

Development of a Genetic Based Management Plan for the Badlands National Park Bison Population

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American bison are the largest mammal to have survived the last glacial period in North America and are a critical keystone species in grassland ecosystems. While more than 500,000 bison exist today, they are nearly all derived from a handful of publicly-managed herds in the U.S. and Canada which represent the genetic “stock” of the bison species. One of these significantly important herds is maintained at Badlands National Park (BNP), which was first established in 1963 with bison originally from Nebraska (Fort Niobrara National Wildlife Refuge lineage) and later supplemented in 1983 with bison from Colorado. Genetic data were collected over a period of 5 years to investigate the levels and patterns of variation within the herd in order to assist with long-term management goals and priorities. Without question, genetic variation is important for long-term population health (viability), and BNP bison have moderately high levels of genetic variation compared with other U.S. federal herds. Like most of the US federal system bison herds, very low levels of domestic cattle DNA (introgression) exist in BNP bison.

Interestingly, two different genetically defined “subpopulations” were discovered in the BNP herd, corresponding to the two historical lineages represented in this herd (Nebraska & Colorado). The observation that bison in this herd preferentially associate with others from their original founding lineage 25 years after the lineages were “mixed” was an unexpected finding by scientists and population managers. In fact, this new discovery could have significant implications for the future management of this national resource and may require more detailed evaluations of hidden population structure and management policies with other important federal bison herds.

Computer simulations were also performed to test the effects of intensive culling, which has been proposed to reduce the herd size from around 850 to 600 bison. While culling duration (years) and intensity are predicted to have only a minimal impact on short-term (less than 10 years) genetic diversity, over longer time periods (100 years) total population size is expected to have a significant impact on genetic diversity. In fact, to maximize levels of genetic diversity in the BNP herd, the results of this study indicate that the herd should be maintained with at least 800-1000 bison. This study underscores the value of using modern biotechnology to help ensure the preservation and conservation of the BNP bison herd for generations to come and could provide valuable insight for future species conservation endeavors.

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Abbreviations key:
NPS, National Park Service
NP, National Park
FWS, U.S. Fish and Wildlife Service
NWR, National Wildlife Refuge
BNP, Badlands National Park
FN, Fort Niobrara National Wildlife Refuge
TRS, Theodore Roosevelt National Park – South Unit
TRN, Theodore Roosevelt National Park – North Unit
 N_A , Total number of alleles
 H_E , Expected (unbiased) heterozygosity
CO-lineage, Colorado lineage
NE-lineage, Nebraska lineage (includes FN and TR populations)

Executive summary

Nearly all of the more than 500,000 bison in existence today are derived from a handful of U.S. and Canadian public herds, but relatively few are currently managed within conservation herds. The long-term preservation of the species, therefore, is dependent upon proper management of these conservation herds, most of which are small and isolated. Among conservation herds, the bison population at Badlands National Park (BNP) has a distinctive history and unique genetic background, and fulfills an essential ecological role as a keystone species and largest herbivore in the Badlands ecosystem. Therefore, prudent and scientifically-sound management strategies are needed to ensure the long-term preservation of genetic diversity and ecological success of this valuable resource. In this study, genotypic data was collected over a period of 5 years (2002-2006) in order to investigate the underlying genetic architecture of the BNP bison population and to evaluate the effects of management practices on both short- and long-term maintenance of genetic diversity. Two closely related, yet distinctive, genetic subpopulations were identified which corresponded to the two lineages known to have contributed to the population. The genetic distinctiveness of the subpopulations appears to have deteriorated over the study period due to cross-breeding, a trend that is likely to continue. Simulation modeling indicates little effect of culling time or intensity on short-term (8 years) retention of genetic diversity, but large differences in long-term (200 years) retention of genetic diversity due to differences in population size.

Study background

As the largest species in North America to have survived the last major glaciation (125,000-500,000 years ago) and one of the first wildlife species to prompt a concerted conservation effort, American bison (*Bison bison*) are an emblem of the Great Plains, Native American culture, and wildlife conservation. While over 500,000 bison exist today, long-term species conservation is not assured (Freese et al. 2007). For example, fewer than 5% of all bison are maintained in conservation populations (Boyd 2003), while the remaining 95% exist in private populations subjected to various levels of artificial selection. The bison maintained in Canadian and U.S. federal populations are a critical germplasm (DNA) resource for species conservation, since the majority of extant bison are derived from these populations (Coder 1975; Boyd 2003). Approximately 8,500 bison are currently maintained as part of the U.S. federal system, and are divided among U.S. Fish and Wildlife Service (FWS, 5 populations) and National Park Service (NPS, 5 populations) units.

Despite estimates of fewer than 1,000 bison at the apex of the population bottleneck in the late 1800s (Coder 1975), most extant bison populations harbor moderate levels of neutral genetic diversity (Wilson and Strobeck 1999; Halbert 2003). Unfortunately, purposeful breeding of bison with domestic cattle (*Bos taurus*) by ranchers (e.g., see Jones 1907; Goodnight 1914) during the apex of the population bottleneck (Coder 1975) lead to the introduction of domestic cattle DNA into the bison genome (introgression). The remnants of these hybridization experiments – small segments of domestic cattle DNA in an otherwise “bison” genome – have been detected in nearly all modern bison populations (Polziehn et al. 1995; Ward et al. 1999; Halbert et al. 2005a; Halbert and Derr 2007a; Vogel et al. 2007; Halbert and Derr, unpublished data on private herds). The presence of domestic cattle introgression is considered undesirable for long-term species conservation, but the total exclusion of populations containing any level of domestic cattle introgression from conservation efforts would also exclude genetically unique

bison lineages with historic and ecological significance. Therefore, conservation priority has been suggested for populations with no detectable domestic cattle introgression, or those populations with low levels of detectable introgression representing unique bison lineages (Freese et al. 2007).

The maintenance of genetic diversity in closed populations is critical to long-term population viability, as it provides a means of adaptability to environmental changes. In general, large effective population sizes (number of effectively breeding individuals) are needed to circumvent the effects of drift and maintain genetic diversity over long periods of time. Therefore, substantial changes in population size, such as those caused by artificial bottlenecks and founder events, can lead to dramatic losses in genetic diversity and should be avoided. Bison have high reproductive rates and nearly all bison conservation populations exist in the absence of natural predators, which would have historically controlled population growth (see Millspaugh et al. 2005 for review). Consequently, most bison populations are subjected to periodic culling to maintain ecologically appropriate census sizes. The most appropriate culling strategies to preserve long-term genetic diversity, maintain the natural social structure of the population, and address landscape-specific ecological limitations are likely to be population-specific and have been largely unevaluated for most bison populations (limited examples include Gross and Wang 2005; Halbert et al. 2005b; Millspaugh et al. 2005).

The Badlands National Park (BNP) population was founded in 1963 with 53 bison from Fort Niobrara National Wildlife Refuge (NWR) and Theodore Roosevelt National Park (NP) (BNP Bison Management Plan 2003). The Theodore Roosevelt NP population was, in turn, derived exclusively from Fort Niobrara NWR bison (Oehler 2007). In 1983, 20 bison were added to the BNP population from the Colorado National Monument, which originated from three bison near Denver, Colorado in 1925 (McClenaghan et al. 1990) and is not known to be related to the Fort Niobrara NWR lineage or represented in any other U.S. federal bison population. Therefore, the BNP population is composed of two lineages: the Nebraska lineage derived from the Fort Niobrara NWR population and the Colorado lineage derived from the Colorado National Monument population (hereafter NE-lineage and CO-lineage, respectively).

The BNP bison range on the 60,000-acre Sage Creek Unit, which has an estimated ecological carrying capacity of 1,500-1,800 bison (BNP Bison Management Plan 2003). However, in order to mitigate the effects of drought to which the region is prone, the current target census size of the population is 600 bison. Culling of BNP bison ceased from 1998-2002 following major damage to the corral system, and the population grew to an estimated maximum of 1,100 bison. From 2002-2006, extensive annual roundups and culling reduced the population to approximately 850 bison (140% of target size). The goals of the current study were to: 1) investigate the genetic architecture of the BNP bison population, 2) model the genetic effects of culling methods to reduce the total census size of the population (i.e., from 850 to 600 bison), and, 3) understand the long-term effects of intensive management on the maintenance of genetic variation and population survival. To accomplish these goals, genetic information was collected from both mitochondrial and nuclear DNA of BNP bison and analyzed using a suite of modern techniques employed to better understand the current and projected genetic structure of the BNP bison population.

Materials and Methods

The BNP population was initially sampled in 2002 as part of a previous study (Derr and Templeton 2000), and initial genetic assessments were performed based on markers developed in our laboratory (Halbert 2003; Gross and Wang 2005; Musani et al. 2006; Halbert and Derr 2007a). Since 2003, park personnel have collected additional samples for genetic evaluation during each annual roundup. A total of 1,616 whole blood samples on Whatman Classic FTA[®] cards and/or hair samples were collected from 2002-2006. An additional 128 samples were collected in October 2007 but were not used as part of this study. Upon receipt, all samples were given unique laboratory identifiers. Demographic and phenotypic data from BNP park personnel was merged into an ACCESS database maintained in our laboratory to allow cross-referencing between datasets. DNA was extracted following previously published protocols (Halbert et al. 2004; Halbert and Derr 2007a).

