

Conservation Genetics and North American Bison (*Bison bison*)

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The many millions of North American bison in the mid-19th century were reduced to near extinction by the middle 1880s. Plains bison, the subspecies found in the United States, were saved from extinction primarily by 5 private ranchers and the survival of a small herd in what is now Yellowstone National Park. This bottleneck resulted in the present-day plains bison population being descended from less than 100 founders. In addition, many conservation herds have cattle ancestry because of hybridization promoted by these ranchers in the late 1800s and early 1900s. Today, although there are around 500 000 plains bison in North America, only 4% (20 000) are in conservation herds. Only 1 conservation herd with no known ancestry from cattle has an effective population size of more than 1000. Here I review and evaluate this situation and provide recommendations for the reduction of cattle ancestry, avoidance of inbreeding depression, and maintenance of genetic variation in the conservation herds of bison.

Key words: *bison*, *effective population size*, *genetic variation*, *hybridization*, *inbreeding*, *mtDNA*

It is generally cited that 60 million North American bison (*Bison bison*), commonly known as buffalo, existed in the Great Plains until the early 1870s (Lott 2002). This estimate was based on the description of a large herd observed by Colonel R. I. Dodge in 1871 along the Arkansas River in Kansas. However, Shaw (1995), in evaluating the 3 approaches used to estimate the ancestral bison population size (direct observations, numbers killed, and environmental carrying capacity), concluded “one may assume with reasonable certainty that the bison population west of the Mississippi River at the close of the Civil War numbered in the millions, probably in the tens of millions. Any greater accuracy seems unlikely.”

It is known from historical and other records that bison ranged from the Arctic Circle and into northern Mexico and nearly across the continent (List et al. 2007; Sanderson et al. 2008). The bison in all this distribution were generally considered the plains bison subspecies (*B. b. bison*) except for the populations in northern Alberta and the Northwest Territories, which had the wood bison subspecies

(*B. b. athabascae*). Wood and plains bison have been described as morphologically different (COSEWIC 2004) although Geist (1991) suggested that this difference might be environmentally caused. However, van Zyll de Jong et al. (1995) found that plains bison raised in both plains and wood bison environments retained plains phenotypes and that wood bison raised in both plains and wood bison environments retained wood bison phenotypes, supporting a genetic basis for the morphological differences between the 2 subspecies.

In the early 1870s, very large numbers of bison were slaughtered, mainly for hides, but also for meat and sport. Further, this slaughter had negative effects on Indians living in buffalo country and was not generally discouraged by government officials. By the middle 1880s, bison were nearly extinct. Plains bison were saved from extinction in the late 1800s by 5 private herds established by ranchers and by a sixth herd at the New York Zoological Park. Altogether, these herds were established with less than 100 wild-caught founders (see below). In addition, a small remnant wild population survived in what is now Yellowstone National Park (NP). This population declined to an official estimate of 25 animals in 1902 (Meagher 1973). In other words, nearly all the present-day plains bison in the United States are descended from a founder population of 100 or less, and probably an effective founder number substantially less than 100, because of the small sizes of the herds in the initial generations (note: I will use the terms “population” and “herd” generally synonymously in this article).

In Canada, wild plains bison were extirpated around 1890 (Roe 1970). Plains bison herds were reestablished with animals from 4 of the 5 private herds that contributed to the recovery of plains bison in the United States (COSEWIC 2004). However, a preponderance of the ancestry in Canadian plains bison herds appears to be from the animals in the Pablo/Allard ranch herd that was shipped to Elk Island NP in 1907. A population of wood bison survived in the area that is now Wood Buffalo NP, Canada, and it reached a low of around 250 individuals by 1900 (Soper 1941). In 1963 and 1965, animals from Wood Buffalo NP were taken to Mackenzie Bison

Sanctuary and Elk Island NP to start new herds of wood bison. The Mackenzie and Elk Island herds were started with 16 and 11 animals, respectively (Wilson and Strobeck 1999)

A number of conservation issues face bison today. In 2002, it was estimated that there were approximately 500 000 plains bison but only 4% (20 000) were in herds managed for conservation, the remainder in herds used for commercial production (Freese et al. 2007). The number of plains bison in conservation herds has stayed relatively constant since the 1930s, whereas the number in commercial herds surpassed the number in conservation herds around 1980 and has grown exponentially since (Freese et al. 2007). There are around 10 700 wood bison today in Canada, both in free-ranging and captive conservation herds (Wilson G, personal communication). However, more than 6000 of these wood bison are in herds with either bovine tuberculosis or brucellosis and are isolated from disease-free conservation herds. In commercial herds, there is often artificial selection for domestication, particularly for ease of handling, and for meat production. In a number of the conservation herds, and nearly all the production herds, there is some cattle (*Bos taurus*) ancestry because of past artificial crossing of these 2 species. Freese et al. (2007) estimated that only 1.5% of the plains bison are free of domestic cattle ancestry. Much of the initial crossbreeding occurred on the private ranches that saved the bison because the ranchers wanted to improve cattle with commercially favorable traits found in bison. Finally, bison presently occupy less than 1% of their historical range, and because of this greatly reduced range and their greatly reduced number, Freese et al. (2007) and Sanderson et al. (2008) state that bison do not fulfill their previous ecological functions.

