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Appendix B

1.Does population size affect genetic diversity? A test with sympatric lizard species lizard.population.size2016
2.Boosting genetic diversity may save vanishing animal populations. But it also may backfire pennisi2019
3.Gross_and_Wang_Effects_of_Population_Control_Strategies_on_Retention_of_Genetic_Diversity_in_National_Park_Service_Bison_Herds
4.Patterns of genetic variation in US federal bison herds HalbertDerr2008

Does population size affect genetic diversity? A test with sympatric lizard species

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Abstract

Genetic diversity is a fundamental requirement for evolution and adaptation. Nonetheless, the forces that maintain patterns of genetic variation in wild populations are not completely understood. Neutral

theory posits that genetic diversity will increase with a larger effective population size and the decreasing effects of drift. However, the lack of compelling evidence for a relationship between genetic diversity and population size in comparative studies has generated some skepticism over the degree that neutral sequence evolution drives overall patterns of diversity. The goal of this study was to measure genetic diversity among sympatric populations of related lizard species that differ in population size and other ecological factors. By sampling related species from a single geographic location, we aimed to reduce nuisance variance in genetic diversity owing to species differences, for example, in mutation rates or historical biogeography. We compared populations of zebra-tailed lizards and western banded geckos, which are abundant and short-lived, to chuckwallas and desert iguanas, which are less common and long-lived. We assessed population genetic diversity at three protein-coding loci for each species. Our results were consistent with the predictions of neutral theory, as the abundant species almost always had higher levels of haplotype diversity than the less common species. Higher population genetic diversity in the abundant species is likely due to a combination of demographic factors, including larger local population sizes (and presumably effective population sizes), faster generation times and high rates of gene flow with other populations.

Introduction

Genetic diversity is the basic currency of evolution: only genetically variable loci are capable of evolutionary change. However, much work remains to be done to understand the factors responsible for generating and maintaining genetic variation in populations (Leffler et al., 2012). Under a neutral model, a population's genetic diversity depends on its effective population size and the gene's mutation rate (Kimura, 1983). Most evolutionary studies default to a neutral expectation as the null model (Kreitman, 1996; Fay and Wu, 2003). However, the apparent lack of a relationship between genetic diversity and population size in wild populations has led some authors to argue that population size and genetic drift are not major factors affecting molecular variation (Lewontin, 1974; Nachman, 1997; Amos and Harwood, 1998; Gillespie, 2001; Bazin et al., 2006).

Central to this criticism is the observation that the range of genetic diversity values from comparisons among taxa with different population sizes is too low to be explained solely by neutral phenomena (Lewontin, 1974). However, many of the comparisons used to bolster this contention are among taxonomically very divergent species, for example, humans vs *Drosophila* sp. vs bacteria or mammals vs mollusks (Lewontin, 1974; Bustamante et al., 2005; Bazin et al., 2006). Although these taxa do differ in population size, they also potentially differ in many other factors that could affect genetic diversity, for example, geographic location, population history, mutation rates and/or population subdivision, making it difficult to determine the effect of population size.

More recently, a handful of studies have increased the taxonomic breadth of sampling efforts. Although most of these studies did not find a convincing relationship between genetic diversity and population size (Nabholz et al., 2008; Leffler et al., 2012; Perry et al., 2012), one did find a significant relationship between diversity and life-history traits that are potentially correlated with population size, such as body mass or propagule size (Romiguier et al., 2014). However, diversity estimates were derived from collections of individuals sampled across large geographic regions, for example, Europe, Canada and Brazil. The conflation of within- and among-population genetic diversity and the lack of control for

different climatic, historical and geological phenomena make drawing conclusions about the ultimate causes of genetic diversity difficult.

In contrast to previous studies, determining which evolutionary mechanisms are actually responsible for genetic diversity is facilitated by comparing more closely related species that differ in significant aspects of life history or ecology (Leffler et al., 2012; Cutter and Payseur, 2013), especially if the species are sympatric. Closely related species are more likely to have similar mutation/substitution rates compared with distantly related species (Martin and Palumbi, 1993; Lynch 2010), and sympatric species are more likely to have been exposed to the same climatic and geological histories than are allopatric species. The reptile community at the Mojave National Preserve (MNP) in San Bernardino County, CA, USA is an excellent model system for the investigation of genetic diversity because of the large number of sympatric species with different population sizes and demographic properties. The goal of this study is to characterize population levels of molecular genetic diversity in single populations of four lizard species sampled over the same small geographic region. Two of these species have larger population sizes than the other two species.

Our laboratory recently conducted a population study of light/dark color polymorphism in a population of side-blotched lizards (*Uta stansburiana*) at the Cima Volcanic Field in the MNP. We examined the cytochrome *b* (*cytb*) and NADH dehydrogenase 4 (*ND4*) mitochondrial loci and the autosomal melanocortin 1 receptor (*MC1R*) locus (Micheletti et al., 2012). Population studies have shown *MC1R* to affect melanin production in other species and the mitochondrial genes were used as potentially non-selected markers. Although the analysis did not find an association among *MC1R* haplotypes and color morphs, we did discover an extraordinary amount of genetic diversity in the population of *U. stansburiana*. Mitochondrial haplotype diversity (*h*) in the *U. stansburiana* sampled across 12 km of the lava field was 0.985, similar to diversity values found in globally sampled marine fish, for example, wahoo and squirrelfish (Micheletti et al., 2012). *MC1R* was also unexpectedly variable in *U. stansburiana*, especially for an autosomal protein-coding region. Micheletti et al. (2012) found 54 unique haplotypes and a haplotype diversity of 0.919 (*N*=220 genes). Because *U. stansburiana* is sympatric with a large number of other squamate reptile species that vary in abundance, comparison of genetic diversity among these lizards and snakes provides a test of the effect of population size on diversity without the confounding effects created by sampling from different localities.

In this study, we sampled four additional lizard species sympatric with *U. stansburiana* at the MNP to document how levels of population genetic diversity vary among different species, two species with large population sizes and two species with smaller population sizes. We seek to relate population size to variation in levels of genetic diversity in order to test the predictions of neutral theory. If population size contributes to the maintenance of genetic diversity, we expect the high-density species to have significantly higher levels of diversity. Alternatively, if population size is not an important determinant of diversity, we expect diversity to vary among species or loci irrespective of each species' population size.

Materials and Methods

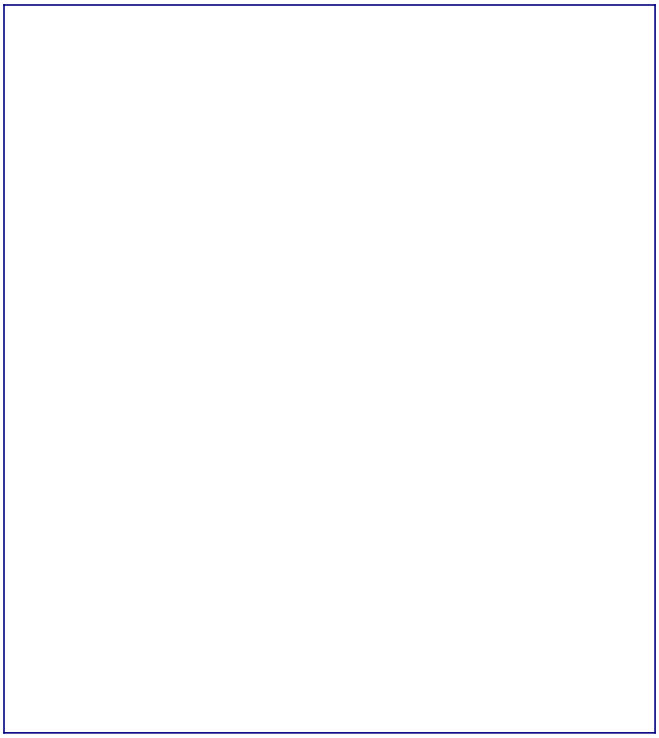
Sampling and choice of species

We sampled four species: zebra-tailed lizards (*Callisaurus draconoides*), western banded geckos (*Coleonyx variegatus*), chuckwallas (*Sauruomalus ater*), and desert iguanas (*Dipsosaurus dorsalis*). Similar to *U. stansburiana*, *C. draconoides* and *C. variegatus* are small-bodied, insectivorous and very abundant at the MNP (Persons and Nowak, 2007). In contrast, *S. ater* and *D. dorsalis* are large-bodied species, primarily herbivorous and less numerous (Persons and Nowak, 2007). In addition, our study site is centrally located within the ranges of all of the species (Jones and Lovich, 2009), so that diversity reduction associated with edge of range effects or marginal habitat is unlikely to be an issue. Moreover, sampling the four species from the same location in MNP increases the likelihood that all the populations have experienced a roughly coincident biogeographic history. With two similar species in each experimental group, our population survey at the MNP is a replicated analysis of variation in the levels of molecular diversity.

We are confident of the designations of these species as high- vs low-density populations. The results of the only survey of the herpetofauna (Persons and Nowak, 2007) are consistent with our classification, as is our own experience catching these lizards at the MNP over the past 19 years. Three of these four species, in addition to *U. stansburiana*, are diurnal lizards that spend most of their activity time basking on rocks, with occasional forays to forage. A typical search for lizards involves walking a transect while scanning rocks and ground for basking lizards. During these searches, we typically encounter 15–20 *U. stansburiana* and *C. draconoides* for every individual of *D. dorsalis* or *S. ater*, even in optimal habitat for the less common species. The nocturnal *C. variegatus* are sampled by driving the main paved road through the MNP and sampling the lizards as they cross the roads. *C. variegatus* is the most common reptile species seen on the road by far, and in normal years a sample of 40 lizards would be easily collected over several nights. Unfortunately, our study was conducted during a drought year, which seemed to depress *C. variegatus* activity, as well as that of other species.

We haphazardly collected individuals from the MNP in the region of the Cima Volcanic Field (Figure 1, data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.g7d1r). Samples consisted of 35 *S. ater*, 21 *D. dorsalis*, 35 *C. draconoides* and 21 *C. variegatus*. Diurnal lizards were captured using a slip-knot noose. Nocturnal *C. variegatus* were captured by hand. A 0.2-cm tail-tip tissue sample was taken from all individuals and preserved in 95% ethanol for genetic analysis.

Figure 1



Satellite imagery of the Cima Volcanic Field region of the Mojave National Preserve with collecting localities. Star on map inset indicates location of the maps.

Full size image

Considering the limited geographic distribution of our samples, we are confident that these samples are from a single population for each species. The largest geographic distances between any two individuals in our sample were found in *C. draconoides* (12 km) and *C. variegatus* (18 km), but most samples were collected over a much more localized area and suitable habitat was continuous between individuals. A few of the samples for *C. draconoides* (4 lizards) and *C. variegatus* (3 lizards) were somewhat geographically separate from the majority of the individuals collected, but reanalysis of the data excluding these individuals had no effect on any of the genetic parameter estimates described below.

Locus choice and molecular methods

For consistency with the *U. stansburiana* study, we surveyed the *cytb* and *MC1R* genes in all four species. For a better depiction of the standing level of genetic diversity, we analyzed an additional autosomal gene: recombination activation gene-1 (*RAG1*). *RAG1* has been successfully used as a marker in higher-level phylogenetic studies, including determining basal divergences in squamate reptiles (Townsend et al., 2004), and therefore is not expected to be highly variable at the within-population level.

Whole genomic DNA was extracted from frozen tissue using the Quick-gDNA MiniPrep Kit (Zymo Research, Irvine, CA, USA). Primers for the *cytb* mitochondrial locus and the *MC1R* and *RAG1* autosomal loci were obtained from previously published studies and developed by aligning sequences from closely related species accessed through GenBank (Supplementary information 1). The target loci were amplified with 20 µl PCR reactions (Supplementary information 1) using AccuPower

PyroHotStart Taq PCR PreMix (Bioneer, Alameda, CA, USA). Each PCR reaction included 18 µl PCR water, 0.5 µl of each primer and 1 µl of the genomic DNA. The PCR product was cleaned using ExoSAP-IT (Affymetrix, Santa Clara, CA, USA), and the purified product was sequenced in both directions by Elim Biopharmaceuticals, Hayward, CA, USA. Sequences were aligned and edited in Geneious 4.8.5 (Biomatters, available from http://www.geneious.com/). To avoid mistaking poor sequence data for high genetic diversity, sequence chromatograms with indistinct peaks or suspicious base changes were resequenced. Heterozygous sites in the autosomal loci were identified by visual inspection and confirmed in both directions of sequencing.

Haplotype linkage phase was inferred computationally with the program PHASE (Stephens et al., 2001). PHASE can sometimes have difficulty resolving low frequency alleles, and omitting such alleles from analyses can lead to artifactual reductions in estimates of molecular diversity in population studies (Garrick et al., 2010). Individuals with alleles that PHASE could not reliably infer at the 90% confidence threshold were resolved by cloning and sequencing the PCR product from that locus. The PCR product of heterozygous individuals was cloned using a TOPO TA Cloning Kit for Sequencing (Life Technologies, Grand Island, NY, USA). Multiple clones were sequenced with the same methods used for sequencing of PCR products from genomic templates in order to determine the true haplotypes for each individual.

Intraspecific genetic diversity

Population genetic analyses were performed with Arlequin 3.5 (Excoffier and Lischer, 2010). Each species was treated as a single population. For each species, we tested for recent population expansion using a pairwise mismatch distribution (Rogers and Harpending, 1992). We tested for significance using the raggedness index (*r*; Harpending, 1994) and the sum of squared deviation test (Rogers and Harpending, 1992). We also tested for departures from neutral expectations using Tajima's *D* (*D*; Tajima, 1989).

To assess molecular diversity, we estimated the number of haplotypes and haplotype diversity (*h*) at each locus (Nei, 1987). We also estimated nucleotide diversity with Watterson's θ (θ; Watterson, 1975) and Nei's θ (π; Watterson, 1975; Tajima, 1983). For each locus, we made pairwise comparisons of haplotype diversity between species. Differences in haplotype diversity between species were deemed significant if the 95% confidence intervals of the two estimates did not overlap by more than half of a one-sided error bar (Cumming and Finch, 2005). We also compared haplotype diversities using a *z*-score test suggested by Nei (1987). To better visualize the genetic diversity at each locus, we constructed haplotype networks for each locus using TCS 1.21 (Clement et al., 2000). Closed loops in the haplotype network were resolved by comparison to a maximum likelihood tree estimated in PAUP* 4.0 (Swofford, 2003).

Results

The results of our tests for population expansion and deviation from neutral model expectations, along with diversity measures, are summarized in Tables 1, 2, 3 and graphically illustrated in Figures 2 and 3. Differences among species in the number of bases sequenced had little-to-no effect on results, based on

comparisons with analyses conducted on data sets confined to only shared nucleotide positions (data not shown).

Table 1 Molecular diversity summary statistics

Full size table

Table 2 Mismatch and neutrality tests

Full size table

Table 3 Pairwise difference in haplotype diversity (*h*) among species and maximum within-species sequence divergence

Full size table

Figure 2

Haplotype networks of the *cytb*, *MC1R* and *RAG1* genes for four species of lizards from the Mojave National Preserve. Circle size is proportional to the number of samples of a given haplotype. Lines between haplotypes represent mutational steps between haplotypes. The solid dots on the lines represent unobserved, inferred haplotypes.

Full size image

Figure 3

Haplotype diversities (*h*) for the *cytb*, *MC1R* and *RAG1* genes for four species of lizards from the Mojave National Preserve. Error bars represent 95% confidence intervals around the point estimates of *h*. *Indicates statistically significantly higher diversities for comparisons between high- and low-density species based on confidence interval overlap (Cumming and Finch, 2005). An additional asterisk indicates statistically significantly higher diversities within high-density species.

Full size image

The mitochondrial locus—*cytb*

The high-density species *C. draconoides* and *C. variegatus* show higher levels of genetic diversity for *cytb* than do the low-density species *S. ater* and *D. dorsalis*. This is true for all measures of diversity. The number of variable sites is 2.5–9.5 times greater in the high-density species (Table 1), resulting in much more complex unrooted haplotype networks (Figure 2). Nei's and Watterson's estimates of θ are also much higher, although not significantly so because of the large 95% confidence intervals. Haplotype diversity is significantly higher for the high-density species (Table 1, Figure 3) and similar to the previously published value for *U. stansburiana*, the other abundant lizard species (Micheletti et al., 2012).

We have no evidence that selection is operating on *cytb*. The ratio of synonymous to nonsynonymous base changes is much higher in all species except *D. dorsalis*, which has only one variable base (Table 1). Tajima's *D* values are not significantly different from 0 for any of the species (Table 2). Pairwise mismatch statistics suggest that only *S. ater* has a stable population (P=0.01) and that *D. dorsalis*, *C. draconoides* and *C. variegatus* are all undergoing population expansion (Table 2). The sum of squared deviation test gave the same results as the raggedness statistic for all loci and is therefore not shown. The lack of significant Tajima's *D* values is not consistent with population expansion. Because the raggedness statistic tests the null hypothesis of population expansion, the inability to reject that hypothesis may reflect a lack of power rather than a true population expansion.

The autosomal loci—*MC1R* and *RAG1*

The high-density species show higher levels of autosomal genetic diversity than do the low-density species for all metrics. Molecular diversity at the *MC1R* locus was surprisingly high for the two high-density lizard species and for *U. stansburiana* (Micheletti et al., 2012), given that autosomal protein-coding loci are normally less variable than mitochondrial loci. In fact, *MC1R* haplotype diversity estimates were greater than the mitochondrial *cytb* values for three of the species, *S. ater*, *D. dorsalis* and *C. draconoides*, although these differences were not statistically significant. As was the case for *cytb*, *C . variegatus* and *C. draconoides MC1R* haplotype diversities were significantly larger than those of both *S. ater* and *D. dorsalis*.

We have no evidence that selection is operating on *MC1R*. The ratio of synonymous to nonsynonymous base changes is much higher in all species (Table 1). As was the case with *cytb*, Tajima's *D* values are not significantly different from 0 for any of the species (Table 2) and only *S. ater* has a significant raggedness statistic for the pairwise mismatch distribution (Table 2).

In lizard studies, *RAG1* is typically used for phylogenetic analyses, including studies of the deeper taxonomic relationships among squamate reptiles (for example, Townsend et al., 2004). Yet we found

relatively high levels of within-population genetic variation at this locus. Levels of genetic diversity for *RAG1* are similar to those of the two other genes but with a few important differences. As with the other two genes, the high-density species have more variable sites and higher point estimates of θ and haplotype diversity (Table 1). However, the low-density species have higher haplotype diversities at *RAG1* compared with the other genes, while *C. draconoides* has a somewhat lower value (h=0.817). As a result, only *C. variegatus* has a haplotype diversity value that is significantly higher than that of the other species (Figure 3). Application of the z-score test does show that haplotype diversity of *C. draconoides* is significantly greater than *D. dorsalis* (P=0.04) using a significance threshold of P=0.05, but the test agrees with the method of Cumming and Finch (2005) if the threshold is Bonferroni corrected for the 18 pairwise comparisons among our species (threshold P=0.028).

For *RAG1*, Tajima's *D* and the value of the raggedness index of the pairwise mismatch distribution are not significantly different from 0 for any species. Unlike the other loci, the number of nonsynonymous base changes exceeds the number of synonymous base changes for three of the species, *D. dorsalis*, *C. draconoides* and *C. variegatus*. For these species, nonsynonymous-to-synonmous ratios of polymorphisms range from 1.4 to 3.5. This may suggest the operation of balancing selection; however, Tajima's *D* was not significantly different from neutral expectations for any species. In most instances, the two amino acids associated with a nonsynonymous mutation shared similar physical, chemical or structural properties, based on the criteria in Taylor (1986). For example, *D. dorsalis* and *C. draconoides* had a limited number of nucleotide positions (three and one, respectively) with mutations that resulted in a switch between two amino acids with different, unique properties. *C. variegatus* was higher, with five nonsynonymous base changes associated with a switch between dissimilar amino acids. The question remains of whether the *RAG1* amino-acid changes are neutral or adaptive.

Discussion

In general, the differences in genetic diversity we observed among species were consistent with the predictions of neutral theory for both mitochondrial and autosomal loci. Patterns of polymorphism were largely consistent with a neutral model of sequence evolution, and the high-density species had significantly higher levels of genetic diversity than the less common ones. These results illustrate that sympatric species can differ, sometimes dramatically, in diversity. The high haplotype diversities observed in *C. draconoides* and *C. variegatus* compared with those of *S. ater* and *D. dorsalis* could be due to several factors. Foremost, the large populations of *C. draconoides* and *C. variegatus* at the MNP are likely an important contributing factor to the high molecular diversity. *Calisaurus draconoides* was one of the most frequently encountered lizard species in a survey of reptiles and amphibians on the MNP (Persons and Nowak, 2007). Espinoza (2009) states that *C. variegatus* '...is among the most frequently encountered desert reptiles in the Southwest'. From our field experience at the collecting site, covering more than 19 years, we are confident that *C. draconoides* and *C. variegatus* are both very abundant in the MNP, especially given the widespread distribution of favorable habitat for both species. These two small-bodied species also have many demographic similarities to *U. stansburiana*, the aforementioned species with high levels of genetic diversity at the same location in the MNP. *U. stansburiana* is by far the most abundant lizard in the MNP (Persons and Nowak, 2007).

Conversely, *S. ater* and *D. dorsalis* are undoubtedly less abundant at the preserve. Despite being large and easily observed, both species had low encounter rates in the MNP inventory (Persons and Nowak, 2007). At the MNP, *S. ater* are found exclusively in rocky areas with large boulders, a habitat which occupies a much smaller geographic area than habitat favored by *C. draconoides, C. variegatus* and *U. stansburiana*. Despite the prominence of suitable habitat for *D. dorsalis* at the site, our observations suggest that the species is much less abundant than the small-bodied lizard species. Daytime encounter rates for *D. dorsalis* are much lower than for *U. stansburiana* and *C. draconoides*.

Consequently, the high- and low-density species almost certainly also differ in effective population size at MNP. Under a neutral model, populations with a larger effective population size (N_e) are expected to have a greater number of neutral mutations (Kimura, 1983). A population's genetic variation at a particular locus is dictated by its effective population size and the gene's mutation rate (μ). The probability of heterozygosity (that is, haplotype diversity) is

where $\theta=4N_e\mu$ (autosomal genes) or $\theta=N_e\mu$ (mitochondrial genes). Effective population size is also the critical variable that determines genetic diversity in coalescent models (Charlesworth, 2009). Although we believe the high-density species have larger effective population sizes than the low-density ones, we do not report coalescent effective population size estimates. This study, along with all studies examining the role of population size on genetic diversity, is constrained to use of census population size as a proxy for the coalescent effective population size. Without long-term pedigree data, the only way to estimate coalescent effective population size is through simulations with empirical genetic diversity data, using models that assume an inherent relationship between genetic diversity and population size (that is, the relationship we seek to test). Therefore, we avoid estimates of effective population size for the purposes of this study. However, if the biogeographic histories of the lizard species at MNP are roughly similar, census population size should be roughly proportional to effective population size by a similar degree for all four species. We believe this is a reasonable assumption, given our samples were collected from sympatric populations.