All samples were examined for the presence of domestic cattle introgression in the mitochondrial (Ward et al. 1999) and nuclear (Halbert et al. 2005a; Halbert and Derr 2007a) genomes following published protocols. The mitochondrial assay is a single-tube PCR with a binary categorical outcome (domestic cattle or bison). The 14 microsatellites used to detect nuclear introgression have non-overlapping allele size ranges defining domestic cattle and bison. These nuclear markers were amplified in 3 PCR assays (Halbert and Derr 2007a) and detected using the methods described below.

The selection of bovine microsatellite markers for population-level genetic diversity studies and description of multiplexed PCR assays has been previously detailed (Halbert *et al.* 2004). From the original group of 54 markers, a total of 26 nuclear autosomal microsatellites (different than the introgression detection panel above) were utilized in this study to evaluate genetic diversity and population structure (Table 1). Two additional markers were used to confirm the sex of each individual, but were not otherwise utilized in this analysis: BMS911 on the X-chromosome and INRA189 on the Y-chromosome. Amplification was performed in 5- μ L volumes in 96-well plates, and PCR products were separated on an ABI 3130xl Genetic Analyzer (Applied Biosystems, Foster City, California). A Rhodamine-X (ROX)-labeled internal size standard (Mapmarker LOW, Bioventures, Inc., Murfreesboro, Tennessee) was utilized for inter-assay standardization. A selected set of reference samples were utilized to standardize allele calling. The reverse primers for BMS410, and BMS527 were 5'-tailed with a viral DNA sequence (GTGTCTT; Brownstein *et al.* 1996) to either facilitate allelic identification or prevent problematic overlapping with other multiplexed markers. The fragment analysis programs Genotyper 3.6 and GeneMapper 3.7 (Applied Biosystems, Foster City, California) were used for allele identification and comparison.

The Microsoft Excel Microsatellite Toolkit (Park 2001) was used to identify and discard duplicate and calculate basic genetic parameters such as unbiased heterozygosity (Nei 1987) and allelic frequencies. Genetic distances were calculated in the program MSA 4.05 (Dieringer et al. 2003), using the proportion of shared alleles (D_{ps}) measure of Bowcock et al. (1994). Neighbor-joining trees were created using the Neighbor program in the PHYLIP 3.7 package (Felsenstein 1993), with randomized input order. Resultant tree topologies were viewed and arranged in the program TreeView 1.6.6 (Page 1996).

Hardy-Weinberg disequilibrium, gametic disequilibrium among marker pairs (Weir 1996), and F-coefficients were measured using the program GENEPOP v. 4.0.7 (Rousset 2008) with the default Markov chain parameters and sequential Bonferroni corrections for multiple tests with a nominal p-value of 0.05. Subpopulation division was also evaluated using the

multilocus Bayesian clustering method in the Structure 2.1 program (Pritchard *et al.* 2000). This method minimizes the presence of Hardy-Weinberg and gametic disequilibrium within a collection of multilocus genotypes by probabilistic assignment of individuals into K populations. After initial testing following the recommendations of Pritchard *et al.* (2000), testing was performed with a burnin period of 10,000 replicates and 40,000 Markov Chain Monte Carlo replicates using the independent allele frequency model including admixture. The default program values were used for all other settings. The most likely number of clusters within the dataset was determined by examining averages and standard deviations at each K for LnP(D) (natural log of the probability of the data fit to the model; Pritchard and Wen 2004) and using the ΔK method (Evanno *et al.* 2005). The modal value of ΔK is based on the second order rate of change of $\ln[\text{Pr}(X | K)]$ with respect to K (Evanno *et al.* 2005). Clusters among different replicates were sorted and aligned using the program CLUMPP 1.0 (Jakobsson and Rosenberg 2007), and the resultant population and individual membership assignments were visualized using the program DISTRUCT 1.1 (Rosenberg 2004).

Population viability analysis was performed using the program VORTEX version 9.72 (Lacy *et al.* 2007), a program designed to model the effects of demographic, environmental, and genetic stochastic events on population size and viability. The input parameters provided in Appendix C were held constant among the various scenarios evaluated, with the demographic rates estimated following analysis by Berger and Cunningham (1994) based on an in-depth 5-year study (1985-1989) of bison at Badlands National Park. Both short-term (8 years) and long-term (200 years) scenarios were evaluated. The short-term model was designed to evaluate various culling strategies to reduce the census population size down from 850 to 600 bison. Reductions were compared between a 2-year period (from 850 to 600 bison in 2 years, followed by 6 years stabilized at approximately 600 bison), a 5-year period (from 850 to 600 bison in 5 years, followed by 3 years stabilized at approximately 600 bison), and an 8-year period (from 850 to 600 bison in 8 years). In the long-term model, the effects of population size were evaluated over a 200-year period, with constant sizes of approximately 600, 800, and 1000 evaluated. The effects of periodic drought were included in 800- and 1000-bison scenarios by the addition of a stochastic environmental effect with a 5% probability (i.e. affecting the population 1 of every 20 years) and causing a 15% reduction in natality rates and a 10% increase in mortality rates across all age classes. Allele frequencies from the 26 nuclear autosomal markers (shown in Appendix C) were used to initialize the model, and the number of alleles (N_A) and unbiased heterozygosity (H_E ; Nei 1987) per marker were measured following each iteration using the program FSTAT 2.9.3.2 (Goudet 1995, 2001).

The culling strategies employed in the VORTEX model to reduce the population size from 850 to 600 bison are recorded in Appendix C. In each case, equal numbers of males and females were removed, and adult culling was proportionate to the number of individuals in each age class. This proportional culling strategy most closely approximates the recent culling strategies employed for this population (BNP Bison Management Plan 2003), and is expected to produce a herd structure representative of the pre-horse estimated bison population (Millspaugh *et al.* 2005). For the stable periods of each scenario (e.g. 2-year reduction, years 3-8), culling was simulated by introducing a carrying capacity at 600 bison, which in effect causes mortality rates (i.e. removals) to increase proportionately across all age classes when the total size of the population exceeds the carrying capacity. Similarly, the long-term scenarios used the target population size (i.e. 600, 800, 1000) as the carrying capacity.

Results

Duplicate sampling was identified by replicate implant numbers and/or genotyping matches; 32 individuals were found to have been sampled twice and 1 individual was sampled 3 times (Appendix A). Therefore, the total number of unique BNP bison samples currently archived at Texas A&M University is 1,582, of which 1,115 were used in the current study (see Appendix B for complete list). A breakdown of samples utilized from each roundup is provided in Table 2.

At least 80% of markers were scored for each individual, with an average genotyping success rate of 98.65%. Individual genotypes for all 40 microsatellite markers (14 nuclear introgression and 26 diversity) are provided in Appendix B. A total of 139 alleles were detected across these markers (23 nuclear introgression and 116 diversity). Four alleles were detected which were not previously identified in the samples from the 2002 collection (Halbert 2003): BM2113, 149-bp allele; BMS410, 91-bp allele; RM372, 138-bp allele; and RM500, 125-bp allele. Two bison were found to harbor the RM372 allele of 138-bp, while each of the other alleles were only detected in a single bison. The bison harboring these alleles were born in 2003 (n=2) or 2004 (n=3). Each genotype was verified through an independent DNA extraction and PCR amplification from two different DNA sources (blood and hair). The finding of “new” rare alleles is not surprising given that the initial study conducted did not include every sample in the population, and thus may have missed rare alleles. Other possibilities which would explain the sudden presence of these alleles is recent genetic mutation or undetected immigration and genetic assimilation, such as from neighboring private bison populations. The BM2113 and RM372 alleles have been identified in other U.S. federal bison populations (Halbert 2003), while the BMS410 allele has been identified in several private bison populations (Halbert and Derr, unpublished data). The 125-bp allele for RM500, a marker developed to detect domestic cattle introgression bison, has not been identified in over 6,800 bison evaluated in our laboratory for this marker, and falls within the range of allele sizes for domestic cattle (Halbert et al. 2005a; Halbert and Derr 2007a). These apparently new alleles were not included in the subsequent analysis (i.e. genotypes were coded as missing from these individuals for these markers).

The mitochondrial haplotype of each sample was successfully determined and no domestic cattle introgression was detected (i.e. all samples contained bison mitochondrial DNA). Following previously published reports (Halbert 2003; Halbert and Derr 2007a), domestic cattle alleles were detected in 2 of the 14 nuclear introgression markers: BM4307, 197-base pair (bp) allele (14.33% frequency); BMS2270, 94-bp allele (3.85% frequency). The frequencies identified for these markers are very similar to those previously reported (Halbert and Derr 2007a). The BM4307 allele of 197-bp has been identified in the Fort Niobrara NWR and the Theodore Roosevelt NP populations. To date, the BMS2270 allele of 94-bp has not been identified in other U.S. federal bison populations, and was most likely introduced with the bison from the Colorado National Monument in 1983 (Halbert and Derr 2007a).

