYell, West Yellowstone; % Genotypic differentiation, frequency of loci that showed significant genotypic differentiation ($p < 0.05$).

differentiated). Pairwise comparisons from West Yellowstone samples were further performed with 8.2%, 8.2%, and 12.2% of the loci differentiated in the 1996 – 97 vs. 1998 – 99, 1996 – 97 vs. 2001 – 02, and 1998 – 99 vs. 2001 – 02 comparisons.

The number of effective migrants between the North (Gardiner) and Central (West Yellowstone) bison subpopulations was estimated at 2.4 for the 1996-97 data and 6.4 for the combined data from all years. For comparison, the same approach was used to estimate the number of effective migrants between the two TR populations ($N_m = 0.3$) and between FN and NS ($N_m = 1.4$) as described in Chapter II.

Posterior probabilities of the $K = 1, 2, 3, 4$, or 5 tested number of subpopulations within the YNP data set including all Gardiner and West Yellowstone samples for 1996 – 97 are shown in Table 21. The probability of 1, 2, or 5 subpopulations within this data set is approximately 0. The most likely number of subpopulations is 3, with an associated 81.7% probability. There is also an approximately 18.2% probability associated with the division of these samples into 4 subpopulations. As discussed in

TABLE 21

Probabilities of various numbers of subpopulations in the 1996-97 YNP bison data

K	ln [*Pr*(*X*|*K*)]	*Pr*(*K*|*X*)
1	-27181.1	0.000000
2	-26697.0	0.000000
3	-26684.0	0.817574
4	-26695.6	0.182426
5	-26705.2	0.000000

A uniform prior on $K = 1 - 5$ is assumed to calculate $Pr(K|X)$ from ln $Pr(X|K)$, as described in Pritchard *et al.* (2000). *K*, predefined number of subpopulations; ln $Pr(X|K)$, estimated natural log of the probability of the data given *K* subpopulations; $Pr(K|X)$, posterior probability of *K* subpopulations.

Pritchard *et al.* (2000), in cases where two modes are found the results should be used for that mode with the highest associated probability. In this case, the associated probability of 3 subpopulations is much higher than that for 4 subpopulations and is therefore the most parsimonious solution given these results. The bimodal results found here may be the result of the sampling of family groups, which would cause a nonrandom distribution of alleles within true subpopulations and lead to an overestimate of *K* in some instances. That is, in some cases, the "true" 3 subpopulations would be further divided into 4 subpopulations simply based on the nonrandomness of the data due to family groups. Similar calculations were made for the entire data set of Gardiner and West Yellowstone shown in Table 19, with the probability of 1 or 2 subpopulations within the data set also being approximately 0. In this analysis, the posterior probability of 4 subpopulations within the dataset was 99.99%, while that for 3 subpopulations was approximately 0.

TABLE 22

Number of YNP 1996-97 samples assigned to various subpopulations

		Number assigned to population:			
K	**Location**	**1**	**2**	**3**	**4**
2	Gardiner	116	50	-	-
	West Yellowstone	3	60	-	-
3	Gardiner	85	36	45	-
	West Yellowstone	2	6	55	-
4	Gardiner	31	27	69	39
	West Yellowstone	6	3	1	53

Assignments were made *a priori* without collection location information. The most likely number of subpopulations based on STRUCTURE analysis is 3, as shown in Table 21.

The assignment of individuals to *K* subpopulations was compared for *K* = 2, 3, and 4 based on both observational and computational probabilities of the existence of 2 – 4 subpopulations, as summarized in Table 22. When *K* = 2, 69.9% of the Gardiner samples are assigned to one population along with 4.8% of the West Yellowstone samples, while 30.1% Gardiner and 95.2% West Yellowstone samples are assigned to the other population. When *K* = 3, two subpopulations are composed mostly of Gardiner samples, while the other subpopulation is again an admixture of Gardiner and West Yellowstone samples. The subpopulation assignments are not robust: when *K* = 2, only 39.8% Gardiner and 49.2% West Yellowstone assignments are based on probabilities of > 80%.

Parentage testing

The total power of exclusion for identifying one unknown parent, without knowledge of the other parent, was 99.99% within each of the 4 groups tested (Table

19). Attempts were not made to find genotyping errors to correct parentage assignments when more than 2 loci were mismatched, even when the assignment carried a high confidence value. As previously discussed, various factors in the sampling of these bison precluded a complete data set of all bison at a particular location on a particular date. As such, the total parent-offspring matches made in each group are considered underestimates of the true number of parent-offspring pairs that likely existed in each location-year group. Attempts were made to detect "cohorts," in this case referring to any related group, and are reported below with maximum inclusion such that the same individual is not represented in more than one group. A summary of the number of parent-offspring matches and cohorts detected in each group is shown in Table 23. From the 166 bison sampled from Gardiner in the 1996 – 97 winter, 29 total parent-offspring matches were confirmed. Of these, 17 involved calves and 12 involved offspring > 1 year old. Of the 12 parent-noncalf matches, 4 included male offspring (three 1 year-olds and one 2 year-old). Within the matches, 7 cohorts were detected. One female 9 year-old was killed on 02/11 along with her 3 year-old female offspring, while her 2 year-old male offspring was killed on 01/16. A female calf killed 01/21 and 1 year-old male killed 01/20 were both offspring of a 5 year-old female killed 01/15. An 8 year-old female killed 01/15 was the dam of a female calf killed 01/20 and a male yearling killed 01/16. A 2 year-old female killed 01/07 and a male calf killed 01/20 were both offspring of a 6 year-old female killed 01/16. One male and one female calf were both determined to be the offspring of an 8 year-old female, all of which were killed on 01/08. The genotypes of the calves do not indicate duplicate sampling,

suggesting either an unlikely error in aging the calves or non-identical twins (precluding clerical error). Bison twins have been noted on rare occasion. Meagher (1973) reported only one known case of twins in YNP through 1966. Another surprising cohort from the 1996 – 97 Gardiner group was a 12 year-old male killed 03/06 who sired 2 minimally half-sib females, ages 2 and 5, killed within two days of each other (01/17 and 01/15). The only multi-generational female cohort detected in this study was led by a 7 year-old female killed 01/24, who was the dam of a 4 year-old female killed 01/16, who was the dam of a 3 year-old female killed 01/17, who was the dam of a male calf killed 01/22.

Three parent-offspring matches were made within the West Yellowstone 1996 – 97 group. The 2 involving calves matched an 11 year-old dam and a 4 year-old dam. The other match was a 15 year-old male with his 6 year-old female offspring killed one month apart (01/27 and 02/27).

All 29 fetal samples from the West Yellowstone 1998 – 99 group matched with confidence to the respective known dams and without conflicting candidate parents (i.e. more than one female with a high probability of parentage). Several other parentage matches were made from this group. In two cases, matches were also made to the sires of the fetuses: one 10 year-old sire was killed 04/01 while the 7 year-old dam and fetus were killed 04/15; one 8 year-old sire was killed 01/08 while the dam and fetus were killed 04/15. In another case, a 5- and 4- year old dam-female offspring pair were killed on the same day and both were pregnant (04/15). Lastly, a 6 year-old pregnant dam was killed on 04/15 at Horse Butte and her 4 year-old male offspring was killed 01/08 at Duck Creek (both locations are in the West Yellowstone area). This last case is the only

TABLE 23

Summary of parentage results by location-year groups

Location	Year	Herd size	Total tested	Dam-calf pairs	Dam-noncalf pairs	Sire-calf pairs	Sire-noncalf pairs	Total parent-offspring pairs	Cohorts detected
Gardiner	1996 - 97	865	166	17	10	0	2	29	7
WYell	1996 - 97	2571	63	2	0	0	1	3	0
WYell	1998 - 99	1846	96	29[a]	2	2[a]	0	33	4[b]
WYell	2001 - 02	2420	103	8	4	0	0	12	1

Herd size estimated for Northern herd for Gardiner samples and for Central herd for West Yellowstone (WYell) samples in the year of collection. Dam- and sire-calf pairs include parentage matches with < 1 year-olds. Dam- and sire-noncalf pairs include parentage matches with > 1 year-olds. Cohorts detected include any groups that were detected through parentage analysis, such as dam and multiple offspring of different ages. Individual cohorts are discussed in the text. [a], from fetal samples; [b], including 2 sire-dam-fetus matches

one in which the offspring-parent pairs were not sampled from the exact same location within the West Yellowstone area.

From the West Yellowstone 2001 – 02 group, 12 parent-offspring pairs were matched. Of these, 8 were calf-dam pairs. One cohort was detected, involving a 5 year-old dam killed 04/25 and 2 of her offspring: a male calf killed 04/25 and a 4 year-old female killed 04/10.

Excluding the 31 total matches made to the 1998 – 99 West Yellowstone fetuses, a total of 46 parent-offspring matches were made. Approximately 35% of these matches were sampled on the same day, while 50% were sampled within 1 day and approximately 83% within one week of each other. The field dates available for these samples correspond to the test date and not necessarily to the capture date, which are generally separated by a maximum of one week (Peter Gogan pers. comm.). It is therefore possible, but not verifiable, that more of the cohorts described above actually exited park boundaries at the same time than are indicated.

Female bison generally reach sexual maturity as 2 year-olds, capable of producing their first calf at 3 years of age (Berger and Cunningham 1994). Although 1 year-old females have been known to breed and produce calves as 2 year-olds, the occurrence is quite rare. Meagher (1973) reported an "occasional" yearling female breeding in the YNP population, while Berger and Cunningham (1994) estimated a 4.1% calving rate for 2 year-olds in the BNP population. Three mother-daughter pairs identified in this study were only 1 year apart (one 4- and 3-year old pair and two 5- and 4-year old pairs). As the production of a calf by a yearling female is biologically not

possible in bison, and the results reported here are quite robust based on the number of polymorphic markers utilized and the high level of statistical confidence required to accept a parent-offspring match, the most likely cause of these 3 discrepancies is in the assignment of ages. Cementum-annuli aging methods in bison have an overall accuracy of only 49%, with a 68% probability of age assignment to within ± 1.06 years of the actual age (Moffitt 1998).

Discussion

After the 1967 adoption of the YNP natural-regulation policy followed by a natural increase in bison numbers, bison began to be killed by non-natural means exclusively when moving beyond YNP boundaries. Until the 1980s, it was necessary to remove relatively few bison by these methods. The combination of several severe winters, competition for winter resources with other grazers, and large census bison population sizes have led to more YNP bison movements to peripheral locations, and across YNP boundaries, in the past 20 years. The current management plan takes into consideration the census size of the bison population and expected population growth rates without regard for possible population substructure. The 1,700 bison minimum, near which point lethal alternatives in the plan are to be minimized in favor of alternative management techniques, is set forth by the IBMP-2000 based on population modeling of the carrying capacity of the Yellowstone ecosystem estimated at 1,700 – 3,500 bison depending on forage availability and weather factors.

Evidence and possible effects of subpopulation structure

The results of this study sufficiently indicate some level of population subdivision with the YNP bison population. The modeling results presented here indicate the Gardiner and West Yellowstone groups may not be true subpopulations given that the Gardiner group appears in part to contain bison with similar genetic background to the West Yellowstone samples (Table 22). That is, the extrinsic grouping of bison by collection location, at least within the 1996 – 97 dataset, is not entirely reflective of underlying genetic structure. There are at least 3 explanations for this observation. First, it is possible that a relatively large number samples from the Central herd (i.e. those that would otherwise have been found at West Yellowstone) migrated north to Gardiner in the winter of 1996 – 97. Although small numbers of bison have been known to migrate from the Central herd in this fashion (IBMP-2000), 45 – 50 bison representing 27 – 30 % of the Gardiner samples would have had to migrate in this fashion to explain these data (assuming $K = 2$ or 3; Table 22). Second, large amounts of gene flow from the Central herd to the Northern herd, but not reciprocally, may have occurred at some point in the past. Third, there may actually be 3 bison subpopulations within this dataset, as indicated by the model used here (Table 21). Meagher (1973) described extensive intermingling of bison from the Pelican Valley and Lamar herds during pre-1970 breeding seasons, and noted the least amount of intermingling between the Mary Mountain herd and the combined Pelican-Lamar herd. The Pelican Valley herd is currently considered part of the Central herd, but is probably not represented in the West Yellowstone samples from 1996 – 97 based on known contemporary winter

movements (IBMP-2000). As such, the current Northern herd may partially consist of germplasm from the Pelican Valley herd due to historic genetic mixing.