Generation time may be another demographic factor contributing to differences in genetic diversity in the four species. Longer generation times mean there are fewer generations between the present and any past causes of reduced genetic variation, such as founder events or population bottlenecks. If such events occurred at similar times in the past for all species, recovery of genetic polymorphism would take longer in the less abundant species. Both *S. ater* and *D. dorsalis* have greater longevity and longer generation times than the more diverse species. Estimates for *S. ater* longevity range up to 15 years in the wild, and juveniles may take up to 3 years to reach sexual maturity (Sullivan and Sullivan, 2012). *D. dorsalis* also has a late maturation, with longevity estimates ranging from 7.5 to 14 years (Krekorian, 1984). The high-density species for which we have genetic data (*C. draconoides, C. variegatus, U. stansburiana*) tend to mature in <1 year and have much lower annual survival than the less genetically diverse species (Tanner and Krogh, 1975). The combination of shorter generation times and larger population sizes are probably important demographic contributors to the higher neutral genetic diversity observed in *C. draconoides, C. variegatus* and *U. stansburiana* compared with *S. ater* and *D. dorsalis*.

Surrounding populations at MNP may also contribute to increased diversity through gene flow and population mixing. High levels of gene flow can produce high within-population diversity by increasing the effective population size of a local population. Because the habitat suitable for *C. draconoides*, *C. variegatus* and *U. stansburiana* is so widespread in this region of the Mojave Desert, there is potential for gene immigration from tens or even hundreds of kilometers from our sample sites. Alternatively, gene flow may be limited in the less abundant species. For example, *S. ater* migration is probably restricted at the MNP owing to the long distances between rocky outcrops. For *D. dorsalis*, estimates of home range in the literature range up to 0.20 hectares (Krekorian, 1976). However, similar to the abundant species, *D. dorsalis* has a large amount of suitable habitat at the MNP and surrounding regions. Therefore, limited gene flow by itself cannot explain the lower genetic diversity in both *S. ater* and *D. dorsalis*. The contribution of gene flow from other populations to genetic diversity can only be evaluated by genetic studies of surrounding populations and estimation of gene flow rates.

Other factors unrelated to demography may also be responsible for the differences in genetic diversity among the lizard species. Two of the high-density/high genetic diversity species (*C. draconoides* and *U. stansburiana*) and the two low-density/low genetic diversity species (*S. ater* and *D. dorsalis*) are all in the Pleurodonta section of the Iguania; however, *U. stansburiana* and *C. draconoides* are in the family Phrynosomatidae and *S. ater* and *D. dorsalis* are in the Iguanidae. Thus population size is confounded with phylogeny at the family level. This is somewhat mitigated by the fact that we also found high genetic diversity in *C. variegatus*, despite this species being in the Gekkota family Eublepharidae, a phylogenetically divergent group in relation to Iguania (Pyron et al., 2013). Ultimately, the effects of phylogeny can only be ruled out if the correlation between population size and diversity is consistent across multiple taxonomic comparisons. At the MNP, we are currently sampling other species in a variety of squamate families, including Phrynosomatidae, Crotaphytidae, Teiidae, Colubridae and Crotalidae, in order to increase taxonomic representation and test whether the relationship between population size and genetic diversity holds across families within the Squamata.

Taken together, our data are consistent with neutral models that predict a relationship between genetic diversity and population size. Alternative evolutionary forces have been proposed as major drivers of differences in population genetic diversity, for example recurrent selection and ‘genetic draft’ (Smith and Haigh, 1974; Gillespie, 2001; Bazin et al., 2006). Although we cannot rule out the importance of other such forces, our data are predominantly consistent with the predictions of neutral theory. Ultimately, no one study can validate the generality of a relationship between genetic diversity and population size. Our current and future work on MNP reptiles must be replicated from taxonomically independent sets of closely related sympatric species sampled from a variety of different higher taxa. Moving forward, studies should also utilize next-generation sequencing technology in order to survey a large number of genes throughout the genome. This study of reptiles represents a single step in the process of resolving the evolutionary forces that maintain genetic diversity in natural populations.

Data archiving

DNA sequences: Genbank accession numbers: KR026343–KR026902. Lizard sample locality data, with associated Genbank numbers, available from the Dryad Digital repository: http://dx.doi.org/10.5061/dryad.g7d1r

References

1. Amos W, Harwood J . (1998). Factors affecting levels of genetic diversity in natural populations. *Philos Trans R Soc Lond B Biol Sci* **353**: 177–186.

   CAS Article Google Scholar

2. Bazin E, Glémin S, Galtier N . (2006). Population size does not influence mitochondrial genetic diversity in animals. *Science* **312**: 570–572.

   CAS Article Google Scholar

3. Bustamante CD, Fledel-Alon A, Williamson S, Nielsen R, Todd Hubisz M, Glanowski S *et al*. (2005). Natural selection on protein-coding genes in the human genome. *Nature* **437**: 1153–1157.

   CAS Article Google Scholar

4. Charlesworth B . (2009). Effective population size and patterns of molecular evolution and variation. *Nat Rev Genet* **10**: 195–205.

   CAS Article Google Scholar

5. Clement M, Posada D, Crandall KA . (2000). TCS: a computer program to estimate gene genealogies. *Mol Ecol* **9**: 1657–1659.

   CAS Article Google Scholar

6. Cumming G, Finch S . (2005). Inference by eye: confidence intervals and how to read pictures of data. *Am Psychol* **60**: 170–180.

   Article Google Scholar

7. Cutter AD, Payseur BA . (2013). Genomic signatures of selection at linked sites: unifying the disparity among species. *Nat Rev Genet* **14**: 262–274.

   CAS Article Google Scholar

8. Espinoza RE . (2009). Western banded gecko. In: Jones LC, Lovich RE (eds). *Lizards of the American Southwest*. Rio Nuevo Publishers: Tucson, AZ, USA, pp 312–315.

   Google Scholar

9. Excoffier L, Lischer HEL . (2010). Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Mol Ecol Resour* **10**: 564–567.

   Article Google Scholar

10. Fay JC, Wu CI . (2003). Sequence divergence, functional constraint, and selection in protein evolution. *Annu Rev Genomics Hum Genet* **4**: 213–235.

    CAS Article Google Scholar

11.Garrick RC, Sunnucks P, Dyer RJ . (2010). Nuclear gene phylogeography using PHASE: dealing with unresolved genotypes, lost alleles, and systematic bias in parameter estimation. *BMC Evol Biol* **10**: 118.

Article Google Scholar

12.Gillespie JH . (2001). Is the population size of a species relevant to its evolution? *Evolution* **55**: 2161–2169.

CAS Article Google Scholar

13.Harpending HC . (1994). Signature of ancient population growth in a low-resolution mitochondrial DNA mismatch distribution. *Hum Biol* **66**: 591–600.

CAS PubMed PubMed Central Google Scholar

14.Jones L, Lovich R (eds). (2009) *Lizards of the American Southwest*. Rio Nuevo Publishers: Tucson, AZ, USA.

Google Scholar

15.Kimura M . (1983) *The Neutral Theory of Molecular Evolution*. Cambridge University Press: Cambridge [Cambridgeshire]; New York, NY, USA.

Google Scholar

16.Kreitman M . (1996). The neutral theory is dead. Long live the neutral theory. *Bioessays* **18**: 678–683.

CAS Article Google Scholar

17.Krekorian CO . (1976). Home range size and overlap and their relationship to food abundance in the desert iguana, *Dipsosaurus dorsalis*. *Herpetologica* **32**: 405–412.

Google Scholar

18.Krekorian CO . (1984). Life history of the desert iguana, *Dipsosaurus dorsalis*. *Herpetologica* **40**: 415–424.

Google Scholar

19.Leffler EM, Bullaughey K, Matute DR, Meyer WK, Ségurel L, Venkat A *et al*. (2012). Revisiting an old riddle: what determines genetic diversity levels within species? *PLoS Biol* **10**: e1001388.

CAS Article Google Scholar

20.Lewontin RC . (1974) *The Genetic Basis of Evolutionary Change*. Columbia University Press: New York, USA.

Google Scholar

21.Lynch M . (2010). Evolution of the mutation rate. *Trends Genet* **26**: 345–352.

CAS Article Google Scholar

22.Martin AP, Palumbi SR . (1993). Body size, metabolic rate, generation time and the molecular clock. *Proc Natl Acad Sci USA* **90**: 4087–4091.

CAS Article Google Scholar

23.Micheletti S, Parra E, Routman EJ . (2012). Adaptive color polymorphism and unusually high local genetic diversity in the side-blotched lizard, *Uta stansburiana*. *PLoS One* **7**: e47694.

CAS Article Google Scholar

24.Nabholz B, Mauffrey J-F, Bazin E, Galtier N, Glémin S . (2008). Determination of mitochondrial genetic diversity in mammals. *Genetics* **178**: 351–361.

Article Google Scholar

25.Nachman MW . (1997). Patterns of DNA variability at X-Linked loci in *Mus domesticus*. *Genetics* **147**: 1303–1316.

CAS PubMed PubMed Central Google Scholar

26.Nei M . (1987) *Molecular Evolutionary Genetics*. Columbia University Press: New York, NY, USA.

Google Scholar

27.Perry GH, Melsted P, Marioni JC, Wang Y, Bainer R, Pickrell JK *et al*. (2012). Comparative RNA sequencing reveals substantial genetic variation in endangered primates. *Genome Res* **22**: 602–610.

CAS Article Google Scholar

28.Persons TB, Nowak EM . (2007) *Inventory of Amphibians and Reptiles at Mojave National Preserve: Final Report*. US Geological Survey: Flagstaff, AZ, USA.

Google Scholar

29.Pyron RA, Burbrink FT, Wiens JJ . (2013). A phylogeny and revised classification of Squamata, including 4161 species of lizards and snakes. *BMC Evol Biol* **13**: 93.

Article Google Scholar

30.Rogers AR, Harpending H . (1992). Population growth makes waves in the distribution of pairwise genetic differences. *Mol Biol Evol* **9**: 552–569.

CAS PubMed PubMed Central Google Scholar

31.Romiguier J, Gayral P, Ballenghien M, Bernard A, Cahais V, Chenuil A *et al*. (2014). Comparative population genomics in animals uncovers the determinants of genetic diversity. *Nature* **515**: 261–263.

CAS Article Google Scholar

32. Smith JM, Haigh J . (1974). The hitch-hiking effect of a favourable gene. *Genet Res* **23**: 23–35.

CAS Article Google Scholar

33. Stephens M, Smith NJ, Donnelly P . (2001). A new statistical method for haplotype reconstruction from population data. *Am J Hum Genet* **68**: 978–989.

CAS Article Google Scholar

34. Sullivan BK, Sullivan KO . (2012). Common chuckwalla (Sauromalus ater) in an urban preserve: persistence of a small population and estimation of longevity. *Herpetol Conserv Biol* **7**: 437–441.

Google Scholar

35. Swofford DL . (2003) *PAUP* Phylogenetic Analysis Using Parsimony (*and Other Methods)*. Sinauer Associates: Sunderland, Massachusetts, USA.

Google Scholar

36. Tajima F . (1983). Evolutionary relationship of DNA sequences in finite populations. *Genetics* **105**: 437–460.

CAS PubMed PubMed Central Google Scholar

37. Tajima F . (1989). The effect of change in population size on DNA polymorphism. *Genetics* **123**: 597–601.

CAS PubMed PubMed Central Google Scholar

38. Tanner WW, Krogh JE . (1975). Ecology of the zebra-tailed lizard *Callisaurus draconoides* at the Nevada Test Site. *Herpetologica* **31**: 302–316.

Google Scholar

39. Taylor WR . (1986). The classification of amino acid conservation. *J Theor Biol* **119**: 205–218.

CAS Article Google Scholar

40. Townsend TM, Larson A, Louis E, Macey JR . (2004). Molecular phylogenetics of Squamata: the position of snakes, amphisbaenians, and dibamids, and the root of the squamate tree. *Syst Biol* **53**: 735–757.

Article Google Scholar

41. Watterson G . (1975). On the number of segregating sites in genetical models without recombination. *Theor Popul Biol* **7**: 256–276.

CAS Article Google Scholar

Download references

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Share

A Florida scrub jay population relies on birds from other groups to sustain its genetic diversity.

Tim Zurowski/SHUTTERSTOCK.COM

Boosting genetic diversity may save vanishing animal populations. But it may also backfire

By Elizabeth PennisiJul. 16, 2019 , 5:55 PM

PROVIDENCE—The expanding global human footprint is dividing the world's flora and fauna into ever-smaller, more isolated populations that could wink out because of inbreeding, disease, or environmental change. For decades, conservationists have proposed revitalizing those holdouts by bringing in new blood from larger populations. But they've wondered whether it really works—and how to do it without swamping the genetic identity and unique adaptations of the group at risk. Last month at Evolution 2019 here, researchers described how genomic tools are refining what is known as genetic rescue.

Although zoos have worked to maintain genetic diversity in endangered species by carefully matching individual animals for breeding, the strategy has rarely been tried in nature. Genetic rescue "should be attempted more frequently," Andrew Whiteley, a conservation genomicist at the University of Montana in Missoula, and his colleagues wrote last week in *Trends in Ecology and Evolution*. But showing that

it works requires tracking multiple generations for years, something few studies have attempted. And researchers have only recently been able to detect what happens on a molecular level. Now, says Sarah Fitzpatrick, an evolutionary biologist at Michigan State University's (MSU's) W. K. Kellogg Biological Station in Hickory Corners, "We have genomic tools to study these populations … in ways we never could before."

Adding new blood to small populations really does help, a long-term experimental evolution study of wild guppies in Trinidad has demonstrated, says Brendan Reid, an MSU conservation biologist who works with Fitzpatrick. Decades ago, researchers seeded the headwaters of two streams in the mountainous country with guppies taken from a distant habitat. In one stream, the displaced fish had to travel a long way and only slowly made their way downstream to a small, isolated population. In the other stream, the fish more quickly joined another isolated group. Every month for 2.5 years, Fitzpatrick and her colleagues caught, marked, and studied all the fish they could find at the isolated groups' territories before returning the fish to the streams. They tracked the growth, survival, and genetic diversity of the fish over about seven generations.

In both streams, the populations increased 10-fold and genetic diversity doubled. Later generations were more fecund, with many of the most fit offspring being hybrids of the local and introduced fish, Reid reported at the meeting. But the findings also sounded a note of caution. In the second stream, the rapid infusion of new fish almost completely eliminated pure residents—an outcome conservationists usually hope to avoid. That result suggests "a slow trickle of immigration might be preferable," Fitzpatrick says.

Another genomic study showed some small populations experience natural genetic rescue—and benefit from it. Nancy Chen, a population geneticist at the University of Rochester in New York, and her team study the threatened Florida scrub jay (*Aphelocoma coerulescens*), whose numbers are down to a few thousand individuals, split among a few hundred sites. For 50 years, researchers have regularly counted and assessed all the jays found at Archbold Biological Station near Lake Placid, Florida. More recently, they've collected blood samples from each bird, which enabled Chen and her colleagues to track genetic changes over time.

The team discovered that the population naturally gets a slow infusion of new blood. Typically, birds trickle in from smaller groups a few kilometers away. The newcomers are less genetically diverse than those already there, but because they are from a different population, they help maintain the resident group's diversity. However, with fewer birds arriving in recent years because of population declines, that diversity is declining, putting the population at risk of dying out. "Gene flow from small populations may be really important," she concluded at the meeting.

Most biologists have assumed that larger populations are better sources of new blood. But Chris Kyriazis, a graduate student at the University of California, Los Angeles, used computer models to study the impact of deleterious mutations hidden in a source population. Because such mutations tend to be harmful only when both parents pass the mutation to offspring, they are likely to be eliminated from historically small, inbred populations and to persist in larger ones. Kyriazis's modeling suggests intermediate-size populations, not the biggest ones, could be the best source for genetic rescues, he reported at the meeting and in a preprint posted 21 June on bioRxiv.

Sometimes, genomic results suggest the rescue strategy may backfire. Just 1000 island foxes (*Urocyon littoralis*) are left on California's Santa Catalina Island, and 60% of them have a cancer that affects their ears. Paul Hohenlohe, an evolutionary biologist from the University of Idaho in Moscow, had identified many genes that make the foxes susceptible to the cancer and wondered whether they were a candidate for genetic rescue. But he found that the Santa Catalina foxes have a genetic advantage over neighboring populations that might be sources of new blood: They have more variation throughout their genome, including in the cancer genes, he reported at the meeting. Furthermore, the Santa Catalina foxes are better adapted to the island's hot, arid climate than the other foxes, many of which live on wetter, cooler islands. So, he recommends letting nature take its course and monitoring whether the foxes eventually evolve resistance to the cancer.

These studies are helping invigorate a strategy that many believe is sorely needed. Fitzpatrick says, "The urgency of the problem and the availability of the tools makes it a really exciting time."

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Elizabeth Pennisi

Effects of Population Control Strategies on Retention of Genetic Diversity in National Park Service Bison (*Bison bison*) Herds

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Table of Contents

Abstract

We simulated the dynamics of bison herds inhabiting National Park Service (NPS) units to evaluate the consequences of management actions on retention of genetic diversity. We used an individual-based model to evaluate the effects of management strategies on the retention of genetic heterozygosity (H_0), retention of alleles, and on herd sex and age structure. To identify general recommendations that could be applied across conditions typical of captive bison herds, we estimated vital rates of herds occupying harsh, average, or good ranges, and we used these vital rates to drive simulations with herd size targets of 200 to 2000 animals. Simulations were initialized with data from observations of microsatellite allele frequencies obtained from NPS bison herds (Halbert 2003). We examined the effects of removal of bison that were young, old, or a random selection of ages, and removals that contained a high proportion of cow-calf groups (24% or 50% of animals removed). We also evaluated the effects of using contraceptives applied to young, old, or a random selection of breeding-age cows. Over the 200-year period of the simulations, herd size accounted for more variation in retention of H_0 and loss of alleles than any other factor. Based on Monte Carlo analysis of 500 replicate simulations, bison herds with more than 400 animals generally met the objective of achieving a 90% probability of retaining 90% of the herd's H_0 for 200 years. Differences in generation time accounted for about 75% of the variation in retention of H_0 in herds of 200-800 bison. When allelic diversity was used as the key criterion for evaluating management alternatives, a population size of about 1000 animals was needed to achieve a 90% probability of retaining 90% of alleles. Under simulated conditions, the choice of population control strategies can have large impact on retention of genetic variation when population sizes are small, but population control strategies have far less influence as population sizes increase. Population control strategies that increase generation time, such as removal or contraception of young animals, most effectively retain genetic variation. Population control strategies had huge effects on the age and sex composition of bison herds.

Introduction

Human activities have profoundly influenced the Earth's natural resources. Foremost among man's effects has been the fragmentation of historically large and contiguous habitats, and the associated transformation of large and extensive populations into a number of smaller, isolated populations. Long-term management of small populations presents special problems associated with random population processes that can lead to skewed sex ratios, genetic drift, founder effects, loss of genetic variation, and expression of deleterious alleles. Populations with fewer than 500 breeding individuals are thought to be especially susceptible to harmful consequences of inbreeding depression and other effects that can be directly traced to the genetic composition of the populations (Frankham 1995; Keller and Waller 2002).

Biologists are concerned about the genetic health of bison (*Bison bison*) herds because all North American herds were founded by few individuals and they have generally been maintained at small population sizes (Boyd 2003). National Park Service (NPS) bison herds were established from groups of about 20 to 50 bison (Halbert 2003:16) and NPS herds have largely been managed to maintain a size of fewer than 1000 animals. The small size and isolation of bison herds has led to concerns about their long-term genetic health. Expressions of inbreeding depression are now well documented in many wild vertebrate populations (Keller and Waller 2002), and considerable attention has been directed towards identifying general guidelines for

the genetic management of small, isolated, and/or intensively managed populations. Key questions focus on the minimum effective population size needed to avoid loss of genetic variation and inbreeding depression, population control strategies to minimize harmful genetic consequences, and on the rates of animal movements between isolated populations needed to achieve an adequate rate of gene flow. General recommendations to managers were based on landmark studies by Wright (1931, 1969) that led to the "one-migrant-per-generation" rule. Further studies suggested that populations with a genetically effective population size (N_e) of 50 to 500 were secure (Meffe and Carroll 1995:171), where N_e is the size of an ideal population composed of randomly breeding individuals (See Hartl and Clark [1997:289] or similar for a more complete, technical definition of N_e).

While the one-migrant-per-generation and minimum size rules have been widely publicized and adopted, these rules remain controversial and difficult to implement. A realistic evaluation of the one-migrant-per-generation rule requires an understanding of the many assumptions on which the rule is based, and it is widely acknowledged that many of these assumptions are unrealistic (Frankham 1995; Mills and Allendorf 1996; Vucetich and Waite 2001; Wang 2004). Most theoretical analyses in population genetics require estimation of N_e, but N_e is notoriously difficult to estimate in real populations because it is strongly affected by variation in population attributes such as sex ratio, age-specific breeding success, and fluctuations in population size (Harris and Allendorf 1989; Shull and Tipton 1987). Since all real populations exhibit variation in these factors, accurate estimation of N_e is usually intractable (Harris and Allendorf 1989). For bison, N_e/N has most commonly been estimated to be between 0.2 and 0.35 (Shull and Tipton 1987; Berger 1996; Wilson and Zittlau 2004), although Shull and Tipton (1987) estimated that N_e/N could be as low as 0.09 in managed herds. Uncertainty in our understanding of mating behaviors of bison, variation in age structure, and other complications result in high degree of uncertainty in estimates of N_e/N. For large ungulates, especially those that exhibit a dominance hierarchy (i.e., high variation in individual breeding success), large uncertainty in estimates of N_e reduces the usefulness of N_e for designing and evaluating realistic management alternatives, such as the one-migrant-per-generation or minimum size rules.

