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Patterns of genetic variation in US federal bison herds

NATALIE D. HALBERT and JAMES N. DERR
Department of Veterinary Pathobiology, Texas A&M University, College Station, TX 77843-4467, USA

Abstract

Like many wide-ranging mammals, American bison (*Bison bison*) have experienced significant range contraction over the past two centuries and are maintained in artificially isolated populations. A basic understanding of the distribution of genetic variation among populations is necessary to facilitate long-term germplasm preservation and species conservation. The 11 herds maintained within the US federal system are a critically important source of germplasm for bison conservation, as they include many of the oldest herds in the USA and have served as a primary resource for the establishment of private and public herds worldwide. In this study, we used a panel of 51 nuclear markers to investigate patterns of neutral genetic variation among these herds. Most of these herds have maintained remarkably high levels of variation despite the severe bottleneck suffered in the late 1800s. However, differences were noted in the patterns of variation and levels of differentiation among herds, which were compared with historical records of establishment, supplementation, herd size, and culling practices. Although some lineages have been replicated across multiple herds within the US federal system, other lineages with high levels of genetic variation exist in isolated herds and should be considered targets for the establishment of satellite herds. From this and other studies, it is clear that the genetic variation represented in the US federal system is unevenly distributed among National Park Service and Fish and Wildlife Service herds, and that these resources must be carefully managed to ensure long-term species conservation.

Keywords: conservation genetics, mammals, population genetics—empirical, wildlife management

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Introduction

Whether directly or indirectly, human population growth and expansion have led to the restriction of many wildlife species on a small portion of their historic ranges. Wide-ranging mammals are particularly susceptible to range contractions, since even large parcels of land may only support small populations. In North America, wide-ranging mammals such as black bear, caribou, elk, grizzly bear, and pronghorn have lost up to 74% of their historic range over the past 150 years (Laliberte & Ripple 2004). With the reduction or complete loss of important natural population-regulating forces such as migration and predation, the population size and range of many wildlife species are limited through active management, such as capturing and moving animals to create or supplement populations, fencing to inhibit movement across landscapes, and implementation of hunting regulations.

Correspondence: Natalie D. Halbert, Fax: 979-845-9972; E-mail: nhalbert@cvm.tamu.edu

Given the continuing growth and expansion of the human population, we are faced with a most serious question: how do we manage wildlife species in discontinuous populations to best promote long-term conservation? On some levels, the answers to this question are undoubtedly species specific. However, we submit that American bison (*Bison bison*) are an ideal model species for evaluating methods to preserve genome integrity and promote long-term species conservation. First, the well-documented decline of bison in the 19th century is similar to that experienced by other species across the world. Unlike many other species, however, bison have made a remarkable recovery in census size in a relatively short period of time (Ceballos & Ehrlich 2002). The entire species recovered from less than 1000 individuals in the late 1800s (Soper 1941; Coder 1975) to more than 500 000 bison today (Boyd 2003). Therefore, understanding the biological factors that led to the recovery of this species will provide insight for recovery efforts in other bottlenecked species. Second, the well-known history of establishment and, in many cases, detailed management records of bison herds across diverse habitats (Sanderson

et al. 2008) provide an opportunity to evaluate factors which have influenced the demographic and genetic recovery. Like many wildlife species, bison are confined to geographically isolated groups (herds) as a result of extreme range contraction, with less than 1% of the historic (*c*. 1500) range currently occupied (Sanderson *et al*. 2008). Furthermore, most bison herds are subjected to various levels of artificial management to control population size and distribution (Boyd 2003); understanding how these strategies affect the retention of genetic diversity is central to the successful management of bison germplasm.

Despite the clearly successful demographic recovery of bison, the long-term preservation of bison germplasm and, thus, conservation of the species, remain threatened. First, fewer than 5% of bison are maintained in conservation herds (Boyd 2003); the remaining 95% exists in private herds subjected to various levels of artificial selection (primarily used for meat production). Second, introgression of domestic cattle DNA into both the mitochondrial (Polziehn *et al*. 1995; Ward *et al*. 1999) and nuclear (Halbert *et al*. 2005; Halbert & Derr 2007) genomes of many bison herds has greatly complicated species conservation efforts. Additionally, infectious diseases prohibit the transfer of bison out of the two oldest and largest free-ranging herds in North America — brucellosis in Yellowstone National Park and both brucellosis and tuberculosis in Wood Buffalo National Park (Boyd 2003). Therefore, the protection of the native bison genome from selection, domestication, introgression, and disease is paramount to the conservation of this species. Human interference has led to similar threats in other wildlife species worldwide, such as the preferential poaching of male saiga antelopes and consequent reproductive collapse in Russia (Milner-Gulland *et al*. 2003), the rapid domestication of wild banteng in southeast Asia (Bradshaw *et al*. 2005), hybridization between domestic dogs and the endangered Ethiopian wolf (Gottelli *et al*. 1994), and canine distempter in the black-footed ferret in the USA (Primack 1993).

The main source of bison germplasm exists in a handful of publicly managed Canadian and US federal herds, from which the majority of extant bison are derived (Soper 1941; Coder 1975). Traditionally, the management of these herds has been left to the discretion of individual unit managers, although more comprehensive efforts have been promoted in recent years through discussions among managers, policy makers, and scientists (Freese *et al*. 2007; Sanderson *et al*. 2008). Most US federal bison have been managed in closed herds over the past 40 to 100 years, but management of these bison as a single metapopulation has been recently considered (Halbert *et al*. 2007) as a means to prevent the erosion of genetic diversity (Margan *et al*. 1998). Clearly, a broad range of issues should be considered before any decision to emulate migration among wildlife populations, including the genetic, environmental, demographic, and health consequences of such manipulation. Although genetic data have been collected from a limited number of individuals and herds (Ward *et al*. 1999; Wilson & Strobeck 1999; Schnabel *et al*. 2000), a comprehensive evaluation of the distribution of genetic diversity among these herds is needed.

In this study, we investigate patterns of neutral genetic variation among US federal bison herds, which are maintained within six National Park Service and five Fish and Wildlife Service units (Table 1). This study is an important step towards understanding the effects of founder events, population size, social structure, and culling strategies on genetic variation in bison herds. Furthermore, assessing the genetic relationships among these herds will be critical to future management decisions and the conservation of bison germplasm.

Materials and methods

Data collection

Samples and DNA were collected as previously described (Halbert & Derr 2007), and are archived at Texas A&M University and the Museum of Southwestern Biology at the University of New Mexico for future reference. The selection of microsatellite markers and description of multiplexed polymerase chain reaction (PCR) assays were previously described (Halbert *et al*. 2004). The panel of markers selected for this study included 48 markers spanning all autosomes except chromosome 24, two markers on the X chromosome, and one marker on the Y chromosome (Appendix S1, Supporting information).

Amplification was performed in 5-μL reactions, and PCR products were separated on an ABI 377, 310, 3100, or 3130*xl* Genetic Analyser (Applied Biosystems). A Rhodomine-X (ROX)-labelled internal size standard (Mapmarker LOW, Bioventures, Inc.) was utilized for inter-assay standardization. A set of reference samples were analysed on each system to standardize allele calling. The fragment analysis programs Genotyper 3.6 and GeneMapper 3.7 (Applied Biosystems) were used for allele identification and comparison.

Basic statistical analysis

The Y chromosome marker INRA189 was used to verify sex phenotypes and calculate the percentage of total alleles detected in each herd. For X chromosome markers BMS6017 and BMS911, genotypes for males were coded as missing data.