The possibility of 4 separate subpopulations within the overall YNP bison population cannot be excluded, but is considered unlikely based on the possible nonrandomness of the dataset due to family groups leading to overestimation of *K*, bias of the data from unpaired collections in various years for the West Yellowstone and Gardiner sites, observational data of only 2 (Central and Northern) or at most 3 (Mary Mountain, Pelican Valley, and Lamar) subpopulations/herds within the overall YNP bison populations, and the tendency of the STRUCTURE program to overestimate *K* (Pritchard *et al.* 2000). However, these analyses do provide sufficient evidence to exclude the possibility of a single, admixed bison population at YNP (Table 21) and are supported by significant genotypic differentiation between the samples collected from Gardiner and West Yellowstone (Table 20).

Estimates of the number of effective migrants between the Gardiner and West Yellowstone "groups" indicate limited gene flow, but at a level sufficient to satisfy the "one migrant per generation" rule to maintain genetic panmixia (Lande and Barrowclough 1987). As expected, the N_m estimates between the YNP groups are higher than the estimates between the two TR populations and the FN and NS populations. The two TR populations were founded from the same stock within years of each other but have been managed in isolation for around 40 years (Table 1). Although the populations have a common ancestry, they have notable genetic differences (Table 4, 6, 7), which are reflected in the low estimated N_m. Alternatively, the FN-NS comparison

was chosen based on the genetic similarity of these populations (Table 6, 7) and recent shared ancestry (although NBR and WM also contributed founders to NS; Table 1). Slatkin's (1985) N_m estimate is based on discrete generations and assuming an equal immigration rate between demes and so should not be considered highly reliable, especially given the results of STRUCTURE modeling and difficulties surrounding non-random sampling of these data.

Although the maintenance of population subdivision theoretically leads to decreased genetic variation within individual subpopulations due to drift, overall population genetic variation is expected to increase due to differential drift of alleles and the establishment of new mutations within subpopulations (Lande and Barrowclough 1987). As such, the maintenance of subpopulations within the YNP bison population may contribute to the relatively high levels of overall genetic variation observed in this population (Table 4; Figure 3, 4). The caveat, however, is that caution must be practiced in the management of populations with substructure to ensure the maintenance of both subpopulation and total population variation. The YNP bison population has not previously been managed with this consideration in mind. For example, 1,084 bison were removed from YNP in the winter of 1996 – 97, representing a 31.5% decrease in total population size. Even more troubling, however, is the inequality in the reductions across the Northern and Central herds. While the Northern herd suffered a loss of approximately 83.9% (726/825), the Central herd was reduced by only around 13.9% (358/2,571; Peter Gogan pers. comm.). If in fact the Yellowstone bison population is

represented by 2 or 3 different subpopulations, disproportionate removals of bison from various subpopulations might have detrimental long-term genetic consequences.

Indications of nonrandom culling

The uncontested matches of 29 fetal samples to their respective dams without *a priori* information provides support for the parentage testing performed here, even when the proportion of sampled parents is assumed to be only 10%. The bison parentage microsatellite panel described by Schnabel *et al.* (2000) consists of 15 total loci, 12 of which are sufficient for most cases when neither parent is known, and all of which were included in this survey (see Chapter II). The current microsatellite panel for domestic cattle recommended by the International Society of Animal Genetics includes 11 loci (Applied Biosystems, Foster City, California). In other species, parentage analysis has been performed with similar numbers of loci, with the number necessary to establish parentage based on number of alleles per locus, heterozygosity within a population or species, and level of resolution sought. For example, while only 7 microsatellite loci were sufficient to establish parentage in armadillos (Prodöhl *et al.* 1998) and cowbirds (Alderson *et al.* 1999), a panel of 21 loci has been established (Anderson *et al.* 2002) and utilized (DeYoung *et al.* 2002) for parentage analysis in deer. In cases where a small proportion of candidate parents are sampled and neither parent is known, higher numbers of microsatellite loci are necessary to provide statistical confidence in parent-offspring matches. In this case, even with a 90% chance of not sampling the true parent of a given

offspring, a complete match at 49 loci gives a 99% exclusionary power, thereby providing confidence in these results.

Bison calves generally remain with their mothers throughout the first year of life (Berger and Cunningham 1994), so it is not very surprising to find cow-calf pairs within the sampled groups. The long-term genetic and ecological effects of killing off cow-calf pairs in this manner are unknown. Within a particular year or group of years, the short-term genetic effects are probably minimal. The population, however, is basically set back one year when a sufficient number of cow-calf pairs are killed through wasted reproductive effort, loss of genetic potential, and use of resources without beneficial gain to the population. In a relatively large population, however, these burdens may be overcome by sheer population size without serious genetic effects.

The parent-offspring matches were not limited to calf-cow pairs. Both male and female 1, 2, and 3 year-old offspring were matched to dams. Several cases of dams with multiple offspring of different ages were found, indicating the presence of family units within the groups analyzed. In one case, a multigenerational matriarchal group was found which spanned 4 generations ranging from a 7 year-old female to a male calf. All of the animals from this group were killed within 8 days of each other from the same location. These analyses indicate is much more likely for sisters or mother-daughter pairs to be sampled from the same location within days of each other, providing evidence of matriarchal groups and corroborating observational data (Seton 1937; Haines 1995). McHugh (1972) wrote off such observational reports of matriarchal groups as "largely guesswork," the idea of which he believed was "introduced on

circumstantial evidence and promulgated by plains visitors who took a fancy to it." Whatever the case in historical bison populations, the results presented here indicate some level of grouping based on relatedness within the YNP bison population. Although the majority of noncalf parent-offspring matches involved dams, 3 sire-offspring matches were found (Table 23). The matches involved only 2 bulls, one each from Gardiner and West Yellowstone in 1996 – 97, which were each killed one month or more apart from their respective offspring.

Concluding remarks

Although subpopulation structure and nonrandom culling with the YNP bison population have been suggested by this study, further investigation is necessary to properly examine each issue. The method of sampling from peripheral locations involved in this study may have precluded the detection of well-defined subpopulations. It is at this point unknown whether 2 or 3 genetic subpopulations exist within the YNP bison population, how these subpopulations are related to each other, and how much interchange occurs between them. A random sampling of bison from various locations within YNP will be necessary to resolve these issues. Furthermore, in the long-term it may be useful to sample bison from several consecutive years in this manner to establish trends in the relationships of subpopulations, such as whether any of the subpopulations are naturally converging or are remaining separate and becoming more divergent.

Although a disconcerting number of parent-offspring pairs and family groups were found in this study, providing evidence of nonrandom culling within the YNP

bison population, the magnitude and long-term genetic and demographic effects of this type of nonrandom culling are unknown. For instance, inadequate sampling and difficulties in establishing groups based on capture dates prohibited testing of average relatedness within cohorts from a single location on a single date against a random sample of bison from the associated subpopulation. The resolution of these issues, including potential long-term genetic impact, will require a complete sampling of bison as they migrate off park boundaries regardless of their eventual status. In this manner, cohorts can be fully investigated, levels of relatedness established, and culled versus non-culled groups compared. The potential impact of these issues on the long-term preservation of YNP bison warrants consideration in the future management of this historically and genetically important bison resource.

CHAPTER VI

CONCLUSION

"It now appears that the conditions of maintenance are so well established that so long as the Government prevails, the American bison will continue to endure."
— Martin S. Garretson, Secretary of the American Bison Society 1938

In the conservation biology field, much energy has been spent recently on understanding the impacts of human influence on wildlife species. In particular, the effects of maintaining protected, isolated animal populations on reserves and in parks have been the subject of much discussion. In general, population isolation will lead to decreased genetic variation within populations and increased genetic differentiation between populations. The theoretical solution to this situation is the artificial movement of a few individuals among populations each generation, thereby creating a panmictic superpopulation. However, other issues such as disease, the adaptation of populations to local environments, and genetic introgression from related species must also be considered in the management of isolated populations.

It is clear that not all bison populations were created equal, and that not all are managed uniformly. Census population sizes, population structure, levels of genetic variation, and the incidence of domestic cattle introgression must all be considered in the management of bison populations. In some cases, such as the Texas State Bison Herd, the probability of population extinction is high enough to warrant the introduction of bison from an unrelated population. In other cases, the movement of bison between

populations is both unwarranted and unwise. With the possible exception of the TRN population, all of the federal bison populations considered here appear to have adequate levels of genetic variation and heterozygosity and high population growth rates, such that no urgency should be placed on mixing bison from different populations.

This study has revealed low levels of domestic cattle introgression in a large number of populations, raising some serious management issues. Obviously, those populations with no detectable domestic cattle introgression should be maintained in isolation. Since both the YNP and WC populations contain high levels of genetic variation and no evidence of domestic cattle introgression, consideration should be given to starting satellite herds using stock from these populations. The establishment of such satellite herds from WC would be considerably easier than from YNP simply due to the brucellosis-free status of the current WC population. The maintenance of satellite herds in this manner will help ensure the future preservation of pure bison germplasm.

The necessary future management of those populations identified as containing domestic cattle introgression is less clear and much more controversial. Possibilities range from population elimination to no management change. The best strategy probably lies somewhere between the two extremes. Most of these populations probably contain unique bison germplasm and many are valuable from a historical perspective. The "hybrid reduction" method described in Chapter III, whereby detectable hybrids at loci identified in this study are eliminated, is one possible solution. This method would effectively reduce the level of detectable hybrids in these populations, but would not create "pure" bison populations. Although additional genetic markers might help this

situation somewhat by identifying other genomic regions with domestic cattle introgression in these populations, it is virtually impossible to recreate "pure" bison populations given the amount of time that has passed since the initial introduction of domestic cattle chromosomal regions into these populations. There may be one exception to this rule, however. A population was formed from NBR bison in Alaska in 1928, as discussed in Chapter I. If the introduction of the observed nuclear domestic cattle introgression into the NBR bison population was post-1928, it may be possible to reconstitute, in part, the original NBR bison population. Sampling of bison from the Delta Junction Herd in Alaska in a manner similar to that performed here might quickly determine if this type of "reconstitution" is possible.

Although attempts were made to sample every U.S. federal bison population, no samples were obtained from the Sully's Hill National Game Preserve. While this population is small, it has a unique historical lineage and has remained a closed population since its foundation (Chapter I). Future investigation is warranted in this case, since it is possible that this small population contains previously unidentified pure bison germplasm. This study was not exhaustive of publicly-maintained sources of pure bison germplasm. Six state (U.S.) bison populations were sampled by Ward (2000), and all had evidence of domestic cattle introgression. All other known state herds are derived from the federal herds considered here or the state herds examined by Ward (2000). However, Ward (2000) did not find evidence of domestic cattle introgression in the few bison tested from Canadian federal populations. Whether or not some of these populations consist of a unique bison subspecies, a genetic survey such as that

undertaken here is needed to determine the genetic constitution of these populations in relationship to the U.S. federal populations and to seek out other bison populations free of domestic cattle introgression.

As with any investigation of this magnitude, a seemingly endless number of new questions have arisen as a result of these analyses, ranging in scope from population-specific to quite general. Still, one noteworthy question stands out among the rest: is the *Bison bison* species a conservation success story? An answer of "no" might be supported by the facts that bison are found only in fragmented populations maintained through human influence, that many of the federally protected populations contain remnants of domestic cattle introgression, and that disease and potentially damaging culling practices are prevalent in one of the few populations with high levels of genetic variation and no evidence of domestic cattle introgression (YNP). However, the alternative answer is substantiated by two facts. First, there is no doubt that without the intervention of a few concerned citizens and the Canadian and U.S. governments in the late 1800s, bison would have suffered the same fate as the passenger pigeon (the single most prevalent bird in North America in the early 1800s, extinct by 1914). Second, the continued involvement of both governments in understanding the current genetic structure and long-term effects of management decisions on federally-maintained bison populations will help ensure the long-term survival of this iconic species, continuing their success story. Bison have made a remarkable recovery from near-extinction in both sheer numbers and relative genetic constitution. The fate of the species, however, still lies in the hands of concerned citizens and the Canadian and U.S. governments.

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APPENDIX A

COMPARATIVE ALLELE SIZES FOR ABI 377, 310, AND 3100 GENETIC ANALYZERS FOR 54 POLYMORPHIC MICROSATELLITES UTILIZED

Locus name and called allele sizes are listed on the first line, followed by designations for type of genetic analyzer. Ranges for allele sizes are approximate and not available in every case. Primers are assumed to be nontailed (nt) and identical to those sequences found at www.sol.marc.usda.gov, unless noted by the "tailed" designation. See Chapter II for details.