Although bison are an icon of conservation success, as indicated by their representation on the emblem of the US Department of Interior, their history and status are somewhat unusual for a conservation species. First, they are not listed as endangered or threatened, mainly because of their large overall numbers. The only exceptions are the wood bison, which are listed as threatened in Canada, and the Yellowstone herd of plains bison, which are under petition for listing as threatened. However, as stated above, only 20 000 bison are in conservation herds, and many of these have been affected by either interspecies hybridization with cattle or artificial selection. Second, in some jurisdictions, bison are not considered a wild animal but are treated as livestock (Freese et al. 2007). As a result, conservation of bison in the Yellowstone population and in Canadian populations have had additional management hurdles. Third, plains bison are the “only wild animal in the United States that is not allowed to live as a wild animal—live outside parks and refuges—anywhere in its original range” (Lott 2002). Finally, bison are the only conservation species (except for some fishes, such as salmon) that has been extensively selected for livestock-related traits, such as docility and meat production, which would be nonadaptive in a wild population. For example, 1

advertisement for bison ranchers promotes “bison people can get along with” and another promotes a bull with an unusually wide rump, “more rump and less hump,” as breeding stock (Lott 2002). These bison provide a potential threat of introducing nonadaptive ancestry if they are ever crossed into conservation populations.

However, it is clear that bison need to be managed as a conservation species because of the potential effects of the low initial numbers of founders, past bottlenecks in various herds, cattle hybridization in a number of conservation herds, artificial selection for nonadaptive traits, isolation of most conservation herds, and the observation of severe inbreeding depression in 1 conservation herd. From a conservation genetics perspective, it is important for bison to keep cattle ancestry at a very low level, avoid detrimental effects of inbreeding and selection for livestock-related traits, and retain sufficient genetic variation for future adaptation. Unfortunately, some of these objectives may be in conflict with each other and may require tradeoffs to achieve the best possible outcome. Although I will present some general information about wood bison and plains bison in Canada, the main thrust of this perspective will be on plains bison in the United States.

Cattle Ancestry in Bison

The first molecular genetic assay of cattle ancestry in bison was by Polziehn et al. (1995) who found cattle mitochondrial DNA (mtDNA) in 2 bison from a sample of 30 from the Custer State Park (CSP) herd in South Dakota. They surveyed 239 other bison in 8 different herds and found only bison mtDNA. Although the founding bison for the CSP herd were purchased from rancher James Philip, who had removed obvious hybrids from his herd, a previous owner of this herd was known to have had cattle–bison hybrids. In the CSP herd and other contemporary conservation herds with low amounts of cattle ancestry, individual bison with cattle ancestry identified by molecular markers have not been reported to be phenotypically different from bison without cattle ancestry, although there are 2 unpublished reports (Halbert N, Derr J, personal communication; Hedgecock D, personal communication) that show an average smaller size for bison with cattle mtDNA (see below).

The *Journal of Heredity* in 1914 published 2 seminal articles by breeders who crossed bison with cattle in efforts to incorporate favorable agricultural traits from bison into cattle, such as meat quality and quantity, hardiness, feed efficiency, and disease resistance (Boyd 1914; Goodnight 1914). The cross between these 2 species was difficult, and they could only cross bison bulls to domestic cows; the reciprocal cross was not possible because bison cows would not mate with domestic bulls (however, see below). From molecular genetic studies, it is estimated that bison and the ancestor of domestic cattle diverged approximately 1 million years ago (Verkaar et al. 2004; Nijman et al. 2008),

suggesting that some reproductive isolation would be expected between these 2 species.

From this first cross, all the offspring were female and there were no male offspring (again, see below). This observation is consistent with Haldane's rule, "When if the F_1 of two different animal races one sex is absent, rare or sterile, that sex is the heterozygous sex" (Haldane 1922). Boyd (1914) suggested that the lack of F_1 males was in part because the large size of male F_1 calves prevented them from passing through the cow's pelvis. However, Charles Goodnight, one of the ranchers that saved bison from extinction, thought that this was mistaken speculation because F_1 s, and even bison calves, were smaller than cattle calves, and they did not develop a hump until after birth (Dary 1974).

Figure 1a is a photograph of an F_1 cow from Boyd (1914), who stated that such F_1 cows, although often barren, were larger in size and produced more meat than cows from either parental species. From 1894 to 1915, Mossom Boyd had "102 successful impregnations of cows by buffalos; there were 63 abortions and 39 births. Of the 39 births, 6 were male, only 2 of which survived more than 24 h, and the one that became adult proved to be sterile" (Dary 1974). Starting initially with Boyd's animals, cattle-bison hybridization experiments were then carried out from 1916 to 1935 at an experimental station near Wainwright, Alberta. From 42 impregnations of domestic cows by bison bulls, 6 calves were born (2 males and 4 females), 20 calves were aborted or stillborn, and 16 cows died (Rorabacher 1970). Interestingly, bison cows and domestic bulls were also successfully crossed at Wainwright. From these crosses, there were 15 impregnations, resulting in 7 male births, 7 female births, and 1 stillbirth (Rorabacher 1970).

F_1 females could then be backcrossed to bison bulls, and nearly all these 75% bison ancestry offspring were females. Sometimes 75% bison ancestry bulls could be produced, but such offspring, although larger than the average bison bull, were often not fertile (Boyd 1914). Most of the backcrosses by these rancher-breeder were to domestic bulls so that commercially favorable bison characteristics could be introgressed into cattle. Figure 1b is a photograph of a bull that is 5/16 bison and 11/16 Hereford and that has a Hereford white face. Animals with a majority cattle ancestry were traditionally called cattelo and are now often called beefalo, a term that refers to animals with $\geq 5/8$ cattle ancestry. However, the development and history of the beefalo stock is complicated (Nichols 2007).