The Microsoft Excel Microsatellite Toolkit (Park 2001) was used to calculate the polymorphic information content value for each marker (Botstein *et al*. 1980) and prepare data sets for downstream analysis. Calculations of allele

Table 1 Population descriptions and sample collection information, sorted by the managing agency within the US Department of Interior

Managing agency	Abbreviation	Herd name†	Location	No. of founders, sources‡‡	Census§§	Collection year(s)	Total sampled	Total males	Total females
Fish and Wildlife Service	FN	Fort Niobrara NWR	Nebraska	21, 4	380	2001–2002	178	86	92
	NBR	National Bison Range	Montana	50, 7	350	2000–2001	179¶¶	98	81
	NS	Neal Smith NWR	Iowa	33, 3	63	2001	62	27	35
	SUH	Sully's Hill NGP	North Dakota¶	19, 5	35	2004	29¶¶	14	15
	WM	Wichita Mountains NWR	Oklahoma	17, 2	600	1999	37¶¶	0	37
National Park Service	BNP	Badlands NP	South Dakota	73, 3	875	2002	328	127	201
	GT	Grand Teton NP‡	Wyoming	32, 2	600	1999–2000	39¶¶	10	29
	TRN	Theodore Roosevelt NP – North Unit§	North Dakota	20, 1	312	2001	309	129	180
	TRS	Theodore Roosevelt NP – South Unit§	North Dakota	29, 1	371	2000	368¶¶	140	228
	WC	Wind Cave NP	South Dakota	20, 2	350	1999–2001	345	139	206
	YNP	Yellowstone NP	Wyoming††	51, 3	3000	1997–2002	505¶¶	221	284
			Total		6936		2379	991	1388

†NP, National Park; NWR, National Wildlife Refuge; NGP, National Game Preserve. ‡Most bison from the Grand Teton NP herd overwinter on the National Elk Refuge (Fish and Wildlife Service); this herd is jointly managed by both federal agencies (2007 Bison and Elk Management Plan: National Elk Refuge/Grand Teton National Park; www.fws.gov/bisonandelkplan/). §Bison at Theodore Roosevelt NP occur on two disjunct units of the park, which are approximately 40 miles (64 km) apart (M. Oehler, personal communication). The herds have been isolated for over 40 years and are, therefore, treated as distinct herds for the purposes of this study. ¶Since the completion of this study, the entire SUH herd was moved into an isolated enclosure within the Fort Niobrara National Wildlife Refuge, and the Sully's Hill National Game Preserve was repopulated with bison from the National Bison Range (T. J. Roffe, personal communication). ††Parts of Yellowstone NP lie within the states of Idaho and Montana. ‡‡Total known number of founding individuals and total number of founding sources for each herd (derived from Halbert *et al.* 2007). The total number of sources was calculated based on the sources known to directly contribute to each herd, and is therefore considered a minimum; it is possible that some of these direct sources were themselves derived from multiple sources. §§Estimated census population size at time of collection, or average over years of collection. Estimates provided by herd managers or field biologists. ¶¶X and Y chromosome microsatellite genotypes used to determine sex of 162 individuals sampled from the following herds: NBR, 47 individuals; SUH, 29 individuals; WM, 37 individuals; GT, 33 individuals; TRS, 3 individuals; YNP, 13 individuals. Of these, 155 determinations were necessary due to an absence of sex phenotypes at collection, while seven were due to discrepancies between the sex phenotype given at collection and that determined by microsatellite analysis. For the remaining 2217 samples, the sex phenotype given at collection matched the sex determined by microsatellite analysis.

Table 2 Summary statistics for 51 microsatellite loci across 11 bison herds

	BNP	FN	GT	NBR	NS	SUH	TRN	TRS	WC	WM	YNP
Percentage of total alleles†	70.7	68.5	63.3	77.8	77.2	56.2	55.2	66.7	75.3	64.2	75.0
N_A‡	4.56	4.40	4.08	5.00	4.96	3.62	3.56	4.30	4.86	4.16	4.84
R_A§	3.86	3.86	3.69	4.29	4.35	3.51	3.16	3.80	4.29	3.85	4.15
H_O¶	57.7	59.3	54.0	64.8	62.1	62.0	53.4	58.2	65.3	57.4	61.5
H_E††	57.8	59.5	56.1	64.7	63.9	56.6	52.2	58.2	65.2	59.1	62.5
Private alleles	2	1	0	6	0	0	0	1	10	2	4
Fixed loci‡‡	0	0	1	0	0	0	2	0	0	0	0

†Percentage of alleles present in each population based on 324 total alleles identified in this study. ‡N_A, average number of alleles per locus, excluding Y chromosome marker INRA189. §R_A, average of allelic richness values across markers, excluding Y chromosome marker INRA189; calculated based on a minimum sample size of 15. ¶H_O, average observed heterozygosity, excluding Y chromosome marker INRA189. ††H_E, average expected heterozygosity, excluding Y chromosome marker INRA189. ‡‡Number of fixed loci excludes the Y chromosome marker INRA189.

frequencies, number of alleles per locus (N_A), allelic richness (R_A; El Mousadik & Petit 1996), observed heterozygosity (H_O), expected heterozygosity (unbiased gene diversity, H_E; Nei 1987), and *F*-statistics (Weir & Cockerham 1984) were performed for each herd-marker combination with the programs FSTAT 2.9.3.2 (Goudet 2001) and MSA 4.05 (Dieringer & Schlötterer 2003). Allelic richness and expected heterozygosity are unbiased estimators of the observed number of alleles per locus and heterozygosity, respectively, which minimize differences due to sample size variances. Each herd-marker combination was tested for Hardy–Weinberg equilibrium in FSTAT 2.9.3.2 and linkage disequilibrium in GenePop 3.1d (Raymond & Rousset 1995) with sequential Bonferroni corrections for multiple tests.

The Kendall rank correlation test (Wessa 2008) was used to evaluate the potential correlation between two measures of genetic diversity (R_A, H_E; Table 2) and the following parameters in a pairwise fashion: number of founding individuals, total number of sources used to establish each herd, and census population size (Table 1).

Analysis of relationships among populations

Sex chromosome markers INRA189, BMS6017, and BMS911 were excluded from each of the following analyses.

While overall allelic variation and heterozygosity values are useful tools for genetic assessment, they do not indicate the amount of genetic variation that is unique to a particular population or how germplasm sources might be prioritized for conservation efforts. To address these issues, the contribution of each population (k) to overall genetic diversity [$C_T(k)$] was calculated based on measures of both unbiased gene diversity and allelic richness (Petit *et al*. 1998). The contribution of each population was further subdivided into components representing the diversity within a population [intrapopulation diversity, $C_S(k)$] and the divergence of that population from other populations [interpopulation differentiation, $C_D(k)$] following the calculations of Petit *et al*. (1998). Since relative genetic contributions are dependent on the relationships of the populations in the analysis, the foundation of some herds from others will tend to underemphasize the contribution of certain lineages to genetic diversity. To study this potential bias, we performed an independent analysis using a set of 'core' herds, which included only those which received bison from at least one source outside of the federal herds: Badlands National Park (BNP), Fort Niobrara National Wildlife Refuge (FN), National Bison Range (NBR), Sully's Hill National Game Preserve (SUH), Wind Cave National Park (WC), Wichita Mountains National Wildlife Refuge (WM), and Yellowstone National Park (YNP) (Halbert *et al*. 2007).

Relationships among herds were assessed using the multilocus Bayesian clustering method in the program Structure 2.1 (Pritchard *et al*. 2000). This method minimizes the presence of Hardy–Weinberg and linkage disequilibrium through probabilistic assignment of individuals into K populations, and is therefore superior to distance-based methods in determining relationships among admixed populations. After initial model evaluation (Pritchard *et al*. 2000), testing was performed with a burn-in period of 10 000 replicates and 40 000 Markov chain Monte Carlo replicates. The data set was examined using the correlated and allele frequency model (Falush *et al*. 2003) assuming admixture, with a standard deviation of alpha (ALPHAPROPSD) of 0.08 to increase mixing. Default parameters were used for all other settings (Pritchard & Wen 2004). Test simulations under different model conditions supported our model parameter choice (data not shown).