AGLA232	155	159	161	165	167	169	173
377			161.43-161.63	165.0-165.58	167.43-167.56	169.4-169.58	173.38-173.55
310			159.02-159.33	162.99-163.37	165.11	167.06-167.1	171.17-171.28
3100	152.72-153.37	156.59-156.87	158.26-159.12	162.09-163.15	164.7-164.92	166.56-167.13	170.64-171.21

BL1036	177	179	181	191	193
377	177.05-177.27	179.15-179.27	180.94-181.27	190.92-191.13	
310	174.47-174.83	176.59	178.27-178.91	188.38-188.81	
3100	173.33-174.97	175.71-176.54	177.39-178.49	187.44-188.77	189.58-190.55

BM1225	239	241	245	247	249	253	265	269	271	273
310	239.88-240.65	241.86-243.43			250.09-250.97	254.01-254.89	266.53-267.27	270.52-271.31	272.44-273.42	
3100	239.37-240.25	241.39-242.66	245.8-245.89	247.78-248.03	249.68-250.16	253.56-254.56	266.04-266.32	269.92-270.92	271.95-272.48	274.13-274.49

BM1706	232	238	252	254	246	250
310	231.41-232.49	237.08-238.56	251.06-252.12	253.03-254.12	245.98	249.44-249.58
3100	230.73-231.66	236.52-237.88	250.55-251.21	252.5-252.93	244.71-245.23	248.76-249.35

BM17132	85	87	89	91	95
310	77.63-78.86	79.62-80.97	81.73-82.79	83.78-84.54	87.72-89.01
3100	77.68-78.27	79.64-80.31	81.76-82.36	83.79-84.38	88.13-88.6

BM1824	178	180	184	190	192	196	198
377		179.27-180.0	183.52-183.85	189.9-190.7	191.95-192.28	196.16-197.22	198.17-199.32
310		178.27-178.68	182.65-182.93	189.04-189.26	191.2-191.46	195.49-195.8	197.61-198.0
3100	175.1-175.65	176.92-178.02	181.52-182.08	187.91-188.41	190.0-190.79	194.26-195.01	195.83-197.22

BM1862	201	202	205	207	211	215
377	201.17-201.35	202.25-202.44	204.76-205.59	207.27-207.66	210.87-211.73	
310		200.93-201.4	203.2-204.26	205.96-206.39	210.08-210.41	
3100	198.55-199.32	199.73-200.3	202.69-203.39	204.55-205.47	208.74-209.45	212.86-213.6

BM188	99	105	109	113	115	117	119	121	123
377	98.11-98.33			112.96-113.63	115.02-115.87	117.22-117.98	119.3-119.78	121.49-121.81	123.51-123.92
310	96.58				113.99-114.19	116.18-116.48	118.58-118.64	120.74-121.04	123.06-123.2
3100	95.33-96.17	101.68-102.32	106.62-106.71	110.52-111.24	112.69-113.48	114.73-115.9	117.42-117.65	119.47-120.24	122.01

BM1905	172	176	182	184
310	170.82-171.65	174.67-175.67	180.66-181.58	183.29-183.43
3100	170.27-171.27	174.11-175.59	180.26-180.92	182.45-183.23

BM2113	127	129	133	135	143	145	149	153
310	123.42-124.01	125.3-126.33	129.38-130.71	131.44-132.25	139.46-140.39	141.53-142.23	145.18-145.91	
3100	123.05-123.47	124.85-125.54	128.79-129.47	130.44-131.49	138.37-140.16	140.53-141.76	144.22-145.08	148.07-148.93

BM2830	142	146	148	150	152	156	158	162	164
377	142.38-142.91	148.24-148.47	150.14-150.35	152.19-152.67	153.97-154.37	158.44-158.5	160-160.41	164.2-164.4	165.92-166.21
3100	139.27-140.15	144.61-145.33	146.73-147.46	148.67-149.22	150.42-151.71	154.67-155.18	156.14-157.04	160.24-160.77	162.06-162.77

BM4028	108	114	116	118	122	126
377	107.57-107.89	113.98-114.28	116.1-116.41	118.27-118.51		
310	105.77-105.93	112.51-112.52	114.43-115.05	116.17-117.63		
3100	104.92-105.29	111.49-111.98	113.7-114.2	116.0-116.56	120.71-120.95	124.79-126.03

BM4107	159	165	173	175	179	181	183	185
377		165.16-165.39			180.0-180.11	182.07-182.24	184.14-184.39	186.26-186.42
310		163.09-163.46			178.04-178.72	180.0-180.43	182.39-182.62	
3100	156.33-156.44	161.98-163.15	171.03-171.41	172.87-174.06	177.13-177.87	179.33-180.23	181.43-182.57	183.86-183.93

BM4311	90	92	96	98	102	104
377	90.57-90.91	92.38-92.84	96.2-96.77	97.88-98.67	101.79-102.99	104.12-104.7
310	86.72-86.79	88.8-88.91	92.69-92.89	94.54-94.78	98.17-98.57	100.0-10.51
3100	85.79-86.33	87.91-88.42	91.94-92.38	93.76-94.3	97.59-98.1	99.22-100.08

BM4440	123	125	127	129	131	133	143
310	123.82-124.28	125.93-126.7	128.21-128.81	130.39-131.16	132.78-133.22	135.23-135.31	145.35-145.7
3100	123.14-123.76	124.98-126.51	127.05-128.28	129.22-130.44	131.45-132.56	133.64-134.14	144.07-144.9

BM47	103	105	107	111
377	103.39-103.64	105.5-105.73	107.65-107.84	
310	102.7-102.94	104.92-105.06		
3100	102.01-102.59	104.36-104.67	106.62-106.81	109.94-110.03

BM6017	104	114	116	118	120	122
377	104.05-104.39	114.22-114.33	115.82-116.45	117.48-118.45	119.78-120.44	122.15-122.44
310	100		112.23-112.5	114.33-114.68	116.42-116.65	118.62-118.73
3100	99.07-99.71	109.32-109.76	111.25-111.9	113.23-113.99	115.47-115.91	117.6-117.95

BM711	161	163	165	167	175	177
377	159.51-160.61		163.85-164.8	166.07-166.45	174.44-174.78	
310	156.9-158.18		161.79-162.08	163.09-164.31	172.43-172.82	
3100	156.58-157.33	158.91-159.26	160.9-161.4	162.71-163.59	171.61-171.95	173.62-174.14

BM720	203	213	223	225	227	229	231	233	235
310	205-205.14	214.51-215.55		224.88-226.01	227.13-228.1	229.26-230.29	231.33-232.58	233.52-234.59	235.63-236.7
3100	203.89-204.75	214.11-214.67	222.37-222.77	224.39-225.7	226.59-227.1	228.62-229.37	230.74-231.54	233.02-234.18	235.16-235.57

BM757	186	188	190	192	194	196	198	200	202
377			190.3-190.42	192.42-192.69	194.39-194.72	196.58-196.82		200.69-201.0	
310				191.49	193.2-193.66	195.44-195.81		199.67-200.1	
3100	183.72-184.74	185.54-185.96	187.89-188.31	189.83-190.41	191.62-192.9	194.11-194.93	196.33-196.78	198.45-199.21	200.57-201.09

BMC4214	175	179	181	185	187	191
377	174.64-175.37	178.82-180.0	180.95-181.6	185.17-185.94	187.27-187.54	
310	173.77-174.01	178.16-178.31	180.32-180.73	184.56-184.78	186.69-187.08	
3100	172.59-173.29	176.56-177.69	178.91-179.84	183.41-184.15	185.46-186.26	189.92-190.08

BMS1001	107	109	111	113	115
377	106.58-106.7	108.12-108.39	110.27-110.5	112.9-113.35	114.86-115.14
310	104.12	105.11-105.8	107.81	110.67-111.09	112.45-112.99
3100	103.25-104.17	104.68-105.55	106.91-107.8	109.72-110.8	111.75-113.03

BMS1074	152	154	156	158	160
377		153.37-153.59	155.24-156.67	157.22-157.4	159.04-159.31
310		150.94-151.27	152.83-153.12	154.61-154.88	156.4-156.8
3100	148.63-148.88	150.19-151.09	152.1-153.08	154.02-154.83	155.87-156.67

BMS1117	89	91	93	99
377	87.94-88.96	89.77-91.03	91.4-92.89	97.69-97.97
310	84.43-85.68	86.48-87.62	88.5-89.61	94.63-94.68
3100	83.79-85.03	85.73-87.07	88.04-89.46	92.97-94.32

BMS1172	86	88	90	92	100	102	104
377 (nt)		86.01-87.12	87.84-89.3	90.0-90.58	97.9-98.89	100-100.83	102.13-102.73
310 (nt)		82.47-83.22	84.22-85.68	87.1-87.67	94.51-95.15	96.53-97.18	98.57-99.01
3100 (nt)		82.16-82.66	84.24-84.64	86.58-86.61	94.26-94.54	96.13-96.35	98.0-98.34
3100 (tail)	87.16	88.99-89.46	90.53-91.38	92.92-93.24	100.39-100.85	102.48-102.78	104.57-104.85

BMS1315	135	137	141	147	149
377	134.3-134.68	136.34-136.84	140.76-141.01	146.99-147.23	
310	133.36-133.67	135.54-135.69	139.9-140.1	146.01-146.14	
3100	131.47-132.8	133.61-134.91	137.79-139.17	144.25-145.58	146.4-147.28

BMS1355	146	148	150	154
377	146.52-147.12	148.57-148.94	150.32-150.8	154.52
310	143.79-144.51	145.62-145.95	147.43-148.0	151.4-151.55
3100	143.05-144.0	144.89-145.74	146.86-147.66	150.78-151.33

BMS1675	85	87	89	91
377	84.89	86.02-87.21	89.12-89.31	91.21-91.6
310		85.84-85.99	88.09-88.27	90.37-90.47
3100	82.7-83.38	84.9-85.55	87.29-87.8	89.45-90.07

BMS1716	185	189	191	193	195	197
377		188.57-188.82	190.0-190.9	192.77-192.86	194.87-195.17	
310		187.93-188.53	190.0-190.54	192.1	194.31-194.9	
3100	182.5-182.96	186.48-187.54	188.55-189.69	190.84-191.8	192.77-193.97	195.47-195.63

BMS1747	89	95	99	101	103
377		94.33-94.75	98.5-99.2	100.58-101.02	102.69-103.24
310		92.9-93.1	97.04-97.3	99.16-99.46	101.28-101.37
3100	85.8-86.11	92.22-92.61	96.44-96.72	98.46-99.37	100.68-100.9

BMS1857	142	146	148	150	156	158	160	162	164	168
377	142.01-142.27			150.09-150.42	156.2-156.65	157.8-158.54	159.8-160.32			168.08-168.41
310	138.75-139.17			146.75-147.08	152.63-152.97	154.1-155.02	156.76-157.21			164.17-164.43
3100	137.82-139.28	141.49-142.56	143.08-144.8	146.22-147.26	152.34-153.36	153.83-155.28	156.19-157.63	158.7-159.55	161.04-161.48	163.3-164.89

BMS1862	142	144	156	158	160	162	164	166	167	168	170
310	140.41-142.09		154.74-155.07	156.1-157.62	158.31-159.6	160.43-161.78	162.06-163.34	164.46-165.49		166.55-167.72	168.63-169.11
3100	139.54-141.15	142.43-142.87	153.98-154.76	155.2-156.63	157.16-159.07	159.84-160.47	161.73-162.82	164.01-164.46	164.99-165.15	166.01-166.9	168.12-169.17

BMS2258	127	134	136	138	140	142	144	146	148	150
377	127.12-127.44				139.89-140.42	141.87-142.49		146.18	148.07-148.75	150.14-150.69
310	125.77-126.22				139.02-139.39	141.03-141.44			147.27-147.47	149.16-149.46
3100	124.61-125.2	131.54-131.7	133.63-134.02	135.52-136.19	137.64-138.43	139.76-140.43	141.84-142.47	143.3-144.55	145.76-146.57	147.91-148.6

BMS2639	168	170	172	174	176	178	186
377	167.43-168.39	169.37-170.36	171.35-172.83	173.46-173.91	175.35-175.9	177.23-177.81	185.31-185.79
310	164.67-165.76	166.96-167.59	168.95-169.49	171.21-171.56	173.19-173.34	175.13-175.45	183.23-183.48
3100	163.75-164.66	165.79-166.56	167.69-168.6	169.93-170.67	171.86-172.68	173.79-174.64	182.0-182.59

BMS410	83	85	89	97	93	95
310 (nt)	80.1-81.0	82.73-83.13	87.14-87.52	95.82-95.99		
310 (tail)	86.2-87.01	88.87-89.09	92.75-93.43	101.41-101.71	97.41-97.51	99.3
3100 (tail)	86.26-87.17	88.71-89.1	92.79-93.53	101.23-101.57	97.25-97.47	99.22-99.64

BMS510	91	92	94	95
377	91.76-92.05	92.69-92.84	94.79-94.97	95.95-96.14
310	89.69-90.0	90.59-90.83	92.69-92.9	93.87-94.12
3100	89.15-90.07	90.22-90.82	92.41-92.73	93.01-93.98