The plains bison (*Bison bison*) is an exemplar species for examining genetic conservation of a charismatic large mammal. Wild bison once roamed vast areas of North America in huge herds and the total population is estimated to have consisted of millions of individuals. These huge populations were decimated by hunters, and by the late 1800s bison were restricted to a few herds with a total of fewer than 1,000 animals (Hornaday 1913; Seaton 1937). This population reduction represents a genetic bottleneck of epic proportions. The subsequent "recovery" of the species is reasonably well documented. Recent analyses of the genetic composition of bison herds have shown that some individuals in most herds contain genetic material that can be unambiguously attributed to hybridization with domestic cattle (Halbert 2003). Bison herds thought to be free of cattle genes are mostly small, and the long-term genetic health of these herds is a serious management concern. Management of captive bison herds is further complicated because some bison herds are infected with *Brucella abortus*, a bacteria that is the causative agent for brucellosis. Recent studies revealed that low levels of inbreeding – levels previously though to be insignificant – were very highly correlated with susceptibility to bacterial disease in sea lions (*Zalophus californianus*) (Acevedo-Whitehouse *et al.* 2003). These results suggest that the effects of genetic depression in wild populations may be much more widespread than previously thought. Bison are hosts to a wide variety of diseases (Williams and

Barker 2001), and transfers of bison between herds have been restricted by regulations designed to inhibit the spread of disease.

Our objectives were to identify options for managing the bison populations inhabiting National Park units and to evaluate the relative consequences of management actions on retention of genetic diversity. We focused our evaluations on population attributes and management strategies that might influence decisions on management in the near future. In addition, we wanted to identify recommendations that applied generally to bison herds and other managed herds of large mammals. To achieve these goals, we constructed an individual-based population model that simulated the dynamics of bison herds and their responses to management actions. We developed sets of model parameters that represented herds in habitats that were harsh, average, or good, and we evaluated interactions between management strategies and herd characteristics.

Methods

Our model operated on an annual time step and explicitly represented breeding, recruitment, removals, contraceptive treatments, natural mortality, and aging. The sex, age, breeding status, number of matings, and genetic composition of each individual were explicitly represented in the model.

Demographic processes and parameter estimation

Our model simulated the demographic processes of birth and death by comparing age and sex-specific probabilities of mating, birth, and death to a random number drawn from a uniform 0 -1 distribution. Breeding was simulated by first determining which cows would breed and then selecting a bull for mating. Age-specific breeding rates of bulls (Figure 1) were estimated from Berger and Cunningham (1994: 189) and Wilson et al. (2002). Data on breeding rates by bulls are extremely limited and we thus developed parameter estimates from available literature and interviews with bison herd managers. We then tested additional, hypothetical breeding rates to examine the sensitivity of model results to changes in this vaguely defined function. For all parameter sets we evaluated, calculations of simulated lifetime breeding rates showed that almost all prime-aged bulls breed, though individuals varied in the number of offspring they sired. In a specific breeding event, the likelihood that a particular bull mated with a cow was determined by the number of breeding-age bulls in the population and the age-specific probability of mating of each male. Data on other factors that may influence lifetime breeding success of bison bulls, such as size, social status, mating group size, etc., are poorly documented and were not included in the model.

For each breeding pair of bison, Mendelian inheritance of selectively neutral alleles was simulated by selecting one allele from each parent at each of the loci simulated. The model was initialized with 3-10 alleles at each of 51 autosomal loci, based on frequencies reported by Halbert (2003). Initial gene frequencies ranged from 0.001 to 1.0 (e.g., from an allele in only one individual to an allele carried by all herd members) based on data from bison herds in National Parks.

Vital rates of bison were estimated from population surveys conducted by NPS biologists and from observed growth rates of bison herds (Meagher 1973; Berger and Cunningham 1994;

Kirkpatrick *et al.* 1996). Although data were not suitable for highly detailed analyses, vital rates clearly differed among herds and we estimated three sets of vital rates that characterized herds in harsh, average, and good habitats, with corresponding rates of fecundity and survival (Figure 1). Vital rates for the harsh, average, and good habitats roughly correspond to observations from the central Yellowstone (YELL) bison herd, Badlands (BADL), and Grand Teton (GRTE) National Parks, respectively. Survival rates at GRTE and BADL appear to be high compared to wild populations, presumably due to supplemental feed (GRTE) and relatively mild winters. Except for YELL and GRTE bison, all bison herds under the jurisdiction of the Department of Interior are subjected to intensive management programs that maintain herd sizes thought to be well below the long-term carrying capacity of the occupied range. In these populations, very high survival and breeding rates indicated that density dependence was of little importance. In YELL, bison respond to increased density mainly by increasing the area used in winter (Taper *et al.* 2000). Because there was considerable uncertainty in estimates of bison vital rates, we conducted a sensitivity analysis to evaluate the potential influence of variation in vital rates on simulation results. When vital rates and management treatments varied within realistic limits, the only significant difference we noted was in the number of animals that needed to be treated to limit population size.

Model treatments

Simulations were conducted to evaluate management strategies that focused on the fundamental decisions that managers confront when developing a strategy to control size of bison populations. Treatments were based on target population size (how many animals?), the type of intervention used to attain the population size target (removal or apply contraceptives?), and which animals to treat (how many males and females, and of what age?).

Removal or contraceptive treatments were simulated by applying rules based on current population size, post-treatment population objective, sex and age of animals to be treated, and the minimum number of animals in each sex/age class that were to remain unaffected by the treatment. To determine the annual control treatment, the population was first compared to size thresholds used to categorize the population as small, normal, or large. If the population size was less than or equal to the lower threshold, it was categorized as small. If the population size was greater than the lower threshold and less than or equal to the higher threshold, it was categorized as normal. If greater than the upper threshold it was considered large.

Removal treatments were categorized as young, old, or random for the age of animals emphasized in the treatment. Some random treatments selected bison randomly with regard to sex or age until the population objective was reached, and some random treatments controlled for sex ratio and selection was completely random only for age. For old animal treatments, the oldest animals in the population were selected first, whereas young treatments first removed the youngest animals first. For both treatments, a minimum of 10 animals (or those left after natural mortality) were left in each yearly age class up to 9 years, and 5 animals in each age class up to 20 years.

The proportion of cows treated with contraceptives varied in response to vital rates of the population. For each contraception treatment, the level of contraception was initially calibrated to achieve a relatively stable population size. The baseline rate of contraceptive treatment of cows was 60% for YELL and 80% for other populations. For all treatments, the application rate

of contraceptives was increased or decreased 15% when population size was less than or greater then the target, respectively. Contraceptive treatments were administered every year and treated cows remained infertile for one year. As with removals, cows were selected for contraceptive treatments based on age, using rules that selected breeding-age cows randomly, or that selected the oldest or youngest cows first.

Cow-calf removal treatments selectively removed cows with their calves. Halbert (2003) estimated that 24% of the bison harvested from YELL were cow-calf pairs, while Shaw and Carter (cited in Shull and Tipton 1987) estimated that roundup procedures for captive populations resulted in capture of about 50% of cows with their calves. Our reference treatments used these rates – 24% cow-calf pairs for YELL and 50% for other herds – and we conducted sensitivity analyses by varying the proportion of cow-calf pairs removed (Appendix 1). The procedure used for reference simulations of cow-calf removals (reported below) was to first calculate the number of animals to be removed, then remove the target proportion of cow-calf pairs from the population. After removal of cow-calf pairs, other animals were selected for removal by following rules for the random removal treatment.

Evaluation of model output

Studies of bison and other species have established a well-accepted relationship between genetic heterozygosity (H_0) and various components of inbreeding depression (Reed and Frankham 2003; for bison: Halbert *et al. in press*). Following Hartl and Clark (1997) we calculated H_0 as

$$H_0 = \frac{\sum_{n=1}^{N} \frac{\text{heterozygotes at locus}_n}{\text{number of individuals}}}{N}$$

where N is the number of loci. Previous studies clearly showed that H_0 can be an insensitive indicator of many changes in genetic resources (Allendorf 1986; Gross 2000), and in particular, many rare alleles can be lost with little change in H_0. We therefore evaluated retention of alleles in response to simulation treatments.

There are currently no quantitative NPS or US Fish and Wildlife Service management objectives for conserving genetic diversity. Gross (2000) used a goal to achieve a 90% probability of maintaining 90% of the selectively neutral genetic variation for 200 years, following recommendations by Soule *et al.* (1986). This goal is consistent with U.S. Bureau of Land Management operational guidelines for wild horse management (Coates-Markle 2000) and we used it as the default evaluation criterion.

Generation time (T), an important variable determining the rate of loss of genetic diversity, can be estimated by a variety of methods. We followed Carey (1993: 86) and estimated generation time as

$$T = \frac{\sum_{x=0}^{\omega} x l_x m_x}{\sum_{x=0}^{\omega} l_x m_x}$$

where x is age (years), ω is the last possible age, l_x is survival from birth to age x, and m_x is the average number of offspring produced by a female in the interval x to $x + 1$.

Initial conditions and simulation procedures

We used data from Halbert (2003) for initial allele frequencies at the 51 autosomal loci simulated in these model experiments. Halbert (2003: 38) reported two to 11 microsatellite alleles per locus, with a total of 350 alleles. Two loci were fixed at THRO-North and one at GRTE, and initial H_0 in National Park bison herds varied from 0.517 – 0.654 (Halbert 2003: 40). We created initial populations that matched population size targets (200-2000 individuals). Observed heterozygosities in these initial populations were mostly within 1% of the values reported by Halbert and all were within 2%.

Senarios were evaluated from the results of 500 (main treatments) or 100 (some sensitivity analyses) Monte Carlo replicates, each lasting 200 years. Each replicate simulation was conducted with a unique set of random variates and the distribution of results was estimated from model outputs. Eight population size objectives were examined: 200, 300, 400, 500, 600, 700, 1000, and 2000 bison. These population objective treatments were crossed with population control treatments (removal or contraceptive) and with age-specific treatments.

Results

In general, results from the six parks varied in a consistent manner and the small differences between parks appeared to be related to the number and frequency distribution of alleles and (more importantly) differences in vital rates. To simplify the presentation of results, we generally report results of simulations using inputs estimated from bison in YELL and Theodore Roosevelt National Park, North Unit (hereafter THRO). Of all National Park bison herds (Halbert 2003: 40), the YELL herd had the highest proportion of all alleles, the second highest H_0, and the most severe environmental conditions. THRO North had the lowest proportion of all alleles, the lowest H_0, and relatively benign environmental conditions. These herds are thus most likely to exhibit the extremes in simulation results.

Treatments and demographic effects

Sets of parameters for vital rates resulted in average annual growth rates (λ) of 1.14, 1.22, and 1.22 for the poor, average, and good habitats, respectively. These growth rates are similar to those reported for the representative parks (YELL, GRTE, and BADL).

Removal and contraception treatments had dramatic and different effects on the age structure of herds (Figure 2). Removal of old animals resulted in populations that consisted almost entirely of animals less than 8 years old, while removal of younger animals resulted in populations with an unusually high proportion of older animals. Contraception treatments led to herds with an extremely even age structure. Target population size had no effect on demographic structure, and herd age structure was only slightly different between the simulations using the three sets of vital rates.

An important consequence of variation in age structure for populations managed by removals was a change in generation time (Figure 3). With a shift to older animals, a greater proportion of young were born to old cows, resulting in an increase in generation time. Generation time of

females ranged from less than 5 years when old animals were removed or treated with contraceptives, to a maximum of 12.7 years when contraceptives were applied only to young animals and virtually all breeding was by very mature, older cows. For contraceptive treatments, age of reproduction was determined by infertility treatment, and age of reproduction was a function of the age structure of the population. Changes in generation time for contraceptive treatments followed logically from treatments – a shift in breeding to younger animals by administering contraceptives to older animals led to a decrease in generation time, and generation time increased when contraceptives were administered to younger animals.

The proportion of the population that had to be removed or treated with contraceptives to achieve a target population size varied between treatments, but not with target population size. For contraceptive treatments, 60-65% of all cows were treated each year in average and good populations, and 40-45% of cows from YELL. In contrast, population control based on removals selecting for cow-calf groups required removal of only 7% to 14% of the population each year (for YELL with 50% cow-calf pairs in harvest, and THRO with 10% cow-calf pairs in harvest, respectively). Removal of animals of random age, old, or young animals required removal of an average of 16%, 13%, or 13% of animals, respectively, except for removal of young animals with a target size of 200. In this case, about 25% of animals were removed each year, apparently due to compromises necessary to maintain a small population while leaving an adequate number of animals in each age class. Differences in the proportion of the population that needed to be "treated" were clearly related to changes in sex and age structure of the population, and to the expected reproductive contribution (reproductive value) of animals removed. Treatments that shifted the sex ratio towards males or that increased the proportion of young (non-breeding) cows led to lower average population growth rates, thereby reducing the need for active population management.

Changes in genetic variation

There were large differences in retention of observed heterozygosity (H_0) between simulated herds with different population sizes and between management treatments (Table 1, Figure 4). Over the range of population sizes and treatments simulated, the effects of population size on retention of genetic variation were large relative to all treatments except contraception of young cows. In general, a minimum population size of about 400 was needed to meet the objective of retaining 90% of selectively neutral variation with a 90% probability for 200 years (Table 1, Figures 4, 5). However, it is important to recognize that these results are based on simulations that precisely implemented management treatments. Under typical field conditions, implementation of treatments will surely be less precise than simulations, and it would be prudent to accommodate the inevitable variation.

Allelic diversity was more sensitive to management treatments than was average H_0 (Figures 6, 7). On average, a high proportion of alleles with an initial frequency of less than 0.05 were lost when herd target sizes were less than 400. In Figure 7, coefficients of variation (CV) were large; after year 100 of simulations CVs exceeded 100% for some treatments. The high uncertainty in simulation results emphasizes the need to use a precautionary approach because our predictive ability is limited. The much greater sensitivity of allelic variation, compared to H_0, is clearly evident by comparing Figures 6 and 7 (note different scales of vertical axes).

When target population size was held constant, differences in generation time accounted for about 75% of the variation in retained H_0 for populations of 200-800 bison for the 200 year period (Figure 8). Remaining variation in loss of genetic diversity is probably due to modification of herd sex and age composition, variation in population growth rates related to specific management strategies, and to stochastic events.

Discussion

Measuring changes in genetic variation

A typical conflict for wildlife managers is a need to maximize population size to avoid loss of genetic variation, and a need to maintain small population sizes to conserve forage or other habitat-related resources. Our simulations show that the choice of a specific population control strategy can have a major influence on the rate of loss of genetic variation in small bison populations, but as population size approaches 1000 animals the effects of population management strategy on genetic variation are small.

Table 1. Proportion of observed heterozygosity (H_0) remaining after 200 years for populations with target sizes of 200-2000, with populations controlled by different population management strategies. Values in table are mean *Ho* after 200 years and 10% lower observation interval (in parentheses). Results (1A) using gene frequencies and vital rates characteristic of Yellowstone National Park (YELL) bison, and (1B) using gene frequencies and vital rates characteristic of Theodore Roosevelt National Park (THRO), North Unit. Bold values indicate those scenarios that did not achieve at least a 90% probability of retaining 90% of H_0.

Table 1A. YELL

Target size	Remove random	Remove cow-calf (0.24)	Remove old	Remove young	Contracept random	Contracept old	Contracept young
200	0.89 **(.86)**	0.90 **(.86)**	0.88 **(.84)**	0.90 **(.87)**	0.91 **(.88)**	0.87 **(.84)**	0.93 **(.90)**
300	0.92 **(.89)**	0.93 **(.90)**	0.92 **(.89)**	0.93 (.91)	0.93 (.91)	0.91 **(.88)**	0.95 (.93)
400	0.94 (.91)	0.94 (.92)	0.94 (.92)	0.94 (.92)	0.94 (.92)	0.93 **(.90)**	0.96 (.94)
500	0.95 (.92)	0.95 (.93)	0.95 (.93)	0.95 (.93)	0.95 (.93)	0.94 (.92)	0.97 (.95)
600	0.95 (.93)	0.96 (.94)	0.96 (.94)	0.96 (.94)	0.96 (.94)	0.95 (.93)	0.97 (.95)
700	0.96 (.94)	0.96 (.94)	0.96 (.94)	0.96 (.95)	0.96 (.94)	0.95 (.93)	0.97 (.95)
1000	0.97 (.95)	0.97 (.96)	0.97 (.95)	0.97 (.96)	0.97 (.95)	0.96 (.94)	0.98 (.96)
2000	0.98 (.97)	0.98 (.97)	0.98 (.97)	0.98 (.97)	0.98 (.96)	0.97 (.96)	0.98 (.97)

Table 1B. THRO, North Unit

Target size	Remove random	Remove cow-calf (0.50)	Remove old	Remove young	Contracept random	Contracept old	Contracept young
200	0.86 **(0.80)**	0.89 **(0.85)**	0.88 **(0.84)**	0.90 **(0.86)**	0.91 **(0.88)**	0.87 **(0.83)**	0.95 (0.92)
300	0.91 **(0.87)**	0.92 **(0.89)**	0.92 **(0.89)**	0.92 **(0.89)**	0.94 (0.91)	0.92 **(0.88)**	0.96 (0.94)
400	0.93 **(0.90)**	0.94 (0.91)	0.94 (0.91)	0.94 (0.91)	0.96 (0.93)	0.94 (0.91)	0.97 (0.95)
500	0.94 (0.91)	0.95 (0.93)	0.95 (0.93)	0.95 (0.93)	0.96 (0.94)	0.95 (0.92)	0.98 (0.96)
600	0.95 (0.93)	0.96 (0.94)	0.96 (0.93)	0.96 (0.94)	0.97 (0.95)	0.96 (0.93)	0.98 (0.96)
700	0.96 (0.94)	0.97 (0.94)	0.96 (0.94)	0.97 (0.95)	0.97 (0.95)	0.96 (0.94)	0.99 (0.97)
1000	0.97 (0.95)	0.98 (0.96)	0.98 (0.96)	0.98 (0.96)	0.98 (0.96)	0.98 (0.96)	0.99 (0.98)
2000	0.99 (0.97)	0.99 (0.97)	0.99 (0.97)	0.99 (0.98)	0.99 (0.98)	0.99 (0.98)	1.00 (0.99)

An important insight from these simulations is the identification of different recommendations that result from evaluations of H_0 versus retention of individual alleles. Most previous studies emphasized H_0, which is most simply defined as the proportion of individuals heterozygous at a locus. H_0 readily lends itself to theoretical analysis of the effects of bottlenecks or small populations sizes on genetic variation. However, under certain conditions, H_0 can be insensitive to the number of alleles at a locus. Allendorf (1986) provided an example to illustrate this point: Consider two populations. The first population (Pop1) has two alleles at equal frequency (0.5) at a particular locus. A second population (Pop2) has seven alleles, one allele with a frequency of 0.7 and the other six alleles with a frequency of 0.05. Our intuitive evaluation is that Pop2 has greater H_0, but this is wrong. For Pop1, $H_0 = 0.500$, whereas $H_0 = 0.495$ for Pop2. While this exact situation will be rare in nature, it illustrates the potential problem of relying on H_0 to evaluate changes in genetic variation. Halbert (2003) reported an average of 4.4 alleles (maximum = 10 alleles) at each locus for the six NPS bison herds. Across all NPS bison herds, 84% of all loci have at least four alleles and H_0 is thus likely to be a relatively insensitive indicator of loss of genetic variation.

If retention of H_0 is the primary aim of management, our simulations suggest that a population objective of about 400 animals is likely to achieve a goal of retaining 90% of currently existing H_0 (Table 1). However, a much larger population objective – on the order of 1000 bison (Figure 8) – is required to achieve a reasonable assurance of retaining 90% of currently existing alleles. In evolutionary terms, H_0 is an index to the overall degree of genetic variance at a locus and it would be expected to reflect the magnitude of short-term responses to artificial or natural selection (James 1971). High allelic diversity will virtually always be correlated with the occurrence of many alleles that have a low frequency in the population. These rare alleles are unlikely to contribute substantially to short-term population responses to selection, but they can be a very important limit to the response to selection over many generations (James 1971; Allendorf 1986). Allelic diversity is thus considered important to the long-term survival of a species, especially where there may be substantial environmental changes, range expansions, or (re)introduction into new sites.

Considerations of the relative merit of management objectives that focus on H_0 or allelic diversity are clearly pertinent to management of NPS bison herds. Halbert (2003) noted that bison in these herds may have retained much of their pre-bottleneck genetic variation, and the genetic composition of NPS bison herds is characterized by the occurrence of many rare alleles (Figure 10).

Nonrandom cow-calf pair removals, as modeled here, are a likely consequence of routine bison removal programs because bison calves generally remain with their mothers throughout the first year of life (Berger and Cunningham 1994). Our results indicate that the short-term genetic effects of cow-calf pair removals is probably minimal compared to other treatments, but we did not explicitly model non-random removal of extended matrilineal groups.

Bison have been reported to naturally assemble into matriarchal groups including several generations of related females and calves (Seton 1937; Haines 1995). In YELL, where culling is primarily through opportunistic selection of bison groups as they exit park boundaries, Halbert (2003) estimated that 24% of the removals were cow-calf pairs, about 50% more cow-calf pairs than we estimated would be removed through a random selection of bison ($p < 0.05$). The extent

of matrilineal group removal from YELL cannot be accurately determined given current limitations in bison sampling as they exit the park. The genetic consequences of non-random removal of matrilineal groups (3 or more generations) was not explicitly considered in this study and it merits further study, although results from simulations with very high levels of cow-calf removals suggest that the effects of matrilineal removals in YELL may be small. While the effect of removal of matrilineal groups from YELL has been most actively discussed, this may be a more important issue in parks where a significant proportion of the herd was traditionally harvested at the same location year after year.

The genetic subpopulation structure of the YELL bison population complicates accurate simulation modeling and the interpretation of the existing simulations. Meagher (1973) reported geographically distinct bison herds within YELL, but as the number of bison in YELL increased some of the herds merged (Taper et al. 2000) . Recent radiotelemetry data indicated little interchange of bison between the northern and central herds (Edward Olexa, personal communication) and historical sightings indicated high densities of bison in several distinct areas of activity (Taper et al. 2000). Recent work revealed genetically distinguishable subpopulations in YELL (Halbert 2003) and cluster analysis of this data (Pritchard et al. 2000) revealed at least 2, and most likely 3, genetically distinguishable subpopulations among those YELL bison sampled (Halbert 2003). Furthermore, statistically significant genetic differentiation between bison collected in different locals (West Yellowstone vs. Gardiner) were observed for between 65 and 78% of the markers analyzed, a result also indicative of subpopulation structure (Halbert 2003). Subpopulation structure serves to reduce N_e from that estimated by the overall population size, and the rate of interchange will need to be considered in the long-term genetic management of YELL bison.

At present, data from YELL are inadequate to accurately estimate rates of genetic interchange between herds, particularly as the total number of bison in YELL varies from 2500 to more than 4000. However, it appears that animal movements between herds are relatively rare (E. Olexa, personal communication), and thus model results should be interpreted as representing a single herd unit (e.g., the northern range herd unit or West Yellowstone). A more complex simulation analysis will be necessary to fully assess the long-term genetic consequences of subpopulation structure and interchange, and non-random removal of matrilineal groups.