Ten tests for each value of K were performed, and K was tested for 1–15 subpopulations. Individual assignments to clusters were compared a posteriori to actual collection sites. The most likely number of clusters within the data set was determined by examining averages and standard deviations of the log of the probability of the data [Ln $P(D)$]

at each K (Pritchard & 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[Pr(X | K)] with respect to K. The height of the modal value of ΔK has been shown to accurately discriminate the true number of clusters in simulations with similar parameters to those considered here: a large number of polymorphic loci, low levels of recent migration, moderate differentiation with F_{ST} values greater than 0.05, and large sample sizes (Evanno *et al*. 2005; Latch *et al*. 2006). Clusters were aligned using the program CLUMPP 1.0 (Jakobsson & Rosenberg 2007) with the *LargeKGreedy* option and 1000 repeats of randomized input order. Resultant assignments were visualized using the program Distruct 1.1 (Rosenberg 2004).

Pairwise F_{ST} values were used to assess the levels of genetic differentiation among clusters using the multilocus estimator in FSTAT 2.9.3.2 (Weir & Cockerham 1984). Genetic distances among clusters were calculated using the chord measure of Reynolds *et al*. (1983), which is appropriate for closely related populations diverging by drift only. The program Convert (Glaubitz 2004) was used to create a gene frequency table for the PHYLIP 3.7 analysis package (Felsenstein 1993), from which a consensus tree with 1000 bootstrap replicates was created using the programs Seqboot, Gendist, Neighbor, and Consense. Input order was always randomized. Resultant tree topologies were evaluated in the program TreeView 1.6.6 (Page 1996).

Results

Basic statistical analysis

A total of 2379 samples from 11 US federal bison herds (Table 1), representing approximately 34% of the bison in these herds, were evaluated using 51 polymorphic microsatellite markers (Appendix S1). An initial goal of sampling 20% of the census size from each herd was exceeded in all except three cases: YNP (16.8%), Grand Teton National Park (GT, 6.5%), and WM (6.17%). When possible, approximately equal proportions of males and females were evaluated. For the GT and WM herds, the ratio of males to females was particularly skewed (0.34 and 0, respectively).

A minimum of 80% of the markers were successfully genotyped for each sample (average 97.6% ± 4.3% SD). Genotyping rates, size ranges, number of alleles identified, and polymorphic information content values for each marker are given in Appendix S1. At least 95% of individuals were genotyped for each marker (range 95.0% to 99.6%, average 97.6 ± 1.2%). The number of alleles detected per locus averaged 6.35 (± 1.96).

Appendix S2 (Supporting information) details allelic frequencies, N_A, R_A, H_O, and H_E for each herd-marker combination, while summary statistics are provided in Table 2. Of the 324 alleles detected in this study, the percentage of alleles present in each herd ranged from 55.2% [Theodore Roosevelt National Park-North Unit (TRN)] to 77.8% (NBR), with an average of 68.2% (± 7.9%). The average number of alleles per locus across herds was 4.39 (± 0.51) and ranged from 3.56 (TRN) to 5.00 (NBR). Similar results were obtained for allelic richness, although the ranking of herds was somewhat different due to sample size corrections, with an average of 3.89 (± 0.36) and range from 3.16 (TRN) to 4.35 [Neal Smith National Wildlife Refuge (NS)]. Likewise, estimates of observed (average 59.60 ± 3.9) and expected (average 59.61 ± 4.1) heterozygosity were similar among herds.

Private alleles were observed in seven herds (Table 2), with 10 of the 26 private alleles found in the WC herd. Excluding INRA189, all markers were polymorphic in each herd with three exceptions: BM757 was monomorphic in GT; BMS1001 and BMS941 were monomorphic in TRN bison (Appendix S2).

None of the herd-locus combinations were rejected for Hardy–Weinberg equilibrium at the nominal 5% level. Linkage disequilibrium was noted for 6.4% of the pairwise marker combinations within the BNP herd; no significant deviations from linkage equilibrium were noted in other herds (nominal $P = 0.01$). The inbreeding coefficient, f (an estimate of F_{IS}), across all loci approached 0 (± 0.04) within each herd except SUH ($f = -0.105$), indicating a modest excess of heterozygotes in the SUH herd. Of the variation detected across samples, the majority (87.8%) was accounted for by differences within herds, while the remainder was distributed among herds (θ, an estimate of $F_{ST} = 0.122$).

A statistically significant correlation was not observed between genetic diversity (R_A or H_E) and the number of founding individuals, the total number of sources used in establishing each herd, or census population sizes (Kendall rank correlation maximum = 0.317).

Relative genetic contributions

The relative contribution of each herd to allelic richness and gene diversity (unbiased heterozygosity) was fractioned into the contributions due to intrapopulation diversity and interpopulation differentiation (Fig. 1, panels A and B). The NBR, WC, WM, and YNP herds had positive overall contributions to allelic richness, with WC and YNP exhibiting comparatively large interpopulation differentiation (over twofold greater than other herds). The contribution of each of the remaining seven herds to allelic richness was at or below 0, although some had positive subcomponents for diversity (NS) or differentiation (GT, SUH, TRN). The WC and WM herds also had positive overall contributions to gene diversity. The contribution of each of the remaining herds to gene diversity was at or below 0, although some had positive subcomponents for diversity (NBR, NS, YNP) or differentiation (GT, SUH, TRN). These results are similar