BMS527	159	163	165	167	171	173	175	177
310 (nt)		159.8-160.11		163.69-163.99		169.54-170.33	171.67-172.36	173.49-174.37
310 (tail)		165.81-166.21	167.83-168.18	169.92-170.61		175.77-176.74	177.72-178.75	179.77-180.83
3100 (tail)	161.51-161.86	165.44-166.01	167.48-167.92	169.33-170.33	173.54-173.73	175.14-176.17	177.14-178.5	179.42-180.07

BMS528	140	146	148	150	152
377	139.89-140.22	145.79-146.44	148.11-148.51	150.19-150.57	152.28-152.25
310	139.9-140.0	145.84-146.19	147.81-148.22	149.89-150.04	151.88-152.06
3100	138.55-139.26	144.67-145.37	146.78-147.22	148.72-149.52	150.83-151.22

BMS601	172	174	176	178	180
377	171.12-171.65	173.21-174.16	175.39-176.13	177.41-178.07	179.68-180.6
310	170.61-171.1	171.95-173.2	174.97-175.4	177.01-178.29	179.45-180
3100	169.5-170.51	171.65-172.52	173.8-174.58	175.96-176.69	178.01-178.89

BMS812	90	96	106	108	110	112	122
377	91.07-91.27		106.51-106.89	108.51-108.78	110.57-110.75	112.52-112.75	
310	88.03-88.26		101.79-102.15	103.54-104.15		107.43-107.75	
3100	87.75-88.52	92.69-93.17	101.83-103.06	103.95-105.04	106.06-106.69	107.37-108.73	116.99-117.76

BMS911	100	102	104	106	112
377	99.06-99.36	100.8-101.39	102.51-103.32	104.75-105.18	
310	96.04-96.24	98.06	99.8-100	101.77-101.87	
3100	95.31-95.88	97.25-97.64	98.95-99.85	100.94-101.48	106.83-107.46

BMS941	81	83	85
377	80.61-80.97	82.01-83.07	85.07-85.14
310	78.38-78.68	80.61-81.09	82.99-83.06
3100	77.82-78.75	79.93-80.92	82.78-83.05

HUJ246	242	252	256	258	260	262	264
377				258.41-258.68	259.47-260.56	261.36-262.55	264.14-264.36
310				256.56-257.66	259.23-259.47	260.43-261.51	262.36-263.27
3100	241.28-242.06	251.13-251.29	254.76-255.59	256.73-257.58	258.01-259.33	259.91-261.66	262.49-263.11

IL4	83	85	89	91	93	95	97	99	103	105
377	83.11-83.35		89.13-90		93.12-93.32		96.93-97.19	98.94-99.12	102.78-103.09	104.79-105.03
310	79.91-80		85.94-86.11		90.09-90.19		93.8-93.99	95.74-95.89	98.99-99.64	101.49-101.59
3100	78.88-80	80.89-81.54	85.2-85.67	87.09-87.73	88.91-89.92	91.12-91.68	93.02-93.62	95.07-95.69	98.82-99.24	100.76-101.95

ILSTS102	113	133	143	145	147	153
377			142.92-143.18	144.84-145.13	146.78-147.04	
310			141.08-141.57	143.18-143.41	145.01-145.33	
3100	110.4-110.86	130.58-131.33	139.7-141.21	141.83-143.14	143.49-145.05	150.22-150.49

INRA037	118	120	122	124	126	132
377	117.72	119.65-120	121.34-122.26	123.66-124.48	126.32-126.35	132.48-133.01
310		115.86-116.19	118.1-118.68	120.43-120.88		129.39-129.44
3100	113.7-114.35	115.84-116.53	118.11-118.83	120.31-121.15	122.72-123.6	129.16-129.68

INRA133	223	227	234	236	238	240
377	223.29-223.43	227.49-227.75		235.79-236.05	237.97-238.07	240.0-240.3
310	222.31	226.24-226.74		234.88-235.3	237.01	239.19-240.31
3100	221.23-222.04	225.0-225.83	231.75-232.04	233.62-234.4	235.89-236.44	237.14-238.7

INRA189	96	100
3100		

INRA194	144	154	156	158	160
377	143.64-144.09	153.24-154.37	155.33-156.49	158.25-158.5	160.32-160.42
310	142.86	152.81-152.98	154.87-155.07	156.93	
3100	141.24-142.03	151.16-152.42	153.4-154.19	155.38-156.13	157.73-158.17

RM372	114	118	128	130	132	134	136	138
377	113.9-113.94	117.68-118.04		129.52-129.67	131.57	133.43-133.57		
310	110.44-111.06	114.1-115.18	124.76-125.43	126.49-127.97	128.86-129.76	130.68-131.79	132.92-134.03	134.89-135.84
3100	110.38-110.72	113.9-115.1	124.48-125.39	126.33-127.4	128.25-129.13	130.21-131.69	132.17-133.16	134.11-135.04

TGLA122	136	140	142	144	148	150
377	135.7-135.88	139.89-140.11	141.94-142.22		148.15-148.63	150.18-150.66
310	134.48-134.67	138.93-139.08	140.99-141.24		147.05-147.27	149.0-149.31
3100	133.61-133.9	137.61-138.41	139.66-140.65	142.39-142.48	145.9-146.74	147.79-148.74

TGLA44	149	151	153	155	157	159
377	148.83-149.22	150.0-151.12	152.92-153.81	154.04-155.27	157.09-157.3	159.06-159.3
310	148.62-148.96	150.62-150.96	152.63-153.16	154.59-155.13	156.67	158.6-158.92
3100	148.02-148.41	149.91-150.53	151.53-152.72	153.2-154.68	155.94-156.7	157.24-158.66

TGLA53	132	134	136	138	140	142
377		133.94-134.7	136.13-136.78		140.62-140.75	
310		131.86-132.3	134.02-134.26			
3100	129.09-129.4	130.97-131.7	133.05-133.93	135.29-135.6	137.22-137.79	139.45-139.77

URB011	139	143	145	147	149	151	153	155
377		142.68-142.89	144.71-144.94	146.77-147.01	148.75-149.12	150.88-151.15	152.81-153.27	
310		141.16	143.18-143.22	145.13-145.23	147.14-147.29	149.18-149.31	151.24-151.83	
3100	136.28-136.36	140.36-140.66	142.27-142.83	144.25-144.73	146.37-146.81	148.42-148.79	150.52-151.15	152.55-153.11

APPENDIX B

ALLELE FREQUENCIES FOR 54 POLYMORPHIC LOCI ACROSS 11 BISON POPULATIONS

Called allele sizes shown in leftmost column with frequencies as percentages.

See Table 1 for population abbreviations. Bold highlights indicate private alleles.

AGLA232	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
155	6.07	8.90			8.16		5.02		15.92		
159											**0.21**
161	14.92	5.21	34.62	60.07	19.39	10.04	19.59	37.50	9.76	52.86	29.63
165	60.98	68.71	15.38	34.90	64.29	70.08	60.50	62.50	47.43	21.43	51.75
167			1.28								2.88
169	5.57	5.21		1.68	4.08	8.07	10.19		9.76	24.29	4.94
173	12.46	11.96	48.72	3.36	4.08	11.81	4.70		17.12	1.43	10.60

BL1036	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
177	4.81	11.14	10.26	4.00	12.24	17.72	9.72		10.17		18.03
179				21.67	5.10				26.03		2.97
181	1.28	13.25	15.38	37.00	30.61		3.13		24.66	55.71	26.84
191	44.07	50.90	42.31	27.33	36.73	27.80	49.53	42.50	19.66	10.00	52.15
193	49.84	24.70	32.05	10.00	15.31	54.48	37.62	57.50	19.48	34.29	

BM1225	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
239	23.86	13.64		2.65	8.51	28.81	8.70		17.91		
241	41.72	60.30	30.26	39.40	65.96	16.73	49.33	45.00	31.03	74.29	43.84
245				**0.99**							
247				**7.62**							
249				7.28	1.06		3.68		5.32		0.82
253	22.73	5.15	7.89	10.60	2.13	10.04	11.87	15.00	20.92	14.29	19.20
265	1.14	2.42							9.75		
269	10.55	18.18	35.53	21.85	15.96	44.42	23.58	40.00	6.21	5.71	9.34
271			26.32				2.84		8.87	5.71	26.80
273		0.30		9.60	6.38						

BM1706	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
232	18.14	13.80	6.41	28.48	18.18	17.78	6.33	12.50	16.43		5.65
238	76.80	67.18	75.64	66.56	71.59	82.22	83.83	77.50	54.24	82.86	76.18
246									1.24	1.43	
250								10.00		1.43	0.62
252	5.07	19.02	17.95	4.97	10.23		9.17		1.41	14.29	14.89
254							0.67		26.68		2.67

BM17132	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
85	3.77	17.37	23.08	16.11	29.17	6.32	12.58	2.50	41.47	72.86	39.22
87	27.87	16.77	44.87	37.92	19.79	51.67	46.12	3.75	36.86	1.43	15.40
89	61.97	53.29	20.51	45.97	39.58	32.34	29.19		21.67	14.29	29.16
91	6.39	12.57	7.69		11.46	9.67	12.11	93.75		11.43	2.67
95			3.85								13.55

BM1824	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
178		6.06			11.46	0.77	3.37				
180	17.50	24.85	29.49	19.93	21.88	37.16	11.35	27.50	49.65	2.94	38.18
184			2.56	13.29	4.17				1.56	47.06	18.41
190			21.79	8.04	3.13						6.07
192	8.17	3.33		18.18	1.04		4.43		29.51		9.10
196	24.67	22.12	12.82	34.97	7.29	7.09	20.57		6.42	22.06	4.18
198	49.67	43.64	33.33	5.59	51.04	54.98	60.28	72.50	12.85	27.94	24.06

BM1862	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
201	13.83	6.97		9.52	15.63	17.66	14.35		11.38	10.29	1.23
202	4.18	0.91		1.36	1.04				5.52	11.76	4.73
205	5.14	13.03	15.79	59.52	30.21	16.17	16.05	77.50	29.66	10.29	38.79
207	45.82	52.12	25.00	4.08	39.58	32.90	29.01	8.75	15.52	44.12	29.32
211	6.11	18.79	59.21	25.51	10.42	2.04	17.28		37.93	17.65	25.93
215	24.92	8.18			3.13	31.23	23.30	13.75		5.88	

BM188	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
99	9.15		3.85		1.02	3.15	0.46	25.00		18.57	14.48
105									**2.25**		
109									1.04	1.43	
113									9.17		0.92
115	10.78	16.27	7.69	30.33	22.45		4.63	6.25	21.80	22.86	29.36
117	75.33	83.13	58.97	38.00	73.47	96.85	91.67	40.00	40.48	54.29	31.72
119				4.00	2.04						4.00
121	4.74	0.60	29.49	27.67	1.02		3.24	28.75	25.26	1.43	17.25
123										1.43	2.26

BM1905	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
172	64.56	83.53	37.18	17.88	66.67	54.85	64.45	5.00	18.73	45.71	6.57
176	35.44	16.47	61.54	61.92	22.92	45.15	31.06	48.75	81.27	35.71	78.95
182			1.28	2.65			2.16				14.48
184				17.55	10.42		2.33	46.25		18.57	

BM2113	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
127	2.11	4.40		4.67	14.44		1.27		4.58	7.81	
129	24.03	18.87	5.13	50.33	14.44	48.13	31.21		26.41	35.94	23.87
133	1.46		1.28	0.33	1.11	5.78	1.27		22.71	23.44	9.36
135									**14.61**		
143	35.06	33.65	91.03	27.33	34.44	39.74	40.13	88.75	17.43	3.13	60.70
145	1.79	14.15			11.11		10.19	11.25	12.32	29.69	
149		0.31		12.67	1.11				1.94		6.07
151							**0.32**				
153	35.55	28.62	2.56	4.67	23.33	6.34	15.61				

BM2830	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
142			3.85								16.94
146	10.91	6.62	1.28	7.43	12.24		4.13		27.40	14.71	4.26
148	2.77	6.62		19.59	3.06	8.08	0.32	52.50	16.44	14.71	4.47
150			21.79	1.35					5.82	14.71	6.55
152	38.44	33.77	15.38	11.15	39.80	15.04	27.46	11.25	15.92	39.71	29.21
156	0.16	7.95		1.35	1.02	0.94	15.40		1.54		1.14
158	29.64	23.84	32.05	12.84	18.37	43.23	29.84	31.25	30.31		20.17
160											**0.10**
162	9.45	3.64		35.14	9.18		12.06		0.68	16.18	5.51
164	8.63	17.55	25.64	11.15	16.33	32.71	10.79	5.00	1.88		11.64