Managing populations and genetic variation

We evaluated a relatively small subset of potential strategies that could be used to control the size of bison herds. Currently, removal (of live or dead animals) is the only available alternative, although there is widespread support for use of contraceptives. Development of contraceptives for bison appears promising (Miller *et al.* in press) and contraceptives may eventually provide a useful management tool. We simulated very simple scenarios that relied on exclusive use of removal or contraception, but it seems likely that many Parks will combine these management tools. Combined use of contraceptives and removals could help mitigate changes in sex or age structure of herds. The combined use of removals and contraceptives was evaluated for wild horses (Gross 2000) and it has been favorably received by horse managers.

When fully developed, contraceptives offer advantages for controlling bison populations, but they may also increase risks. Application of contraceptives would presumably result in a smaller number of cows in estrus at any one time, thus one or a few bulls may be more able to dominate

breeding. Our understanding of breeding behavior in bison limits our ability to forecast the effects of management options. There are no data for evaluating breeding behavior with the use of contraceptives, and potential changes in breeding behaviors were not accounted for in simulations. The magnitude of effect that they could have on loss of genetic diversity is unknown. Any application of contraceptives should be accompanied by studies that evaluate both the effectiveness of contraceptives to control population size, and changes in behavior and breeding success of individual males. Ideally, genetic markers would be used to determine parentage.

An obvious strategy for maintaining or enhancing genetic diversity of NPS bison herds is to move animals between herd units, thereby supplementing the gene pool and managing herds as an extended metapopulation. Wright (1931) postulated the simple "one-migrant-per-generation" rule, showing that (in theory) a low rate of migration was sufficient to prevent inbreeding depression, regardless of population size. More recent analyses have clearly shown that more information is required to estimate a migration (or transfer) rate needed to meet explicit goals for retaining genetic variation. For example, small or fluctuating population sizes can greatly increase the number of migrants necessary to avoid an increase in inbreeding coefficient, as does a small ratio of N_e to census population size (N)(Vucetich and Waite 2000, 2001). Wang (2004) considered a wide range of population characteristics, including N_e/N, variation in population size, and skewed sex ratios. Based on these considerations, transfer of about 10 individuals of either sex per generation should be adequate to maintain an acceptable level of similarity in subpopulations. However, Wang (2004) noted that a more accurate estimation requires an understanding of the factors that lead to a small N_e/N.

Simulation modeling could be used to estimate the number of migrants needed to maintain genetic variation across a number of bison herds. However, the implementation of a credible simulation approach requires clear identification of a limited number of realistic management scenarios and clear definition of evaluation criteria. For NPS bison herds, this is currently a difficult challenge due to hybridization of bison with cattle (Halbert 2003), occurrence of infectious diseases, and the enormous number of permutations defined by the animals moved (sex, age, number), frequency of movement, source, and target herds. Allendorf (1994) and Halbert *et al.* (*in press* b) conducted very simple simulation experiments to examine the potential benefits of transferring animals into small populations. Allendorf (1994) forecast a considerable reduction in the rate of loss of genetic heterozygosity by introducing two individuals every generation into a small grizzly bear population. Halbert *et al.* (*in press* b) simulated introduction of bison from YELL into the highly inbred Texas bison herd. A one-time introduction of 3-9 bison from YELL would dramatically enhance heterozygosity and increase allelic diversity in that inbred herd. These results demonstrate the case-specific nature of simulation analyses of animal transfers, and they emphasize the need to clearly identify a limited set of realistic scenarios for analysis.

This study emphasized the ability of managers to alter rates of loss of genetic diversity through selection of population control treatments whose effects are mediated primarily by altering generation time. Other alternative strategies may also be available to retain genetic diversity. Frankham et al. (2002: 441) reviewed the potential use of reproductive technologies such as artificial insemination, cryopreservation, cloning, and genome resource banks for preserving genetic material. Robison et al. (1998) examined the potential application of reproductive

technologies to conservation of genetic material from brucellosis-infected bison herds, and provided preliminary data demonstrating the practicality of this strategy. Technologies investigated by Robison et al. (1998) might permit transfer of genetic material between bison herds, circumventing some problems related to disease and breeding success. Similarly, Derr and Halbert (personal communication) suggested the use of cryopreservation of bison tissues. For example, eggs or sperm might be frozen for an extended period and then reintroduced into the same herd or a different herd. Presumably, the increase in generation time would be proportional to the time between sample collection and reintroduction and the number of transfers.

Interpreting model results

Any interpretation of simulation model results must consider the quality of the data used to drive the model, the assumptions on which the model is founded, and the sensitivity of model results to uncertainty in model inputs and assumptions. Sensitivity analyses showed that our model results were relatively insensitive to realistic variation in vital rates, initial population structure, and initial genetic composition of herds. In this model, sensitivity analysis showed that a potentially realistic variation in male breeding success could significantly affect results, primarily in populations with fewer than about 600 animals. We identified complicated interactions between variation in male breeding success, population control strategy, and target population size. In general, greater levels of variation in male breeding success affected treatments that removed old animals to a greater extent than those that removed young. There are extremely few reliable data available to estimate variation in lifetime breeding success of bison, or for that matter, any other large ungulate (Wilson et al. 2002; McEligott and Hayden 2000; Roed et al. 2002; Coltman et al. 1999). The reliability of simulation model predictions for some treatments could be significantly increased by incorporating data on paternity analysis based on genetic samples from herds of interest. At present, there are no data from bison herds that can be used to estimate how herd size, sex ratio, habitat characteristics (e.g., open vs closed), age structure, or other factors influence variation in male success. The absence of this information constrains our ability to realistically forecast the effect of population control measures on retention of genetic diversity.

Comparisons of results from simulations initialized with genetic data from different NPS bison herds exhibited small differences in retention of H_0 (Appendix 1). We suggest that model results be interpreted conservatively. The model used in this study has explicit random variation and no two sets of 500 runs will be exactly the same. Stochastic models better reflect the variation seen when observing actual populations, but they also complicate evaluation of results.

Summary and recommendations

Because there are inherent uncertainties in model assumptions, input data, and our ability to properly interpret model results, the most appropriate use of these results is to support general recommendations on management of NPS bison units. Management actions can be simulated with a much higher degree of precision than they can be implemented under field conditions. Given these caveats, there are several clear conclusions:

1. For small bison herds (say, fewer than 500 animals), removal or contraception of young animals can significantly enhance retention of genetic variation. Other treatments that significantly increase generation time will yield similar results.

2. Bison herds with fewer than about 400 animals are unlikely to meet a long-term goal of achieving a 90% probability of retaining 90% of genetic heterozygosity for 200 years.

3. A moderate bison population size - about 1000 animals – is necessary to meet a long-term goal of achieving a 90% probability of retaining 90% of allelic diversity for 200 years.

4. Goals described in 2 & 3 can be achieved with much smaller herd sizes if animals can be moved between herds. Development and evaluation of a set of realistic management strategies that involves transferring animals between herds requires knowledge of individual herd characteristics, including genetic composition and disease status, and a clear statement of management objectives. A similar result might be obtained by other treatments not identified or evaluated by this study (e.g., preserving and reintroducing sperm or eggs).

5. In particular, the absence of reliable data on and understanding of variation in male lifetime reproductive success is a constraint to developing more specific management recommendations.

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References

Acevedo-Whitehouse, K., F. Gulland, D. Greig, and W. Amos. 2003. Disease susceptibility in California sea lions. Nature 422:35.

Allendorf, F. W. 1986. Genetic drift and the loss of alleles versus heterozygosity. Zoo Biology 5:181-190.

Allendorf, F. W. 1994. Genetically effective sizes of grizzly bear populations. Pages 155-156 in: G. K. Meffe and C. R. Carroll, eds. Principles of Conservation Biology. Sinauer Associates, Sunderland, MA.

Berger, J. 1996. Scenarios involving genetics and population size of bison in Jackson Hole. Report to National Park Service.

Berger, J. and C. Cunningham. 1994. Bison: Mating and conservation in small populations. Columbia University Press, New York.

Boyd, D. 2003. Conservation of North American bison: status and recommendations. Master's Degree Thesis, University of Calgary, Calgary, Canada. 222 pages.

Briskie, J. V. and M. Mackintosh. 2004. Hatching failure increases with severity of population bottlenecks in birds. Proceedings of the National Academy of Sciences of the United States of America 101:558-561.

Carey, J. R. 1993. Applied demography for biologists. Oxford University Press, New York.

Coates-Markle, L. 2000. Summary recommendations - BLM wild horse and burro population viability forum. Resource Notes 35:1-4.

Coltman, D. W. and J. Slate. 2003. Microsatellite measures of inbreeding: a meta-analysis.

Evolution 57:971-983.
Coltman, D. W., J. Smith, D. Bancroft, J. Pilkington, A. MacColl, T. Clutton-Brock, and J. Pemberton. 1999. Density-dependent variation in lifetime breeding success and natural and sexual selection in Soay rams. American Naturalist 154:730-746.
Couvet, D. 2002. Deleterious effects of restricted gene flow in fragmented populations. Conservation Biology 16:369-376.
Dobson, A. and M. Meagher. 1996. The population dynamics of Brucellosis in the Yellowstone National Park. Ecology 77:1026-1036.
Eberhardt, L. L. 1985. Assessing the dynamics of wild populations. Journal of Wildlife Management 49:997-1012.
Frankham, R. 1995. Conservation genetics. Annual Review of Genetics 29:305-327.
Frankham, R., J. D. Ballou, and D. A. Briscoe. 2002. Introduction to Conservation Genetics. Cambridge University Press, New York.
Gross, J. E. 2000. A dynamic simulation model for evaluating the effects of removal and contraception on genetic variation and demography of Pryor Mountain wild horses. Biological Conservation 96:319-330.
Haines, F. 1995. The buffalo: the story of American bison and their hunters from prehistoric times to the present. University of Oklahoma Press, Norman.
Halbert, N. D. 2003. The utilization of genetic markers to resolve modern management issues in historic bison populations: implications for species conservation. Ph.D. Dissertation, Texas A&M University, College Station.
Halbert, N. D., W. A. Grant, and J. N. Derr. *In press* a. Genetic and demographic consequences of importing animals into a small population: a simulation model of the Texas state bison herd (U. S. A.). Ecological Modelling.
Halbert, N. D., T. Raudsepp, B. P. Chowdhary, and J. N. Derr. *In press* b. Conservation genetic analysis of the Texas state bison herd. Journal of Mammalogy.
Harris, R. B. and F. W. Allendorf. 1989. Genetically effective population size of large mammals: an assessment of estimators. Conservation Biology 3:181-191.
Hartl, D. L. and A. G. Clark. 1997. Principles of population genetics. Sinauer Associates, Sunderland, Massachusetts.
Hoelzel, A. R., J. Halley, S. J. Obrien, C. Campagna, T. Arnbom, B. Leboeuf, K. Ralls, and G. A. Dover. 1993. Elephant seal genetic-variation and the use of simulation models to investigate historical population bottlenecks. Journal of Heredity 84:443-449.
Hornaday, W. T. 1889. The extermination of the American bison, with a sketch of its discovery and life history. Report to the U.S. National Museum, 1886-1887. Part 2: 367-548.
Hornaday, W. T. 1913. Our Vanishing Wildlife: Its Extermination and Preservation. New York Zoological Society, New York.
James, J. W. 1971. The founder effect and response to artificial selection. Genetical Research 12:249-266.
Keller, L. F. and D. M. Waller. 2002. Inbreeding effects in wild populations. Trends in Ecology & Evolution 17:230-241.
Kirkpatrick, J. F., J. C. McCarty, D. F. Gundermuth, S. E. Shideler, and B. L. Lasley. 1996. An assessment of the reproductive biology of Yellowstone bison (*Bison bison*) subpopulations using noncapture methods. Canadian Journal of Zoology 74:8-14.
Lacy, R. C. 1987. Loss of genetic diversity from managed populations: interacting effects of drift, mutation, immigration, selection, and population subdivision. Conservation Biology

1:143-158.
Lande, R. 1995. Breeding plans for small populations based on the dynamics of quantitative genetic variance. Pages 318-340 in: J. D. Ballou, M. Gilpin, and T. J. Foose, eds. Population Management for Survival and Recovery. Columbia University Press, New York.
Lynch, M. 1996. A quantitative-genetic perspective on conservation issues. Pages 471-501 in: J. C. Avise and J. L. Hamrick, eds. Conservation Genetics: Case Histories from Nature. Chapman and Hall, New York.
McEligott, A. G. and T. J. Hayden. 2000. Lifetime mating success, sexual selection and life history of fallow bucks (*Dama dama*). Behavioral Ecology and Sociobiology 48:203-210.
Meagher, M. 1986. *Bison bison*. Mammalian Species 266:1-8.
Meagher, M. M. 1973. The bison of Yellowstone National Park. National Park Service Scientific Monograph Series 1:1-161.
Meffe, G. K. and C. R. Carroll. 1994. Principles of Conservation Biology. Sinauer Associates, Inc., Sunderland, MA.
Miller, L. A., J. C. Rhyan, and M. Drew. 2005. Contraception of bison by GnRH vaccine: a possible means of decreasing transmission of brucellosis in bison. Journal of Wildlife Diseases In press:
Mills, L. S. and F. W. Allendorf. 1996. The one-migrant-per-generation rule in conservation and management. Conservation Biology 10:1509-1518.
Nunney, L. and K. A. Campbell. 1993. Assessing minimum viable population size: demography meets population genetics. TREE 8:234-239.
Peterson, M. J., W. E. Grant, and D. S. Davis. 1991. Bison-brucellosis management: simulation of alternative strategies. Journal of Wildlife Management 55:205-213.
Peterson, M. J., W. E. Grant, and D. S. Davis. 1991. Simulation of host-parasite interactions within a resource management framework: impact of brucellosis on bison population dynamics. Ecological Modelling 54:299-320.
Pritchard, J. K., M. Stephens, and P. Donnelly. 2000 Inference of population structure using multilocus genotype data. Genetics 155: 945-959.
Reed, D. H. and R. Frankham. 2003. Correlation between fitness and genetic diversity. Conservation Biology 17:230-237.
Reynolds, H. W, R. D Glaholt, and A. W. L Hawley. 1982. Bison. Pages 972-1007 in J. A Chapman and Feldhamer, G. A. Wild Mammals of North America. John Hopkins University Press, Baltimore.
Robison, C. D., D. S. Davis, J. W. Templeton, M. Westusin, W. B. Foxworth, M. J. Gilsdorf, and L. G. Adams. 1998. Conservation of germ plasm from bison infected with *Brucella abortus*. Journal of Wildlife Diseases 34:582-589.
Roed, K. H., O. Holand, M. E. Smith, H. Gjostein, J. Kumpula, and M. Nieminen. 2002. Reproductive success in reindeer males in a herd with varying sex ratio. Molecular Ecology 11:1239-1243.
Ryman, N., R. Vaccus, C. Reuterwall, and M. H. Smith. 1981. Effective population size, generation interval, and potential loss of genetic variability in game species under different hunting regimes. Oikos 36:257-266.
Seton, E. T. 1937. Lives of game animals. Literary Guild of America, New York.
Shull, A. M. and A. R. Tipton. 1987. Effective population size of bison on the Wichita Mountains Wildlife Refuge. Conservation Biology 1:35-41.
Soule, M. E., M. Gilpin, W. Conway, and T. Foose. 1986. The millennium ark: how long a

voyage, how many staterooms, and how many passengers? Zoo Biology 5:101-114.
Starfield, A. M., H. A. Rowley, H. Quadling, and T. J. Foose. 1995. Genetic risk analysis of a small wild lion population. Pages 155-179 in: J. D. Ballou, M. Gilpin, and T. J. Foose, eds. Population Management for Survival and Recovery. Columbia University Press, New York.
Taper, M. L., M. Meagher, and C. L. Jerde. 2000. The phenology of space: spatial aspects of bison density dependence in Yellowstone National Park. Final report to United States Geological Service, Biological Resources Division, Bozeman, Montana. 1-114.
Vucetich, J. A. and T. A. Waite. 2000. Is one migrant per generation sufficient for the genetic management of fluctuating populations? Animal Conservation 3:261-266.
Vucetich, J. A. and T. A. Waie. 2001. Migration and inbreeding: the importance of recipient population size for genetic management. Conservation Genetics 2:167-171.
Vucetich, J. A., T. A. Waite, and L. Nunney. 1997. Fluctuating population size and the ratio of effective to census population size. Evolution 51:2017-2021.
Wang, J. 2004. Application of the one-migrant-per-generation rule to conservation and management. Conservation Biology 18:332-343.
Williams, E. S. and I. K. Barker. 2001. Infectious diseases of wild mammals. Iowa State University Press, Ames.
Wilson, G. A., W. Olson, and C. Strobeck. 2002. Reproductive success in wood bison (*Bison bison athabascae*) established using molecular techniques. Canadian Journal of Zoology 80:1537-1548.
Wilson, G. A. and C. Strobeck. 1999. Genetic variation within and relatedness among wood and plains bison populations. Genome 42:483-496.
Wilson, G. A. and K. Zittlau. 2004. Management strategies for minimizing the loss of genetic diversity in wood and plains bison populations at Elk Island National Park. Unpublished report, 41 pages.
Woodworth, L. M., M. E. Montgomery, D. A. Briscoe, and R. Frankham. 2002. Rapid genetic deterioration in captive populations: causes and conservation implications. Conservation Genetics 3:277-288.
Wright, S. J. 1931. Evolution in Mendelian populations. Genetics 16:97-159.
Wright, S. J. 1969. Evolution and the genetics of populations. Volume 2. The theory of gene frequencies. University of Chicago Press, Chicago, Illinois.

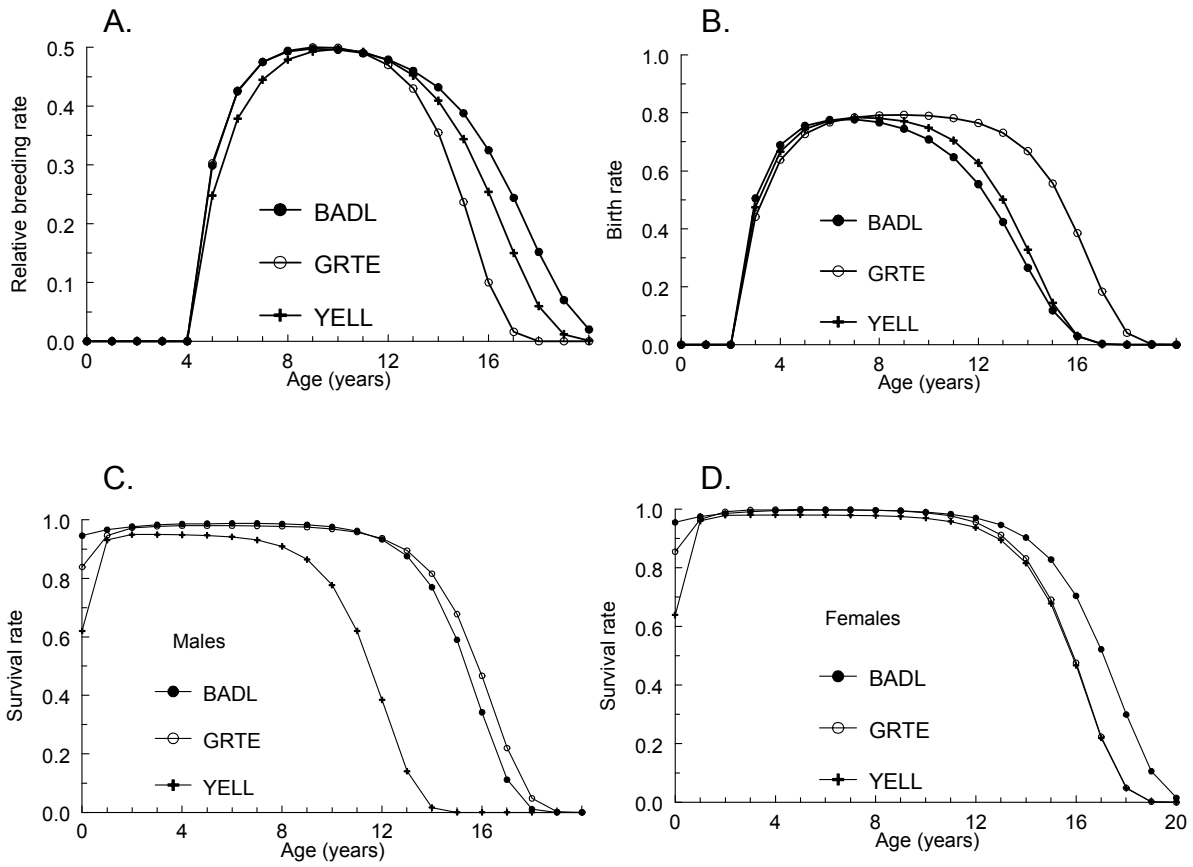

Figure 1. Baseline age-specific vital rates used in simulations. (A) Age-specific probability of mating for males (these are relative – see text), (B) birthing rates for females, (C) survival rates for males, and (D) survival rates for females. Estimated from observations of bison in BADL, GRTE, and YELL.