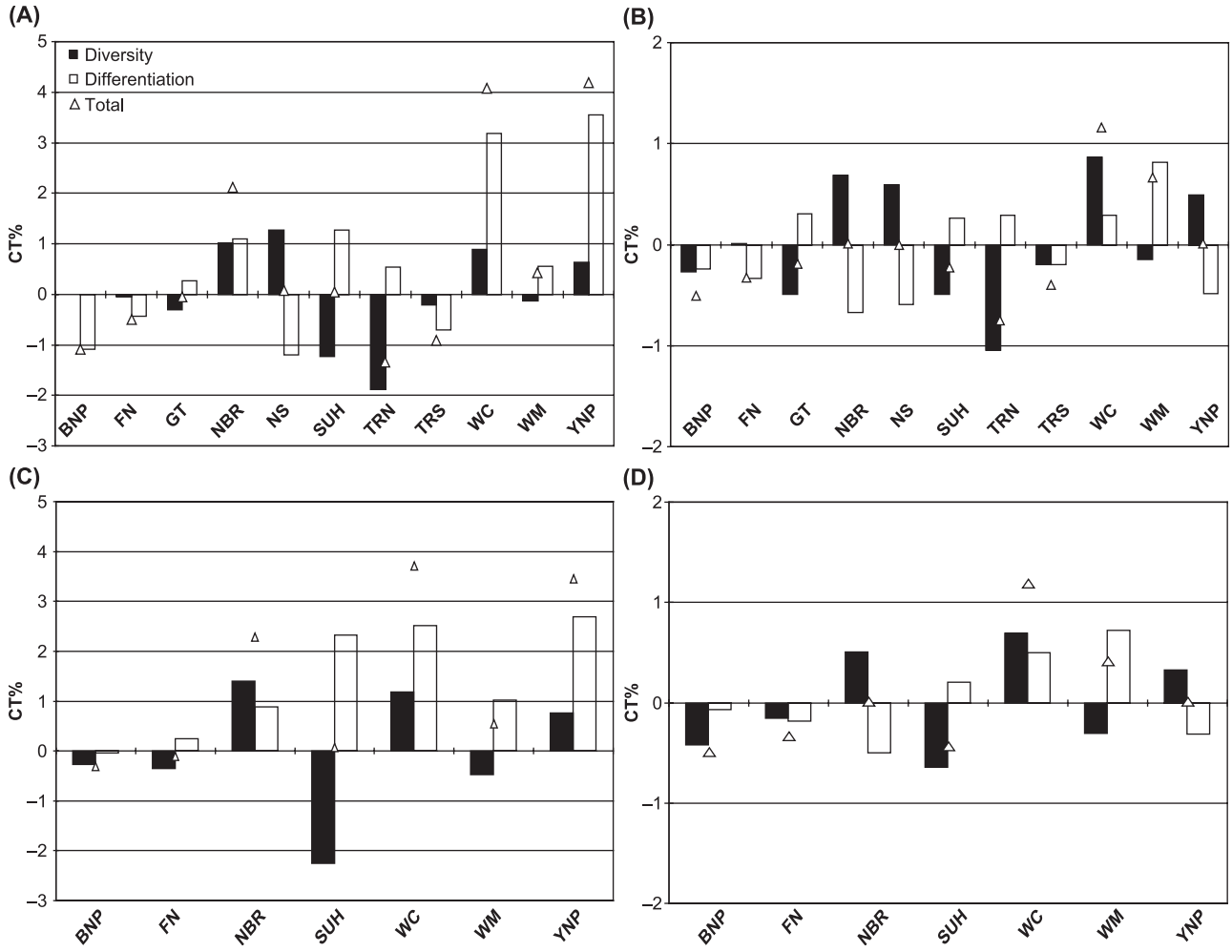

Fig. 1 Relative genetic contribution of each of the 11 federal bison herds to overall allelic richness (panel A) and gene diversity (panel B) based on 48 autosomal markers. An independent analysis with only the seven core herds was similarly performed to measure overall contributions to allelic richness (panel C) and gene diversity (panel D). Allelic richness was calculated based on a minimum sample size of 28 diploid individuals. Overall genetic contributions, which are marked with open triangles, were further fractionated into the contributions due to intrapopulation diversity (open bars) and interpopulation differentiation (filled bars).

to those produced by analyzing only the seven core herds with respect to magnitude and direction of contributions (Fig. 1, panels C and D). The overall contributions of the BNP, FN, and SUH herds to allelic richness and gene diversity were still approximately zero, most likely due to the establishment of the BNP and SUH herds in part from the FN herd (Halbert *et al*. 2007).

Genetic relationships among herds

Evaluation of Ln $P(D)$ (Pritchard & Wen 2004) and ΔK (Evanno *et al*. 2005) calculations from multiple Structure simulations indicate the data set most likely represents eight genetically defined clusters (Fig. 2). The average proportion membership of each geographical herd into the eight clusters is shown in Fig. 3a. From this analysis, only two clusters are representative of single herds: WC (cluster 1) and BNP (cluster 5). An additional six herds had more than 90% membership in a single cluster (shared with at least one other herd): FN, NBR, TRN, TRS, WM, and YNP. The remaining three herds — GT, NS, and SUH — appear to represent admixed groups with at least 15% membership in more than one cluster. The membership assignments for these herds were variable across models and at different values of K, possibly due to sampling error (GT) or recent admixture (NS and SUH; Fig. 3a). Individuals from WM, and less frequently YNP, were occasionally assigned to multiple clusters (Fig. 3b); Ln $P(D)$ values, however, indicate the data fit to the model were better when WM and YNP were assigned to single clusters.

To further assess the choice of K_8 and investigate the division of the GT, NS, and SUH herds into multiple

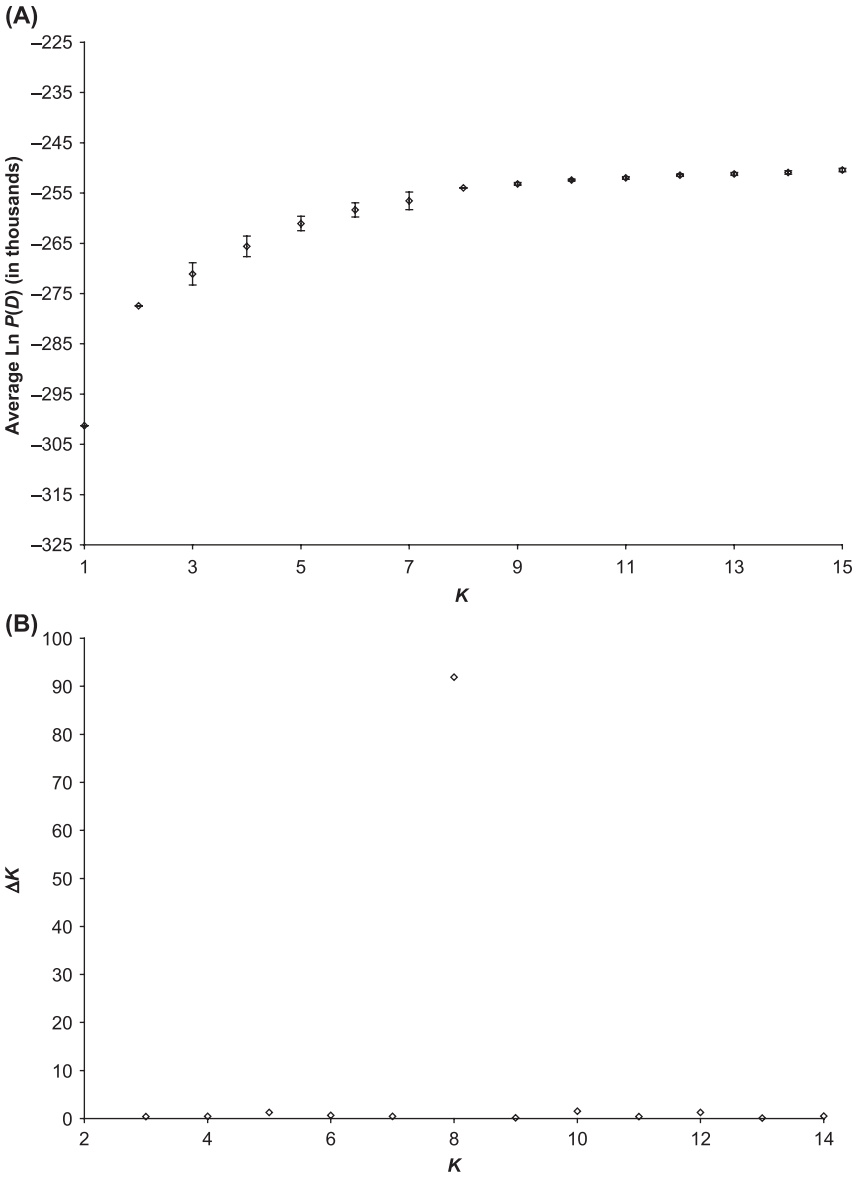

Fig. 2 Evaluation of Structure clustering for K values ranging from 1 to 15. In panel A, averages and standard deviations for Ln $P(D)$ values based on 10 simulations for each value of K are shown. Corresponding ΔK values are shown in panel B, following the calculations of Evanno *et al*. (2005). The most likely model to fit the data set includes eight genetically defined clusters based on the following observations: (i) large average Ln $P(D)$ for K_8 compared with smaller values of K and a plateau of average Ln $P(D)$ values for $K_n > K_8$ (panel A); (ii) comparatively small standard deviation of Ln $P(D)$ for K_8 compared with smaller values of K (panel A); and, (iii) a ΔK peak at K_8 (panel B). Although the ΔK value for K_2 was even larger ($\Delta K = 1522.4$) than that for K_8 ($\Delta K = 91.9$), K_2 is not the best fit for the data based on the following: (i) the inflated ΔK value for K_2 is due to the poor fit of the data for K_1, resulting in a large difference in average Ln $P(D)$ between K_1 and K_2; and, (ii) the average Ln $P(D)$ for K_2 is low compared with that for other values of K (panel A). To maintain a reasonable y-axis scale, the high ΔK value for K_2 is not shown in panel B.

clusters, individual membership proportions at K_2 were used to divide the data set into two metapopulations, which were then re-analysed independently following the methods outlined by Rosenberg *et al*. (2001). Metapopulation A contained 1268 individuals, including the BNP, FN, TRN, and TRS herds as well as individuals with at least 50% membership from the GT ($n = 24$), NS ($n = 38$), and SUH ($n = 23$) herds; this metapopulation roughly corresponds to herds derived from the FN lineage. Metapopulation B contained 1111 individuals, including the NBR, WC, WM, and YNP herds as well as individuals with at least 50% membership from the GT ($n = 15$), NS ($n = 24$), and SUH ($n = 6$) herds. Simulations were performed using the parameters previously described for K from one to eight. Four clusters were identified in metapopulation A, each of which corresponded to one of the clusters from the global analysis at K_8. In contrast, three clusters were identified in metapopulation B; one of these clusters represented the combination of cluster 1 (WC) and cluster 2 (WM) from the global analysis, while the remaining assignments were congruent with previous results.