BM4028	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
108	1.97			0.34			0.63		19.07		12.60
112				**0.34**							
114	62.30	68.24	57.69	21.77	37.76	91.60	41.77		16.30	4.29	13.22
116	2.79	7.86	6.41	45.24	19.39	0.20	5.85	7.50	58.89	55.71	36.26
118	30.00	23.90	35.90	32.31	42.86	8.20	51.74	20.00	5.74	40.00	37.91
122	**2.95**										
126								**72.50**			

BM4107	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
159										**15.71**	
165	19.07	60.18	29.49	37.42	61.22	10.07	25.47	68.75	29.55	48.57	51.13
173		0.60		0.66	2.04					2.86	
175	45.67	13.77	30.77	16.56	9.18	27.99	47.03		17.53	7.14	
179		0.30	1.28	18.87	3.06		3.59	10.00	13.92	10.00	16.29
181	13.46	12.57	6.41	18.54	10.20	34.89	12.34		12.54	2.86	9.22
183	21.79	12.57	32.05	7.95	14.29	27.05	11.56	21.25	26.46	12.86	22.54
185											**0.82**

BM4311	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
90	7.28	2.40	2.56		3.06	15.37	15.17	25.00	8.77	4.29	6.67
92	5.96	2.69		38.26	1.02				18.07	4.29	7.49
96	0.17	3.29	2.56	0.67	1.02				33.33	2.86	9.03
98	36.42	19.76	41.03	45.64	30.61	34.81	29.26	33.75	7.89	14.29	37.78
102	23.68	32.34	25.64	9.06	17.35	22.96	37.31		0.53		9.34
104	26.49	39.52	28.21	6.38	46.94	26.85	18.27	41.25	31.40	74.29	29.67

BM4440	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
123	3.58	19.76	14.10	6.25	19.39	5.37	19.50		0.17		4.93
125	28.50	20.96	12.82	19.08	12.24	6.11	34.50	100.00	35.32	5.71	54.11
127	19.06	16.47	44.87	37.17	16.33	20.00	10.50		4.78	28.57	25.98
129	36.48	35.63	6.41	13.16	36.73	39.26	17.50		56.14	38.57	13.66
131	12.38	7.19	21.79	21.38	13.27	29.26	16.83		1.88	7.14	1.33
133							1.17		0.17		
143				2.96	2.04				1.54	20.00	

BM47	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
103	70.32	48.08	67.95	62.84	57.45	97.93	84.74	100.00	77.05	34.29	88.63
105	29.68	42.31	32.05	34.80	32.98	2.07	14.49		12.84	65.71	10.76
107		9.62			9.57		0.78		10.10		0.61
111				**2.36**							

BM6017	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
104	6.92	0.80		38.53	18.18		7.39		21.26	22.06	6.97
114	2.44			12.39	2.60			21.31	9.98	22.06	
116	64.97	52.99	23.53	27.52	40.26	21.65	53.60	67.21	10.20	14.71	16.32
118	24.66	46.22	70.59	11.93	36.36	78.35	39.02	11.48	58.57	38.24	68.82
120			1.47	9.17	2.60					2.94	5.66
122			4.41	0.46							2.24

BM711	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
161	5.52	2.99	61.54	36.00	13.27	12.92	9.94	38.75	24.73	27.14	63.48
163		4.79		6.00						8.57	
165				18.00	6.12	0.94			15.41		8.02
167	94.48	92.22	38.46	40.00	79.59	86.14	90.06	61.25	55.73	61.43	22.02
175											**6.48**
177					1.02				4.12	2.86	

BM720	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
203	8.93	10.61		3.62	13.83	5.66	5.87				
213		1.52	3.85	3.29					20.92		15.64
223				**2.63**							
225	13.96	13.03	3.85	15.46	13.83		7.55	40.00	9.57	48.57	13.27
227	2.27				2.13	0.19			20.57	10.00	0.21
229	36.20	46.67	62.82	11.18	28.72	46.42	45.13		10.46	2.86	25.72
231	37.99	27.88	28.21	54.61	41.49	47.74	41.44	3.75	24.82	38.57	33.44
233	0.49	0.30		9.21				56.25	13.65		3.09
235	0.16		1.28								8.64

BM757	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
186									**1.04**		
188									9.34		0.10
190	0.16	0.92		2.03			2.86		3.98	5.71	0.62
192									0.69		1.23
194	77.94	71.78	100.00	68.24	77.17	91.53	87.46	77.50	34.95	32.86	58.62
196	6.54	12.58			14.13	5.44	8.25		5.36	2.86	13.86
198									**3.11**		
200	15.36	13.80		29.05	8.70	3.02	1.43	22.50	39.62	58.57	25.56
202		0.92		0.68					1.90		

BMC4214	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
175	1.13	3.01	2.56	26.57	2.27				9.39		17.98
179	21.77	22.89	32.05	12.59	20.45	14.31	25.08		8.87	23.53	2.38
181	22.42	20.48	42.31	10.14	26.14	21.76	23.06	100.00	24.06	23.53	12.40
185	2.74	22.59	5.13	19.23	20.45	0.59	2.02		22.35	44.12	34.09
187	51.94	31.02	17.95	31.47	30.68	63.33	49.83		35.32	5.88	33.16
191										**2.94**	

BMS1001	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
107									6.66		1.13
109	3.53		3.85	19.41	3.06				7.34	17.14	34.91
111	3.21			1.32			0.46		5.63	1.43	1.95
113	16.35	10.18	16.67	26.97	12.24		8.95	100.00	9.39	10.00	24.33
115	76.92	89.82	79.49	52.30	84.69	100.00	90.59		70.99	71.43	37.68

BMS1074	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
152				6.85	2.04						
154	14.79	12.05	14.47	20.89	13.27	8.18	23.61		10.07	22.06	13.45
156	5.14	21.08	6.58	30.14	26.53		4.48	21.25	45.05	25.00	14.78
158	56.59	60.24	56.58	33.56	53.06	60.78	52.31	32.50	14.85	29.41	12.53
160	23.47	6.63	22.37	8.56	5.10	31.04	19.60	46.25	30.03	23.53	59.24

BMS1117	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
89	56.25	46.95	19.23	29.53	51.02	36.79	36.02	8.75	43.75	55.88	25.37
91	34.29	27.13	20.51	19.80	22.45	44.72	39.44	91.25	45.66	32.35	21.35
93	8.33	23.17	57.69	50.67	26.53	0.75	22.20		10.59	11.76	53.28
99	1.12	2.74	2.56			17.74	2.33				

BMS1172	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
86				**12.33**							
88	2.24	6.93		28.33	12.24	8.17	16.46		38.49	11.76	3.33
90	80.13	69.28	82.05	29.33	64.29	77.82	61.80	2.50	28.35	57.35	62.58
92	16.35	16.87			13.27	3.31	17.08			5.88	
100		6.93	6.41	13.33	8.16	10.70	4.66	1.25	16.32	8.82	1.46
102			1.28	10.67				35.00			22.97
104	1.28		10.26	6.00	2.04			61.25	16.84	16.18	9.67

BMS1315	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
135	56.89	44.61	10.26	60.86	72.45	36.43	42.72	12.50	47.07	74.29	53.59
137	2.40		2.56	14.14	6.12			87.50	8.97	5.71	15.98
141	2.72	0.60	20.51	9.21	1.02				21.38	12.86	20.18
147	37.66	45.81	66.67	13.49	18.37	63.57	49.23		22.59	7.14	10.25
149	0.32	8.98		2.30	2.04		8.05				

BMS1355	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
146	36.06	24.25	48.72	39.47	52.04	79.96	15.78	83.75	39.25	54.41	78.79
148	7.37	10.18	46.15	10.86	7.14	4.31	28.44		19.28	7.35	13.52
150	56.57	65.57	5.13	45.39	40.82	15.73	55.78	16.25	29.01	35.29	7.68
154				4.28					12.46	2.94	

BMS1675	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
85	12.54	12.95	7.89	18.42	21.43	6.85	18.52		24.57	8.57	
87	66.08	50.30	86.84	76.64	34.69	37.59	52.93	1.25	58.70	62.86	72.23
89	7.40	26.20		1.32	31.63	17.96	4.94	80.00	9.73	7.14	10.96
91	13.99	10.54	5.26	3.62	12.24	37.59	23.61	18.75	7.00	21.43	16.80

BMS1716	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
185									**0.85**		
189	22.03	14.46	3.85	35.81	26.53	8.55	4.78		16.55	5.88	14.27
191	30.55	48.19	64.10	17.91	39.80	16.91	18.98	100.00	44.03	36.76	75.15
193	10.93	13.55	7.69	1.35	15.31	20.45	12.96			5.88	0.62
195	36.50	23.80	24.36	41.22	18.37	54.09	63.27		38.57	51.47	9.96
197				**3.72**							

BMS1747	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
89	3.59				1.04					11.43	
95	48.53	41.82	41.03	36.75	31.25	73.75	60.78	67.50	59.90	44.29	27.66
99		7.27	21.79	59.60	17.71		6.41	26.25	12.12	42.86	33.40
101	47.88	50.91	16.67	3.64	50.00	26.25	32.81		27.99	1.43	31.15
103			20.51					6.25			7.79

BMS1857	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
142	8.65	1.20	2.56	6.38	1.04	4.66	25.39		8.42	4.29	11.89
146								**71.25**			
148	10.42	30.12	42.31	7.72	25.00	34.89	2.51		24.05	51.43	
150			23.08				2.51		5.15		16.19
156	17.79	11.75	3.85	13.42	17.71	29.29	10.34		21.31	25.71	12.40
158	33.01	34.94	20.51	24.50	38.54	1.31	15.52		18.56	10.00	27.36
160	19.39	18.07	7.69	18.46	15.63	24.44	31.35		22.51	8.57	24.49
162	10.74	3.92			2.08	5.41	12.38				
164				**22.82**							
168				6.71				28.75			7.68

BMS1862	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
142	50.00	57.20	50.00	17.89	41.94	86.51	61.11		40.26	5.88	31.83
144					4.84					10.29	
156				26.42	1.61					11.76	1.05
158	9.24	6.40	9.46	1.63	17.74		6.79		27.02	7.35	16.81
160	3.30	3.20		1.63	11.29	0.99	4.63	73.75	21.69	25.00	
162	16.34	2.00		5.69	6.45	11.51	12.19		2.21	25.00	8.19
164	16.67	20.80	1.35	22.36	11.29	0.79	8.49	11.25	1.29	2.94	5.67
166			4.05	0.81	1.61				3.31	10.29	22.16
167	**2.31**										
168	2.15	9.20	35.14	23.58	3.23	0.20	6.79		4.23		9.56
170		1.20						15.00		1.47	4.73

BMS2258	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
127	6.73	5.39	20.51	38.82	10.20			46.25	11.26		17.21
134										**11.76**	
136	**2.72**										
138	21.96	27.54	28.21	1.97	33.67	30.97	31.46	11.25		36.76	
140	14.10	34.73	8.97	23.03	35.71	13.62	13.86	18.75	26.62	4.41	39.04
142	4.81	1.50	2.56	1.64	1.02	1.12	14.95				19.88
144									**2.39**		
146	38.94	14.07	17.95	2.30	5.10	44.78	31.00		17.41	1.47	
148	10.74	16.77	20.51	7.89	4.08	9.51	8.72	23.75	20.65	30.88	13.73
150			1.28	24.34	10.20				21.50	14.71	10.14
152									**0.17**		

BMS2639	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
168	41.51	26.06	16.67	7.05	26.53	47.81	34.63	47.50	24.22	24.24	16.63
170	8.97	16.06	15.38	27.18	15.31	2.99	13.66		29.79	30.30	38.95
172	6.09	13.94	52.56	11.74	21.43		2.33	5.00	36.59	10.61	40.84
174	19.23	19.70		43.96	14.29	2.99	4.50				
176	8.81	16.06	8.97	8.39	13.27	1.79	27.02	6.25	0.17	28.79	
178	14.74	8.18	6.41		9.18	44.42	17.86		9.23		
186	0.64			1.68				41.25		6.06	3.58

BMS410	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
83	16.67	43.11	67.95	60.67	54.08	54.94	17.49	13.75	59.59	42.65	44.66
85				5.33	3.06				13.18	16.18	
89	80.23	49.70	16.67	26.00	27.55	44.68	72.14	52.50	27.23	29.41	22.38
93	3.10	7.19			13.27	0.38	10.37				
95				8.00	2.04			33.75		11.76	0.21
97			15.38								32.75

BMS510	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
91	53.76	33.94	25.64	35.71	42.39	54.63	35.91	26.25	61.38	77.94	41.17
92	12.42	14.85	42.31	32.31	22.83	16.99	21.21	73.75	20.00	16.18	29.67
94	13.24	19.39	5.13	6.46	16.30		3.41		8.28	5.88	7.39
95	20.59	31.82	26.92	25.51	18.48	28.38	39.47		10.34		21.77