Genes with different modes of inheritance have different representations in the progeny of these crosses, and Table 1 gives the expected proportion of cattle ancestry for autosomes, mtDNA, and Y chromosomes. For the cross between bison bulls and domestic cows, the offspring have 50% autosomal cattle ancestry, 100% cattle mtDNA, and because there are generally no male offspring, 0% cattle Y ancestry. For the backcross progeny of a bison bull to F_1 cow, there is 25% autosomal cattle ancestry, again 100% mtDNA cattle, and 0% cattle Y ancestry. In other words,

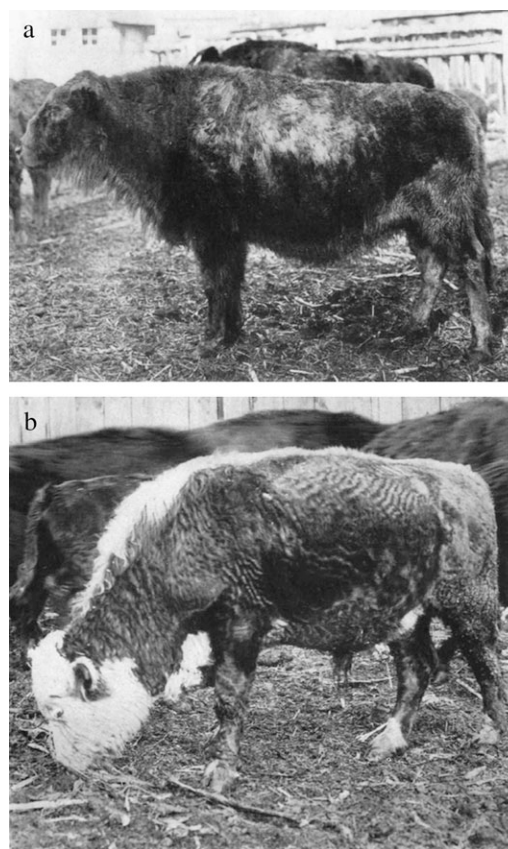


Figure 1. (a) F_1 hybrid cow offspring from a bison bull and a domestic polled Angus cow and (b) a bull that is 5/16 bison and 11/16 Hereford with a Hereford white face (Boyd 1914).

these first crosses result in a predicted excess of cattle mtDNA ancestry and a deficiency of cattle Y chromosomal ancestry compared with autosomal ancestry.

Table 2 summarizes the published estimates of cattle ancestry from 10 herds of plains bison and 3 herds of wood bison. As predicted, none of these herds, even those with cattle ancestry for autosomal genes and mtDNA, have any cattle ancestry from Y chromosome genes. Also, as predicted, the overall level of cattle mtDNA ancestry is higher than that for autosomal genes. As extreme examples, the herds from Santa Catalina Island (SCI) and Williams Ranch (WR) had mtDNA estimates of cattle ancestry of 44.9% (Vogel et al. 2007) and 100% (Ward et al. 1999), respectively, whereas estimates of cattle ancestry from autosomal genes for these 2 herds were 0.06% (SCI, Penedo C, personal communication) and 0% (WR, Halbert, Ward, et al. 2005), respectively.

The estimates for autosomal ancestry are the average of 14 independent, diagnostic loci for which cattle and bison have nonoverlapping sets of alleles (Halbert, Ward, et al. 2005). On the other hand, the estimate for mtDNA is for the D-loop and indicates ancestry of a single genetic unit, which as a result would be expected to have a much higher variance than the autosomal estimate. Although 5 of the 10

Table 1. The proportion of cattle ancestry from (a) interspecific crosses between bison and cattle and (b) for progeny of backcrosses to bison for genes on autosomes, mtDNA, and Y chromosomes

Cross	Male	×	Female	Cattle ancestry		
				Autosomal	mtDNA	Y
(a) Interspecies	Bison	×	Cattle	0.5	1.0	— (no male offspring)
	Cattle	×	Bison	—	— (no offspring)	—
(b) Backcross to bison	Bison	×	F ₁	0.25	1.0	0.0 (few males)
	F ₁ no males	×	Bison	—	— (no offspring)	—

plains bison herds have autosomal cattle ancestry, the average estimated ancestry is less than 1%.

Finally, the 3 wood bison herds do not appear to have any cattle ancestry, as would be expected if they are descended from the wild herd that survived. However, it appears that these wood bison populations hybridized with the large number of plains bison that were moved to Wood Buffalo NP from 1925 to 1928. In other words, it appears that these wood bison populations, and indeed all wood bison populations, are wood-plains hybrids and not pure wood bison (see discussion and data in Wilson and Strobeck 1999; COSEWIC 2004).

Understanding the difference in mtDNA and autosomal cattle ancestry and their modes of inheritance can be useful. If males are translocated from a herd with high mtDNA cattle ancestry and apparently low autosomal cattle ancestry, then the mtDNA ancestry would not be transmitted. For example, males from the Santa Catalina Island herd, if used in other herds, would not transmit cattle mtDNA.