While most individuals were repeatedly assigned to the same cluster, 57 (2.4%) had a maximum membership of less than 55% into any one cluster. Most of these individuals were from the GT ($n = 18$) and SUH ($n = 16$) herds and may have been difficult to assign due to sampling error or admixture. Additionally, three (0.1%) individuals appeared to be assigned to the wrong cluster: two samples from TRN were assigned to cluster 1 (WC) and one sample from TRS was assigned to cluster 5 (BNP). These misassignments

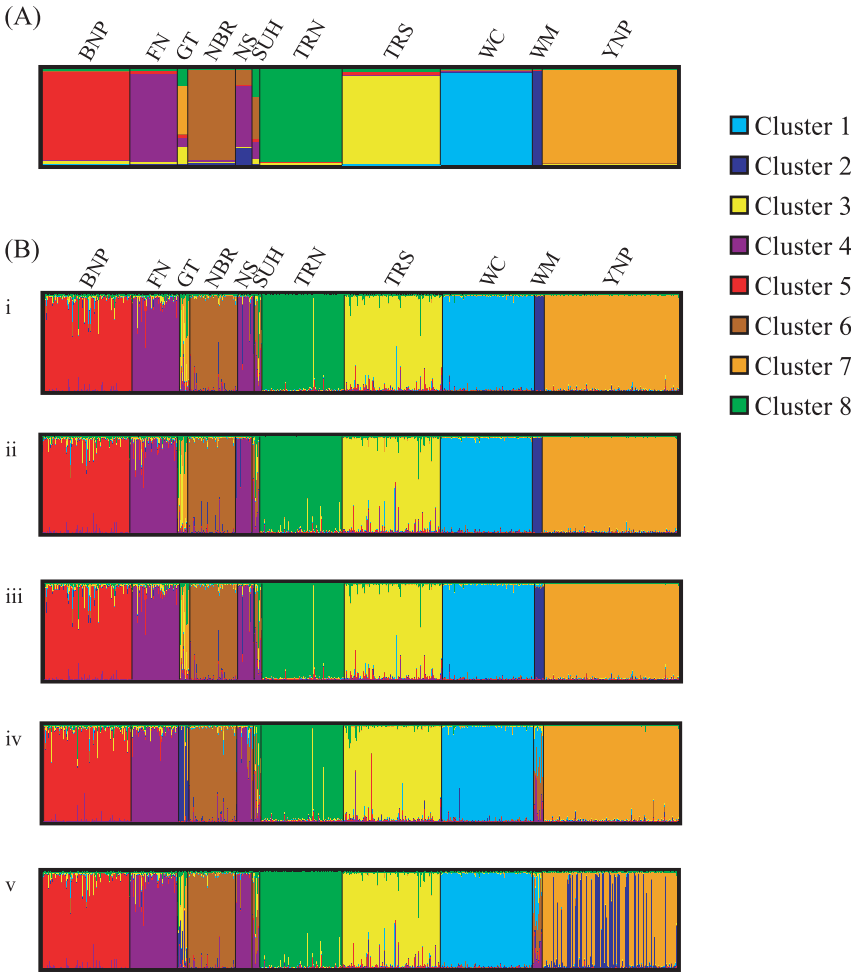

Fig. 3 A comparison of estimated population structure across 11 geographically defined populations of bison into eight clusters (identified by colour). In panel A, average cluster assignments across 10 independent iterations with K_8 are indicated for each of 11 geographically defined herds. In panel B, individual membership proportions into the eight clusters are compared among five independent iterations (subpanels i–v). The order of individuals (thin vertical lines) and herds (separated by thick vertical black lines) is identical across iterations. The frequency of panels presented here is not indicative of individual assignment frequencies. Subpanels i–iii illustrate the general reproducibility of individual assignments. Assignments of individuals from the GT, NS, and SUH herds were often unstable, while individuals from the WM and YNP herds were less frequently assigned to more than one cluster (e.g. subpanels iv and v).

were likely due to sample labelling error, genotyping error, or genotypes which are by chance frequent in the assigned clusters (natural migration is not possible between these herds). An association between the 60 samples with ambiguous or implausible assignments and genotyping success rates was not apparent. To prevent bias, these samples were excluded from the F_{ST} and distance calculations (n = 2319).

Pairwise F_{ST} values averaged 0.1249 (± 0.042) across all clusters, with clusters 3 (TRS) and 5 (BNP) representing the least differentiated pair (lowest F_{ST} value = 0.0414) and clusters 2 (WM) and 8 (TRN) representing the most differentiated pair (highest F_{ST} value = 0.2131; Table 3). Genetic distances among paired clusters, as shown in Table 3, averaged 0.1275 (± 0.042), with the smallest distance between clusters 3 and 5 (0.0428) and the largest distance between clusters 2 and 8 (0.2111). These distance values resulted in the tree topology shown in Fig. 4, in which the herds outside of the FN lineage (NBR, WC, WM, and YNP) fall into a distinctly separate clade (clusters 6, 1, 2, and 7) from the herds derived from the FN lineage; these results are congruent with the metapopulations assigned through Structure analysis.

Discussion

Factors influencing genetic diversity in bison

Despite the dramatic and well-documented bottleneck to which bison were subjected in the late 19th century (Soper 1941; Coder 1975), the species has recovered demographically (Boyd 2003) and retains relatively high levels of genetic diversity compared with other mammals which have survived similar bottleneck events (Bradshaw *et al*. 2007). In fact, many of the herds included in this study harbour only slightly lower levels of diversity compared with some breeds of domestic cattle (Fig. 5). While it has long been presumed that bottleneck events will lead to reduced genetic diversity (Nei *et al*. 1975), many exceptions have been noted (Amos & Balmford 2001). Several factors may have contributed to the retention of high levels of genetic diversity in bison.