The entire BNP dataset was compared with bison genotypes from the Fort Niobrara NWR (n = 178), Theodore Roosevelt NP-North Unit (n = 309), and Theodore Roosevelt NP-South Unit (n = 368) populations (Halbert 2003), representing exclusively NE-lineage populations (i.e., derived from Fort Niobrara NWR). Of the 139 alleles in the BNP population, 132 (95.0%) were previously identified in at least one of the NE-lineage populations. The remaining 7 (5.0%) alleles, which include the 197-bp allele from marker BMS2270 described above, were most likely introduced into the BNP population with the bison from the Colorado National Monument in 1983 and are presumed to be specific to the CO-lineage. It should also be

noted, however, that at least 3 other explanations would account for the presence of these alleles in the BNP population: 1) alleles originally present in Fort Niobrara NWR bison, but subsequently lost from that population (and both the north and south units of Theodore Roosevelt NP, presuming they were also present in one or both of these populations); 2) alleles derived from undocumented immigration of bison into the BNP population; 3) alleles arising through new mutation. These possibilities are considered less likely than the derivation of these alleles from the CO-lineage. The identified alleles and their frequencies are reported in Table 4. With the exception of the BMS2270, 197-bp allele, each of the other alleles presumptively derived from the CO-lineage have been previously identified in at least one other U.S. federal bison population (Halbert 2003). Of the 1,115 bison evaluated, 259 (23.3%) harbored at least one of these 7 alleles, and the breakdown of the number of alleles per individual is shown in Figure 1. The percentage of individuals detected with CO-lineage alleles across birth cohorts from 2000-2006 does not follow an obvious pattern of increase or decrease, but rather appears to be randomly fluctuating, with levels ranging from 18.18% to 36.36% of individuals with CO-lineage alleles per year (Figure 2).

To assess changes in genetic diversity of the BNP population from 2002-2006, average heterozygosity (unbiased gene diversity; Nei 1987) and allelic diversity (number of alleles per locus) were calculated for the bison in the population before and after each cull (pre- and post-cull) from 2002-2006. While this comparison does not include all individuals in the population at each time point, our sampling scheme included at least 50% of the estimated population for each time point (Table 3). Levels of heterozygosity and allelic diversity were nearly identical across groups (Table 3), indicating that the culling strategies employed to abate population growth over this 5-year period did not substantially affect overall genetic diversity.

The post-cull 2002 ($n = 391$) and post-cull 2006 ($n = 503$) data sets were evaluated for evidence of Hardy-Weinberg and gametic disequilibrium. In each data set, all 26 loci appear to be in Hardy-Weinberg equilibrium. Of the 325 possible paired marker combinations, gametic disequilibrium was noted for 18 (5.5%) combinations in the post-cull 2002 data set and 20 (6.2%) of the post-cull 2006 data set.

To assess evidence for subpopulation structure, the entire dataset ($n = 1115$) was evaluated using a multilocus Bayesian model in the program Structure for each K from 1 to 5 with 10 replicate iterations at each value of K . The averages and standard deviations of $\text{LnP}(D)$ for each K are shown in Figure 3a. The value of K following the largest increase in average $\text{LnP}(D)$, and in which the standard deviation is low, is generally taken as an *ad hoc* estimate of the true number of subpopulations in a dataset (Pritchard and Wen 2004). In this case, $K = 2$ appears to be the most likely number of subpopulations in the dataset. Another proposed method for determining the true value of K is to take the highest value of ΔK , the second order rate of change of $\text{LnP}(D)$ (Evanno et al. 2005). The plots in figure 3b indicate that the highest value of ΔK for this dataset also occurs when $K = 2$. Taken together, these data indicate that two genetically-distinct subpopulations may exist within the BNP bison population. To evaluate the possibility of bias from evaluating the entire dataset, where large family relationships might influence the structure of the dataset, the entire analysis was repeated for the post-cull 2006 dataset ($n = 503$). The results of this analysis are not shown, but were similar to that with the entire dataset and also indicated subpopulation structure two likely subpopulations; the initial analysis based on the entire dataset is considered hereafter.

Assignment probabilities of individuals into each of the two clusters were highly consistent across iterations, as illustrated in Figure 4. Assignment probabilities were averaged

across the 10 iterations. Individuals with at least an average of 60% probability of assignment to a cluster were considered likely to belong to that cluster; individuals with an average of less than 60% probability of assignment into either cluster were not classified into a cluster and were considered to be of mixed lineage. Overall, approximately 62% (691/1115) of the individuals were assigned to cluster 1, 33% (373/1115) were assigned to cluster 2, and 4.6% (51/1115) were of mixed lineage. To evaluate the amount of genetic differentiation among these clusters, F coefficients representing the amount of genetic differentiation due to subpopulation division (F_{ST}) and inbreeding (F_{IS}) were measured in each post-cull dataset from 2002-2006, including only individuals with assignment probabilities greater than 60%. Overall, F_{ST} decreased from 0.0381 to 0.0275, and F_{IS} increased from -0.0255 to -0.0175. Within each cluster, inbreeding decreased over this time period. In cluster 1, F_{IS} increased from -0.0065 to 0.0005 while in cluster 2, F_{IS} increased from -0.0663 to -0.0499. The magnitude of difference in F_{IS} values indicates a higher level of inbreeding within cluster 2 compared with cluster 1.

From 2002-2006, the percentage of individuals assigned to cluster 1 decreased (Figure 5a), with 72.05% of the 2002 and 61.52% of the 2006 pre-cull populations assigned to cluster 1. Likewise, the percentage of individuals assigned to cluster 2 increased over this period (Figure 5a), with 25.59% of the 2002 and 33.91% of the 2006 pre-cull populations assigned to cluster 2. The number of individuals classified as mixed lineage also increased slightly over this period, with 2.36% of the 2002 and 4.58% of the 2006 population classified as mixed lineage (Figure 5a).

The dataset was further subdivided such that assignments with greater than 90% probability into either cluster were considered strong and compared to the more moderate assignments in the 60-90% range. Interestingly, the proportion of individuals with moderate association in cluster 1 (>60% and <90% assignment probability) remained relatively constant (14.57% in 2002 vs. 16.17% in 2006) while the proportion of individuals with strong association in cluster 1 (>90% assignment probability) declined more dramatically (57.48% in 2002 vs. 45.35% in 2006), indicating a deterioration of the genetic distinctiveness represented by cluster 1 (Figure 5a). In contrast, the proportion of individuals with moderate association in cluster 2 increased (4.92% in 2002 vs. 11.30% in 2006) while the proportion of individuals with strong association in cluster 2 remained relatively constant (20.67% in 2002 vs. 22.60% in 2006). Overall, a large but apparently decreasing proportion of the population was strongly associated with one of the two clusters (Figure 5b strong assignments; 78.15% in 2002 vs. 67.95% in 2006), while a smaller but apparently increasing proportion of the population had less distinctive associations with either cluster (Figure 5b moderate/mixed assignments; 21.85% in 2002 vs. 32.04% in 2006).

The numbers of presumed CO-lineage alleles harbored by each individual were compared across clusters *a posteriori*. Of the 259 individuals in the entire dataset harboring one or more CO-lineage alleles, 238 (91.9%) were successfully assigned to a cluster (i.e. assignment probability >60%). Of these, an overwhelming majority - 90.3% (215/238) - were assigned to cluster 2. Of the 373 individuals assigned to cluster 2, this represents 57.6% of the cluster (215/373). In comparison, only 3.3% (23/691) of the individuals assigned to cluster 1 harbored CO-lineage alleles. Not surprisingly, the number of CO-lineage alleles per individual were highly skewed across clusters, with individuals harboring more CO-lineage alleles most often assigned to cluster 2 (Figure 6). These data indicate that the genetically-distinct subpopulation defined by cluster 2 is derived from, and contains genetic “signatures” of, the introduction of bison from the Colorado National Monument in 1983.

To further assess the nature of the genetic clusters identified by the above analysis, pairwise individual distances were used to generate a graphical representation (tree diagram) of the relationships among individuals in the population. This analysis was performed independently of the STRUCTURE analysis above, using the post-cull 2006 data set (n = 503) to avoid potential bias due to large family groups and to allow evaluation of the most recent possible data set. Patterns based on clustering and the presence of CO-lineage alleles were assessed *a posteriori* from the resultant tree topologies (Figure 7).

The effect of genetic clustering on body size measurements was assessed using data from the 2006 roundup for calves (born in 2006) and yearlings (born in 2005). Accounting for differences due to birth year and sex, neither weight (pounds) nor height (inches) was different among the clusters (Figure 8).