BMS527	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
159				5.48	1.04						
163	1.16	10.67	2.56	13.01	15.63		0.46		1.38		4.73
165	2.98			7.19							1.75
167	11.09	29.57	20.51	0.68	25.00	28.07	11.30	41.25	2.07	7.35	4.42
171				**2.05**							
173	26.49	26.52	29.49	50.00	22.92	42.94	48.45	5.00	23.62	33.82	23.77
175	50.83	27.74	44.87	20.21	26.04	25.65	31.11	50.00	17.59	41.18	47.63
177	7.45	5.49	2.56	1.37	9.38	3.35	8.67	3.75	55.34	17.65	17.70

BMS528	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
140	48.69	13.19	41.03	50.99	19.77	38.13	31.19		40.92	30.00	23.77
146	22.71	55.83	8.97	29.14	37.21	23.74	12.38	17.50	34.76	31.43	20.78
148	3.27	9.51	6.41		11.63		6.90	2.50		5.71	37.04
150	24.84	21.47	16.67	9.27	29.07	38.13	49.53	80.00	22.77	32.86	10.08
152	0.49		26.92	10.60	2.33				1.54		8.33

BMS601	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
172	10.74	15.57	34.62	18.75	9.18	16.42	9.65	83.75	34.47	61.76	22.78
174	14.10	17.66	8.97	37.83	27.55	16.42	15.82	6.25	24.06	5.88	33.40
176	2.72	0.30	1.28				0.16		24.23		13.61
178			1.28	14.47	2.04				16.21	23.53	23.61
180	72.44	66.47	53.85	28.95	61.22	67.16	74.37	10.00	1.02	8.82	6.60

BMS812	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
90	13.89	24.53	8.97	13.31	18.37	22.92	11.36	100.00	1.71	24.24	10.60
96									**8.73**		
106	52.45	43.08	84.62	23.38	53.06	35.42	41.48		50.00	28.79	47.84
108	3.59	2.52	1.28	16.19	5.10		3.31		21.75	42.42	31.07
110	3.43			10.79	1.02				14.04	4.55	4.94
112	20.59	25.47	5.13	36.33	18.37	23.48	38.33		3.77		5.56
122	6.05	4.40			4.08	18.18	5.52				

BMS911	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
100	4.36	11.55		39.82	20.51	6.21	3.62		1.49	47.06	17.72
102				14.03					4.69		3.41
104	58.02	47.41	67.65	31.67	42.31	27.92	43.62	100.00	65.67	36.76	69.03
106	9.70		10.29						21.96		9.84
112	27.92	40.64	22.06	14.48	37.18	65.87	52.76		6.18	16.18	
114		0.40									

BMS941	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
81	3.25	7.06	3.85	2.00	14.58		6.43	87.50	50.69	50.00	39.14
83	96.75	92.94	96.15	44.33	78.13	100.00	93.57	12.50	33.45	50.00	60.04
85				53.67	7.29				15.86		0.82

HUJ246	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
242								**52.50**			
252								**8.75**			
256	15.76	30.42	1.32	13.42	15.31	6.69	11.88		12.50	10.29	
258	12.86	27.11	51.32	1.68	22.45	11.34	33.64				27.10
260	17.52	11.14	3.95	15.44	3.06	26.21	6.94		6.51	25.00	6.26
262	47.43	28.61	43.42	62.75	55.10	37.55	43.52	11.25	73.63	64.71	60.99
264	6.43	2.71		6.71	4.08	18.22	4.01	27.50	7.36		5.65

IL4	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
83	0.50	2.41	2.56	1.67		1.69	8.52				4.38
85	18.50	3.92	11.54	0.67	7.29	4.68	5.05		4.04		
89		5.12	6.41	26.00	6.25				12.28		34.69
91	40.50	32.23	20.51	35.67	31.25	49.06	30.60		8.60	4.41	0.21
93	1.50			6.00	11.46			3.75	20.70	45.59	2.71
95	3.67	5.12			7.29	16.48	3.15			13.24	
97	24.50	30.72	25.64	6.33	13.54	26.97	43.69		27.19	5.88	14.69
99		0.30	2.56	0.33							8.44
103	10.83	20.18	21.79	14.67	20.83	1.12	8.99	96.25	27.19	27.94	29.79
105			8.97	8.67	2.08					2.94	5.10

ILSTS102	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
113	6.41	2.99			4.08	0.19	25.16	15.00		17.14	
133	9.78	0.90		2.96	3.06	16.92	8.91		4.47		
143	44.39	48.20	34.62	69.08	43.88	34.02	26.41	85.00	51.55	34.29	47.44
145	26.28	32.34	48.72	24.34	44.90	23.12	25.63		30.41	47.14	25.61
147	13.14	15.57	16.67	3.62	4.08	25.75	13.91		11.86	1.43	26.95
153									**1.72**		

INRA037	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
118	0.32	4.49			6.12				11.64	16.18	
120	3.21			13.82						2.94	8.71
122	68.75	65.57	47.44	16.45	47.96	29.00	30.47	70.00	17.29	39.71	44.06
124	3.53	17.96	44.87	63.82	28.57	41.64	26.25	26.25	57.36	10.29	42.83
126	2.24	6.89			16.33		25.31	3.75	13.70	30.88	
132	21.96	5.09	7.69	5.92	1.02	29.37	17.97				4.41

INRA133	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
223								5.00			1.45
227		7.78	19.23	60.74	28.05	4.37	1.71		31.82	20.00	11.80
234				1.68						24.29	
236	26.17	17.66	6.41	14.09	13.41	18.25	16.93		8.50		11.80
238	0.34	0.30		0.67			0.93	15.00	2.96	22.86	0.52
240	73.49	74.25	74.36	22.82	58.54	77.38	80.43	80.00	56.72	32.86	74.43

INRA189	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
96	100.00	52.00	100.00	92.21	61.11	100.00	100.00	100.00	100.00		100.00
100		48.00		7.79	38.89						

INRA194	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
144			2.56	1.97					4.79		11.27
154	51.95	37.12	57.69	42.11	24.49	36.75	63.77	67.50	66.78	70.00	46.11
156	43.32	60.74	35.90	54.61	65.31	62.69	25.95	30.00	24.14	5.71	33.81
158	4.72	2.15	3.85	0.99	5.10	0.56	10.28		4.28	5.71	7.38
160				0.33	5.10			2.50		18.57	1.43

RM372	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
114				0.33					1.91		1.54
118	9.71	28.44	3.85	7.33	36.17	33.02	15.37		29.86		12.53
128	1.46			6.67				6.25	12.67	11.76	
130	5.66	12.81	25.64	53.67	42.55	3.24	13.82	17.50	25.52	70.59	28.44
132	25.73	8.75	5.13		1.06	27.67	25.00		2.95	1.47	18.38
134	30.26	28.13	51.28	32.00	10.64	19.47	30.59	76.25	12.85	11.76	37.47
136	27.18	21.88	14.10		9.57	16.60	15.22		9.03	4.41	1.64
138									**5.21**		

TGLA122	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
136			1.28								4.34
140			2.56					41.25	1.77		20.87
142	50.33	32.93	44.87	37.00	41.84	64.31	61.61	38.75	22.44	84.29	18.80
144	0.50										0.10
148	19.70	33.23	43.59	53.67	32.65	11.34	15.79	20.00	50.35	14.29	30.37
150	29.47	33.83	7.69	9.33	25.51	24.35	22.60		25.44	1.43	25.52

TGLA44	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
149			16.67								9.79
151			3.85	9.00					6.87		2.99
153		0.90	5.13	28.33	9.18	1.86	2.52	1.25	37.11		33.30
155	27.54	53.61	23.08	45.33	48.98	29.74	25.87	98.75	43.30	88.24	43.30
157	30.33	10.24	2.56	6.00	13.27	28.62	14.67		9.97	11.76	3.51
159	42.13	35.24	48.72	11.33	28.57	39.78	56.94		2.75		7.11

TGLA53	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
132	1.14			4.05							
134	37.30	17.72	66.67	37.84	12.24	35.28	41.40	16.25	36.70	37.50	43.42
136	17.75	37.03	8.97	30.07	50.00	19.15	28.90	55.00	52.13	29.69	54.22
138	0.98			17.91	6.12			28.75		21.88	
140	42.83	45.25	24.36	6.76	30.61	45.56	29.71		11.17	10.94	2.37
142				3.38	1.02						

URB011	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
139										**11.76**	
143	7.21	1.50		35.00	4.17	0.79	4.39				2.38
145			1.28	0.71			1.57		16.72		7.14
147	56.73	30.24	19.23	3.57	15.63	47.05	43.26	23.75	30.31	52.94	18.32
149	17.63	39.52	44.87	45.00	43.75	38.39	20.22	76.25	26.31	30.88	37.16
151	0.80	15.27	1.28	11.07	22.92		6.74		15.16		14.70
153	17.63	13.47	33.33	4.64	13.54	13.78	23.82				20.29
155									11.50	4.41	

APPENDIX C

AVERAGE STATISTICS FOR 54 POLYMORPHIC LOCI ACROSS 11 BISON POPULATIONS

Average number of alleles (N_A), allelic richness (A_R), observed heterozygosity (H_O), and expected heterozygosity (H_E) are shown. Averages and standard deviations (Std Dev) given for each population. N_A and A_R calculated across all samples (ALL). See Table 1 for population abbreviations.

N_A	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP	ALL
AGLA232	5	5	4	4	5	4	5	2	5	4	6	7
BL1036	4	4	4	5	5	3	4	2	5	3	4	5
BM1225	5	6	4	8	6	4	6	3	7	4	5	10
BM1706	3	3	3	3	3	2	4	3	5	4	5	6
BM17132	4	4	5	3	4	4	4	3	3	4	5	5
BM1824	4	5	5	6	7	4	5	2	5	4	6	7
BM1862	6	6	3	5	6	5	5	3	5	6	5	6
BM188	4	3	4	4	5	2	4	4	6	6	7	9
BM1905	2	2	3	4	3	2	4	3	2	3	3	4
BM2113	6	6	4	6	7	4	7	2	7	5	4	9
BM2830	7	7	6	8	7	5	7	4	8	5	10	10
BM4028	5	3	3	5	3	3	4	3	4	3	4	7
BM4107	4	6	5	6	6	4	5	3	5	7	5	8
BM4311	6	6	5	5	6	4	4	3	6	5	6	6
BM4440	5	5	5	6	6	5	6	1	7	5	5	7
BM47	2	3	2	3	3	2	3	1	3	2	3	4
BM6017	4	3	4	6	5	2	3	3	4	5	5	6
BM711	2	3	2	4	4	3	2	2	4	4	4	6

N_A	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP	ALL
BM720	7	6	5	7	5	4	4	3	6	4	7	9
BM757	4	5	1	4	3	3	4	2	9	4	6	9
BMC4214	5	5	5	5	5	4	4	1	5	5	5	6
BMS1001	4	2	3	4	3	1	3	1	5	4	5	5
BMS1074	4	4	4	5	5	3	4	3	4	4	4	5
BMS1117	4	4	4	3	3	4	4	2	3	3	3	4
BMS1172	4	4	4	6	5	4	4	4	4	5	5	7
BMS1315	5	4	4	5	5	2	3	2	4	4	4	5
BMS1355	3	3	3	4	3	3	3	2	4	4	3	4
BMS1675	4	4	3	4	4	4	4	3	4	4	3	4
BMS1716	4	4	4	5	4	4	4	1	4	4	4	6
BMS1747	3	3	4	3	4	2	3	3	3	4	4	5
BMS1857	6	6	6	7	6	6	7	2	6	5	6	10
BMS1862	7	7	5	8	9	5	6	3	7	9	8	11
BMS2258	7	6	7	7	7	5	5	4	7	6	5	11
BMS2639	7	6	5	6	6	5	6	4	5	5	4	7
BMS410	3	3	3	4	5	3	3	3	3	4	4	6
BMS510	4	4	4	4	4	3	4	2	4	3	4	4
BMS527	6	5	5	8	6	4	5	4	5	4	6	8
BMS528	5	4	5	4	5	3	4	3	4	4	5	5
BMS601	4	4	5	4	4	3	4	3	5	4	5	5
BMS812	6	5	4	5	6	4	5	1	6	4	5	7
BMS911	4	4	3	4	3	3	3	1	5	3	4	6
BMS941	2	2	2	3	3	1	2	2	3	2	3	3
HUJ246	5	5	4	5	5	5	5	4	4	3	4	7
IL4	7	8	8	9	8	6	6	2	6	6	8	10
ILSTS102	5	5	3	4	5	5	5	2	5	4	3	6
INRA037	6	5	3	4	5	3	4	3	4	5	4	6
INRA133	3	4	3	5	3	3	4	3	4	4	5	6
INRA189	1	2	1	2	2	1	1	1	1	-	1	2
INRA194	3	3	4	5	4	3	3	3	4	4	5	5