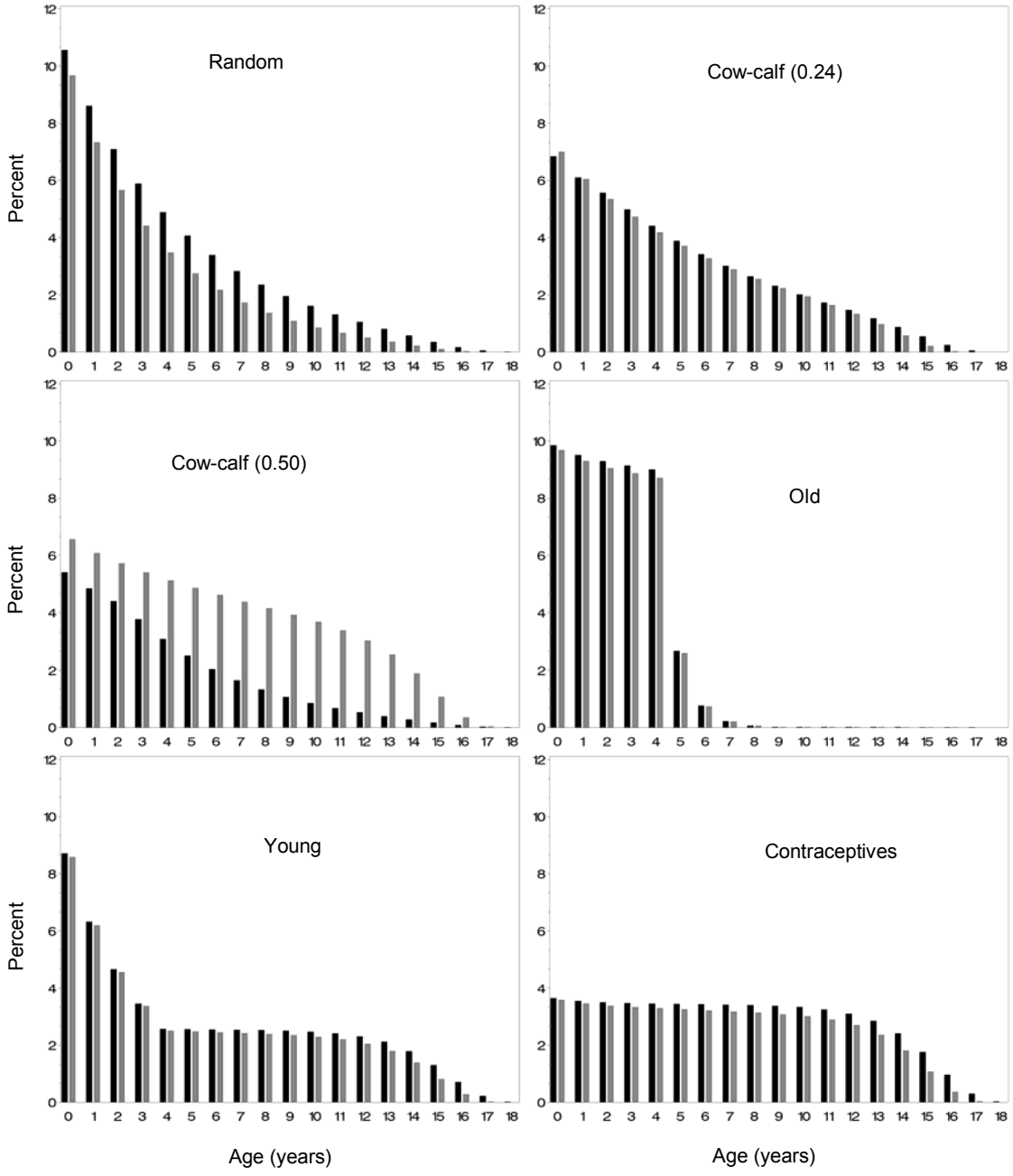

Figure 2. Age structure of bison herds subjected to population controls based on removal of individuals of random (Random), old (Old), or young (Young) ages, or removal of cow-calf groups (either 24% or 50% of removals (Cow-calf (0.24)) or (Cow-calf (0.5)), respectively), or contraceptive treatment (Contraceptive) of cows. All contraceptive treatments resulted in very similar age distributions. Population size did not affect age structure. Results in this figure are from simulations of THRO, except results of Cow-calf (0.24) are from YELL.

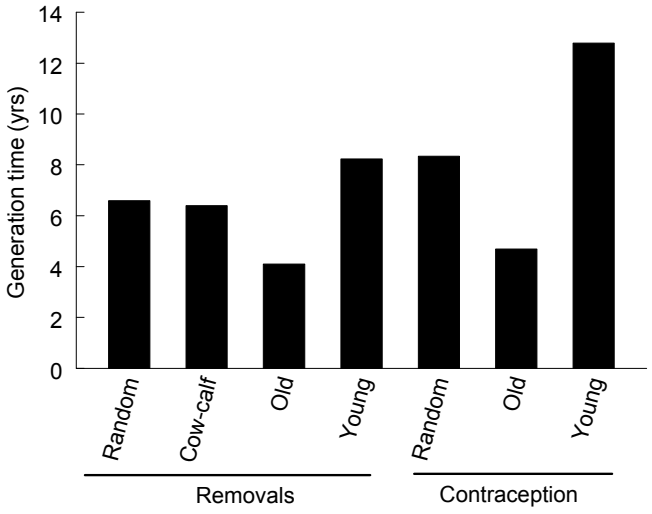

Figure 3. Average generation time of cows in bison herds subjected to population controls based on removal of individuals of old, young, or random ages, or removal of cow-calf groups (either 24% or 50% of removals), or contraceptive treatment of cows of old, young, or random ages. Generation time did not vary with population target size; data from simulations of BADL.

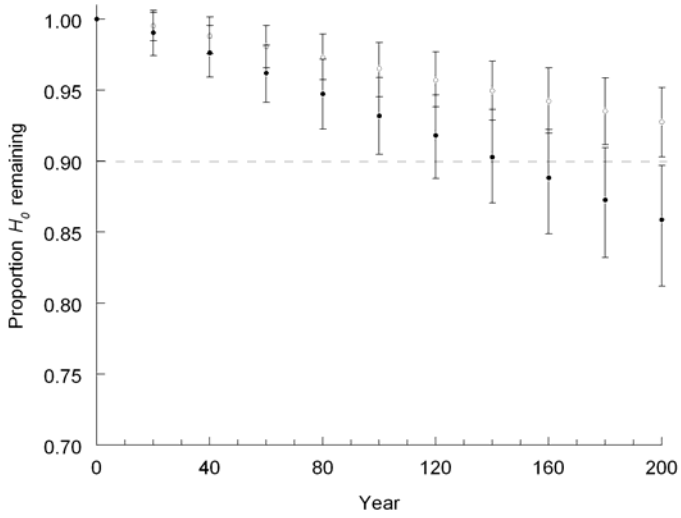

Figure 4. Simulated persistence of genetic heterozygosity (H_0) for target populations of 200 (filled circle) and 400 (open circle), controlled by removal of individuals of random ages. Symbols are averages, and error bars display the range of 10% and 90% observation intervals of simulation results. Initial H_0 based on allelic frequencies observed for bison from WICA.

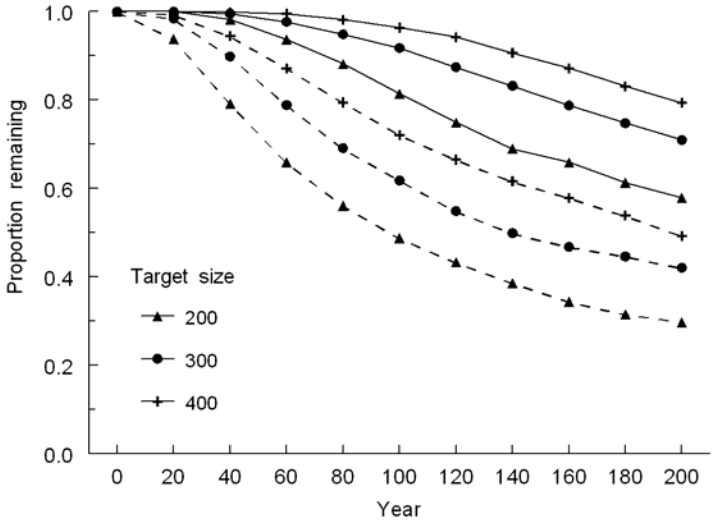

Figure 5. Average persistence of alleles with an initial frequency of 0.02 (broken lines) or 0.05 (solid lines), for bison herds managed to different population target sizes by removal of a random selection of animals. Results from simulations of the YELL bison herd.

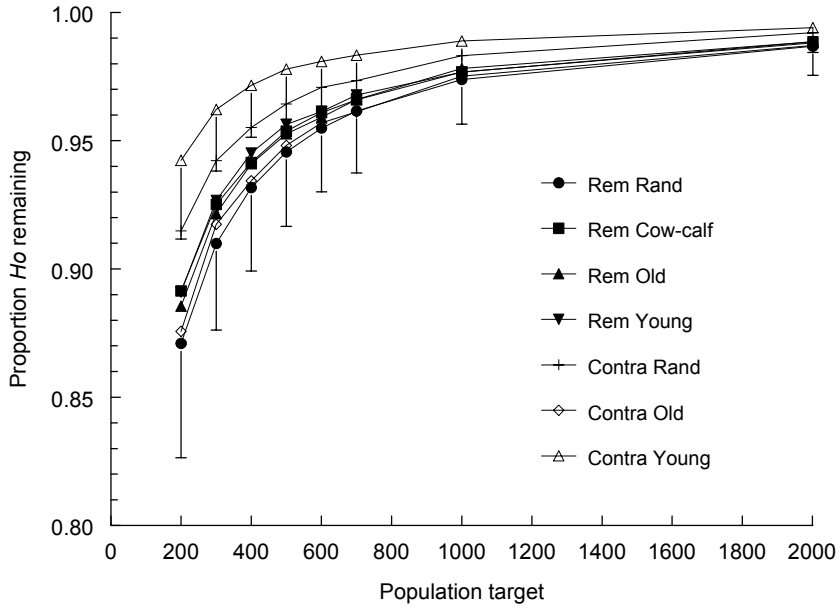

Figure 6. Average proportion of initial heterozygosity (H_0) remaining at year 200 for simulations of bison herds. Simulations were initialized with allele frequencies observed for the BADL bison herd (Halbert 2003). In legend, Rem = removal, Contra = contraceptive treatment. See text for detailed explanation of management treatments. Vertical lines show lower 10% observation interval for removal-random and contraceptive-young treatments.

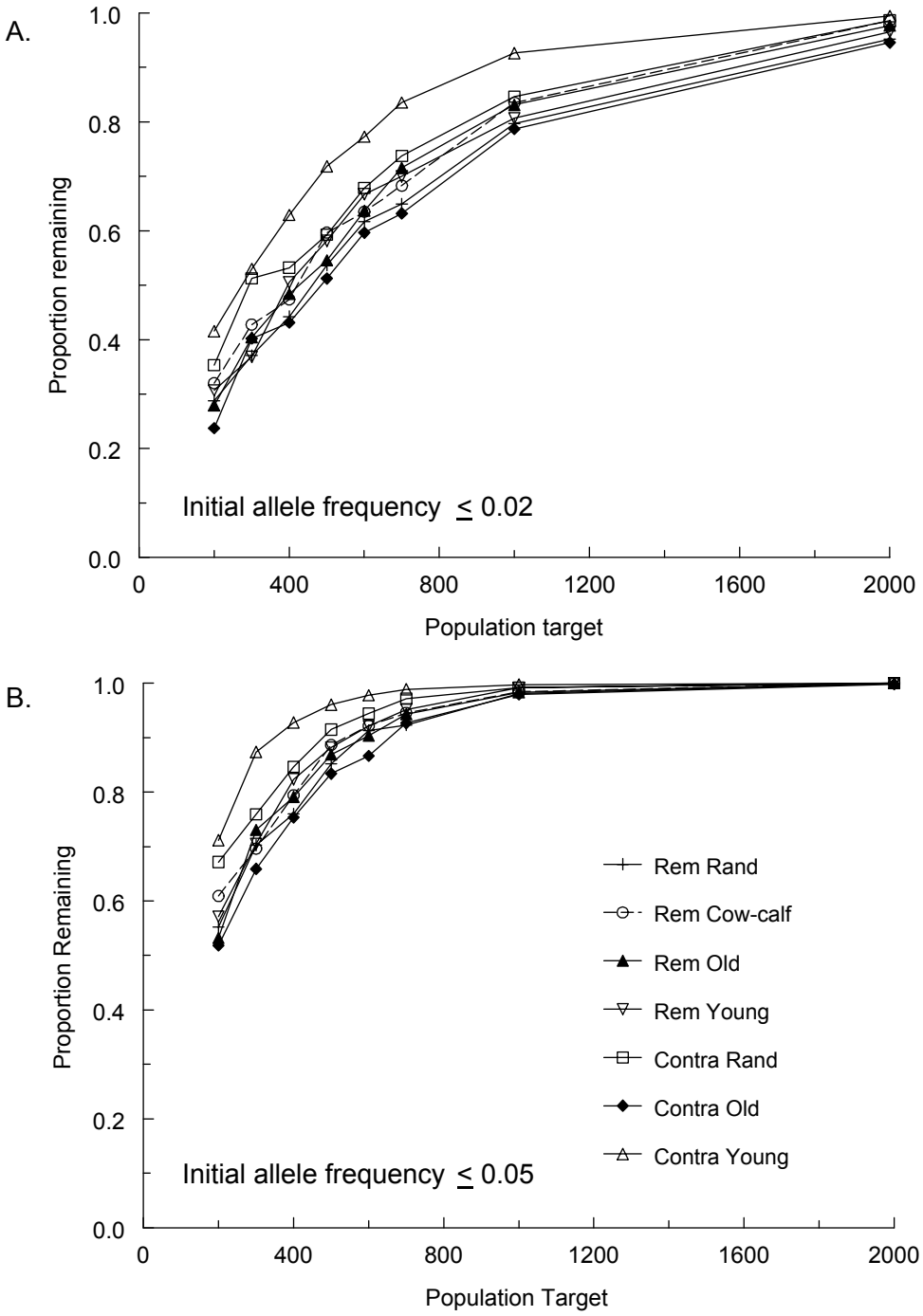

Figure 7. Average proportion of alleles remaining at year 200 for simulations of bison herds, for alleles with an initial frequency of (A) ≤ 0.02 or (B) ≤ 0.05. Simulations were initialized with allele frequencies observed for the YELL bison herd (Halbert 2003). Simulated bison herds were subjected to different management treatments and with different population size targets. In legend, Rem = removal, Contra = contraceptive treatment. See text for detailed explanation of management treatments.

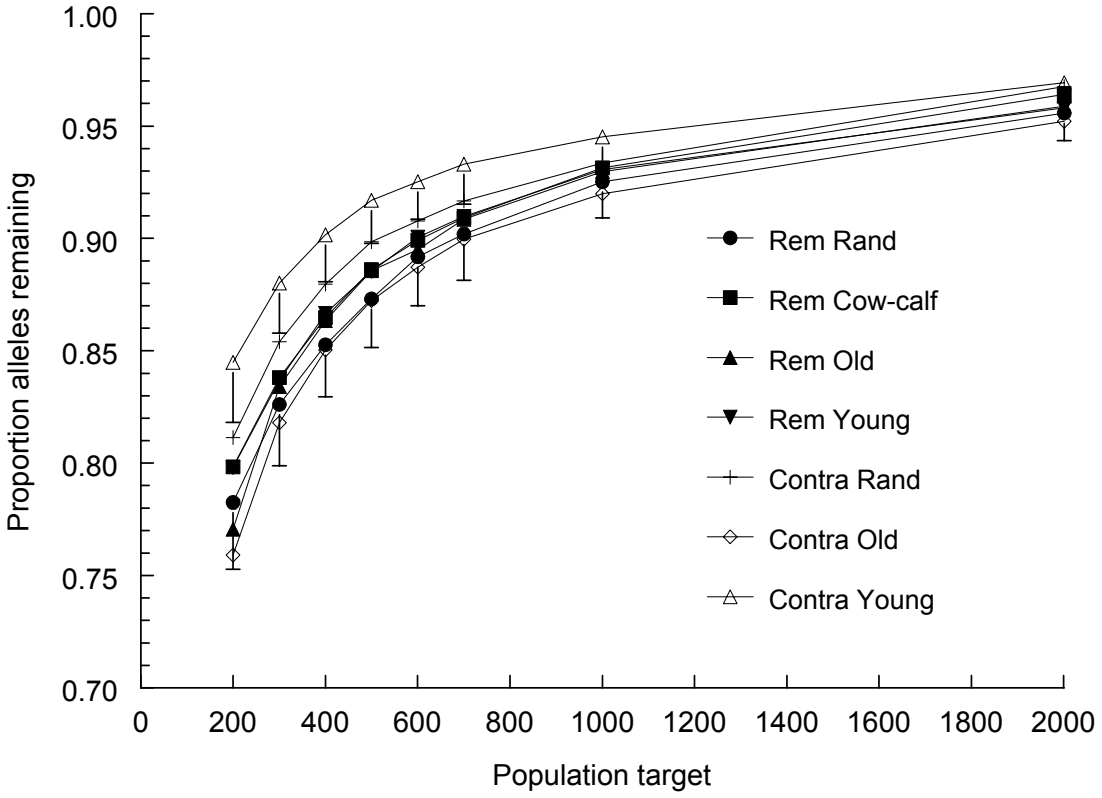

Figure 8. Average proportion of all alleles remaining at year 200 for simulations of bison herds. Simulations were initialized with allele frequencies observed for the BADL bison herd (Halbert 2003). Error bars indicate lower 10^{th} percentile of results from simulations of random removals animals and for contraception of young cows. In legend, Rem = removal, Contra = contraceptive treatment. See text for detailed explanation of management treatments.

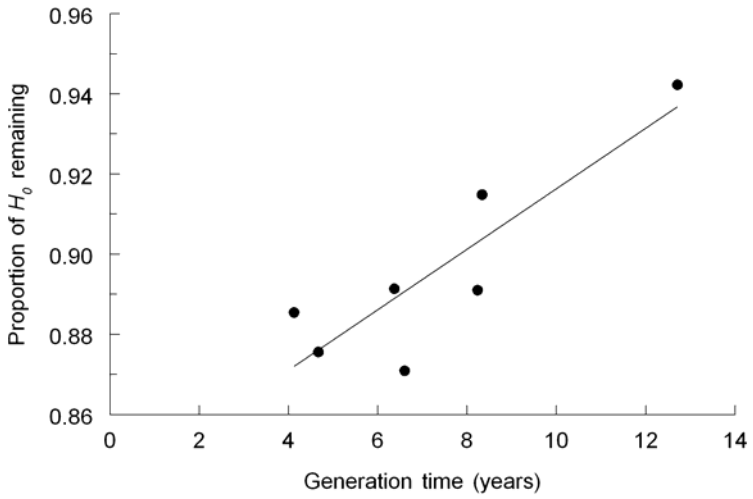

Figure 9. Relationship between generation time of bison cows and average heterozygosity (H_0) remaining after 200 years for simulations of a bison herd with a target size of 200. Simulations used allele frequencies and vital rates based on observations of the BADL bison herd. The regression was highly significant ($r^2 = 0.76$). Generation time explained a similar amount of variation in H_0 for population target sizes of fewer than 1000 animals, although the slope diminished with increasing target size.

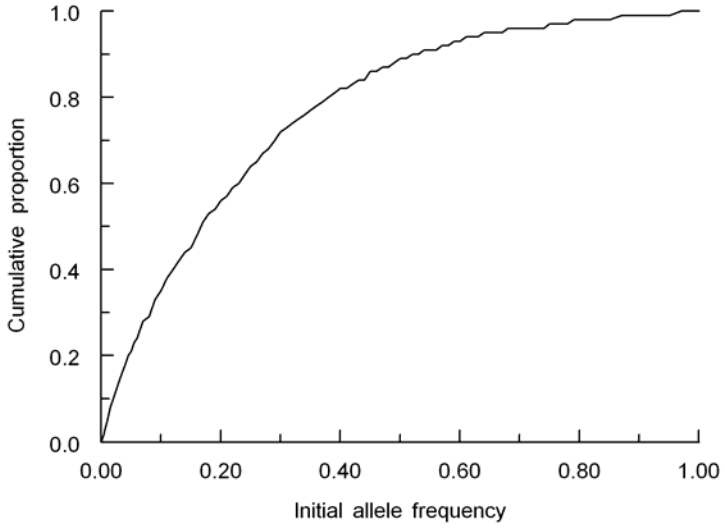

Figure 10. Cumulative frequency distribution of bison alleles across all National Park units, showing that more than 20% of all alleles occurred with a frequency of less than 0.05. More than 50% of all alleles occurred at a frequency of 0.17 or less. Data from Halbert (2003).

Appendix 1. Sensitivity analyses: effects of sex ratio and proportion of cow-calf pairs in removals on retention of genetic diversity

In addition to the population control treatments described in the main body of the report, we conducted sensitivity analyses to examine interactions between herd sex ratio, population control treatment, and the relative strength of effect of vital rates and allelic composition of herds on retention of genetic diversity. These sensitivity analyses were designed to address three questions:

1. How does removal of cow-calf pairs influence herd sex ratio when 'non cow-calf' removals are random with regard to sex and age?
2. What effect does herd sex ratio have on retention of genetic diversity when animals are randomly removed or when cow-calf pairs are selectively removed?
3. What are the effects of variation in the genetic composition and vital rates of NPS herds on retention of genetic diversity?

The first question relates to the effect of removing a prescribed proportion of cow-calf pairs. Because equal numbers of male and female calves are born, the sex ratio of cow-calf pairs will be female-biased (i.e., all cow-calf pairs include the cow, and on average ½ of the calves will be female). Thus the average sex ratio of this proportion of the removals will be three females for every male (0.5 + 0.5*0.5). Sex-ratio biases due to harvesting will increase with increases in the proportion cow-calf pairs harvested, while efforts to harvest nearly the same number of males and females will obviously compensate for this effect. In general, males had slightly higher mortality rates than females, and difference in mortality thus contributed to unequal numbers of males and females.

The second question follows from a desire to understand potential interactions between effects due to biased sex ratio and those attributable to population control strategy. Stated in a different way, are differences in the rate of loss of genetic diversity due to direct effects of the treatment under investigation (i.e., harvesting strategy), or are they due to the indirect effect of changes in sex ratio that result from a treatment effect? We explored this question by explicitly controlling sex ratio in those treatments where the proportion of each sex could diverge from nearly equal numbers of males and females.

Cow-calf removals, sex ratio, and H_0

We conducted simulations using parameters from THRO (used here to represent THRO North Unit) and YELL and we varied the proportion of the harvest consisting of pre-selected cow-calf pairs from 0% to 50% of all animals removed. The composition and number of animals harvested was determined by the following process. First, the number of animals to be removed was determined by comparing the current herd size to the objective herd size. Next, the target proportion of cow-calf pairs was removed. For treatments where only the proportion of cow-calf pairs was controlled, the remaining animals to be removed were selected randomly with regard to sex and age. For treatments where both sex ratio and the proportion of cow-calf pairs removed were controlled, cow-calf pairs were removed first and an attempt was then made to remove animals of each sex in the quantity needed to achieve the desired sex ratio, subject to the constraint that no additional animals were removed once the target population size was achieved. Thus the criterion for target size was given a higher priority than that for sex ratio. Selection of animals to be removed was independent of age.

The proportion of males in simulated bison herds increased with the proportion cow-calf pairs removed (Table 1.1). Cow-calf removals had a more pronounced effect on sex ratio in THRO than in YELL because bison in THRO exhibited a greater growth rate, which therefore required removal of a larger proportion of the population to maintain population size. Sex-biased removals had a direct effect on sex ratio of the herd. Age-specific survival rates of males (resulting from both natural and harvest-related mortality) were positively related to the proportion of cow-calf pairs harvested, and this shifted the age structure of, especially, the male component of herds to older-aged bull (Figures 1.1, 1.2). With the increased proportion of older bulls, the average age of mating bulls increased, which was reflected by changes in generation time. Both age structure and generation time of cows was also influenced by the degree of selection for cow-calf pairs, but in a direction opposite to that of bulls (Table 1.1, Figure 1.1). Population growth rates increase with the proportion of reproductively active females in the herd, thus the proportion of the population harvested when removals were comprised of 10-50% cow-calf pairs ranged from 0.10 to 0.07 for YELL and 0.14 to 0.09 for THRO.

The proportion cow-calf pairs harvested had a small effect on retention of H_0 over a 200 year period, especially when compared to effects of population target size (Figure 1.3).