Although conservation herds have been managed as pure bison, it has become clear in recent years that even conservation bison herds have some cattle ancestry resulting from the early experimental crosses. In a comprehensive survey of 11 federal conservation herds, Halbert and Derr (2007) used 14 diagnostic, nuclear microsatellite loci and mtDNA markers to estimate the amount of cattle ancestry

(Table 3). Seven of these herds had evidence of low amounts of nuclear cattle ancestry (average of 0.84%).

This cattle ancestry appears to be the result of early crosses between cattle and bison in combination with more recent movement of animals that has spread this ancestry between populations. For example, 5 of these populations have the cattle microsatellite allele BM4307-197 in frequencies ranging from 0.115 to 0.163. However, 4 of these populations, Badlands NP, Neal Smith National Wildlife Refuge (NWR), Theodore Roosevelt NP-S, and Theodore Roosevelt NP-N, were founded entirely or in part with animals from Fort Niobrara NP, the only other federal conservation herd where this allele was detected. The Fort Niobrara population was established in 1913 in part by animals from a private ranch (Halbert and Derr 2007). A likely explanation is that this cattle microsatellite allele was by chance in high frequency on the private ranch because of small population size and that the similar high frequency in all the 5 descendant herds reflected this initial high frequency. Similarly, cattle microsatellite allele BM7145-166 was found both in the National Bison Range herd and the Neal Smith NWR herd, which was partially founded from animals from the National Bison Range.

Only 4 of these federal conservation herds do not have detectable cattle ancestry (Grand Teton NP, Sully's Hill NGP, Yellowstone NP, and Wind Cave NP). However,

Table 2. Estimated cattle ancestry for autosomal genes, mtDNA, and Y chromosome genes for 10 plains bison herds and 3 wood bison herds (summarized from Ward et al. 1999, 2001; Halbert et al. 2004; Halbert, Ward, et al. 2005; Vogel et al. 2007; Penedo C, personal communication)

Subspecies	Herd name	Location	Cattle ancestry		
			Autosomal	mtDNA	Y
Plains	Antelope Island SP	Utah	0	0.011	0
	Custer SP	South Dakota	0.015	0.206	0
	Elk Island NP	Canada	0	0	0
	Finney GR	Kansas	0.018	0.038	0
	Henry Mountains	Utah	0	0	0
	Maxwell GR	Kansas	0.011	0.180	0
	National Bison Range	Montana	0.003	0.018	0
	Santa Catalina Island	California	0.006	0.449	—
	TSBH	Texas	0	0.167	—
	Williams Ranch	Texas	0	1.000	0
Wood	Elk Island NP	Canada	0	0	0
	Mackenzie Bison Sanctuary	Canada	0	0	0
	Wood Buffalo NP	Canada	0	0	0

SP, State Park; GR, Game Refuge; —, not evaluated. Elk Island NP has both subspecies in separate areas.

Table 3. Eleven federal bison herds and the estimated proportion of autosomal cattle ancestry for mtDNA and 4 cattle alleles and overall estimated cattle ancestry found in these herds (Halbert and Derr 2007)

Herd name	Location	Cattle mtDNA or allele					Cattle ancestry
		mtDNA	BM314-157	BM4307-197	BM7145-166	BMS2270-94	
Badlands NP	S Dakota	0	0	0.136	0	0.032	0.011
Fort Niobrara NWR	Nebraska	0	0	0.135	0	0	0.009
Grand Teton NP	Wyoming	0	0	0	0	0	0
National Bison Range	Montana	0.018	0	0	0.038	0	0.004
Neal Smith NWR	Iowa	0	0	0.135	0.016	0	0.010
Sully's Hill NGP	N Dakota	0	0	0	0	0	0
Theodore Roosevelt NP-N	N Dakota	0	0	0.163	0	0	0.011
Theodore Roosevelt NP-S	N Dakota	0	0	0.115	0	0	0.008
Wichita Mountains NWR	Oklahoma	0	0.090	0	0	0	0.006
Wind Cave NP	S Dakota	0	0	0	0	0	0
Yellowstone NP	Wyoming	0	0	0	0	0	0

NGP, National Game Preserve; N, north unit; S, south unit.

given other historical evidence, Halbert and Derr (2007) suggested that they are reasonably confident that the herds are free of cattle ancestry only for Yellowstone NP and Wind Cave NP, due to the large samples examined and the known ancestry of these populations. Because the Grand Teton NP population was partially founded from the Theodore Roosevelt NP herd, which has cattle ancestry (the rest of the population was descended from Yellowstone animals), they suggested that a larger sample from Grand Teton NP (39 animals were surveyed) might potentially uncover cattle ancestry. Likewise, a substantial proportion of the founders of the Sully's Hill NGP population came from herds with known cattle ancestry, such as Fort Niobrara NP (note that the current census number in the Sully's Hill population is only 35 females). Only 1 of these 11 populations, National Bison Range, had cattle mtDNA ancestry (1.8%) even though these populations were exhaustively sampled (except in Grand Teton NP). Although Y chromosome cattle ancestry was not specifically evaluated in these populations (except for the National Bison Range where it was 0%), from the results discussed above, it is assumed to be 0%.

As mentioned above, there are 2 unpublished reports that bison with cattle mtDNA have smaller body size than bison with bison mtDNA (Halbert N, Derr J, personal communication; Hedgecock D, personal communication). From these data, the lack of known phenotypic differences between bison with cattle and bison ancestry at particular genes may be because there has not been detailed study of the appropriate comparisons. In other words, it is possible that cattle ancestry in bison may have important undesirable phenotypic effects.