First, while fewer than 1000 bison were in existence at the apex of the bottleneck, these individuals were distributed across a large portion of North America (Coder 1975), and likely represented a substantial cross-section of the

Table 3 Pairwise F_{ST} (above diagonal) and Reynolds *et al*. (1983) genetic distance (below diagonal) measures among clusters assigned by Structure analysis (n = 2319). The total number of individuals assigned to each cluster is shown in parenthesis in the first column. Cluster numbering corresponds to Fig. 3

	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	Cluster 7	Cluster 8
Cluster 1 (345)	—	0.1095	0.1419	0.1274	0.1380	0.0951	0.0855	0.1616
Cluster 2 (47)	0.1190	—	0.1614	0.1369	0.1557	0.1307	0.1360	0.2131
Cluster 3 (364)	0.1429	0.1661	—	0.0656	0.0414	0.1456	0.1502	0.0696
Cluster 4 (214)	0.1301	0.1434	0.0672	—	0.0483	0.1335	0.1315	0.0965
Cluster 5 (326)	0.1395	0.1601	0.0428	0.0500	—	0.1478	0.1464	0.0687
Cluster 6 (190)	0.0973	0.1397	0.1455	0.1355	0.1478	—	0.0975	0.1807
Cluster 7 (519)	0.0863	0.1447	0.1522	0.1342	0.1488	0.0988	—	0.1811
Cluster 8 (314)	0.1637	0.2111	0.0713	0.0973	0.0703	0.1789	0.1856	—

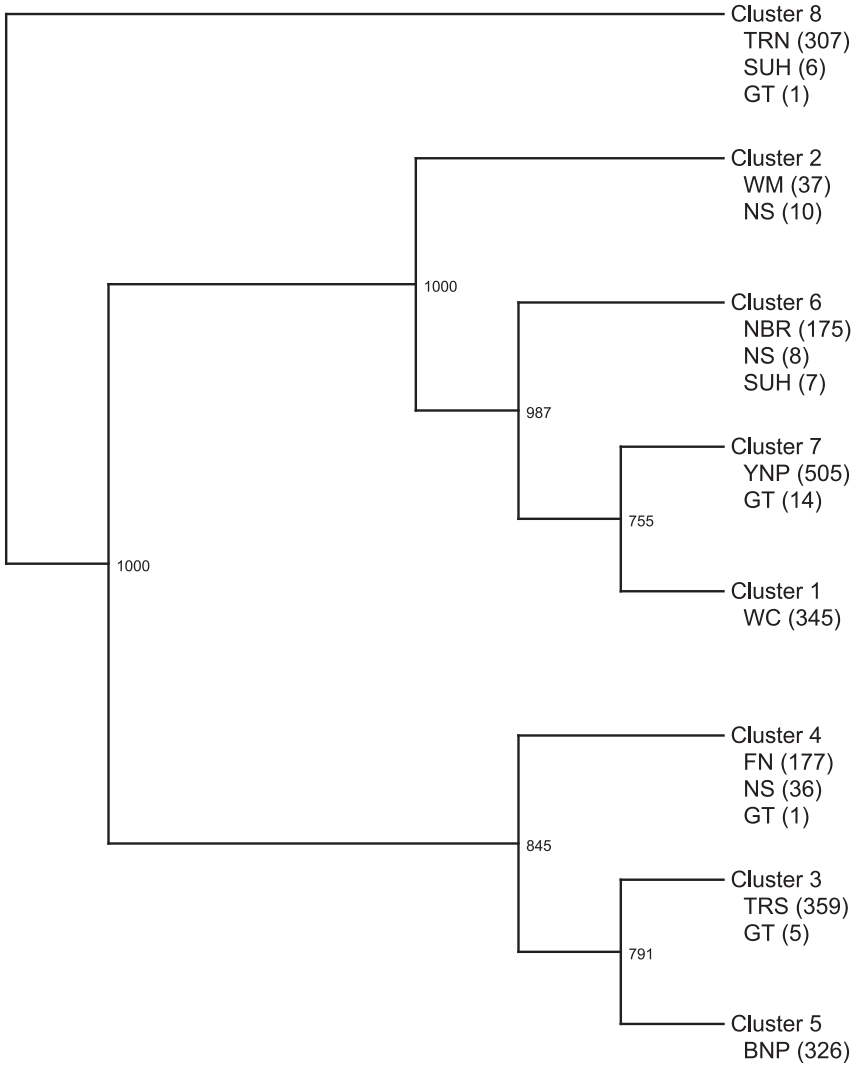

Fig. 4 UPGMA tree diagram based on Reynolds *et al*. (1983) genetic distances. Cluster numbers correspond to Fig. 3.

species' diversity. Similarly, relatively high levels of genetic diversity have been observed in populations derived from multiple sources, even those from endangered species (Uphyrkina *et al*. 2002). In comparison, species recovery programmes based on a single source population have resulted in markedly lower levels of genetic diversity (Wisely *et al*. 2002; Luenser *et al*. 2005).

Second, the census size of the bison population rapidly increased following the bottleneck (Coder 1975), which limited the potential for genetic drift and inbreeding (Nei

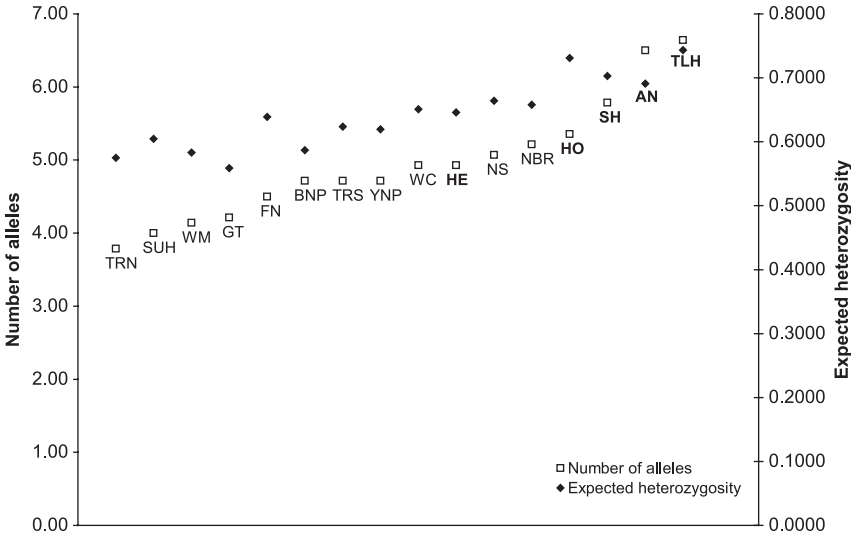

Fig. 5 Average number of alleles per locus and average expected heterozygosity across 14 microsatellite markers for 11 bison herds (Table 2) and 5 domestic cattle (*B. taurus*) breeds (AN, Angus; HE, Hereford; HO, Holstein; SH, Shorthorn; TLH, Texas Longhorn). Domestic cattle breed data from Schnabel *et al*. (2000). It should be noted that the markers used for this comparison were chosen on the basis of having a large number of alleles in bison (Schnabel *et al*. 2000), and a more random marker selection method might indicate greater differences between the species. Markers reported: BM1225, BM1706, BM17132, BM1905, BM2113, BM4440, BM720, BMS1117, BMS1172, BMS2639, BMS410, BMS510, BMS527, RM372.

et al. 1975). Rapid population growth has been linked to the maintenance of high levels of genetic diversity following bottleneck events (Zenger *et al*. 2003), while slow population growth likely contributed to the loss of genetic variation in other cases (Williams *et al*. 2002). Several factors led to the rapid increase in the bison population, including the adaptability of the species to a wide range of environments (Sanderson *et al*. 2008) and long generation times coupled with high reproductive rates (Berger & Cunningham 1994). Species with long generation times are less sensitive to demographic stochasticity (Legendre *et al*. 1999) since the lifetime breeding success rate per individual is high, which in turn permits retention of high levels of genetic diversity following population bottlenecks (Dinerstein & McCracken 1990; Hailer *et al*. 2006).

It is also plausible that genetic introgression as a result of interspecies hybridization with domestic cattle during the late 1800s contributed to the diversity detected across these bison herds. Indeed, some electromorphs identified in this study are the same size as those in cattle (Appendix S2; Schnabel *et al*. 2000), although it is currently unknown whether these similarities are due to genetic introgression, symplesiomorphy, or convergence. However, the possibility that introgression has played a significant role in increasing bison genetic diversity is considered small, since overall levels of introgression in these herds are low (Halbert & Derr 2007) and those herds in which no introgression has been previously detected (WC, YNP) harbour high levels of diversity (Table 2).