Population viability analysis was performed for 10 iterations across each of 6 scenarios (3 short-term and 3 long-term) using the program VORTEX. Across the long-term scenarios, the exponential growth rate ranged from 19.62% to 20.15%, which approximates the growth rates previously reported for this population (Berger and Cunningham 1994; Millspaugh et al. 2005). The population remained viable in all 60 iterations with a 0% probability of extinction. Furthermore, the levels of inbreeding remained stable and low in each scenario, with the final number of estimated lethal alleles per individual ranging from 1.0 to 1.59 compared with the starting value of 1.57. Changes in genetic diversity were measured based on the total number of alleles and average unbiased heterozygosity across 26 nuclear markers, and final measures of these parameters were averaged across iterations and compared with the initial values for the BNP population (based on post-cull 2006 data, 116 alleles and 60.03% heterozygosity). Small differences in projected levels of genetic diversity were noted in the short-term scenarios, with the 8-year reduction scenario corresponding to the highest levels of estimated genetic diversity (Figure 9). The long-term models, however, indicated large differences in projected levels of genetic diversity corresponding with the total population size (Figure 9).

Discussion

Previous studies have indicated that the BNP population contains moderately high levels of genetic variation in comparison to other bison populations and some unique genetic polymorphisms (Halbert 2003). Low levels of domestic cattle DNA (introgression) were also previously detected in the nuclear - but not mitochondrial - genome of BNP bison (Halbert and Derr 2007a). The BNP population has a distinctive history and unique genetic background, and fulfills an essential ecological role as a keystone species and largest herbivore in the Badlands ecosystem. Therefore, prudent and scientifically-sound management strategies are needed to ensure the long-term conservation of germplasm (genetic makeup) and ecological success of this valuable resource.

Genotypes were collected from 2 panels of markers recently developed in our laboratory, and were employed in this study to examine the genetic architecture of the BNP population. The first panel was used to assess levels of domestic cattle introgression and includes markers from both the mitochondrial (1) and nuclear (14) genomes. Genetic analysis of genotypes from these markers confirmed previously published results from this population based on samples collected in 2002 (Halbert and Derr 2007a), indicating exclusively bison mitochondrial DNA and domestic cattle nuclear DNA in 2 different regions (denoted by markers BM4307 and BMS2270). The history of introductions and genetic evaluations in other U.S. federal herds indicate these

domestic cattle regions were introduced from the NE- and CO-lineages, respectively (Halbert and Derr 2007a). In addition, a single individual was identified with an allele (genetic variant) at marker RM500 which falls within the range identified for domestic cattle and has not been previously identified in any other bison herds evaluated to date (Halbert et al. 2005a; Halbert and Derr 2007a; Halbert and Derr, unpublished data on private herds). The origin of this allele is unknown, and we are currently sequencing this region to determine whether the allele is a bison or domestic cattle variant (i.e., arising through mutation or introgression, respectively).

The second panel of 26 microsatellite markers (Table 1) is part of a larger group of markers designed to study bison population genetics (Schnabel et al. 2000; Halbert 2003; Halbert et al. 2004). Microsatellites occur in non-coding regions of the genome and are a preferred marker type for analyses of population-level genetic variation due to their neutrality (or near-neutrality) and high mutation rates. Two complimentary measures of genetic variation were utilized in this study: unbiased heterozygosity (or gene diversity) and allelic diversity. An individual is heterozygous at a marker when 2 different alleles are inherited (one on each of the 2 chromosomes), and is homozygous when the same allele is inherited from both parents. Unbiased heterozygosity is a measure of the number of heterozygous individuals in a population (# heterozygotes/total population), corrected for differences in sample size to allow direct comparisons among groups. Heterozygosity is an indicator of past breeding in a population, and is affected by non-random mating. For example, inbreeding, or the preferential breeding of closely-related individuals, leads to decreased heterozygosity. Allelic diversity was measured by directly counting the total number of alleles detected for the 26 markers in this study (in some cases, this number was averaged across markers). Allelic diversity is a measure of genetic variation present in a population, and is most dramatically affected by genetic drift (random loss of alleles), especially in small populations.

The short-term genetic effects of recent culling practices employed to control the size of the BNP population were investigated by comparing overall genetic diversity before and after each of the cull events from 2002-2006. Significant differences were not identified within any year (pre-cull vs. post-cull) or across years (Table 3), indicating that recent culling of BNP bison has not substantially altered the overall genetic makeup of the remaining population.

The overall genetic architecture of a population can influence the long-term erosion of genetic diversity over time, such as through non-random mating or population substructure. Several analysis methods were employed to evaluate the genetic architecture of the BNP bison population. First, Hardy-Weinberg and gametic disequilibrium were evaluated. Hardy-Weinberg equilibrium is indicated by stable allelic and genotypic frequencies from one generation to the next, and is expected in the absence of forces which alter allele frequencies, such as non-random mating (e.g. inbreeding), strong selection, migration, or genetic drift (such as due to a very small breeding population size). In this study, none of the markers were found to be in Hardy-Weinberg disequilibrium. Gametic disequilibrium is the nonrandom association of allele frequencies at unlinked loci, and can be produced by migration of individuals of a distinguishable genetic background into a population. Gametic disequilibrium is expected to decrease rapidly over time (i.e. towards equilibrium) following such migration if mating is random; inbreeding will delay the rate of decay of gametic disequilibrium. In this study, 5.5-6.2% of the marker combinations were in gametic disequilibrium. In contrast, no significant deviations from gametic equilibrium were noted in any of the other 9 U.S. federal bison populations (Halbert 2003; Halbert and Derr unpublished data). These results indicate some

level of population subdivision or nonrandom mating has previously and/or is currently affecting the BNP bison population.

To further investigate the genetic architecture of this herd, subpopulation division was evaluated using the program Structure, which indicated that BNP bison comprise 2 genetically distinct subpopulations (Figures 3, 4). Furthermore, by identifying and tracking alleles that are not found in extant populations representing the NE-lineage (i.e., alleles likely derived from the CO-lineage) and comparing the distribution of these alleles among the 2 subpopulations, we found that clusters 1 and 2 correspond roughly to the NE- and CO-lineages, respectively (Figure 6). A comparison of cluster assignments from 2002-2006 indicates a modest increase in the percentage of mixed-lineage individuals (Figure 5b), corresponding to a decrease in the percentage of individuals with high-probability assignments in cluster 1 (predominantly NE-lineage). These findings suggest the subpopulation division observed among BNP bison is deteriorating in an asymmetrical manner, at least during this study period. That is, it appears that genetic exchange has occurred from the CO-lineage into the NE-lineage, causing the NE-lineage to become more dilute over time. Conversely, the CO-lineage is to some extent genetically isolated, as approximately 20% of the overall BNP herd remained strongly associated with cluster 2 (predominantly CO-lineage) over the study period (Figure 5a). In comparison, around 6.7% (or less) of the population was represented by the CO-lineage in 1983 (20 bison introduced into a population of no more than 300 NE-lineage bison; Berger and Cunningham 1994).

A distinct and complimentary method of evaluating the population substructure based on individual pairwise genetic distance measures was employed to further evaluate the magnitude of subpopulation division among BNP bison. It should be noted that distance-based measures are known to be less sensitive than assignment tests in identifying subpopulation structure (Pritchard et al. 2000; Rosenberg et al. 2001). The bush-shaped tree morphology suggests small genetic differences among groups, which was confirmed by F_{ST} comparisons (see below). The branching patterns are not strongly correlated with either cluster assignments (Figure 7a) or the presence of CO-lineage alleles (Figure 7b), indicating lineage mixing has occurred broadly across the population.

F-coefficients were used to measure the level of genetic differentiation between clusters (higher F_{ST} values indicate more genetic difference) and the level of inbreeding within clusters (small, negative F_{IS} values indicate higher levels of inbreeding). A previous analysis of the genetic structure of all DOI bison populations indicated that the most closely related groups were defined by BNP (entire 2002 dataset) and the Theodore Roosevelt NP South Unit/Grand Teton NP group (F_{ST} 0.0378 based on the same markers and assignment probability exclusion criteria; Halbert and Derr, unpublished data). In 2002, the two BNP clusters had a nearly equivalent amount of genetic differentiation (F_{ST} 0.0381). However, all other clusters within the DOI bison dataset were less similar (F_{ST} range 0.0518 to 0.1778; overall DOI cluster average 0.117 ± 0.037 S.D.; Halbert and Derr, unpublished data). The analysis from this study also indicates that the BNP clusters are becoming more similar over time (2006 post-cull dataset F_{ST} 0.0275). A higher level of inbreeding was detected in Cluster 2 (CO-lineage) than in cluster 1 (NE-lineage), although the levels of inbreeding within both clusters appear to be decreasing over time (F_{IS} cluster 1: -0.0065 in 2002 to 0.0005 in 2006; F_{IS} cluster 2: -0.0663 in 2002 to -0.0499 in 2006). This information compares favorably with previous reports, as Berger and Cunningham (1995) also predicted more inbreeding within the CO-lineage in comparison to the NE-lineage based on the historic sizes and levels of mixing within the lineages.