Inbreeding Depression

Determining inbreeding levels, and inbreeding depression, for individuals in which specific pedigrees are not known is difficult. However, given a thorough population sampling, detailed genetic information, and known mother-offspring

pairs, paternity can be inferred and accurate pedigrees from wild populations constructed (Pemberton 2008). To be confident of estimates of inbreeding and inbreeding depression, a detailed pedigree of at least several generations is generally required and reliable information on fitness measures, such as individual mortality, reproduction, and mating success, or of fitness surrogates, such as measurements of body size, is necessary.

The formerly large population size, and presumably large ancestral effective population size for bison, suggests that there was substantial detrimental genetic variation segregating in bison, assuming equilibrium. Further, the rapid reduction in population size from many millions to an effective founder number of less than 100 in plains bison suggests that some of these detrimental variants became fixed or increased in frequency by chance, resulting in a lowered population fitness (genetic load) and/or increased inbreeding depression (Hedrick 2005). In cattle, a longer time period of lower numbers during which they were domesticated and breeds developed may have allowed many detrimental variants to be purged. At this point, inbreeding depression has only been documented in the Goodnight herd (discussed below) and suggested for the population in Badlands NP (Berger and Cunningham 1994). However, this does not mean that it has not been present in other herds, only that it has not been demonstrated.

Charles Goodnight, one of the ranchers who worked to save bison from extinction in the late 1800s, began his herd with 5 wild-caught calves from Texas in the mid 1880s. Records indicate that his herd had 13 animals in 1887, 125 in 1910, and 200–250 in 1920s (Haley 1949). After Goodnight's death in 1929, the herd changed ownership several times, and in 1997, the remaining 36 animals were donated to Texas State Parks and Wildlife and moved to Caprock Canyons State Park (Halbert et al. 2004). These animals are now known as the Texas State Bison Herd (TSBH). The contemporary animals in this herd appear to be directly and exclusively descended from the bison herd originally assembled by Charles Goodnight. Six of the 36 bison

Table 4. The average age, natality (offspring per adult female), and early mortality from the first 6 years of the TSBH (Halbert, Grant, and Derr 2005) (*N* indicates sample size)

Year	Average age	Natality (<i>N</i>)	<1 Year mortality (<i>N</i>)
1997	3.56	0.19 (21)	0.75 (4)
1998	4.59	0.24 (17)	0.50 (4)
1999	5.35	0.73 (15)	0.64 (11)
2000	5.73	0.24 (17)	0.25 (4)
2001	6.23	0.62 (16)	0.30 (10)
2002	6.20	0.33 (15)	0.80 (5)
Average for TSBH		0.376 (101)	0.526 (38)
Other herds		0.560	0.042

donated in 1997 had cattle mtDNA, reflecting the crosses with cattle made by Goodnight (Halbert et al. 2004).

In 2002, the TSBH herd was still only 40 animals and had not shown any population growth. Other conservation herds have often had very rapid population growth, for example, the Badlands NP herd studied by Berger and Cunningham (1994) grew 10–20% per year. Table 4 gives the age, natality, and early mortality over the first 6 years of the TSBH (Halbert, Grant, and Derr 2005). Over this short time period, the average age in the herd increased by nearly 3 years, the natality rate (births per cow) was only 67% that in comparison herds, and the mortality in the first year was 12.5 times as high as other herds. This low natality and high early mortality explains the lack of growth of the population and its increasing age. In 2001, 15 of the 18 cows were pregnant but only 5 calves were born in 2002, and 4 of these died in the first year, so only 1 calf was produced for 15 cows. In addition, of 8 mature bulls (>3 years old) tested in 2000 for sperm motility and morphology, 4 had significant sperm abnormalities including low motility, bent tails, and detached heads (Halbert et al. 2004).

The TSBH was started from a small number of animals and is thought to have been through several bottlenecks during different owners and has had a small population size throughout most of its history. Although detailed records have been kept of this herd since 1997, there is no pedigree from earlier years from which to calculate inbreeding. As a general surrogate for these early records, we can compare the heterozygosity calculated by Halbert et al. (2004) for 54 microsatellite loci in 2001 in the TSBH (0.38) to the average in the Yellowstone and Theodore Roosevelt NP herds for the same loci (0.60). Using these estimates as the heterozygosity before and after genetic drift over a number of generations, a general estimate of the effect of small population size on heterozygosity in this population is

$$H_t = H_0 \prod_{i=1}^t \left(1 - \frac{1}{2N_{ei}}\right), \quad (1a)$$

where H_t is the heterozygosity in the t th generation and N_{ei} is the effective population size in the i th generation (Hedrick

2005). We can then assume that the overall effect on reducing heterozygosity is approximately

$$1 - f \approx \prod_{i=1}^t \left(1 - \frac{1}{2N_{ei}}\right)$$

and

$$f \approx 1 - \frac{H_t}{H_0}, \quad (1b)$$

where f is an estimate of the inbreeding coefficient. Therefore, assuming that $H_0 = 0.60$ and $H_t = 0.38$, the approximate level of inbreeding is $f \approx 0.367$. In other words, the reduced fitness observed in the TSBH appears to be equivalent to that expected from substantial inbreeding, on the order of 2 generations of full-sib mating (Hedrick 2005) although the loss of genetic variation probably took place unevenly over the last century (over 12 generations assuming a generation length of 8 years, see below).