Table 1.1. Average sex ratio (proportion males; std in parentheses) and generation time (yrs; std in parentheses) of cows and bulls from simulations of THRO and YELL where the proportion of cow-calf pairs harvested (cow-calf pairs) varied from 0 (i.e., random removal) to 0.50. After the targeted proportion of cow-calf pairs was removed, additional animals were removed by harvest of a random selection of animals (i.e., no selection by sex or age). Standard deviations were calculated from overall means of each level of cow-calf removal.

	Theodore Roosevelt			Yellowstone		
Cow-calf pairs	Proportion males	Generation time (yr) cows	Generation time (yr) bulls	Proportion males	Generation time (yr) cows	Generation time (yr) bulls
0.00	0.43 (0.008)	6.67 (0.002)	7.06 (0.093)	0.41 (0.001)	7.02 (0.005)	8.31 (0.013)
0.10	0.54 (0.008)	6.62 (0.009)	7.66 (0.092)	0.45 (0.003)	7.00 (0.003)	8.47 (0.023)
0.20	0.60 (0.004)	6.56 (0.007)	7.98 (0.064)	0.48 (0.003)	6.97 (0.004)	8.61 (0.020)
0.30	0.63 (0.004)	6.52 (0.008)	8.17 (0.052)	0.50 (0.003)	6.95 (0.003)	8.70 (0.018)
0.40	0.65 (0.003)	6.47 (0.006)	8.31 (0.047)	0.52 (0.003)	6.93 (0.006)	8.78 (0.017)
0.50	0.67 (0.002)	6.43 (0.004)	8.42 (0.040)	0.53 (0.002)	6.91 (0.002)	8.84 (0.011)

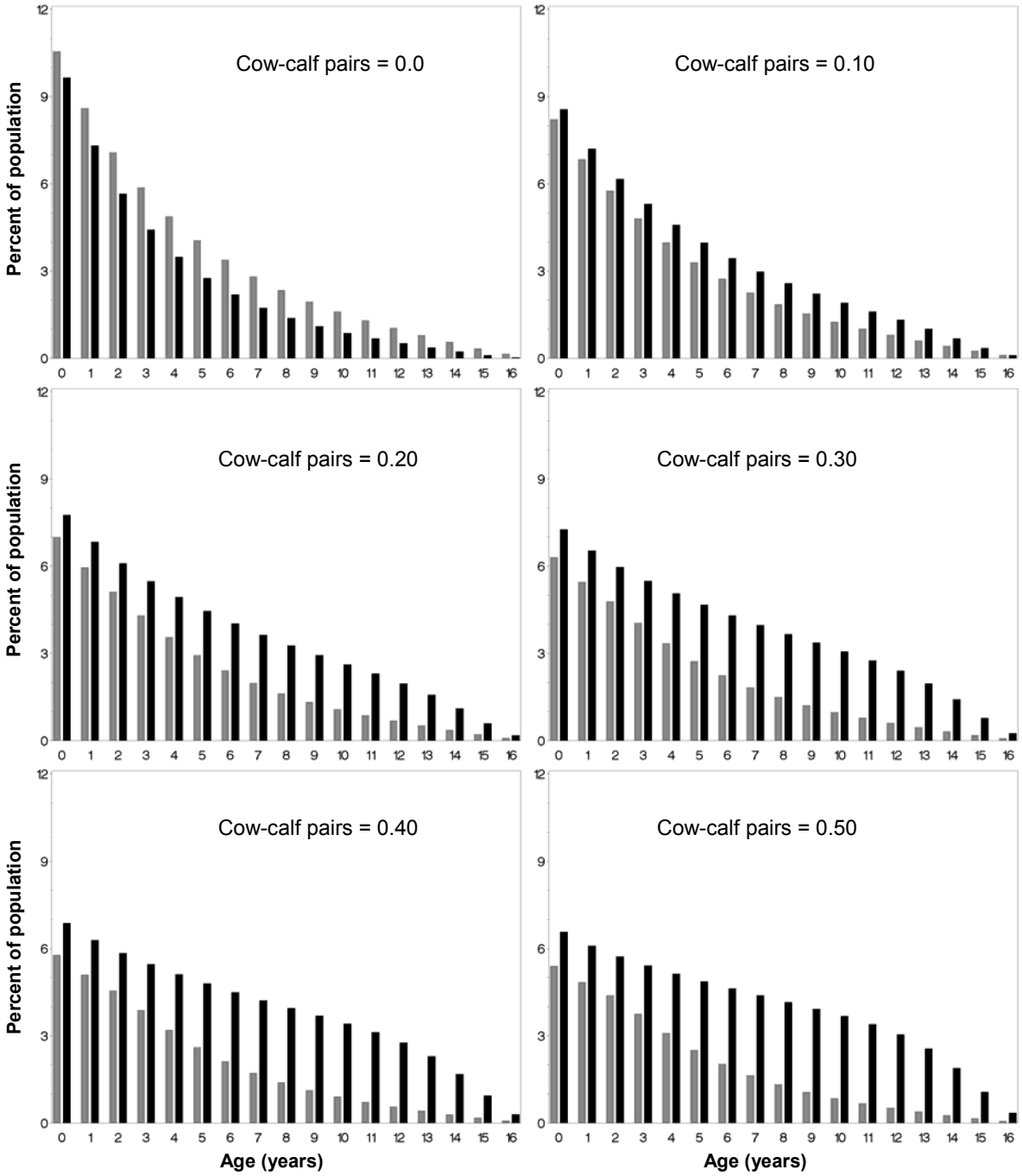

Figure 1.1. Age structure of simulated bison herds where cow-calf pairs constituted 0 to 50% of animals removed (other bison removed randomly with respect to sex and age). Based on vital rate parameters for THRO. Gray bars are females, black bars are males. Age structure did not vary with population size.

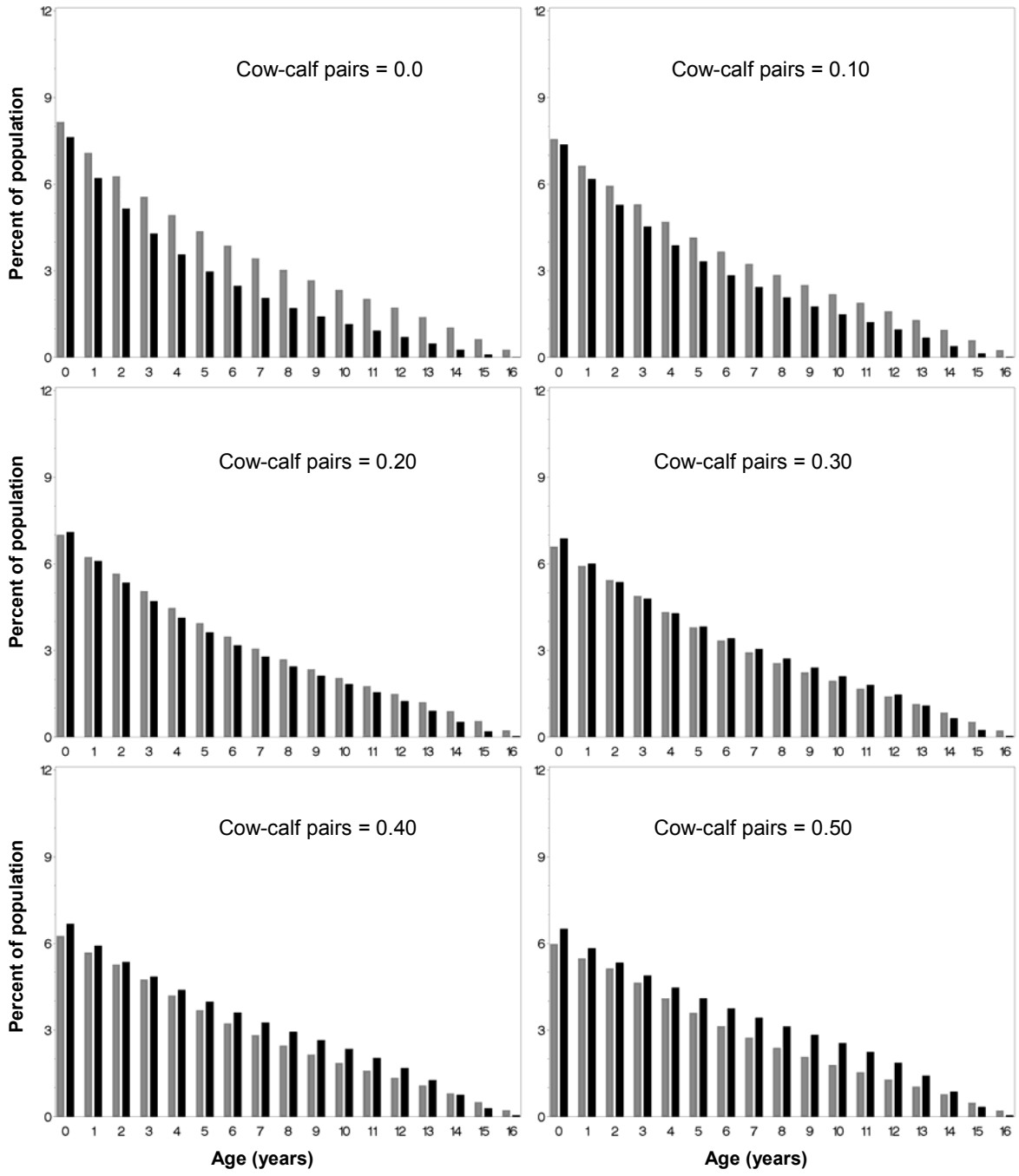

Figure 1.2. Age structure of simulated bison herds where cow-calf pairs constituted 0 to 50% of animals removed (other bison removed randomly with respect to sex and age). Based on vital rate parameters for YELL. Gray bars are females, black bars are males. Age structure did not vary with population size.

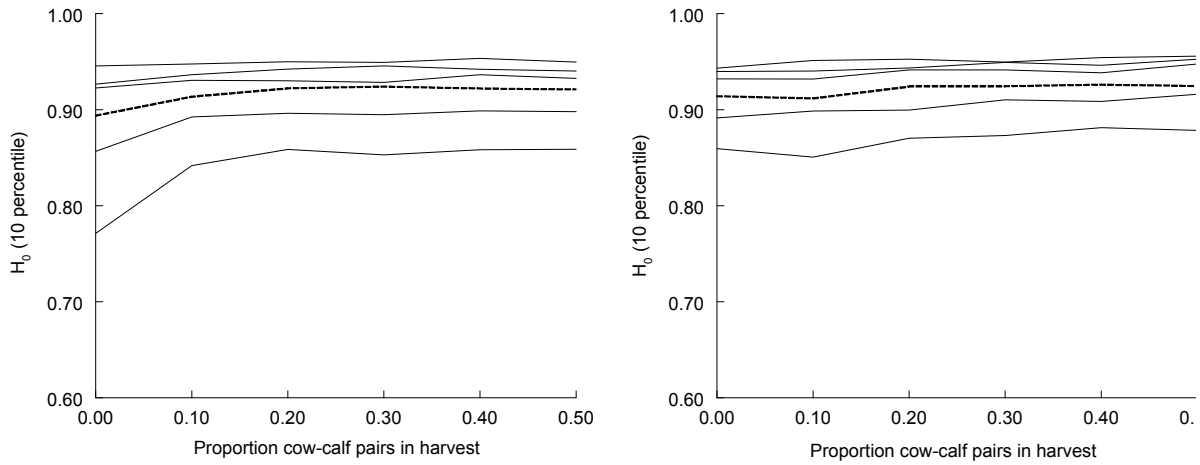

Figure 1.3. Lower 10 percentile of retained heterozygosity at year 200 as a function of the proportion of cow-calf pairs selected for harvest in THRO (left plot) or YELL (right plot). Lines, from bottom to top, are results for population target sizes of 200, 300, 400 (dashed), 500, 600, and 700.

Effects of sex ratio - cow-calf removals

We evaluated the effects of controlling sex ratio in cow-calf treatments from 100 simulations for each parameter set with target population sizes of 200, 300, 400, 500, 600 and 700. Because target population size had a higher priority than sex ratio, target sex ratios were not always achieved in simulations with a target proportion of males greater than 0.60 (Table 1.2). Herds with a low proportion of males (< 0.50) retained less H_0 than herds with a higher proportion of males (Figure 1.6). This effect was more pronounced for THRO than YELL.

Effects of sex ratio – random removals

In simulations conducted for this project, random removals represented a 'null model' for treatment effects. Random removal treatments did not control or bound changes in age structure or sex ratio, and these population-level attributes thus varied a result of vital rates and the

Table 1.2. Target and average (std) achieved sex ratios for random and cow-calf removal treatments using YELL and THRO vital rates. Averages calculated from years 20-200, across population targets of 200, 300, 400, 500, 600, and 700. There were no differences in achieved sex ratios between removal strategies.

Target	THRO	YELL
0.2	0.20 (0.000)	0.18 (0.000)
0.3	0.30 (0.000)	0.28 (0.000)
0.4	0.40 (0.000)	0.39 (0.000)
0.5	0.50 (0.000)	0.49 (0.000)
0.6	0.59 (0.000)	0.58 (0.002)
0.7	0.69 (0.001)	0.60 (0.001)
0.8	0.74 (0.003)	0.60 (0.001)

sampling error inherent to processes in small populations (demographic stochasticity). However, herd managers may set an objective for a prescribed herd sex ratio and we thus conducted simulation experiments to examine the likely consequences of managing for both a prescribed herd size and sex ratio. We conducted a limited set of simulations where target sex ratios were to achieve herds composed of 20% to 80% males and sex ratio was held constant. Cows and bulls of random ages were selected for harvest.

Treatments that resulted in highly biased herd sex ratios had profound effects on the age structure of the herd, especially when there were few males (Figure 1.4, 1.5). Retention of H_0 was much lower in strongly female-biased herds (Figure 1.6). Two obvious factors that contributed to this result were (1) very high growth rates that required annual removal of a relatively large proportion of the herd to maintain the target size, and (2) the very small proportion of breeding-age males in these herds.

Loss of H_0 was much greater when sex ratios were female biased, while a strong male bias in sex ratio had relatively little effect (Figure 1.6). Effects of a strong male bias were greater for simulations of THRO than for YELL, which reflected the influence on male age structure in these herds and the resulting greater numbers of breeding-age males (Figure 1.4, 1.5). The very small proportion of breeding-age males in some cow-calf removal treatments were accompanied by very high variation in individual male breeding success, especially in THRO (Figure 1.7). Trends in retention of H_0 were also related to changes in generation time of males, but the effects of changes in variation in individual breeding success clearly had a much strong influence on H_0 (Table 1.3).

Table 1.3. Average generation time (years) of cow and bull bison from simulations where removal treatment (cow-calf removals, random removals) and the target proportion of males ('Target males') in the population varied. Maximum achieved proportion of males varied by treatment and park, and the maximum for YELL and THRO were about 0.60 and 0.73, respectively. Values are means (std) across populations of 200, 300, 400, 500, 600, and 700. There was very little variation in generation time between population sizes.

Target	YELL Cow-calf removal (0.24)		YELL Random removals		THRO Cow-calf removal (0.50)		THRO Random removals	
males	Cows	Bulls	Cows	Bulls	Cows	Bulls	Cows	Bulls
0.2	6.9 (0.01)	6.6 (0.05)	7.0 (0.00)	4.8 (0.01)	6.3 (0.00)	5.6 (0.03)	6.7 (0.02)	6.4 (0.07)
0.3	6.9 (0.00)	7.3 (0.02)	7.0 (0.00)	5.5 (0.01)	6.3 (0.01)	6.1 (0.01)	6.7 (0.01)	6.9 (0.04)
0.4	7.0 (0.00)	7.7 (0.01)	7.0 (0.01)	6.0 (0.01)	6.4 (0.00)	6.6 (0.01)	6.7 (0.01)	7.4 (0.02)
0.5	7.0 (0.01)	8.1 (0.01)	7.0 (0.01)	6.5 (0.01)	6.4 (0.00)	7.0 (0.00)	6.7 (0.01)	7.7 (0.01)
0.6	7.0 (0.01)	8.3 (0.01)	7.0 (0.01)	6.8 (0.01)	6.4 (0.00)	7.4 (0.00)	6.7 (0.01)	8.0 (0.01)
0.7*	7.0 (0.01)	8.5 (0.31)	7.0 (0.01)	6.9 (0.01)	6.5 (0.00)	7.7 (0.00)	6.7 (0.01)	8.3 (0.01)
0.8*	7.0 (0.01)	8.6 (0.29)	7.0 (0.01)	7.0 (0.01)	6.5 (0.01)	7.8 (0.00)	6.7 (0.01)	8.4 (0.01)

* These targets were not always achieved – see Table 1.2.

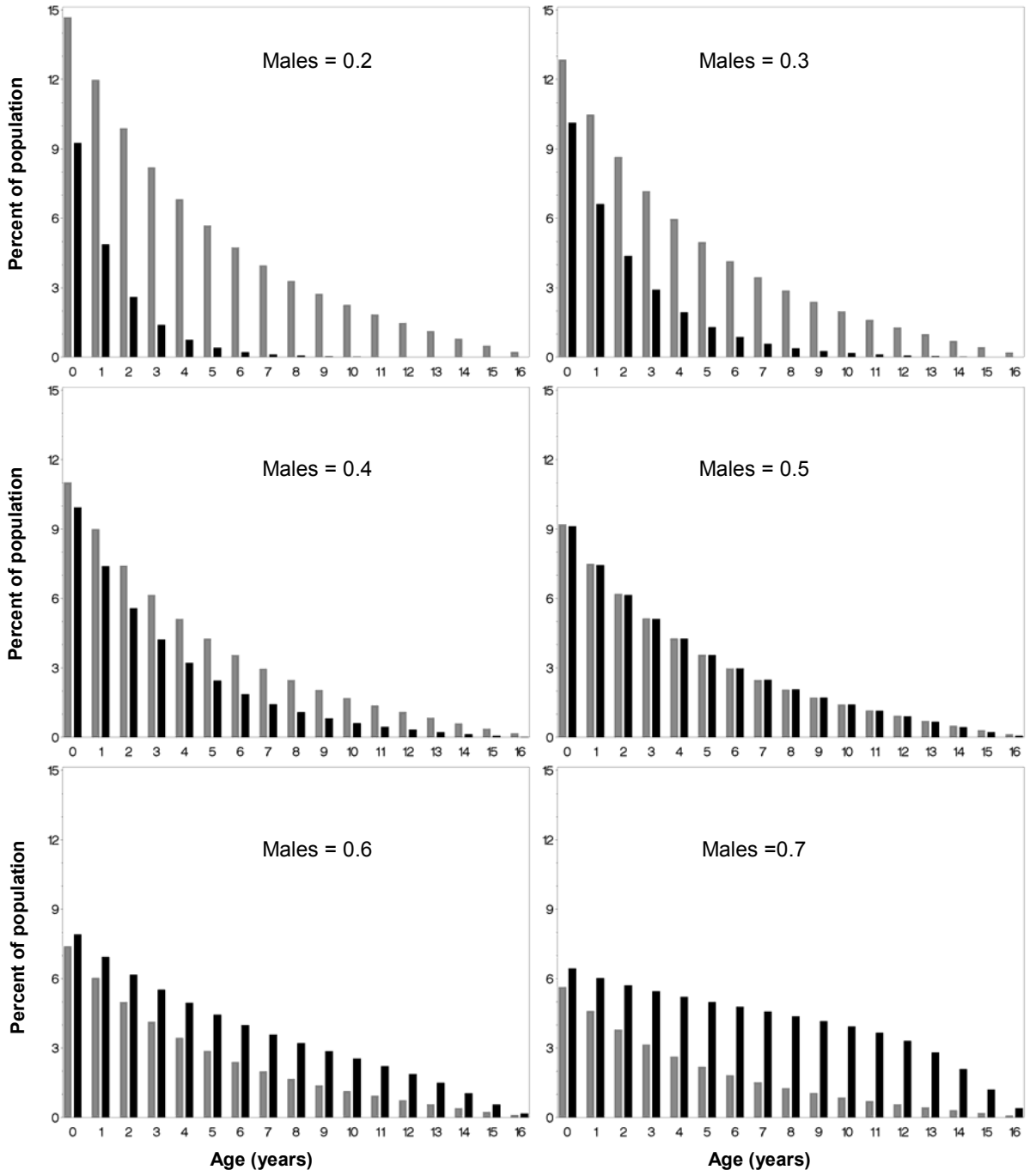

Figure 1.4. Age structure of simulated bison herds subjected to random-age removals where sex ratio was controlled, and the target proportion of males in the population varied from 0.2 to 0.7. Based on vital rate parameters for THRO. Gray bars are females, black bars are males. Age structure did not vary with population size.

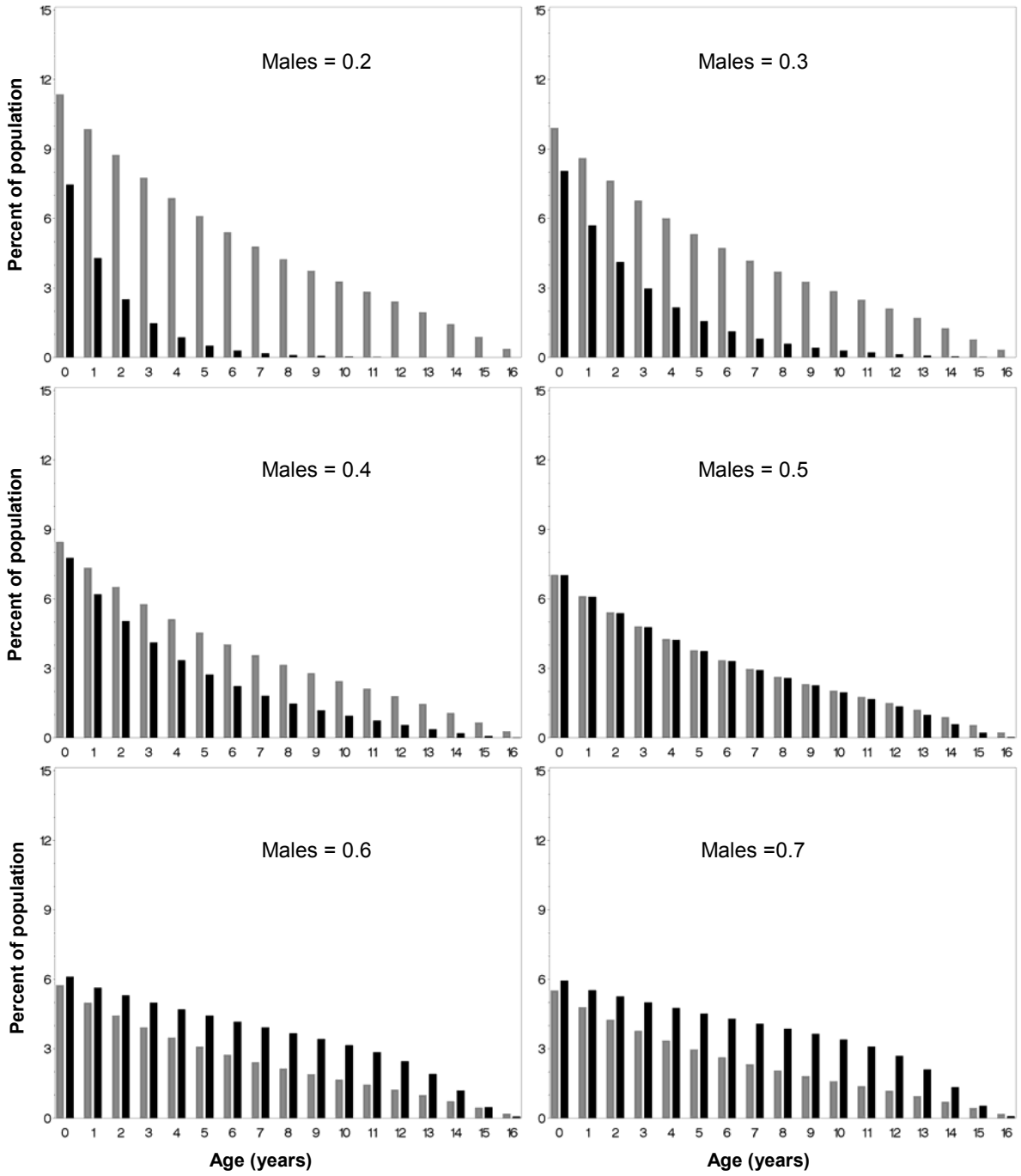

Figure 1.5. Age structure of simulated bison herds subjected to random-age removals where sex ratio was controlled, and the target proportion of males in the population varied from 0.2 to 0.7. Based on vital rate parameters for YELL. Gray bars are females, black bars are males. Age structure did not vary with population size.