The identification of subpopulation division within the BNP bison population is a significant finding, and is somewhat unexpected given that geographic barriers are not known to influence bison movement (Berger and Cunningham 1994). Lineage sorting, or line breeding, is the most obvious explanation for the findings of gametic disequilibrium and population subdivision correlating with lineage-specific alleles in the BNP population. That is, these results might be expected if the bison introduced from the Colorado National Monument in 1983 preferentially bred with each other and did not mix with the FN-lineage herd already present at BNP, or only began mixing after a number of years of line breeding. Additionally, given the potential for female bison to successfully produce offspring beyond 20 years of age (Wilson et al. 2002; McHugh 1958; Halbert and Derr 2007b), it is likely that some of the bison sampled during this study are only 1 or 2 generations removed from the Colorado introduction, and that ample time has not elapsed to allow complete genetic mixing between the lineages. The social structure of bison may also hinder lineage mixing and, consequently, the erosion of gametic disequilibrium. The 20 bison introduced from Colorado apparently comprised an intact herd which had been isolated for nearly 60 years prior to their movement to BNP (Berger and Cunningham 1994). This small, inbred group of bison likely remained socially isolated from the resident BNP (NE-lineage) bison for some period of time. Whatever the cause(s), a large but slowly declining proportion (about 67%) of the BNP population remains strongly associated with one of the two subpopulations (Figure 5b).

Maintenance of genetic diversity in closed populations is critical to long-term population viability, as it provides a means of adaptability to environmental changes

The population viability model VORTEX (Lacy et al. 2007) was employed to evaluate the effects of various management strategies on genetic diversity. First, the effects of culling strategies designed to reduce the total population from 850 to 600 bison over various time periods (2 vs. 5 vs. 8 years) were evaluated. All ages of bison were culled from the model at each time step, although the culling was not proportionate with respect to age during the reduction periods, when it was necessary to cull a disproportionately larger number of young (1 year-old) bison to reduce herd size while maintaining individuals in older age classes (see Appendix C for details on the number of bison culled for each age class). To allow for direct comparisons among these models, the 2-year and 5-year reduction models were followed by maintenance of the herd at approximately 600 bison to 8 years (i.e. 6 years and 3 years, respectively), during which time culling was proportionate within each age and sex class. Therefore, the 2-year reduction scenario resulted in 2 years of intensive culling (Appendix C) followed by 6 years of proportionate culling and stable population size. In comparison, the 5-year reduction scenario resulted in 5 years of comparatively less intense culling (Appendix C) followed by 3 years of proportionate culling and stable population size. Likewise, the 8-year reduction scenario included the least intensive of culling scenarios. Despite these differences in intensity of culling and reduction time, only small differences were detected among these 3 scenarios in the maintenance of genetic diversity (Figure 9), indicating minimal short-term genetic effects of reducing the BNP population from 850 to 600 bison regardless of time period.

Long-term effects of BNP bison population size on the maintenance of genetic diversity and population survival were also evaluated using methods similar to those developed for the short-term analysis (Appendix C). In the long-term scenarios, the population was modeled over a 200-year period at approximately stable sizes of 600, 800, or 1000 bison. Culling was employed in a manner proportionate to age and sex class when the population exceeded the target size. This “proportionate” culling strategy was employed based on the following: 1) this

strategy most closely approximates that most recently employed for this population (BNP Bison Management Plan 2003); and, 2) compared with other culling strategies, this strategy is expected to produce a herd structure representative of the pre-horse estimated bison population (Millspaugh et al. 2005). While the estimated ecological carrying capacity for the BNP population is 1,500-1,800 bison (BNP Bison Management Plan 2003), the current target census size of the population is only 600 bison. This reduction in target size is based on limited resources during drought periods, and is a conservative estimate of a population size likely to remain stable (i.e., without increased mortality rates) during drought periods (BNP Bison Management Plan 2003). Therefore, the estimated effects of drought on natality and mortality rates were only included in the scenarios with 800 and 1000 bison (see Appendix C for details). This model predicted that the population would survive (0% extinction probability) equally well under any of the 3 scenarios, but that the maintenance of genetic diversity over the 200-year period was largely influenced by population size. Even with the stochastic effects due to periodic drought, the proportion of genetic diversity after 200 years is predicted to be substantially higher in a simulated population of 800 or 1000 bison (Figure 9). Similarly, Gross and Wang (2005) estimated a population size of 1000 bison was necessary for a 90% probability of retaining at least 90% of the initial heterozygosity and allelic diversity (from Halbert 2003) over a 200-year period. Furthermore, the retention of genetic diversity in a population of 1000 or greater is less sensitive to the choice of culling method (e.g., removal of young vs. old vs. random removals), though culling method does influence demographic structure (Gross and Wang 2005; Millspaugh et al. 2005).

The time and intensity of short-term culling strategies to reduce herd size appear to influence genetic diversity much less than the long-term population size (Figure 9). Genetic diversity is expected to be lost at a rate inversely proportional to effective population size due to random genetic drift (Nei et al. 1975; Senner 1980; Lacy 1987). A minimum effective population size of 500 has been suggested to maintain long-term genetic diversity (Franklin 1980). However, effective population size is negatively influenced by variances in family size, which are expected in polygamous species such as bison and are not considered in the minimum number of 500 given above (Lande and Barrowclough 1987). Given the age and sex structure of the BNP herd, the effective population size is at best approximately 290 when the census size is 600 bison and approximately 486 when the census size is 1000 bison. These figures are conservative estimates, since the variance in family size is unknown and not used in these calculations. Regardless, the effective population size of the BNP population is much less than the suggested size of 500 when the census size of the population is only 600.

These findings warrant careful consideration of possible strategies to increase the effective size of the BNP bison population. As the population is currently limited to only a portion of the total BNP landscape, it may be possible to expand the bison range or to use adjacent federal land, such as within the Buffalo Gap National Grasslands (2,300 km² U.S. Forest Service land). Even without expanded range, however, the results of this study indicate a larger population (e.g. 800-1000 bison) affected by seasonal drought and consequent reduction in population growth or size will maintain higher levels of genetic diversity than a small population (600 bison) unaffected by such drought. As with any management decision, other factors such as effects on plant and animal communities must be considered.

The BNP bison population is among the most thoroughly studied of all bison populations. The extensive observational data on behavior and reproduction accumulated by Berger and Cunningham (1994), the previous inter-population genetic comparisons (Halbert 2003; Halbert

and Derr 2007), and the population-specific analyses performed in this study provide an unprecedented volume of biological, demographic, and genetic information on BNP bison from which scientifically-informed management decisions can be made to help preserve this valuable resource.

Future directions

As previously mentioned, we are in the process of sequencing the 125-bp RM500 allele to investigate the origin of this aberrant allele. While outside the scope of the initial project, these results will be shared with park biologists as they become available.

The results of this study indicate slow mixing (genetic exchange) between two genetically-defined clusters has occurred, and we speculate that mixing will continue to occur until the clusters are no longer detectable. However, continued monitoring of the substructure of this herd is advised to ensure complete mixing does occur, since management implications are much different for two (stable) subpopulations than for a single metapopulation. Such monitoring need not be as frequent or extensive as the data collected in this study (e.g. data collection every 5-10 years is sufficient so long as new population-level issues do not arise). This type of monitoring is likewise critical if the population census size is kept at the current target of 600 bison to help circumvent large-scale losses in genetic diversity.

At least one manuscript will be prepared for publication in a peer-reviewed scientific journal based on these findings.

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Table 1. Microsatellite marker summary information.

Locus	Chromosome (Position)^a	Size range	# bison alleles^b	# BNP alleles
BL1036	14 (78.7)	177-193	5	4
BM1225	20 (8.0)	239-273	10	5
BM1706	16 (80.6)	232-254	6	3
BM17132	19 (58.6)	85-95	5	4
BM1862	17 (86.3)	201-215	6	6
BM1905	23 (64.3)	172-184	4	2
BM2113	2 (106.2)	127-153	9	6
BM4107	20 (52.4)	159-185	8	4
BM4311	6 (89.7)	90-104	6	6
BM4440	2 (55.0)	123-143	7	5
BM47	23 (9.1)	103-111	4	2
BM711	8 (83.6)	161-177	6	2
BM720	13 (38.6)	203-235	9	7
BMS1001	27 (5.1)	107-115	5	4
BMS1074	4 (74.9)	152-160	5	4
BMS1315	5 (31.8)	135-149	5	5
BMS1675	27 (64.1)	85-91	4	4
BMS1716	11 (47.7)	185-197	6	4
BMS1857	29 (0.9)	142-170	9	6
BMS410	12 (0.0)	83-97	6	3
BMS510	28 (22.1)	91-95	4	4
BMS527	1 (55.9)	159-177	8	6
HUJ246	3 (67.9)	256-264	5	5
ILSTS102	25 (6.5)	113-153	6	5
RM372	8 (19.1)	114-138	8	6
TGLA122	21 (67.3)	136-150	6	4
Average			6.23	4.46

^a chromosomal positions (cM) as reported in the USDA cattle gene mapping database
(<http://www.marc.usda.gov>, last accessed 03-31-07)

^b number of bison alleles across 11 US federal populations (including BNP) from Halbert (2003) and Halbert and Derr (manuscript in prep. 2008)

Table 2. Samples utilized in this study, subdivided by collection year, culling status, and sex.