Factors influencing differences in genetic diversity among herds

While the 19th century bottleneck may not have had a significant impact on the neutral genetic variation across this species, levels of genetic diversity varied across the geographically defined herds. The highest levels of diversity were detected in the NBR, NS, and WC herds, while the lowest were found in the SUH, GT, and TRN herds (Table 2). In contrast, samples from four of these herds were evaluated in a previous microsatellite-based study (Wilson & Strobeck 1999), which resulted in different rankings in terms of average number of alleles (WM, FN, NBR, YNP) and expected heterozygosity (WM, YNP, NBR, FN) compared to this study (Table 2). The most likely source of this discrepancy is sampling bias, as the prior study sampled a much smaller number of individuals from each herd (21 to 36 individuals; Wilson & Strobeck 1999).

Overall, the observed differences in genetic diversity among herds are not explained simply by differences in the number of founding individuals, the total number of sources, or census population sizes. It is most likely that genetic diversity in these herds has been influenced by a combination of forces including levels of genetic diversity present in the founders, relative genetic contribution of founders, differences in culling strategies, and effective population sizes over time (Primack 1993). For example, while the SUH herd was derived from several sources (Table 1), the herd has low levels of diversity (Table 2) compared with other herds founded with similar numbers of individuals and fewer sources (FN, WC), most likely due to the continuous maintenance of the SUH herd with a small number of bison (C. Dixon, personal communication.). In contrast, the NS herd harbours higher levels of diversity (Table 2) despite having a small census size (Table 1). However, the effects of drift in the NS herd, which was only recently established (1996–1998), are not comparable to herds which have been closed for longer periods.

Sequential founder events are expected to lead to decreased genetic diversity (Nei *et al*. 1975), and likely

contributed the low levels of diversity observed in the TRN herd. The TRN herd was derived from the TRS herd, which was in turn founded with bison from the FN herd (Halbert *et al*. 2007). The loss of allelic diversity due to these founder events is traceable: of 237 alleles, 93.7% (222) are found in the FN herd, 91.1% (216) in the TRS herd, and 75.5% (179) in the TRN herd (Appendix S2). Within this lineage, 7.6% (18) of the private alleles were identified in the FN herd, 5.1% (12) in the TRS herd, and 0.4% (1) in the TRN herd (Appendix S2). Similarly, expected heterozygosity was reduced by 2.2% following the first founder event and 10.3% following the second founder event (Table 2). These results are not easily extrapolated to similar situations in other species since the magnitude of change in diversity following sequential founder events is influenced by numerous factors including the number of founders, the genetic variability of the source population, and population growth rates (Broders *et al*. 1999; Taylor & Jamieson 2008). In general, however, it is evident that sequential founder events, particularly those involving small numbers of founders, should be avoided whenever possible to minimize the loss of genetic variation.

Drastically different management approaches are used to maintain the herds this study. For instance, nearly all bison culled from the WC herd each year are from a single juvenile age class (National Park Service 2003), while bison from the FN herd are culled across all age classes (Fish & Wildlife Service 2003). The comparison of these two herds is indicative of the importance of culling strategies on the maintenance of genetic variation: both herds were founded around the same time (WC in 1916, FN in 1913) and have been maintained with similar census sizes (Table 1), but in this study we detected substantially higher levels of genetic variation in WC bison (Table 2). This finding is somewhat surprising considering that the WC herd has been a closed population for over 90 years while the FN herd received several supplementations through the 1950s (Halbert *et al*. 2007). The observed levels of diversity in these herds most likely reflect differences in management strategies. For instance, FN bison were artificially selected for size and conformation over a period of at least 20 years, which may have concomitantly reduced genetic diversity (Coltman 2008). Differences in management strategies are expected to influence effective population sizes and levels of genetic variation among herds (Frankham 1996). Breeding structure parameters such as inter-individual variation in offspring number and sex-dependent reproductive age ranges likely differ among herds but have been largely unevaluated (however, see Berger & Cunningham 1994; Kirkpatrick *et al*. 1996; Helbig *et al*. 2006). Therefore, classical calculations of effective population sizes among these herds are not feasible at this time (Lande & Barrowclough 1987). Simulation modelling based on the data collected in this study is currently being used to evaluate the impact of management policies on effective population sizes and the maintenance of genetic variation.

With the continuous expansion of human populations and disruption of wildlife migration patterns, supplementation of existing wildlife populations has become an increasingly important conservation tool. However, the success of supplementations is rarely followed and reported (Fischer & Lindenmayer 2000). Bison represent a valuable case study in this regard, as multiple simultaneous experiments in population supplementation were performed and recorded over the past century. Based on the results of this study, translocation of bison among US federal herds has resulted in mixed levels of success (i.e. mixture of germplasm from the original and translocated individuals). The primary determinants of the success of translocated bison are likely social influences, such as mate competition and social structuring within herds (females and juveniles in mixed groups, older males in bull groups or solidarity; Berger & Cunningham 1994). For instance, maternal presence is important to the social integration of juvenile bison, and aggressive behaviour of resident bison towards young translocated bison has been noted (Coppedge *et al*. 1997).

In this study, we found that the genetic contribution of multiple translocations of male FN bison into the SUH herd was lower than expected (Fig. 3; see also discussion below); this finding is likely the result of unsuccessful mating competition by the translocated bulls and may have been influenced by genetic drift in the continuously small SUH herd. In contrast, levels of genetic admixture in the NS herd indicate an approximately equal contribution of translocated NBR bison compared with resident bison (Fig. 3; see also discussion below). In this case, the introduced bison may have been socially accepted and sexually competitive with the resident bison due to a lack of social structure in the NS herd, which had only existed for 1 year at the time of translocation (Halbert *et al*. 2007).

Translocation of family units, as opposed to unrelated individuals, has been linked to the successful establishment of new populations in socially structured species (Shier 2006). Likewise, social structure among translocated individuals may influence population supplementation efforts. For instance, social structure likely existed among the bison translocated into the BNP herd from Colorado in 1983, as these bison made up a small but long-existing herd (Berger & Cunningham 1994). Although over 25 years have passed since the Colorado bison were introduced, linkage disequilibrium (LD) is still prevalent among nonsyntenic markers. The deterioration of LD in this herd may be inhibited by continuous lineage sorting, although the cause and rate of erosion of LD in the BNP herd remain to be investigated. Few studies have evaluated LD due to admixture in wildlife populations (Slate & Pemberton 2007). However, this phenomenon may become more common as population supplementation efforts increase, and the long-term effects

of LD on genetic diversity and evolutionary potential should be considered.

The translocation of bison among herds continues to be a popular management tool, and is generally presumed to result in enhanced genetic diversity. The equal contribution of translocated bison into the germplasm of the resident herd, however, is critical to meeting the goal of increased genetic diversity. These results underscore the importance of careful planning and monitoring, such as through parentage testing, to ensure the success of population supplementations (Fischer & Lindenmayer 2000).

Relationships among herds

The identification of genetic structure among populations is a primary goal in conservation genetics (Waples & Gaggiotti 2006). Geographical origin is commonly used to define populations, but does not always correlate with genetic relationships (e.g. Funk *et al*. 2007). One way to circumvent this problem is to conduct a *posteriori* comparisons of genetic cluster assignments to collection site information. Using this method, we found the cluster assignments produced in the program Structure strongly correlated with historical records of herd establishment and multiple translocations among the US federal herds (Halbert *et al*. 2007), thereby demonstrating the utility of cluster-based analyses in species with unknown histories or cryptic population structure (Rosenberg *et al*. 2001). For instance, the two metapopulations identified by cluster analysis are not equally distinctive (Table 3): clusters assigned to metapopulation A are more similar to each other (average F_{ST} 0.065 ± 0.019 SD) than the clusters representing metapopulation B (average F_{ST} 0.109 ± 0.020 SD). These observations are congruent with the common history of the herds in metapopulation A as part of the FN lineage (FN, BNP, TRN, TRS). The relationships among the herds represented by metapopulation B are more indirect in nature (NBR, WC, WM, YNP): while translocations have occurred among some of these herds, none share an exclusive relationship (Halbert *et al*. 2007).