Relative effects of vital rates and genetic constitution

We examined the relative effects of high (THRO) and low (YELL) reproductive and survival rates for bison and of high (YELL) and low (THRO) levels of extant genetic diversity by crossing model inputs for these factors and comparing retention of H_0. To do so, we initialized the model with populations that used vital rates from one population and genetic data from the other. We simulated random age removals using all four combinations of parameter sets (YELL-YELL, YELL-THRO, THRO-THRO, THRO-YELL) with target proportions of males of 0.20 to 0.80. Simulations were conducted as described above.

Differences in genetic composition of YELL and THRO had a small but consistent effect on retention of H_0 (Figure 1.8). Simulated populations initialized with genetic data from YELL consistently lost slightly more H_0 than those initialized data from THRO, reflecting the greater number of rare alleles in YELL and initial greater H_0. The effects of genetic composition were more pronounced for simulations using vital rates from THRO than YELL, which is consistent with higher growth rates of THRO (and thus a decreased generation time, higher reproductive variance, and harvest of a larger proportion of the population). However, the effects of differences in genetic composition were small compared to other factors, especially population size.

Vital rates had a strong influence when sex ratios were highly female-biased, but relatively little effect when herd sex ratios were near unity (Figure 1.9). Similarly, the effects of vital rates were more pronounced in small populations. When there was an effect, higher survival and reproductive rates led to more rapid losses of H_0, but population size had a greater effect than vital rates.

These results clearly show that decisions on management of population size can have a profound effect on genetic diversity in small populations. As population size increases, the consequences of a biased sex ratio, harvest strategy, and variance in individual reproductive success are much reduced, and for very large bison herds (say, > 1500), management decisions are unlikely to significantly affect retention of genetic variation.

Our simulations assumed that individual bison in herds mixed randomly and that herds were relatively homogeneous. Population substructures can result in reduced rates of genetic recombination and in non-random harvest of animals. Results in this report are thus more appropriately applied, for example, to the YELL northern range herd or the YELL central herd, rather than to the entire YELL bison population. Similarly, spatial structuring in a park like BADL may lead to highly non-random removals, thereby increasing the loss of genetic diversity relative to these simulations.

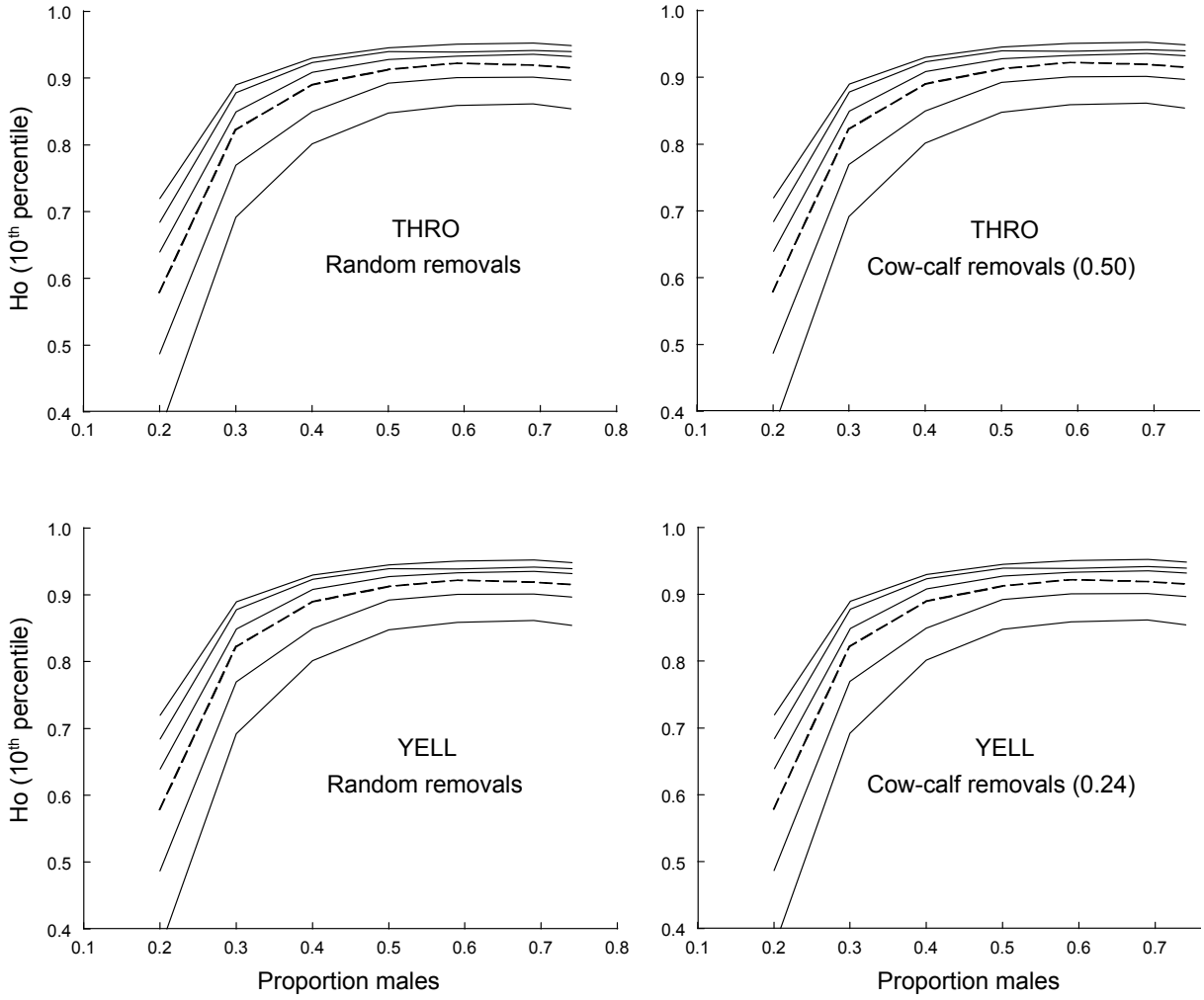

Figure 1.6. Observed heterozygosity (*Ho*; lower 10 percentile) at year 200 for simulations using vital rates and genetic data (Halbert 2003) from YELL and THRO. See text for treatments; cow-calf removal rates were 50% and 24% for THRO and YELL, respectively. Lines, from bottom to top, show results for population sizes of 200, 300, 400 (dashed line), 500, 600, and 700.

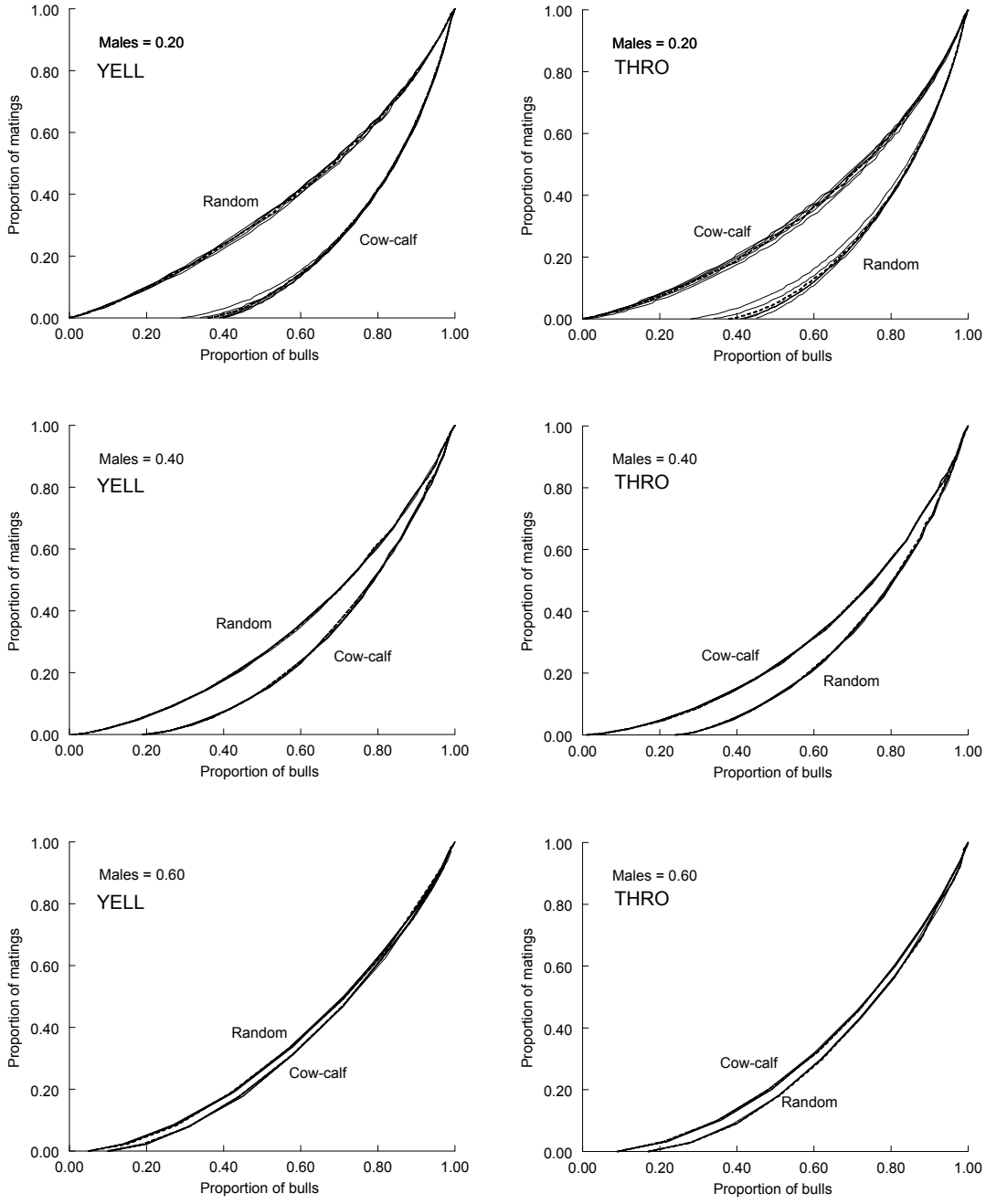

Figure 1.7. Cumulative distribution of individual male breeding success for random removals (Random) and removal of cow-calf pairs (Cow-calf) for YELL (left column) and THRO (right column). Note contrasting effects of treatments for on variation in breeding success. Lines are for population sizes of 200, 300, 400 (dashed line), 500, 600, and 700.

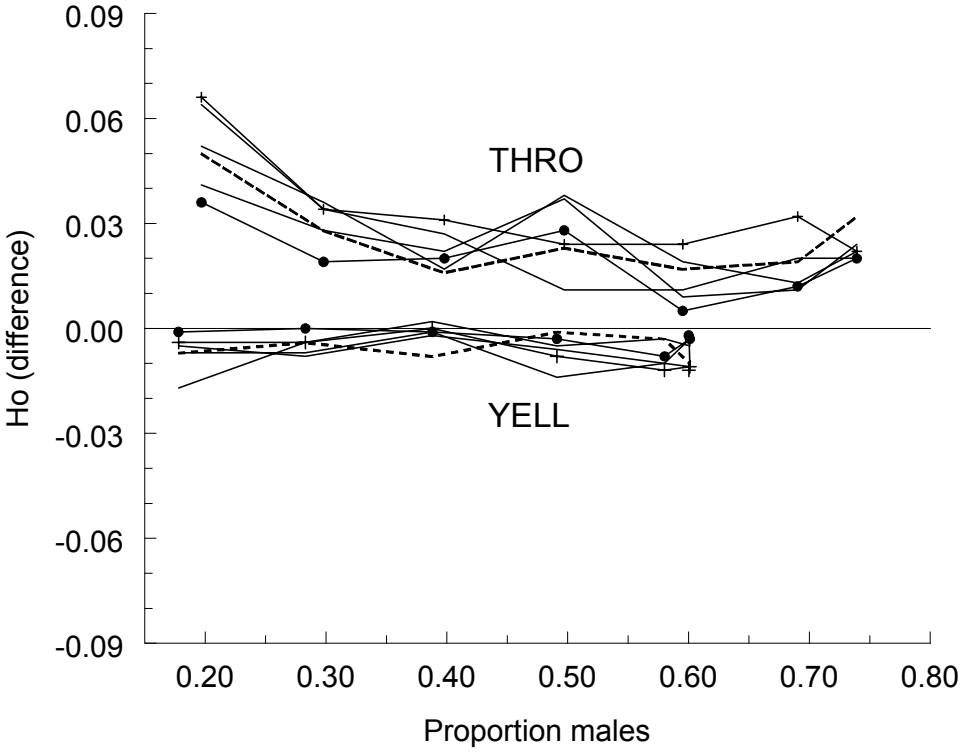

Figure 1.8. Plot showing the small effect of genetic constitution on retention of Ho. Vertical axis is the difference in *Ho* (lower 10 percentile) at year 200 for simulations of cow-calf removals using vital rates and genetic data (Halbert 2003) from YELL and THRO. Top set of lines (THRO) are results from simulations that used THRO vital rates and bottom set of lines (YELL) used YELL vital rates. Top lines were obtained by subtracting results from simulations initialized with YELL genetic data from those using the THRO genome. Bottom lines were obtained by subtracting results from simulations using THRO genetic data from those using the YELL genome. See text for treatments; cow-calf removal rates were 50% and 24% for THRO and YELL, respectively. Lines are results for population sizes of 200 (line with +), 300, 400 (dashed line), 500, 600, and 700 (line with filled circle).

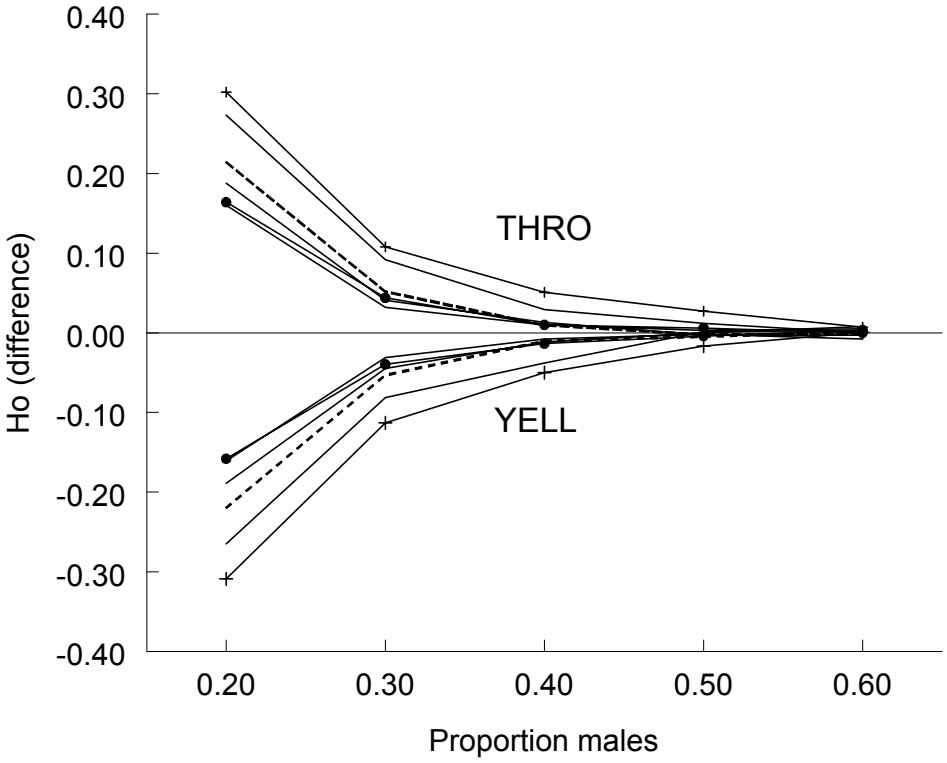

Figure 1.9. Plot showing effects of differences in vital rates on *Ho* for a relatively diverse herd (YELL) and a relatively homogeneous herd (THRO). The vertical axis is the difference in observed heterozygosity (*Ho*; lower 10% percentile) at year 200 for simulations of cow-calf removals, with controlled sex ratio, using vital rates and genetic data (Halbert 2003) from YELL and THRO. Top set of lines (THRO) are results from simulations that used THRO genetic composition and bottom set of lines (YELL) used genetic data from YELL. Results displayed in the upper set of lines are differences obtained by subtracting results from simulations that used THRO vital rates from those using YELL vital rates; bottom lines were obtained by subtracting results from simulations using YELL vital rates from those using THRO vital rates. Cow-calf removal rates were 50% and 24% for THRO and YELL, respectively. Lines are results for population sizes of 200 (line with +), 300, 400 (dashed line), 500, 600, and 700 (line with filled circle). Results reported only for simulations were average sex ratios were approximately equal across treatments (20% to 60% males; Table 1.2).

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Patterns of genetic variation in US federal bison herds

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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.

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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

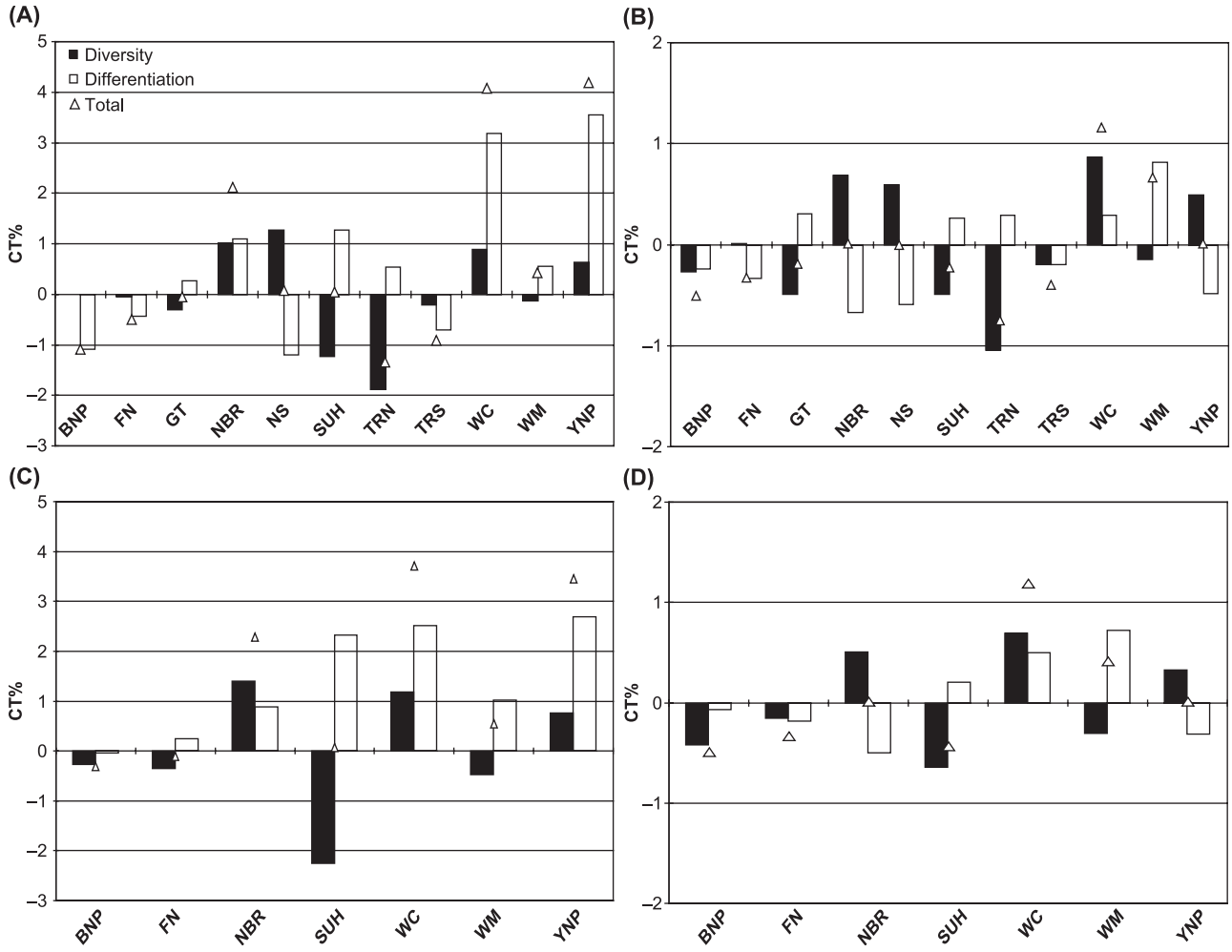

Fig. 1 Relative genetic contribution of each of the 11 federal bison herds to overall allelic richness (panel A) and gene diversity (panel B) based on 48 autosomal markers. An independent analysis with only the seven core herds was similarly performed to measure overall contributions to allelic richness (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