Year	Total samples	Total analyzed	Culled		Non-culled	
			Female	Male	Female	Male
2002	496	324 ^a	121	72	79	52
2003	345	257	86	75	54	42
2004	230	165	66	42	24	33
2005	298	193	47	72	48	26
2006	247	176	7	24	61	84
Sum	1616	1115	327	285	266	237

^asamples collected in 2002 were genotyped as part of a previous study of bison on NPS lands (Derr and Templeton 2000). These genotypes were initially reported by Halbert (2003).

Table 3. Pre- and post-cull population genetic diversity measures across 26 nuclear polymorphic markers for BNP bison, 2002-2006.

	Population size ^a	Sample size (% of population)	Average heterozygosity ^b	Average allelic diversity ^c
pre-cull 2002	876	508 (58.0%)	0.595 ± 0.032	4.46 ± 1.36
post-cull 2002	739	391 (52.9%)	0.596 ± 0.031	4.46 ± 1.36
pre-cull 2003	915	543 (59.3%)	0.596 ± 0.031	4.46 ± 1.36
post-cull 2003	785	516 (65.7%)	0.597 ± 0.031	4.46 ± 1.36
pre-cull 2004	935	658 (70.3%)	0.598 ± 0.031	4.46 ± 1.36
post-cull 2004	756	551 (72.9%)	0.598 ± 0.031	4.46 ± 1.36
pre-cull 2005	965	734 (76.1%)	0.598 ± 0.031	4.46 ± 1.36
post-cull 2005	696	569 (81.8%)	0.599 ± 0.031	4.46 ± 1.36
pre-cull 2006	850	699 (82.2%)	0.599 ± 0.032	4.46 ± 1.36
post-cull 2006	626	503 (80.4%)	0.600 ± 0.032	4.46 ± 1.36
all samples		1115	0.598 ± 0.032	4.46 ± 1.36

^aEstimated population sizes based on aerial survey and roundup data.

^bUnbiased gene diversity (H_E , Nei 1987), shown as average across markers ± standard deviation.

^cAllelic diversity (number of alleles (N_A) per locus), shown as average across markers ± standard deviation.

Table 4. Alleles identified which are presumed to be specific to the CO-lineage.

Marker	Allele (bp)	Frequency
BM720	235	0.0045
BMS1001	109	0.0414
BMS1315	137	0.0310
BMS2270	94	0.0385
BMS527	165	0.0334
RM372	128	0.0204
TGLA122	144	0.0115

Figure 1. Distribution of CO-lineage alleles among BNP bison.

Figure 1

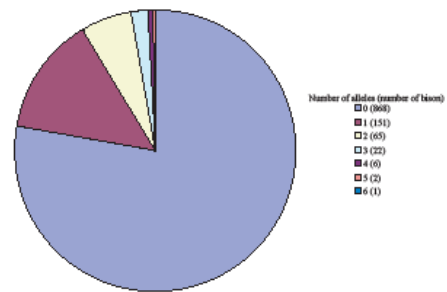


Figure 2. Levels of Colorado lineage alleles (% of individuals evaluated) across birth cohorts from 2000 to 2006. The detected percentage of individuals in each cohort harboring alleles presumed to be specific to the Colorado lineage are shown.

Figure 2

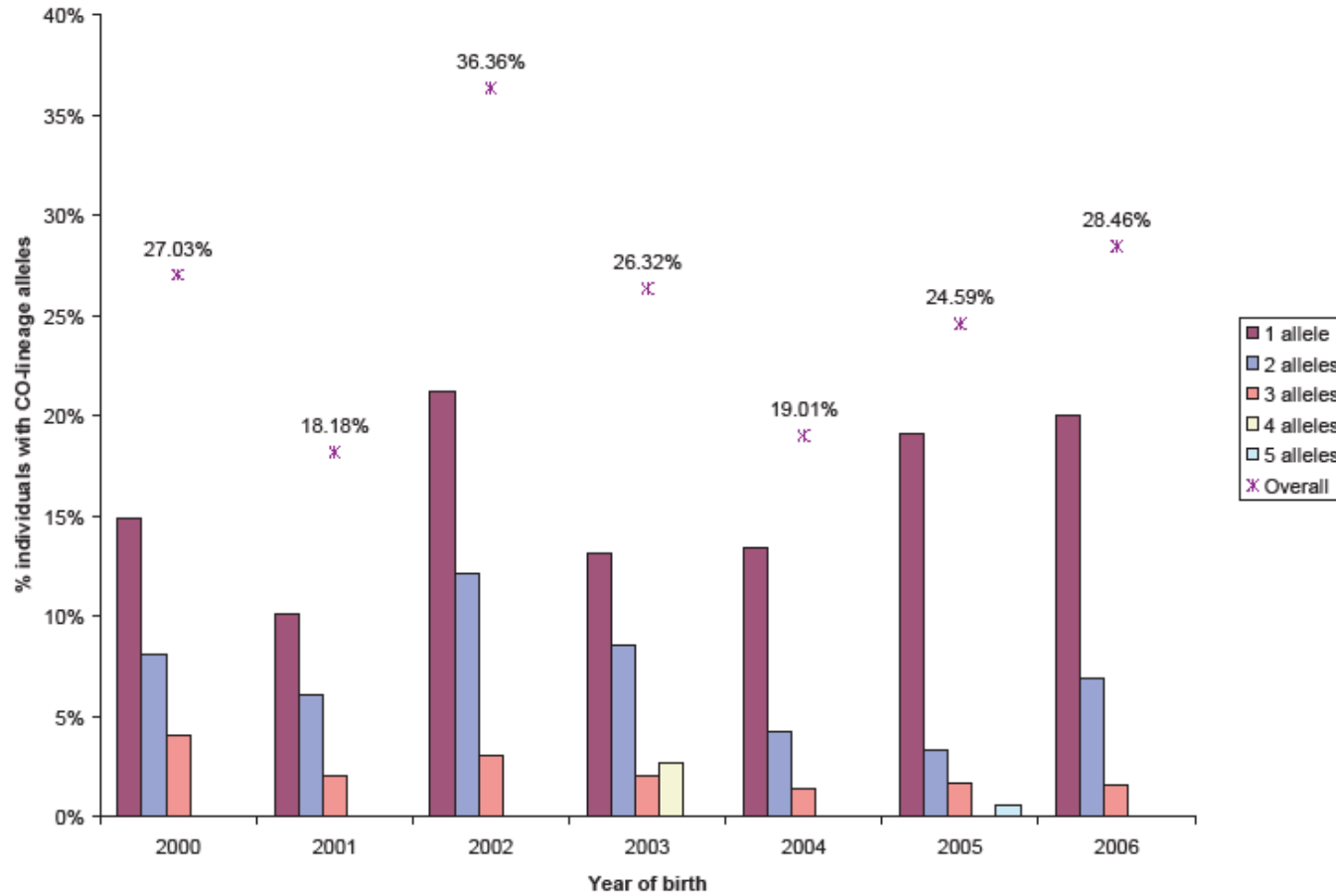


Figure 3. Evaluation of STRUCTURE clustering for K values ranging from 1 to 5. In panel A, averages and standard deviations for $\text{LnP}(D)$ values based on 10 simulations for each value of K are shown. Corresponding ΔK values are shown in panel B, following the calculations of Evanno et al. (2005).