Additional cluster analysis revealed eight of the 11 herds were sufficiently differentiated to be assigned to individual clusters (Fig. 3). These results suggest that the existence of bison in (mostly) small, isolated herds has led to substantial genetic drift in a short period of time. Rapid genetic drift and differentiation as a consequence of short-term population isolation has been indicated in other wildlife species (Broders *et al*. 1999; Whitehouse & Harley 2001), demonstrating the importance of routine genetic monitoring to identify and mitigate the loss of diversity across populations.

Bison from the remaining three herds were assigned to multiple clusters, reflecting both recent (NS) and more distant (SUH, GT) admixture based on recorded translocations into these herds. In the case of the NS herd, admixture is clear from both historic records and genetic analysis (Fig. 3). The variation within the NS herd was divided among three clusters shared with FN (cluster 4, 64%), WM (cluster 2, 17.8%), and NBR (cluster 6, 15.4%). These cluster proportions are remarkably similar to the estimated contribution of these lineages to the NS herd (Halbert *et al*. 2007). Cluster assignments for the SUH herd also corresponded to herds from which translocations were derived (FN, NBR, TRN; Halbert *et al*. 2007). The largest membership proportions were in clusters shared with NBR (43.6%) and TRN (28.7%), which represent the two most recent sources of translocation into the SUH herd. Interestingly, only 17.3% of the SUH membership was assigned to the same cluster as FN (Fig. 3a) despite records indicating that more individuals from the FN herd were added to the SUH herd than from any other single source (seven over nearly 40 years; Halbert *et al*. 2007). These observations suggest that the translocated bison did not equally contribute to the genetic make-up of this herd. Additionally, the GT herd was consistently assigned to multiple clusters (Fig. 3b), although the membership proportions and number of assigned clusters were unstable. Conversely, the WM herd was occasionally split into two clusters, one of which was shared with WC (Fig. 3b, panels ii and v) and might be explained by the common historic link through the New York Zoological Park herd (Coder 1975). However, given the small number of samples obtained from the GT and WM herds (Table 1), these results are tentative at best. Known transfers among other herds were not detected with this method, most likely due to either minimal genetic contribution by the introduced bison or sufficient mixing of the gene pools such that admixture is not apparent.

Management implications

Unfortunately, only a small number of samples were available from the GT and WM herds (Table 1). Larger sample sizes are necessary to accurately evaluate the variation present within these herds and make reliable comparisons with other herds. Therefore, management implications regarding these herds are not further considered here.

The identification and prioritization of germplasm resources is critical to planning and implementing species conservation programmes. By assessing the contribution of individual herds to overall levels of genetic diversity (allelic richness and gene diversity), three herds were identified as critical germplasm resources: NBR, WC, and YNP (Fig. 1). Seven of the remaining herds were wholly or in part derived from the FN lineage (Halbert *et al*. 2007), likely explaining the low or negative genetic contribution of these herds to overall allelic richness and gene diversity. Collectively, the analyses presented in this study indicate that the FN lineage has been widely dispersed and replicated within the US federal herds compared with the NBR, WC,

and YNP lineages. It is also evident that levels of allelic diversity and heterozygosity alone are not useful indicators of conservation priority targets (Petit *et al*. 1998): the FN, BNP, and TRS herds all have moderate levels of genetic diversity (Table 2) and yet are closely related to each other as part of the FN lineage.

Of the three critical germplasm sources identified in this study, the WC and YNP herds are also among the few known sources of germplasm from which domestic cattle introgression has not been detected (Ward *et al*. 1999; Halbert *et al*. 2005; Halbert & Derr 2007). The creation of satellite herds from these sources therefore should be a conservation priority for this species to mitigate the effects of genetic drift and protect against the catastrophic loss of critical germplasm (Margan *et al*. 1998). Reportedly, state-managed satellite herds of exclusively NBR (Alaska; Coder 1975) and YNP (Utah; J. Karpowitz, personal communication.) germplasm are already in existence, although the source(s) and levels of genetic diversity within these have not been verified to our knowledge. However, bison from the WC herd have been recently used to establish two small, privately managed herds for the purposes of germplasm conservation, and genetic analyses have been performed for source verification and monitoring of diversity (N.D. Halbert, unpublished data).

As fragmented populations are generally believed to be more susceptible to inbreeding, loss of genetic diversity, and extinction (Frankham 2003), the proper management of isolated populations is imperative for long-term conservation. The movement of individuals between populations is a proposed management alternative to mitigate these effects (Margan *et al*. 1998). Even in a species such as bison with seemingly plentiful numbers of individuals and populations, however, the potential benefits of such transfers may not outweigh the costs (e.g. financial considerations, risk of disease transfer, dilution of native germplasm, unequal or lack of genetic contribution by translocated individuals). In fact, given the current body of scientific evidence, the management of the US federal bison herds as a metapopulation is not warranted. First, domestic cattle introgression has been detected in many, but not all (WC, YNP), of these herds (Halbert & Derr 2007). Obviously, bison from sources with domestic cattle introgression should not be moved into these herds. Mixing of bison from different introgression sources is also not advisable, as this would increase the number of introgressed segments in the recipient herd in an additive manner. Second, it does not appear that there is currently a critical need to initiate a broad-scale metapopulation management programme. Genetic diversity was much higher in each of the 11 herds in this study compared with a small, isolated herd likely suffering from inbreeding depression in Texas, which was found to have an average of 2.56 alleles/locus and 38% observed heterozygosity for the same markers (Halbert *et al*. 2004). With the possible exceptions of the TRN and SUH herds, relatively high levels of genetic diversity indicate that the US federal herds have not suffered from extreme drift or inbreeding depression (Table 2). Furthermore, to our knowledge, demographic indicators of inbreeding such as low natality rates and high juvenile mortality rates (Frankham 2003) have not been observed in any of these herds.

It is important, however, to consider supplementation of isolated populations where justified. In this study, it appears that translocations should be considered among the FN, TRS, and TRN herds. These herds are derived exclusively from the same lineage, appear to be free of infectious diseases (Boyd 2003), and harbour domestic cattle introgression from the same source (Halbert & Derr 2007). The translocation of bison among these herds would help preserve the FN lineage by increasing low diversity in the TRN herd and reintroducing lost diversity into the FN herd from the TRS herd. In fact, the identification of the genetic relationships among these herds exemplifies the importance of maintaining multiple small populations from a single source to counteract the effects of drift (Margan *et al*. 1998): without the replication of the FN lineage in the TRN and TRS herds, an estimated 5% of the allelic diversity of this lineage would be unrecoverable today since no other exclusive sources of FN germplasm are known.

Even with the relatively large amount of historical demographic information available for many bison herds, this study has emphasized the importance of population surveys in understanding the interplay of variables known to influence genetic diversity (e.g. germplasm sources, length of isolation, effective population size). The genetic variation identified in this study is unevenly distributed among National Park Service and Fish and Wildlife Service herds, and must be cautiously and cooperatively managed to ensure the long-term integrity of the bison genome. The techniques utilized in this study can be easily applied to other important sources of bison germplasm, such as those maintained by Parks Canada and private conservation groups, in order to gain insight into patterns of genetic variation and identify additional conservation priorities.

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N. Halbert is a research assistant professor with interests in mammalian population genetics, conservation genetics, and natural resistance to infectious diseases. J. Derr has broad research interests in conservation genetics, population genetics, gene mapping of economically important traits, and the genetics of disease resistance in domesticated livestock and wildlife.

Supporting Information

Additional supporting information may be found in the online version of this article:

Appendix S1 Summary information for loci used in this study.

Appendix S2 Summary statistics and allele frequency data for 51 microsatellite markers utilized in this study. Allele sizes are given in second column, and private alleles are in boldface type. See Table 1 for herd abbreviations.

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