N_A	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP	ALL
RM372	6	5	5	5	5	5	5	3	8	5	6	8
TGLA122	4	3	5	3	3	3	3	3	4	3	6	6
TGLA44	3	4	6	5	4	4	4	2	5	2	6	6
TGLA53	5	3	3	6	5	3	3	3	3	4	3	6
URB011	5	5	5	6	5	4	6	2	5	4	6	8
N_A Average	**4.50**	**4.37**	**4.06**	**4.98**	**4.78**	**3.52**	**4.24**	**2.54**	**4.80**	**4.25**	**4.83**	**6.48**
N_A Std Dev	**1.48**	**1.39**	**1.34**	**1.54**	**1.46**	**1.19**	**1.27**	**0.91**	**1.55**	**1.22**	**1.51**	**2.03**

A_R	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP	ALL
AGLA232	4.96	4.95	3.80	3.60	4.97	4.00	4.92	2.00	5.00	3.89	4.93	5.31
BL1036	3.53	4.00	4.00	4.94	4.99	3.00	3.87	2.00	5.00	3.00	3.86	4.98
BM1225	4.53	4.98	4.00	7.33	5.55	4.00	5.76	3.00	6.95	4.00	4.41	7.09
BM1706	2.97	3.00	3.00	2.97	3.00	2.00	3.34	3.00	4.17	3.77	4.13	4.24
BM17132	3.91	4.00	4.99	3.00	4.00	3.99	4.00	2.94	3.00	3.89	4.82	4.86
BM1824	4.00	4.89	4.96	5.98	6.59	3.39	4.84	2.00	4.63	3.99	5.92	6.32
BM1862	5.89	5.46	3.00	4.56	5.60	4.74	5.00	3.00	4.98	6.00	4.50	5.83
BM188	3.96	2.34	3.99	3.94	4.13	1.88	3.10	4.00	5.27	5.66	6.14	5.66
BM1905	2.00	2.00	2.80	3.85	3.00	2.00	3.55	3.00	2.00	3.00	2.99	3.76
BM2113	5.06	5.15	3.75	5.13	6.38	3.97	5.32	2.00	6.68	5.00	3.98	7.25
BM2830	5.94	6.90	5.79	7.22	6.59	4.46	6.12	4.00	6.69	5.00	8.43	8.67
BM4028	4.43	3.00	3.00	3.42	3.00	2.12	3.32	3.00	3.98	3.00	4.00	4.81
BM4107	4.00	4.52	4.80	5.37	5.82	4.00	4.91	3.00	5.00	6.98	4.41	5.36
BM4311	5.08	5.56	4.92	4.36	5.22	4.00	4.00	3.00	5.29	4.99	5.98	5.98
BM4440	4.91	4.99	5.00	5.86	5.87	4.96	5.54	1.00	4.52	5.00	4.54	5.50
BM47	2.00	3.00	2.00	2.81	3.00	1.75	2.40	1.00	3.00	2.00	2.33	2.90
BM6017	3.68	2.25	2.93	5.30	4.86	2.00	2.95	3.00	3.99	4.86	4.53	4.53
BM711	1.98	2.84	2.00	3.99	3.63	2.46	2.00	2.00	3.94	3.99	3.98	4.34
BM720	5.15	4.84	4.78	6.67	4.89	3.10	3.98	2.99	6.00	3.99	5.99	7.57
BM757	3.09	3.94	1.00	3.14	3.00	2.85	3.45	2.00	7.35	3.99	3.94	4.89
BMC4214	4.36	4.88	4.96	5.00	4.92	3.32	3.74	1.00	5.00	4.99	4.79	5.02
BMS1001	3.79	2.00	2.99	3.60	2.95	1.00	2.26	1.00	4.96	3.89	4.23	4.20

A_R	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP	ALL
BMS1074	3.97	3.99	4.00	4.99	4.86	3.00	3.95	3.00	4.00	4.00	4.00	4.27
BMS1117	3.52	3.85	3.96	3.00	3.00	3.39	3.79	2.00	3.00	3.00	3.00	3.85
BMS1172	3.34	3.99	3.80	5.99	4.87	3.89	3.96	3.73	4.00	5.00	4.49	6.37
BMS1315	3.82	3.34	3.96	4.80	4.50	2.00	3.00	2.00	4.00	4.00	4.00	4.74
BMS1355	2.99	3.00	3.00	3.95	3.00	2.95	3.00	2.00	4.00	3.99	2.99	3.72
BMS1675	3.99	4.00	3.00	3.52	4.00	3.99	3.96	2.78	3.99	4.00	3.00	4.00
BMS1716	4.00	4.00	3.99	4.54	4.00	4.00	3.96	1.00	3.43	4.00	3.33	4.21
BMS1747	2.91	2.99	4.00	2.92	3.65	2.00	2.99	3.00	3.00	3.89	3.99	4.12
BMS1857	6.00	5.50	5.95	6.98	5.52	5.51	6.62	2.00	5.97	5.00	5.99	8.92
BMS1862	6.43	6.23	4.83	6.81	9.00	3.02	5.93	3.00	6.18	8.91	7.42	8.63
BMS2258	6.78	5.62	6.76	6.23	6.61	4.52	5.00	4.00	5.90	5.91	5.00	7.51
BMS2639	6.32	6.00	5.00	5.68	6.00	4.43	5.74	4.00	4.11	5.00	3.90	6.69
BMS410	2.87	2.99	3.00	3.98	4.82	2.22	3.00	3.00	3.00	4.00	3.12	5.23
BMS510	4.00	4.00	4.00	3.99	4.00	3.00	3.90	2.00	4.00	3.00	3.99	3.99
BMS527	5.39	4.98	4.92	6.74	5.65	3.89	4.26	3.99	4.34	4.00	5.58	5.76
BMS528	4.16	4.00	5.00	4.00	4.92	3.00	3.99	2.95	3.64	4.00	5.00	4.89
BMS601	3.84	3.19	4.59	4.00	3.87	3.00	3.10	3.00	4.49	4.00	4.99	4.99
BMS812	5.79	4.78	3.79	5.00	5.61	4.00	4.86	1.00	5.59	4.00	4.94	6.41
BMS911	3.87	3.23	3.00	4.00	3.00	2.95	2.81	1.00	4.30	3.00	3.81	4.57
BMS941	1.88	1.99	1.99	2.75	3.00	1.00	1.99	2.00	3.00	2.00	2.41	2.98
HUJ246	4.99	4.85	3.81	4.68	4.94	4.99	4.92	4.00	3.98	3.00	3.96	5.54
IL4	5.82	6.88	7.92	7.24	7.88	5.16	5.84	1.99	5.93	5.99	6.86	9.12
ILSTS102	4.99	4.34	3.00	3.80	4.92	4.12	5.00	2.00	4.63	3.89	3.00	5.08
INRA037	4.75	4.92	3.00	3.99	4.63	3.00	4.00	2.99	4.00	4.99	3.94	5.65
INRA133	2.20	3.18	3.00	4.07	3.00	2.95	3.13	3.00	3.86	4.00	3.89	4.11
INRA189	1.00	2.00	1.00	1.82	2.00	1.00	1.00	1.00	1.00	-	1.00	1.61
INRA194	2.96	2.78	3.95	3.45	3.99	2.31	3.00	2.95	3.90	4.00	4.60	4.24
RM372	5.59	5.00	4.99	4.19	4.66	4.89	5.00	3.00	7.55	4.91	5.28	6.50
TGLA122	3.28	3.00	4.76	3.00	3.00	3.00	3.00	3.00	3.69	2.89	5.01	4.50
TGLA44	3.00	3.46	5.95	4.99	4.00	3.71	3.81	1.78	4.83	2.00	5.75	5.56
TGLA53	4.00	3.00	3.00	5.85	4.63	3.00	3.00	3.00	3.00	4.00	2.78	4.18

A_R	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP	ALL
URB011	4.40	4.64	4.59	5.28	4.99	3.41	5.58	2.00	5.00	4.00	5.78	6.64
A_R Average	**4.11**	**4.06**	**3.96**	**4.60**	**4.60**	**3.26**	**3.99**	**2.52**	**4.49**	**4.21**	**4.44**	**5.33**
A_R Std Dev	**1.28**	**1.24**	**1.31**	**1.34**	**1.37**	**1.09**	**1.19**	**0.90**	**1.30**	**1.21**	**1.31**	**1.54**

H_O	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
AGLA232	0.587	0.497	0.564	0.517	0.561	0.484	0.602	0.350	0.712	0.686	0.636
BL1036	0.567	0.639	0.692	0.713	0.796	0.575	0.586	0.450	0.759	0.629	0.600
BM1225	0.724	0.582	0.632	0.788	0.553	0.688	0.706	0.675	0.823	0.400	0.694
BM1706	0.425	0.528	0.385	0.477	0.500	0.319	0.267	0.400	0.587	0.286	0.409
BM17132	0.505	0.611	0.692	0.644	0.729	0.628	0.693	0.125	0.696	0.486	0.706
BM1824	0.647	0.655	0.667	0.776	0.708	0.594	0.582	0.350	0.688	0.559	0.757
BM1862	0.698	0.721	0.447	0.517	0.583	0.758	0.802	0.425	0.728	0.706	0.708
BM188	0.369	0.265	0.462	0.700	0.388	0.056	0.062	0.850	0.706	0.486	0.828
BM1905	0.469	0.257	0.513	0.596	0.500	0.507	0.422	0.675	0.283	0.714	0.357
BM2113	0.688	0.717	0.179	0.673	0.778	0.586	0.678	0.225	0.746	0.531	0.558
BM2830	0.775	0.795	0.821	0.811	0.755	0.703	0.756	0.625	0.764	0.706	0.813
BM4028	0.475	0.440	0.513	0.714	0.714	0.152	0.582	0.200	0.600	0.543	0.669
BM4107	0.708	0.581	0.718	0.781	0.449	0.713	0.691	0.475	0.780	0.771	0.633
BM4311	0.689	0.617	0.590	0.691	0.531	0.748	0.672	0.725	0.747	0.429	0.731
BM4440	0.707	0.784	0.667	0.763	0.776	0.730	0.790	0.000	0.556	0.714	0.606
BM47	0.439	0.577	0.385	0.453	0.596	0.041	0.249	0.000	0.373	0.571	0.197
BM6017	0.553	0.452	0.517	0.750	0.621	0.335	0.515	0.476	0.580	0.824	0.513
BM711	0.104	0.150	0.564	0.680	0.224	0.247	0.180	0.375	0.620	0.686	0.541
BM720	0.679	0.721	0.487	0.697	0.702	0.532	0.644	0.425	0.798	0.571	0.788
BM757	0.363	0.436	0.000	0.399	0.326	0.153	0.232	0.400	0.754	0.371	0.567
BMC4214	0.661	0.801	0.615	0.776	0.727	0.573	0.670	0.000	0.758	0.647	0.711
BMS1001	0.394	0.144	0.333	0.625	0.265	0.000	0.170	0.000	0.509	0.343	0.639
BMS1074	0.624	0.536	0.553	0.781	0.531	0.518	0.623	0.750	0.717	0.853	0.571
BMS1117	0.535	0.701	0.513	0.550	0.673	0.642	0.677	0.175	0.590	0.441	0.615
BMS1172	0.308	0.482	0.308	0.767	0.612	0.335	0.565	0.475	0.739	0.500	0.559
BMS1315	0.538	0.611	0.590	0.513	0.449	0.483	0.542	0.200	0.648	0.457	0.637