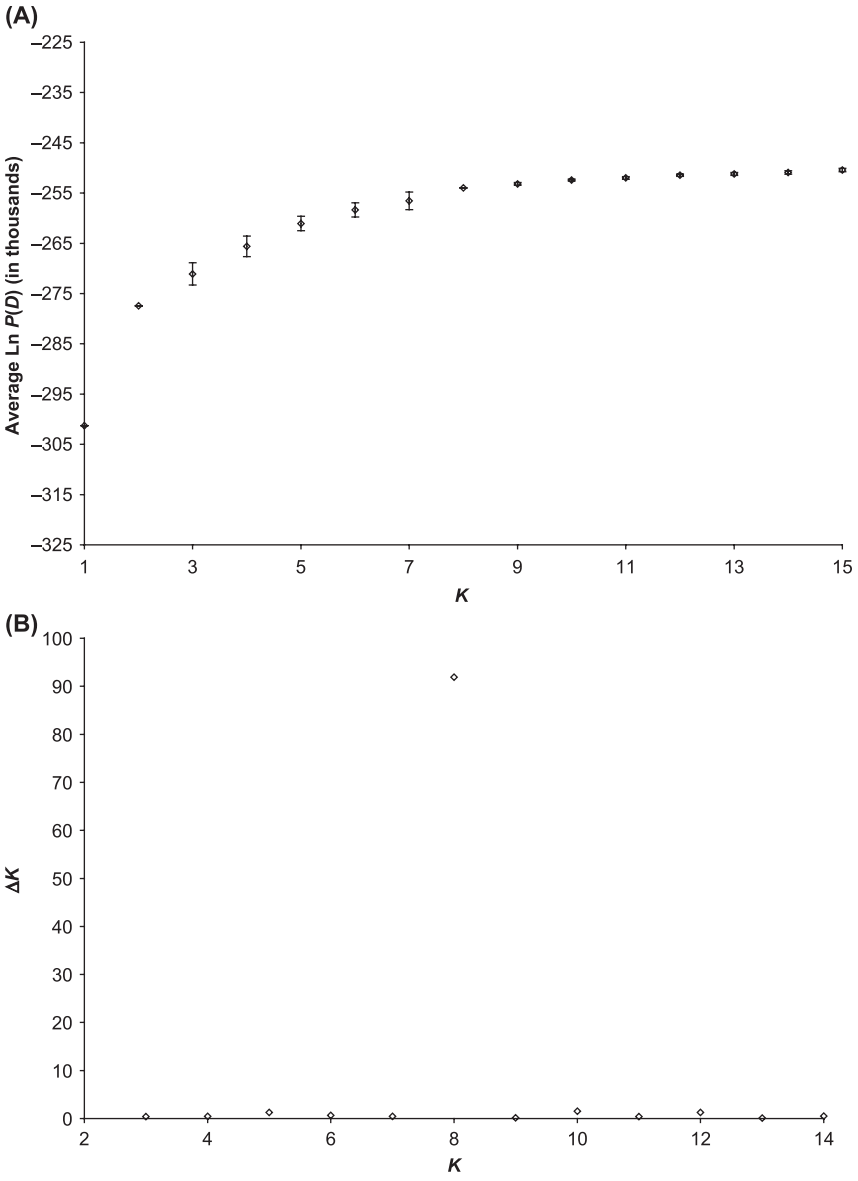

Fig. 2 Evaluation of Structure clustering for K values ranging from 1 to 15. In panel A, averages and standard deviations for Ln $P(D)$ values based on 10 simulations for each value of K are shown. Corresponding ΔK values are shown in panel B, following the calculations of Evanno *et al*. (2005). The 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

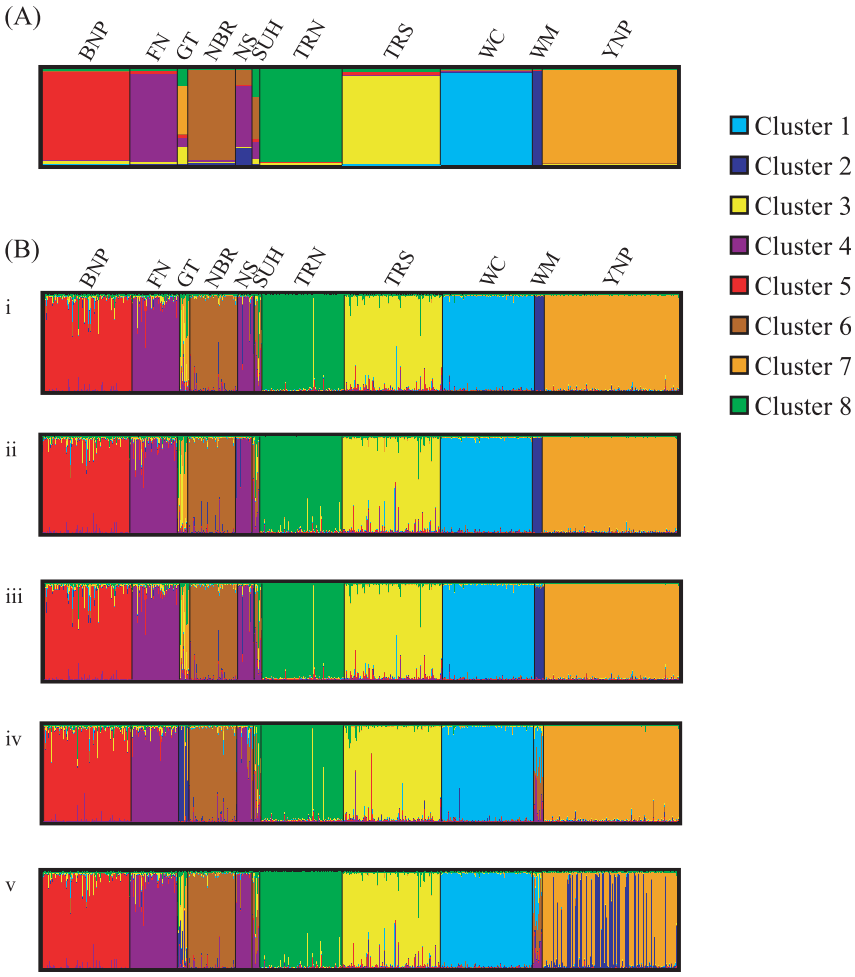

Fig. 3 A comparison of estimated population structure across 11 geographically defined populations of bison into eight clusters (identified by colour). In panel A, average cluster assignments across 10 independent iterations with K_8 are indicated for each of 11 geographically defined herds. In panel 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	—

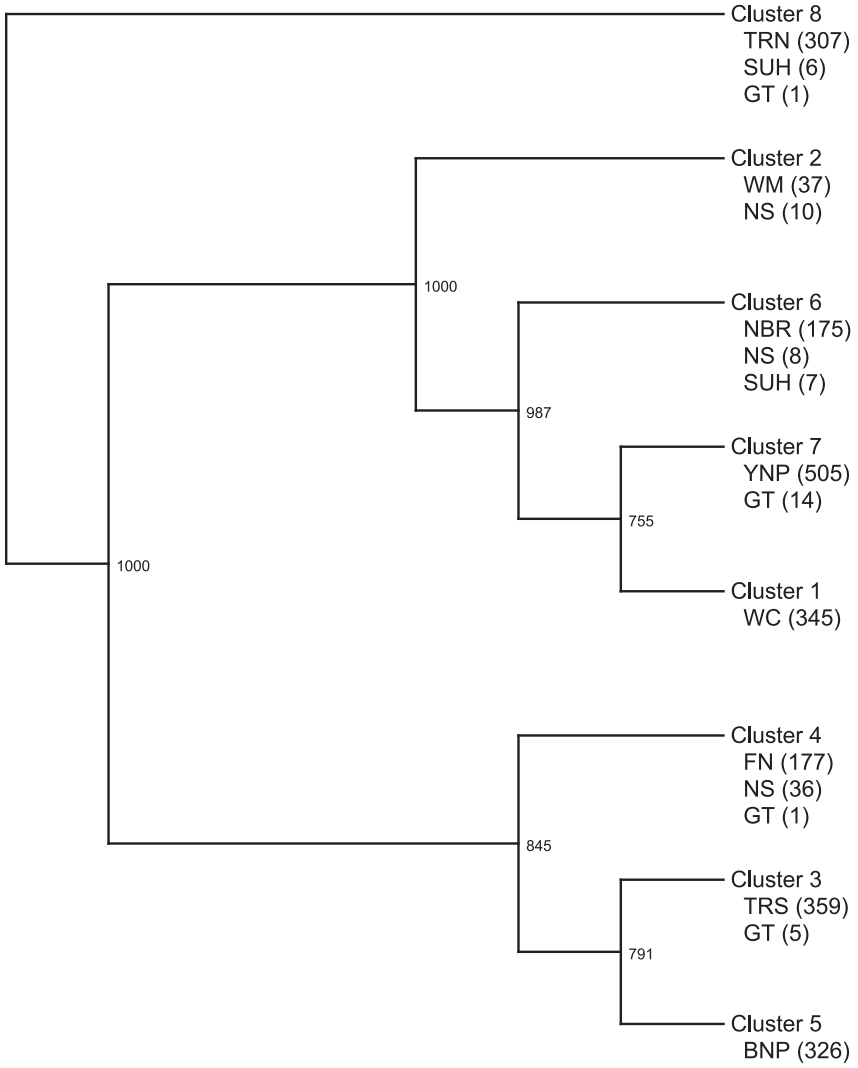

Fig. 4 UPGMA tree diagram based on Reynolds *et al*. (1983) genetic distances. Cluster numbers correspond to Fig. 3.

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

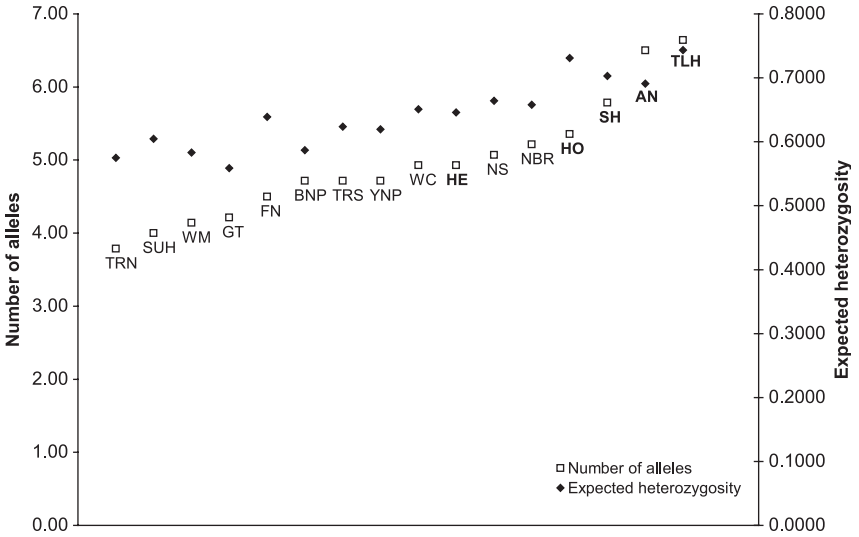

Fig. 5 Average number of alleles per locus and average expected heterozygosity across 14 microsatellite markers for 11 bison herds (Table 2) and 5 domestic cattle (*B. taurus*) breeds (AN, Angus; HE, Hereford; HO, Holstein; SH, Shorthorn; TLH, Texas Longhorn). Domestic cattle breed data from Schnabel *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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References

Amos W, Balmford A (2001) When does conservation genetics matter? *Heredity*, **87**, 257–265.

Berger J, Cunningham C (1994) *Bison: Mating and Conservation in Small Populations*. Columbia University Press, New York.

Botstein D, White RL, Skolnick M, Davis RW (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American Journal of Human Genetics*, **32**, 314–331.

Boyd DP (2003) *Conservation of North American bison: status and recommendations*. MS Thesis. University of Calgary, Alberta, Canada.

Bradshaw CJA, Isagi Y, Kaneko S, Bowman DMJS, Brook BW (2005) Conservation value of non-native banteng in Northern Australia. *Conservation Biology*, **20**, 1306–1311.

Bradshaw CJ, Isagi Y, Kaneko S *et al*. (2007) Low genetic diversity in the bottlenecked population of endangered non-native banteng in northern Australia. *Molecular Ecology*, **16**, 2998–3008.

Broders HG, Mahoney SP, Montevecchi WA, Davidson WS (1999) Population genetic structure and the effect of founder events on the genetic variability of moose, *Alces alces*, in Canada. *Molecular Ecology*, **8**, 1309–1315.

Ceballos G, Ehrlich PR (2002) Mammal population losses and the extinction crisis. *Science*, **296**, 904–907.

Coder GD (1975) *The national movement to preserve the American buffalo in the United States and Canada between 1880 and 1920*. PhD Thesis. The Ohio State University, Columbus, Ohio.

Coltman DW (2008) Molecular ecological approaches to studying the evolutionary impact of selective harvesting in wildlife. *Molecular Ecology*, **17**, 221–235.

Coppedge BR, Carter TS, Shaw JH, Hamilton RG (1997) Agnostic behaviour associated with orphan bison (*Bison bison* L.) calves released into a mixed resident population. *Applied Animal Behaviour Science*, **55**, 1–10.

Dieringer D, Schlötterer C (2003) Microsatellite analyser (MSA): a platform independent analysis tool for large microsatellite data sets. *Molecular Ecology Notes*, **3**, 167–169.

Dinerstein E, McCracken GF (1990) Endangered greater one-horned rhinoceros carry high levels of genetic variation. *Conservation Biology*, **4**, 417–422.

El Mousadik A, Petit RJ (1996) High level of genetic differentiation for allelic richness among populations of the argan tree [*Argania spinosa* (L.) Skeels] endemic to Morocco. *Theoretical and Applied Genetics*, **92**, 832–839.

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software Structure: a simulation study. *Molecular Ecology*, **14**, 2611–2620.

Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics*, **164**, 1567–1587.

Felsenstein J (1993) PHYLIP *(Phylogeny Inference Package)*. Distributed by the author. Department of Genetics, University of Washington, Seattle, Washington.

Fischer J, Lindenmayer DB (2000) An assessment of the published results of animal relocations. *Biological Conservation*, **96**, 1–11.

Fish & Wildlife Service (2003) Ft. Niobrara-Valentine National Wildlife Refuge Complex Bison Briefing Paper. Fish & Wildlife Service, Valentine, Nebraska.

Frankham R (1996) Relationship of genetic variation to population size in wildlife. *Conservation Biology*, **10**, 1500–1508.

Frankham R (2003) Genetics and conservation biology. *Comptes Rendus Biologies*, **326**, 22–29.

Freese CH, Aune KE, Boyd DP *et al*. (2007) Second chance for the plains bison. *Biological Conservation*, **136**, 175–184.

Funk CW, Mullins TD, Haig SM (2007) Conservation genetics of snowy plovers (*Charadrius alexandrinus*) in the Western Hemisphere: population genetic structure and delineation of subspecies. *Conservation Genetics*, **8**, 1287–1309.

Glaubitz JC (2004) Convert: a user-friendly program to reformat diploid genotypic data for commonly used population genetic software packages. *Molecular Ecology Notes*, **4**, 309–310.

Gottelli D, Sillero-Zubiri C, Applebaum GD *et al*. (1994) Molecular genetics of the most endangered canid: the Ethiopian wolf *Canis simensis*. *Molecular Ecology*, **3**, 301–312.

Goudet J (2001) FSTAT, *A Program to Estimate and Test Gene Diversities and Fixation Indices*. Available from URL: http://www.unil.ch/izea/softwares/fstat.html, updated from Goudet (1995).

Hailer F, Helander B, Folkestad AO *et al*. (2006) Bottlenecked but long-lived: high genetic diversity retained in white-tailed eagles upon recovery from population decline. *Biology Letters*, **2**, 316–319.

Halbert ND, Derr JN (2007) A comprehensive evaluation of cattle introgression into US federal bison herds. *Journal of Heredity*, **98**, 1–12.

Halbert ND, Raudsepp T, Chowdhary BP, Derr JN (2004) Conservation genetic analysis of the Texas State bison herd. *Journal of Mammalogy*, **85**, 924–931.

Halbert ND, Ward TJ, Schnabel RD, Taylor JF, Derr JN (2005) Conservation genomics: disequilibrium mapping of domestic cattle chromosomal segments in North American bison populations. *Molecular Ecology*, **14**, 2343–2362.

Halbert ND, Gogan PJP, Hiebert R, Derr JN (2007) Where the buffalo roam: the role of history and genetics in the conservation of bison on U.S. federal lands. *Park Science*, **24**, 22–29.

Helbig L, Woodbury MR, Haigh JC, Barth AD (2006) The onset of puberty in North American bison (*Bison bison*) bulls. *Animal Reproduction Science*, 1–13.

Jakobsson M, Rosenberg NA (2007) CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics*, **23**, 1801–1806.

Kirkpatrick JF, McCarthy JC, Gudermuth DF, Shideler SE, Lasley BL (1996) An assessment of the reproductive biology of Yellowstone bison (*Bison bison*) subpopulations using noncapture methods. *Canadian Journal of Zoology*, **74**, 8–14.

Laliberte AS, Ripple WJ (2004) Range contractions of North American carnivores and ungulates. *Bioscience*, **54**, 123–138.

Lande R, Barrowclough GF (1987) Effective population size, genetic variation, and their use in population management. In: *Viable Populations for Conservation* (ed. Soulé ME), pp. 87–123. Cambridge University Press, Cambridge, UK.

Latch E, Dharmarajan G, Glaubitz J, Rhodes O (2006) Relative performance of Bayesian clustering software for inferring population substructure and individual assignment at low levels of population differentiation. *Conservation Genetics*, **7**, 295–302.

Legendre S, Clobert J, Moller AP, Sorci G (1999) Demographic stochasticity and social mating system in the process of extinction of small populations: the case of passerines introduced to New Zealand. *The American Naturalist*, **153**, 449–463.

Luenser K, Fickel J, Lehnen A, Speck S (2005) Low level of genetic variability in European bisons (*Bison bonasus*) from the Bialowieza National Park in Poland. *European Journal of Wildlife Research*, **51**, 84–87.

Margan SH, Nurthen RK, Montgomery ME *et al*. (1998) Single large or several small? Population fragmentation in the captive management of endangered species. *Zoo Biology*, **17**, 467–480.

Milner-Gulland EJ, Bukreeva OM, Coulson T *et al*. (2003) Conservation: reproductive collapse in saiga antelope harems. *Nature*, **422**, 135.

National Park Service (2003) Bison Management Strategy Wind Cave National Park, National Park Service, Hot Springs, South Dakota.

Nei M (1987) *Molecular Evolutionary Genetics*. Columbia University Press, New York.

Nei M, Maruyama T, Chakraborty R (1975) The bottleneck effect and genetic variability in populations. *Evolution*, **29**, 1–10.

Page RDM (1996) TreeView: an application to display phylogenetic trees on personal computers. *Computer Applications in the Biosciences*, **12**, 357–358.

Park SDE (2001) *Trypanotolerance in West African cattle and the population genetic effects of selection*. PhD Thesis. University of Dublin, Dublin, Ireland.

Petit RJ, Mousadik AE, Pons O (1998) Identifying populations for conservation on the basis of genetic markers. *Conservation Biology*, **12**, 844–855.

Polziehn RO, Strobeck CM, Sheraton J, Beech R (1995) Bovine mtDNA discovered in North American bison populations. *Conservation Biology*, **9**, 1638–1643.

Primack RB (1993) *Essentials of Conservation Biology*. Sinauer & Associates Inc., Sunderland, Massachusetts.

Pritchard JK, Wen W (2004) *Documentation for Structure Software, Version 2*. http://pritch.bsd.uchicago.edu.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.

Raymond M, Rousset F (1995) GenePop (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.

Reynolds J, Weir BS, Cockerham CC (1983) Estimation of the coancestry coefficient: basis for a short-term genetic distance. *Genetics*, **105**, 767–779.

Rosenberg NA (2004) Distruct: a program for the graphical display of population structure. *Molecular Ecology Notes*, **4**, 137–138.

Rosenberg NA, Burke T, Elo K *et al*. (2001) Empirical evaluation of genetic clustering methods using multilocus genotypes from 20 chicken breeds. *Genetics*, **159**, 699–713.

Sanderson EW, Redford KH, Weber B *et al*. (2008) The ecological future of the North American bison: conceiving long-term, large-scale conservation of wildlife. *Conservation Biology*, **22**, 252–266.

Schnabel RD, Ward TJ, Derr JN (2000) Validation of 15 microsatellites for parentage testing in North American bison, *Bison bison* and domestic cattle. *Animal Genetics*, **31**, 360–366.

Shier DM (2006) Effect of family support on the success of translocated black-tailed prairie dogs. *Conservation Biology*, **20**, 1780–1790.

Slate J, Pemberton JM (2007) Admixture and patterns of linkage disequilibrium in a free-living vertebrate population. *Journal of Evolutionary Biology*, **20**, 1415–1427.

Soper JD (1941) History, range and home life of the northern bison. *Ecological Monographs*, **11**, 349–410.

Taylor SS, Jamieson IG (2008) No evidence for loss of genetic variation following sequential translocations in extant populations of a genetically depauperate species. *Molecular Ecology*, **17**, 545–556.

Uphyrkina O, Miquelle D, Quigley H, Driscoll C, O'Brien SJ (2002) Conservation genetics of the Far Eastern leopard (*Panthera pardus orientalis*). *The Journal of Heredity*, **93**, 303–311.

Waples RS, Gaggiotti O (2006) What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. *Molecular Ecology*, **15**, 1419–1439.

Ward TJ, Bielawski JP, Davis SK, Templeton JW, Derr JN (1999) Identification of domestic cattle hybrids in wild cattle and bison species: a general approach using mtDNA markers and the parametric bootstrap. *Animal Conservation*, **2**, 51–57.

Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population structure. *Evolution*, **38**, 1358–1370.

Wessa P (2008) *Free Statistics Software, version 1.1.23-r2. Office for Research Development and Education*. http://www.wessa.net/.

Whitehouse AM, Harley EH (2001) Post-bottleneck genetic diversity of elephant populations in South Africa, revealed using microsatellite analysis. *Molecular Ecology*, **10**, 2139–2149.

Williams CL, Serfass TL, Cogan R, Rhodes OE (2002) Microsatellite variation in the reintroduced Pennsylvania elk herd. *Molecular Ecology*, **11**, 1299–1310.

Wilson GA, Strobeck CM (1999) Genetic variation within and relatedness among wood and plains bison populations. *Genome*, **42**, 483–496.

Wisely SM, Buskirk SW, Fleming MA, McDonald DB, Ostrander EA (2002) Genetic diversity and fitness in black-footed ferrets before and during a bottleneck. *Journal of Heredity*, **93**, 231–237.

Zenger KR, Richardson BJ, Vachot-Griffin AM (2003) A rapid population expansion retains genetic diversity within European rabbits in Australia. *Molecular Ecology*, **12**, 789–794.

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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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