Figure 3

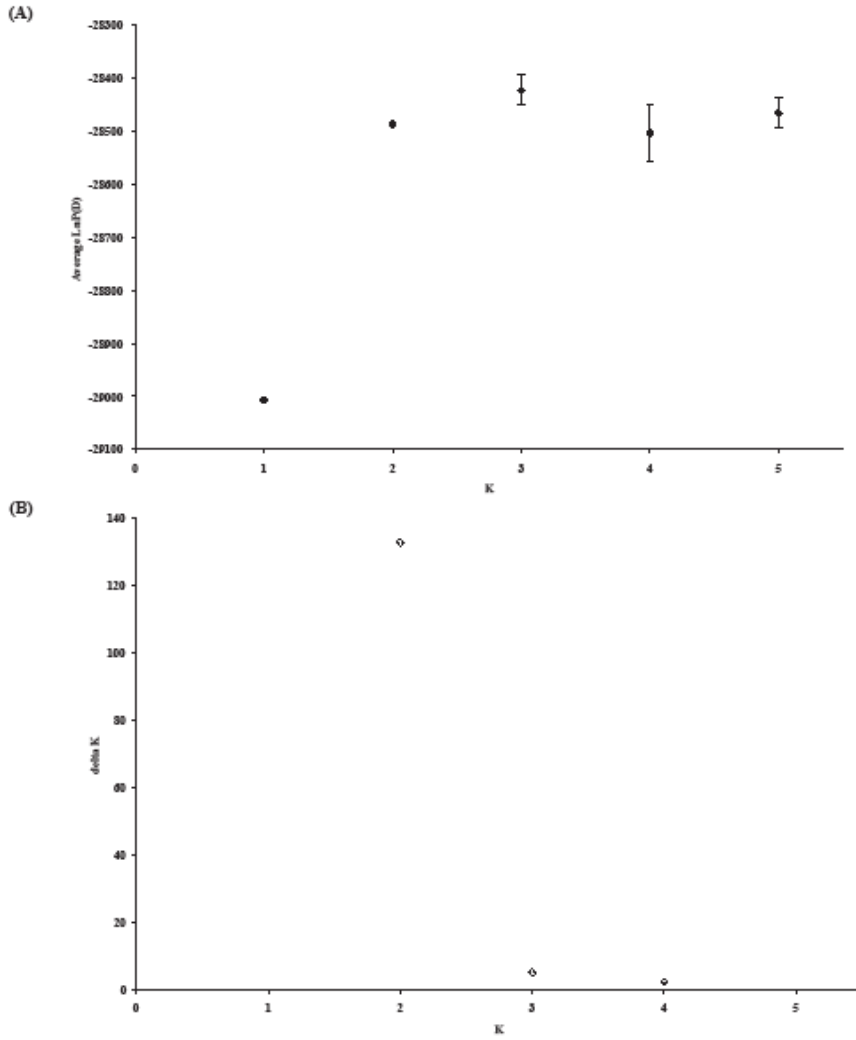


Figure 4. Consistency of assignment probabilities of individual BNP bison. Three of 10 iterations with $K = 2$ are shown. In the top panel, individuals were sorted in descending probability of assignment into cluster 1. In the middle and lower panels, individuals are in the same order as in the top panel.

Figure 4

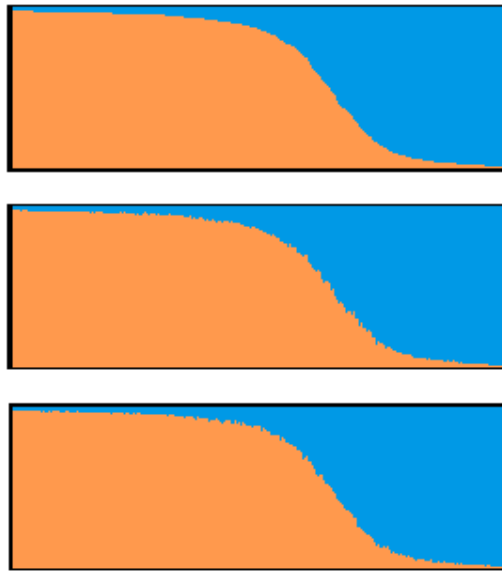


Figure 5. Trends in number of individuals assigned to each of two clusters from 2002-2006. Percentages are taken over the number of individuals in the pre-cull population for each year as shown in Table 3. A) Categories labeled as “strong” or “moderate” refer to assignment probabilities of at least 90% in the cluster or between 60% and 90% in the cluster, respectively. Individuals with less than 60% assignment probability into any one cluster are shown in the category labeled “mixed lineage.” B) Overall change in percentages of individuals assigned to cluster extremes. All categories with “strong” assignment probabilities in panel A, regardless of cluster, are grouped together, while the “moderate” and “mixed lineage” categories in panel A are grouped together.

Figure 5

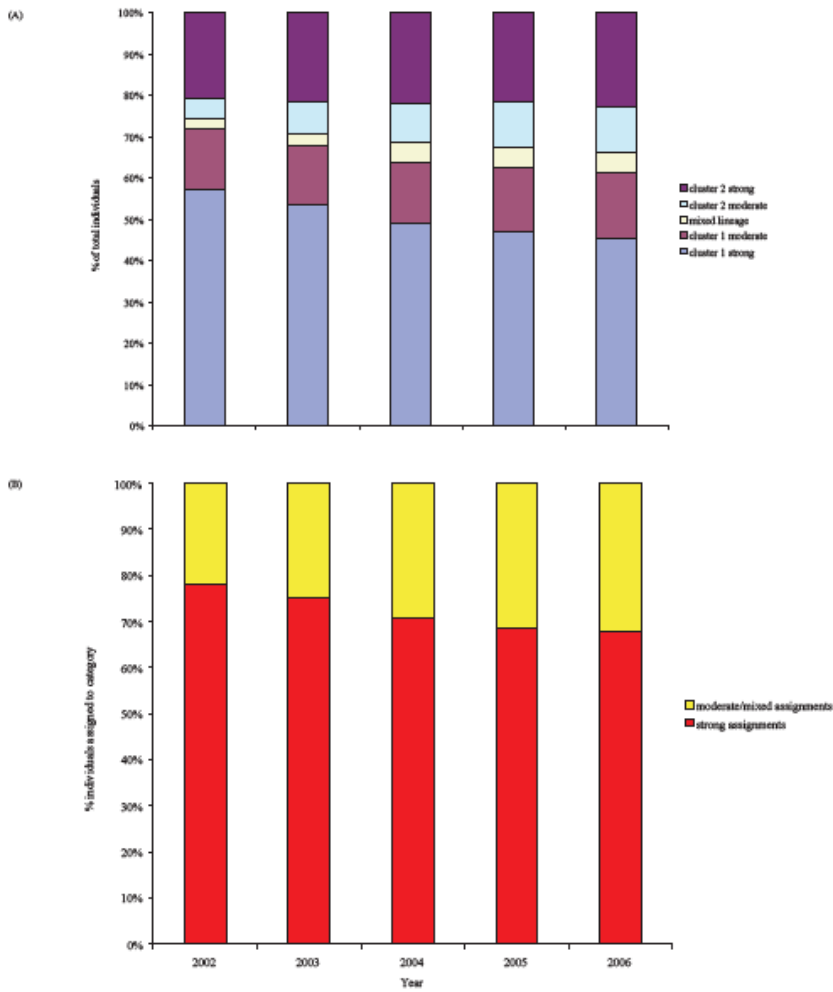


Figure 6. Distribution of individuals with CO-lineage alleles across two genetically distinct clusters. To prevent bias, only individuals with at least 60% probability of assignment into a cluster are shown (n = 1064).

Figure 6

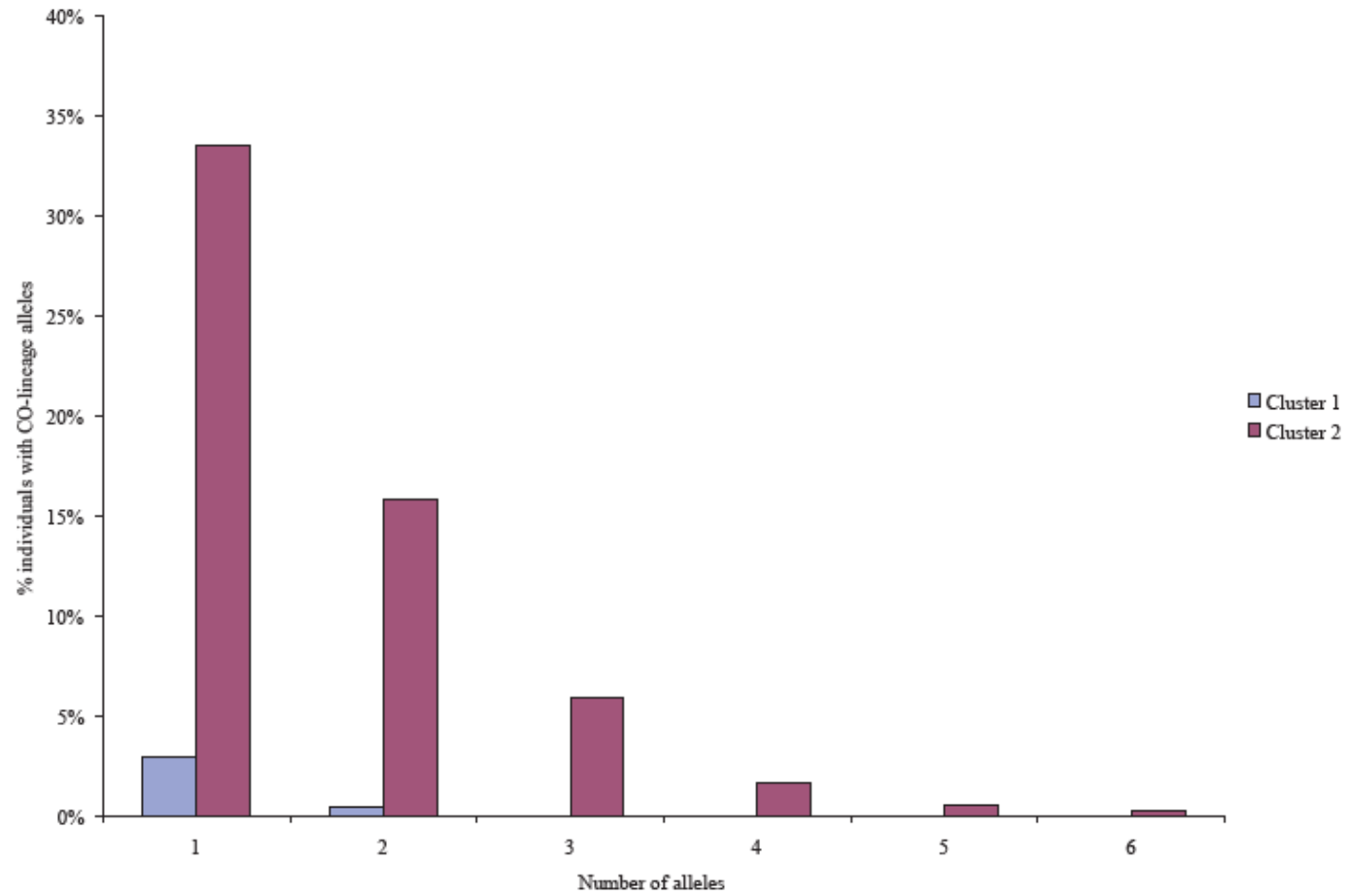


Figure 7. Individual-based genetic distance (proportion of shared alleles; Bowcock et al. 1994) trees of post-cull 2006 dataset. In panel A, asterisks indicate individuals assigned to cluster 2, while all other branches represent individuals assigned to cluster 1. In panel B, crosses indicate individuals with one or more CO-lineage alleles, while all other branches represent individuals in which CO-lineage alleles were not detected.