H_O	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
BMS1355	0.545	0.485	0.615	0.618	0.633	0.356	0.550	0.325	0.672	0.559	0.316
BMS1675	0.502	0.620	0.211	0.388	0.735	0.715	0.623	0.275	0.611	0.657	0.418
BMS1716	0.714	0.657	0.487	0.682	0.776	0.636	0.503	0.000	0.618	0.618	0.394
BMS1747	0.542	0.485	0.821	0.517	0.521	0.402	0.538	0.500	0.577	0.629	0.732
BMS1857	0.792	0.729	0.718	0.832	0.708	0.762	0.755	0.375	0.842	0.714	0.783
BMS1862	0.667	0.336	0.514	0.618	0.548	0.206	0.583	0.425	0.739	0.765	0.756
BMS2258	0.740	0.790	0.692	0.783	0.857	0.690	0.763	0.725	0.788	0.794	0.734
BMS2639	0.760	0.788	0.667	0.691	0.939	0.614	0.767	0.725	0.679	0.545	0.619
BMS410	0.304	0.605	0.564	0.560	0.673	0.551	0.440	0.675	0.521	0.647	0.626
BMS510	0.657	0.764	0.641	0.701	0.696	0.606	0.641	0.325	0.607	0.382	0.674
BMS527	0.619	0.695	0.692	0.678	0.708	0.677	0.663	0.650	0.603	0.588	0.687
BMS528	0.614	0.650	0.667	0.669	0.744	0.728	0.705	0.300	0.644	0.800	0.730
BMS601	0.147	0.222	0.154	0.428	0.184	0.172	0.117	0.150	0.720	0.588	0.658
BMS812	0.601	0.648	0.231	0.755	0.673	0.765	0.688	0.000	0.654	0.576	0.547
BMS911	0.596	0.560	0.448	0.696	0.517	0.474	0.567	0.000	0.489	0.529	0.471
BMS941	0.065	0.129	0.077	0.480	0.313	0.000	0.129	0.250	0.590	0.429	0.453
HUJ246	0.730	0.735	0.632	0.577	0.571	0.773	0.707	0.650	0.445	0.500	0.585
IL4	0.690	0.771	0.821	0.800	0.729	0.685	0.691	0.075	0.814	0.765	0.708
ILSTS102	0.740	0.695	0.667	0.487	0.612	0.774	0.772	0.300	0.653	0.629	0.602
INRA037	0.474	0.515	0.564	0.605	0.633	0.658	0.759	0.350	0.582	0.647	0.592
INRA133	0.315	0.329	0.462	0.490	0.390	0.365	0.258	0.100	0.514	0.629	0.395
INRA189	-	-	-	-	-	-	-	-	-	-	-
INRA194	0.570	0.509	0.436	0.526	0.571	0.459	0.509	0.450	0.476	0.486	0.658
RM372	0.731	0.750	0.564	0.553	0.660	0.779	0.783	0.400	0.840	0.471	0.708
TGLA122	0.641	0.689	0.564	0.553	0.653	0.550	0.529	0.800	0.664	0.314	0.789
TGLA44	0.646	0.596	0.590	0.680	0.673	0.632	0.571	0.025	0.677	0.235	0.664
TGLA53	0.671	0.741	0.538	0.716	0.633	0.641	0.659	0.550	0.603	0.813	0.475
URB011	0.631	0.731	0.692	0.636	0.667	0.630	0.680	0.425	0.794	0.529	0.760
H_O Average	**0.565**	**0.576**	**0.531**	**0.639**	**0.605**	**0.517**	**0.564**	**0.371**	**0.654**	**0.580**	**0.615**
H_O Std Dev	**0.17**	**0.18**	**0.18**	**0.12**	**0.16**	**0.22**	**0.20**	**0.24**	**0.12**	**0.15**	**0.14**

H_E	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
AGLA232	0.584	0.502	0.628	0.518	0.545	0.479	0.581	0.476	0.702	0.624	0.631
BL1036	0.556	0.652	0.693	0.732	0.737	0.596	0.604	0.496	0.786	0.569	0.623
BM1225	0.707	0.583	0.717	0.767	0.533	0.683	0.678	0.622	0.805	0.428	0.691
BM1706	0.375	0.495	0.397	0.475	0.448	0.293	0.285	0.378	0.608	0.297	0.394
BM17132	0.534	0.644	0.705	0.621	0.713	0.616	0.673	0.121	0.646	0.441	0.719
BM1824	0.656	0.696	0.748	0.780	0.677	0.555	0.579	0.404	0.647	0.662	0.750
BM1862	0.702	0.666	0.571	0.572	0.725	0.738	0.786	0.377	0.729	0.747	0.695
BM188	0.411	0.283	0.566	0.688	0.414	0.061	0.157	0.698	0.717	0.629	0.761
BM1905	0.458	0.276	0.489	0.555	0.497	0.496	0.488	0.551	0.305	0.637	0.352
BM2113	0.693	0.750	0.170	0.654	0.781	0.604	0.708	0.202	0.811	0.735	0.563
BM2830	0.736	0.784	0.768	0.794	0.765	0.678	0.785	0.619	0.778	0.763	0.821
BM4028	0.521	0.473	0.541	0.645	0.642	0.155	0.555	0.437	0.588	0.535	0.692
BM4107	0.690	0.589	0.721	0.759	0.592	0.718	0.685	0.478	0.778	0.716	0.653
BM4311	0.734	0.700	0.695	0.635	0.663	0.732	0.720	0.661	0.745	0.429	0.743
BM4440	0.734	0.760	0.721	0.760	0.776	0.715	0.774	0.000	0.558	0.732	0.619
BM47	0.418	0.583	0.442	0.485	0.558	0.041	0.261	0.000	0.380	0.455	0.203
BM6017	0.541	0.505	0.454	0.747	0.693	0.313	0.547	0.485	0.605	0.744	0.496
BM711	0.104	0.147	0.478	0.677	0.350	0.242	0.179	0.482	0.604	0.547	0.539
BM720	0.698	0.678	0.530	0.656	0.714	0.555	0.616	0.530	0.815	0.614	0.772
BM757	0.365	0.451	0.000	0.451	0.382	0.159	0.228	0.353	0.707	0.555	0.572
BMC4214	0.633	0.760	0.693	0.770	0.762	0.532	0.636	0.000	0.752	0.701	0.726
BMS1001	0.380	0.184	0.343	0.618	0.270	0.000	0.172	0.000	0.475	0.458	0.677
BMS1074	0.601	0.576	0.613	0.743	0.635	0.529	0.631	0.642	0.676	0.757	0.594
BMS1117	0.560	0.653	0.596	0.619	0.625	0.634	0.666	0.162	0.590	0.580	0.607
BMS1172	0.331	0.483	0.316	0.788	0.552	0.376	0.560	0.508	0.718	0.631	0.546
BMS1315	0.534	0.585	0.508	0.585	0.442	0.464	0.570	0.222	0.675	0.429	0.637
BMS1355	0.545	0.502	0.553	0.627	0.562	0.335	0.584	0.275	0.710	0.582	0.355
BMS1675	0.523	0.652	0.240	0.378	0.726	0.682	0.628	0.329	0.582	0.553	0.438
BMS1716	0.714	0.674	0.530	0.670	0.721	0.631	0.545	0.000	0.631	0.602	0.405
BMS1747	0.535	0.563	0.722	0.510	0.629	0.388	0.520	0.477	0.549	0.616	0.710
BMS1857	0.793	0.741	0.727	0.824	0.741	0.729	0.787	0.415	0.803	0.659	0.804

H_E	BNP	FN	GT	NBR	NS	TRN	TRS	TSBH	WC	WM	YNP
BMS1862	0.686	0.619	0.626	0.793	0.775	0.239	0.594	0.426	0.716	0.844	0.801
BMS2258	0.762	0.755	0.807	0.732	0.740	0.677	0.757	0.690	0.798	0.742	0.750
BMS2639	0.751	0.818	0.669	0.709	0.821	0.573	0.755	0.604	0.712	0.767	0.653
BMS410	0.328	0.564	0.492	0.557	0.618	0.499	0.439	0.598	0.554	0.703	0.644
BMS510	0.637	0.726	0.690	0.701	0.715	0.593	0.670	0.393	0.567	0.368	0.690
BMS527	0.654	0.753	0.677	0.686	0.793	0.671	0.649	0.582	0.607	0.691	0.682
BMS528	0.650	0.618	0.729	0.637	0.732	0.654	0.638	0.333	0.661	0.709	0.747
BMS601	0.444	0.505	0.595	0.720	0.550	0.497	0.414	0.290	0.739	0.560	0.759
BMS812	0.658	0.689	0.277	0.761	0.653	0.735	0.665	0.000	0.675	0.688	0.659
BMS911	0.582	0.576	0.526	0.709	0.640	0.488	0.533	0.000	0.514	0.628	0.492
BMS941	0.063	0.132	0.075	0.517	0.367	0.000	0.120	0.221	0.607	0.508	0.487
HUJ246	0.700	0.741	0.553	0.561	0.627	0.741	0.678	0.636	0.433	0.516	0.548
IL4	0.730	0.756	0.829	0.771	0.822	0.658	0.698	0.073	0.786	0.700	0.758
ILSTS102	0.704	0.640	0.622	0.463	0.608	0.737	0.775	0.258	0.626	0.640	0.637
INRA037	0.477	0.530	0.575	0.545	0.665	0.658	0.743	0.446	0.610	0.721	0.614
INRA133	0.392	0.413	0.411	0.561	0.570	0.367	0.325	0.342	0.570	0.753	0.418
INRA189	-	-	-	-	-	-	-	-	-	-	-
INRA194	0.541	0.494	0.545	0.526	0.513	0.473	0.516	0.460	0.492	0.476	0.655
RM372	0.757	0.771	0.657	0.602	0.675	0.749	0.779	0.389	0.802	0.479	0.729
TGLA122	0.622	0.669	0.610	0.568	0.660	0.515	0.545	0.646	0.632	0.272	0.763
TGLA44	0.656	0.579	0.687	0.692	0.659	0.672	0.588	0.025	0.660	0.210	0.686
TGLA53	0.647	0.628	0.494	0.730	0.644	0.632	0.658	0.596	0.582	0.721	0.518
URB011	0.612	0.713	0.658	0.662	0.720	0.613	0.710	0.366	0.776	0.619	0.761
H_E Average	**0.574**	**0.590**	**0.560**	**0.647**	**0.631**	**0.513**	**0.574**	**0.373**	**0.653**	**0.599**	**0.627**
H_E Std Dev	**0.158**	**0.161**	**0.177**	**0.106**	**0.128**	**0.210**	**0.178**	**0.215**	**0.114**	**0.138**	**0.135**

APPENDIX D

COMPARATIVE ALLELE SIZES FOR ABI 377, 310, AND 3100 GENETIC ANALYZERS FOR 15 DISCRIMINATORY MICROSATELLITES

Locus name and called allele sizes are listed on the first line, followed by designations for type of genetic analyzer. Ranges for allele sizes are approximate and not available in every case. Primers are assumed to be nontailed (nt) and identical to those sequences found at www.sol.marc.usda.gov, unless noted by the "tailed" designation. See Chapter III for details.

AGLA17	215
377	215.64 - 215.88
310	212.96 - 214.33
3100	212.51 - 213.76

AGLA293	218	220
3100	217.67 - 218.65	219.92 - 220.17

BM1314	137	157
377 (nt)	136.68 - 136.93	
310 (nt)	134.49 - 135.30	
310 (tail)	140.66 - 141.93	161.29 - 161.62
3100 (tail)	140.62 - 141.09	160.99 - 161.29

BM4307	185	187	197
3100	180.94 - 181.70	182.99 - 183.65	193.00 - 193.63

BM4513	132	134
377	131.71 - 132.00	133.66 - 133.95
310	129.50 - 130.29	131.59 - 132.49
3100	129.26 - 129.72	131.27 - 131.71

BM7145	108	110	116
377	106.80 - 107.17	108.82 - 109.16	
310	103.32 - 104.64	105.33 - 106.97	112.03 - 112.76
3100	103.13 - 103.83	105.31 - 105.67	111.92 - 111.94

BMC3224	176
377 (nt)	175.84 - 177.19
310 (nt)	175.66 - 176.37
310 (tail)	181.68 - 182.98
3100 (tail)	181.54 - 182.05

BMS2270	66	68	70	94
377 (nt)	62.84 - 63.75	64.57 - 66.00	68.00 - 68.00	
310 (nt)	58.33 - 58.98	59.53 - 61.79	62.48 - 62.69	86.23 - 86.58
3100 (tail)	72.09 - 73.57	74.31 - 74.68	76.14 - 76.59	100.3 - 100.38

BMS4040	75
377	73.18 - 74.41
310	70.80 - 72.37
3100	70.00 - 71.48

CSSM36	158
377	159.58 - 159.80
310	157.62 - 159.24
3100	157.17 - 157.95

CSSM42	167	169	171
377	169.06 - 169.33	170.96-171.22	172.84-173.21
310	167.02 - 168.16	169.11-169.97	170.84-172.16
3100	166.60 - 167.27	168.59-169.03	170.50-171.09

RM185	92
3100	91.57-92.56

RM500	123
377	122.75-123.00
310	120.24-121.21
3100	120.00-120.71

SPS113	128	130	132
377		130.06-130.35	132.07-132.32
310	126.41-126.95	128.34-129.55	130.33-131.51
3100		128.08-128.61	130.2-130.59

TGLA227	72	73
377 (nt)	72.69-72.94	73.67-74.41
310 (nt)	70.47-70.78	71.31-73.80
310 (tail)	76.48-77.02	77.55-78.38
3100 (tail)	76.19-76.70	77.35-77.96

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Publications

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