In 2003, 3 bison bulls from Ted Turner's Vermejo Ranch in New Mexico were donated to the TSBH. The Vermejo Ranch herd is the only known private herd that does not appear to have cattle ancestry (Freese et al. 2007). Initial unpublished information suggests that the negative fitness effects in the TSBH have been overcome by this introduction (Swepston D, personal communication), a potential example of genetic rescue (Tallmon et al. 2004).

Genetic Variation

As general biological background related to understanding the amount of genetic variation in bison, we can use information on reproductive success from Berger and Cunningham (1994) and Wilson et al. (2002) in plains and wood bison, respectively. In plains bison, fecundity was highest for females aged 3–13 years, whereas the highest male success was for males aged 7–12 years (Berger and Cunningham 1994). Similarly, Wilson et al. (2002) found the highest success for females aged 3–12 years and the highest success for males aged 7–9 years. A general idea of generation length can be obtained as the average of these data on age of reproduction as approximately 8 years (or somewhat less if age-specific mortality is included). In addition, in both subspecies, the variance in reproductive success was significantly higher in males than females (Berger and Cunningham 1994; Wilson et al. 2002). Using the data from plains bison at Badlands NP, Berger and Cunningham (1994) estimated the ratio of effective population size (N_e) to census population size (N), N_e/N , as between 0.3 and 0.45 over different years and estimation approaches.

Before the identification of cattle ancestry in bison, the major conservation genetic concern in bison was the potentially low genetic variation, mainly because of low initial founder numbers but also because of subsequent bottlenecks and genetic isolation. For example, the 5 original ranch herds were each founded by a very small number of individuals. From the historical literature (Dary 1974;

Wilson and Strobeck 1999; Halbert, Ward, et al. 2005), it appears that the Goodnight herd (Texas) was descended from 5 founders, the Alloway–McKay herd (Canada) from 5 founders, the Dupree–Philip herd (South Dakota) from 6 or 7 founders, and the Pablo–Allard herd (Montana) from 6 founders. Although the Jones herd (Kansas and Oklahoma) appears to have had a number of founders, it is known to have contributed only 1 animal to the New York Zoological Gardens population and a small number of founders to other private herds. In other words, the total number of independent founders that these 5 herds contributed to the present population appears to be less than 50 and may have been only 30.

Although the wild Yellowstone herd is thought to have had substantial population numbers in some years, it had official census estimates of only 25–50 for the 16 years from 1896 to 1912 (Meagher 1973), suggesting a 2-generation bottleneck for this population. Further, the official estimates were only 25 in 1901, 1902, and 1907 and in 1902 only 22 were observed in the main herd (1 other animal was also observed). Because of these low numbers in the wild Yellowstone herd, 18 cows from the Pablo–Allard herd and 3 bulls from the Goodnight herd were introduced into a fenced area in Yellowstone NP in 1902 (Meagher 1973). In 4 of the first 5 years, only 2 of these males were present (1 died after the first year), and in 3 of the first 5 years, only 17 of the 18 females were present. Using a standard formula for effective population size (Hedrick 2005), the effective number of founders for this group is unlikely to be more than $N_e = 4N_f N_m / (N_f + N_m) = (4)(17)(2) / (17 + 2) = 7.2$. This population was kept separate from the wild population until 1915–1920 and later Meagher (1973) suggested that it constituted 60–70% of the ancestry of the total Yellowstone population. Thus, it appears that a majority of the Yellowstone ancestry may be descended from a small effective founder number of animals from the Pablo–Allard and Goodnight herds, which may have reduced overall genetic variation in the Yellowstone herd.

Wilson and Strobeck (1999) examined variation at 11 microsatellite loci in a number of herds and looked for correlations of genetic variation with founder number and number of founder sources and found a positive correlation between the number of founders and the average number of alleles. Halbert and Derr (2008) examined variation at 51 microsatellite loci in the 11 federal herds; Table 5 summarizes their results and information about the founding of these herds (average number of alleles is given rather than the standardized allelic richness because a very high proportion of nearly all the populations were sampled). For example, 2 of the herds with the highest genetic variation, National Bison Range and Yellowstone NP, had many founders and multiple founder sources. The Neal Smith herd also had high genetic variation but was only established recently, and so has not experienced much genetic drift. On the other hand, the lowest variation was observed in the Theodore Roosevelt NP-N, which was founded from 20 animals from the Theodore Roosevelt NP-S in 1962. The Sully's Hill herd, which also has low variation,

has a low census number and has been kept at a low number for many years.

Most of these federal herds have been managed separately except for the translocation of animals to establish new herds and infrequently to augment herds. In other words, many of them have independently undergone genetic drift over a number of generations so that some genetic differentiation between them would be expected. Halbert and Derr (2008) examined these herds for genetic differentiation using several different approaches and suggested that there are 5 different clusters (Table 5). Four of these clusters are single populations that show genetic differences from other herds, National Bison Range, Wichita Mountains, Wind Cave, and Yellowstone (average F_{ST} of 0.135). The other cluster (A) is composed of the 5 other populations that are connected by translocations and show lower differentiation between them (average F_{ST} of 0.065). The 2 other herds from Grand Teton and Neal Smith do not fit well into these categories, presumably because of their founding history from several sources.