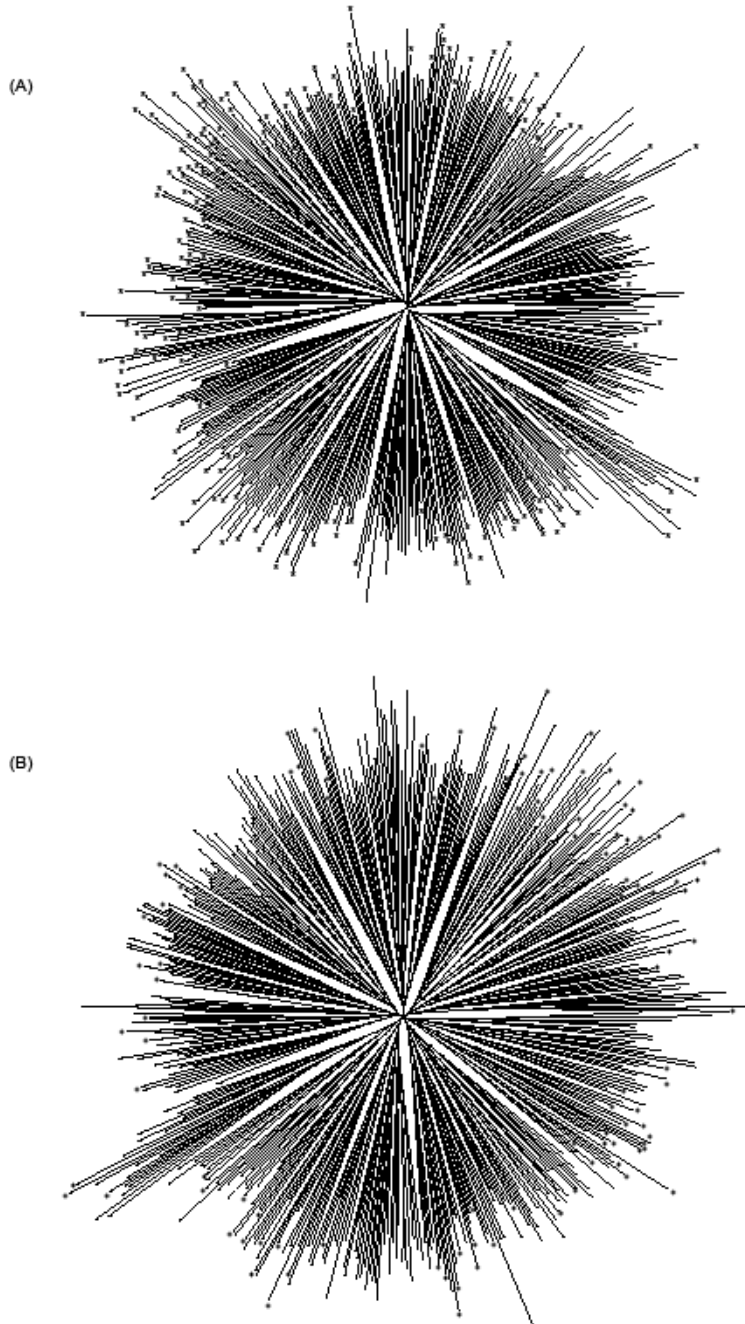


Figure 8. Evaluation of clustering on differences in body size. Weight (pounds) and height (inches) measurements were taken during the 2006 roundup for calves (born in 2006) and yearlings (born in 2005) of both sexes. Only individuals with at least 60% assignment probability into any one cluster were included in this analysis (n = 293). Averages are shown with vertical standard deviation bars. Differences between clusters within a given age and sex class are very small and within the range of the standard deviation.

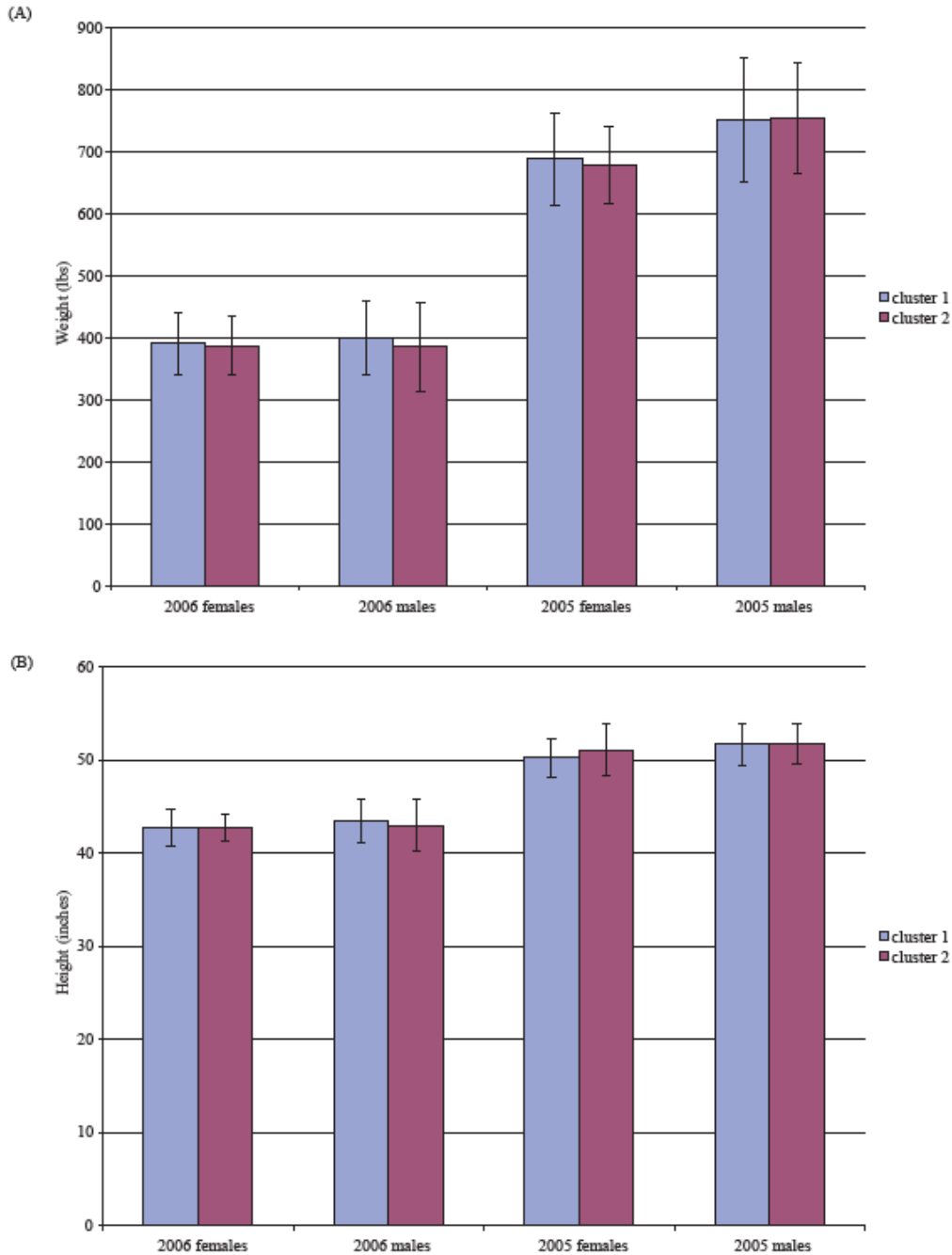


Figure 9. Effects of management strategies on genetic diversity using the population viability model VORTEX. The short-term (3 categories on x-axis to the right) and long-term (3 categories to the left) scenarios are described in the text. Genetic diversity was measured as average unbiased heterozygosity and total number of alleles for 26 nuclear markers (Table 1). Initial values are indicated by horizontal lines. Vertical standard deviation bars for 10 iterations of each scenario are indicated.

Figure 9