Conservation Genetic Perspective and Recommendations

Cattle Ancestry in Bison

Much of the focus of conservation genetics in bison in recent years has been to identify herds with cattle ancestry (Halbert, Ward, et al. 2005; Halbert and Derr 2007). For example, Halbert and Derr (2007) suggested “the apparent success of the bison recovery efforts over the past 150 years is threatened by domestic cattle introgression. Hybrid species do not have taxonomic status and are not protected by the Endangered Species Act (ESA).” However, in Canada, only wood bison are listed as threatened, and the only petition for listing as threatened in the United States is for the Yellowstone herd of plains bison, and neither of these show evidence of cattle ancestry. Further, there does not appear at present to be an official policy to provide guidelines for dealing with hybrids under the US ESA (Allendorf et al. 2004), so how the ESA would be applied to bison with cattle ancestry is not clear.

As discussed above, in the federal herds that have cattle ancestry, the level appears quite low, less than 1%. Turning this around, it appears that more than 99% of the ancestry in these bison herds is from bison. Except for the 2 unpublished reports of the effect of cattle mtDNA on bison body size, animals with molecular evidence of cattle ancestry have not been identified as being morphologically, behaviorally, or in other phenotypic ways different than bison without identified cattle ancestry. Reducing cattle ancestry from <1% to 0% may not have a substantial positive impact on bison fitness. On the other hand, cattle ancestry could be different in kind than ancestry in most other hybridization situations because cattle were domesticated up to 10 000 years ago (Bruford et al. 2003). Since then, cattle have been selected for agricultural traits, making their ancestry potentially very detrimental in a wild species such as bison.

Table 5. Eleven federal bison herds (for abbreviations, see Table 3) and the number of founders, number of founder sources, and years of introduction for them (Halbert and Derr 2007)

Herd	Founder			Census	H_E	Number of alleles	Cluster
	Number	Sources	Years				
Badlands NP	73	3	1963, 1983	875	0.578	4.56	A
Fort Niobrara NWR	21	4	1913–1952	380	0.595	4.40	A
Grand Teton NP	32	2	1948, 1964	600	0.561	4.08	—
National Bison Range	50	7	1908–1984	350	0.647	5.00	6
Neal Smith NWR	33	3	1996–1998	63	0.639	4.96	—
Sully's Hill NGP	19	5	1919–1997	35	0.566	3.62	A
Theodore Roosevelt NP-N	20	1	1962	312	0.522	3.56	A
Theodore Roosevelt NP-S	29	1	1956	371	0.582	4.30	A
Wichita Mountains NWR	17	2	1907, 1940	600	0.652	4.85	2
Wind Cave NP	20	2	1913, 1916	350	0.591	4.16	1
Yellowstone NP	46 ^a	3	1902	3000	0.625	4.84	7

Also given is the current census estimate (total number of individuals), level of heterozygosity (H_E), and average number of alleles for 51 microsatellite loci and cluster number from STRUCTURE analysis (Halbert and Derr 2008) (— not in cluster).

^a About 25 from the surviving wild population.

In general, hybridization between endangered species and common related species is considered to be a potential threat to endangered species (Rhymer and Simberloff 1996; Allendorf et al. 2001). However, the greatest threat is thought to be from high levels of contemporary mating between an endangered species and a related nonendangered species. The cattle ancestry in conservation herds of bison is not the result of contemporary mating but is the result of artificial crosses, mostly made 100 or more years ago. Crossing cattle with bison is difficult, even in containment, and there is no evidence that crosses between these species occur naturally.

The low level of autosomal cattle ancestry in conservation herds of bison suggests that either the initial level when no more cattle ancestry was introduced was low or selection has resulted in a decline of cattle ancestry over time. There does not seem to be an imminent danger for the swamping of the bison gene pool by cattle ancestry. Although there are no temporal data on cattle ancestry in conservation herds of bison, it is entirely possible that the level of cattle ancestry is declining over time. If there is detrimental cattle ancestry, such as cattle mtDNA that appears to reduce body size, maintaining a large effective population size (so genetic drift is not important) would allow selection to reduce this detrimental ancestry without further human intervention.

For estimation of cattle ancestry, the microsatellite loci (and mtDNA D-loop) are appropriate and probably reflect neutral differences between the genomes of bison and cattle. However, it is important to put the estimate of <1% autosomal cattle ancestry from these markers in perspective. First, bison and cattle probably share more than 99% of their DNA sequence, as do other closely related species. In other words, the <1% cattle ancestry is probably <1% different from bison, or it results in <0.01% difference in the genomes of conservation herds with and without cattle ancestry.

Second, from the known cattle ancestry (Halbert, Ward, et al. 2005; Halbert and Derr 2007), there does not appear to be evidence that specific regions of the cattle genome have been positively selected in bison. In fact, the distribution of cattle regions in bison appears consistent with that expected by chance due to genetic drift (Halbert N, Derr J, personal communication). Even the very high cattle mtDNA ancestry in the Santa Catalina and Williams Ranch herds may be a chance effect of genetic drift.

How did the proportion of autosomal cattle ancestry become so low in bison herds with cattle ancestry? If backcrosses to bison occurred over multiple generations (as shown for 1 generation in Table 1), then the autosomal ancestry is reduced by half each generation. Or the expected cattle ancestry for t generations of backcrossing is $(1/2)^t$. For 6 and 7 generations of backcrossing, the cattle ancestry would be reduced to 1.56% and 0.78%. This is not inconsistent with what has been observed. Or, if additional purebred bison were added to the herd, then this would decrease the level of cattle ancestry as well. Perhaps instead of this scenario, some of the cattle regions of the genome may have been selected against resulting in a decline in their frequency over time.

With these caveats, several recommendations about reduction of cattle ancestry in conservation bison herds seem reasonable.

- (1) Bison from populations with evidence of cattle ancestry should not be introduced into populations with no evidence of cattle ancestry.
- (2) Introduction of animals from herds with no evidence of cattle ancestry into herds with cattle ancestry is appropriate when surplus animals are available. This could, for example, result in a decrease of inbreeding depression, an increase of genetic variation, or even genetic swamping of cattle ancestry. In addition, excess animals from these herds without evidence of cattle

ancestry could be used to establish new conservation herds by public and private stakeholders.

- (3) Translocation between herds with similar levels of cattle ancestry is potentially appropriate because this would not increase the overall level of cattle ancestry. However, to make sure that herds have similar levels of cattle ancestry, a more accurate estimate of cattle ancestry, based on more microsatellite loci or perhaps single nucleotide polymorphisms, is recommended. In addition, further detailed examination of the potential phenotypic effects of cattle ancestry in bison is recommended.
- (4) Reduction of cattle ancestry by culling animals with known cattle mtDNA is generally appropriate and could eliminate cattle mtDNA from herds. However, such culling should not be assumed to reduce the nuclear cattle ancestry because the mtDNA and nuclear cattle ancestries are not expected to be associated, that is, to be in linkage disequilibrium. In herds with high cattle mtDNA levels, great care should be taken to retain bison variation at nuclear loci if there is selective culling to reduce cattle mtDNA.
- (5) Reduction of the frequency of specific nuclear cattle alleles from a population by culling is also possible, but it is likely that cattle ancestry will remain at other unidentified genetic regions in these herds.

Avoiding Inbreeding Depression and Maintaining Genetic Variation

Although some population sizes of the conservation bison herds are not small, these numbers should be compared with the very high numbers present 150 years ago. When the total number for plains bison was in the many millions and there was generally gene flow throughout the subspecies, there presumably was high variation for genes having detrimental, neutral, and advantageous effects. It is not known whether the variation today reflects this historic variation. Examination of museum or historic samples before the great bison decline, from 1850 or earlier, could be used to compare the present-day variation with ancestral variation. For example, if the variation at neutral loci or sites is lower today than historically, this may indicate significant bottleneck effects and a consequent potential for increase in some detrimental variants.

As discussed above, the TSBH has substantially lowered fitness for several different traits. Although no other herds have as low genetic variation as the TSBH, the Neal Smith herd, for example, has relatively low heterozygosity. The amount of variation in other isolated conservation herds, besides the federal herds, may also potentially be low.

Although Halbert and Derr (2008) identified 5 different clusters with substantial differentiation between them, it is likely that these differences have been generated primarily by genetic drift since the founding of the herds. It is possible that some of the differences reflect ancestral differences present in bison herds before their near extinction, although

the large ancestral population size and high amounts of ancestral gene flow make this unlikely.

Several general recommendations on inbreeding depression and genetic variation for the conservation bison herds seem reasonable if considered in balance with the previous recommendations on reducing cattle ancestry:

- (1) In order to minimize inbreeding depression and maintain genetic variation in populations, regular exchange between bison populations is recommended. It appears that natural bison populations were composed of large, intermixing groups, and reestablishment of this situation is recommended. Of course, considerations beyond genetics, such as disease transfer, different state laws on bison, and success of translocated animals, must also be considered.
- (2) Monitoring of fitness-related traits, such as mortality, natality, and mating success is recommended so that fitness levels (and adaptation) can be documented.
- (3) Individual herds or clusters should have an effective population size of 1000 (census number of 2000–3000) to avoid inbreeding depression and maintain genetic variation. If it is not possible to have this primary herd in 1 location, then it could be in 2 or 3 locations with significant genetic exchange between them. Note that this is larger than any of the plains bison herds except for Yellowstone NP and any of the wood bison herds except for Wood Buffalo NP and Mackenzie Bison Sanctuary in Canada.
- (4) Identified differentiated populations (clusters) should be replicated with at least one other physically separated population with an effective population size of 1000. If it is not possible to have this replicated population in 1 location, then it could be in 2 or 3 locations with significant genetic exchange between them.
- (5) Pedigrees of the populations should be established. The molecular data already collected could form the basis of identifying paternity and potentially other relationships in these pedigrees. In addition, from these data, the contemporary effective population size N_e and the ratio of N_e to the census number, N_e/N , could be estimated.
- (6) Estimation of effective population size in recent generations using analysis of linkage disequilibrium and variance in allele frequency, and past effective population size using sequence data, is recommended (Schwartz et al. 2007).
- (7) Estimation of the ancestral level of genetic variation from museum samples or other historical samples is recommended. This should help identify genetic patterns over space that were present before the great reduction in bison numbers and large changes in allele frequency that may have occurred due to the changes in population numbers.
- (8) There is no justification to select for preservation of specific rare bison microsatellite alleles in populations. Selection for the increase of the frequency of specific rare alleles has been shown to have a significantly greater cost than benefit because of the loss in genetic variation in the rest of the genome (Hedrick and Miller 1994).

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