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Evaluation of alternative management strategies for maintenance of genetic variation in wildlife populations

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Abstract

Wildlife management strategies are often designed around a population's demographic goals, but such strategies also can inadvertently impact genetic variation. For species like bison *Bison bison*, where management includes the regular removal of individuals to maintain restricted population sizes on constrained landscapes, management actions can be tailored to address genetic diversity retention in addition to simply maintaining a target population size. In this study, we provide an assessment of alternative culling strategies for maintenance of genetic variation in managed wildlife populations. Our primary goal was to compare the long-term retention of genetic variation and accumulation of inbreeding among three types of culling strategies, including one that considered genetic variation directly by measuring variation at a suite of variable loci [mean allele frequency (MAF) strategy], one that used genome-wide measures of variation [mean kinship (MK) strategy] and one that relied solely on demographic information (sex and age; RANDOM). To achieve this goal, we built an individual-based model, parameterized in accordance with bison biology, to project levels of genetic variation and inbreeding over time under each of the three management strategies. Our results suggest wildlife management strategies that incorporate goals for retaining genetic variation (MAF and MK strategies) are better suited to preserving the evolutionary potential of wildlife populations than those that focus solely on a target size and demographic stability (RANDOM). In particular, the MK culling strategy performed the best at maximizing the retention of genome-wide variation. These results extend previous work demonstrating the utility of pedigree-based mate selection strategies in captive population management, and show that such strategies maximize the retention of genome-wide variation under culling practices as well. These models will aid in the long-term management of bison, and can be adapted to other managed wildlife species.

Introduction

Wildlife management is an old practice, with Egyptian hunting records dating as far back as 2500 BCE (Leopold, 1933; Gilbert & Dodds, 2001). Today, wildlife management programs aim to maintain self-sustaining populations that are viable over the long term. Historically, this goal has been met by focusing on actions to maintain demographic stability, mainly by enforcing hunting and trapping restrictions such as bag limits or closed harvest seasons. As habitats are increasingly altered and wildlife populations are more heavily impacted by human activities, the intensity of wildlife management has increased, with more species dependent on regular monitoring and intervention to ensure their persistence. Management practices such as moratoria, anti-poaching efforts, predator removal, culling, health care and disease management are often undertaken at the scale of the individual animal, especially for small populations.

Small, isolated populations are not only less demographically stable than large populations, but they are also more susceptible to erosion of genetic variation by genetic drift (Wright, 1931). In the absence of gene flow, the loss of genetic variation through drift is not mitigated. A lack of genetic variation not only makes a population more susceptible to inbreeding depression (Ralls, Brugger & Ballou, 1979; Crnokrak & Roff, 1999; Keller & Waller, 2002), but also less able to adapt to changing environmental conditions (Falconer, 1981; Keller *et al.*, 1994; Willi, Van Buskirk & Hoffmann, 2006; Markert *et al.*, 2010). Preserving genetic variation has become a priority for management, particularly

for small and isolated populations, in order to maintain long term viability (McNeely *et al.*, 1990; Lacy, 1997).

A number of different strategies exist to maintain genetic variation in small, isolated populations. Indirect methods aim to maximize the exchange of genetic variation from generation to generation, and include maintaining balanced sex ratios (Komers & Curman, 2000; Harris, Wall & Allendorf, 2002; Peek *et al.*, 2002; Wedekind, 2002), avoiding fluctuations in population size over time (Caballero & Toro, 2000) and extending mean generation length (Foose & Ballou, 1988). For example, removal or contraception of young animals results in a greater proportion of offspring born to older females, an increase in generation time and higher retention of genetic variation (Gross, 2000; Gross & Wang, 2005; Hailer *et al.*, 2006). These indirect approaches are typically straightforward to implement because they do not require genetic data to be obtained for individuals.

Management strategies can also be designed to manage genetic variation directly. If genetic data are available for individual animals, strategies designed to maximize the retention of alleles could maintain variation in small and isolated populations (Wayne *et al.*, 1986). When rare alleles are found in individuals with underrepresented ancestry, an allele retention strategy could preserve rare and potentially important variation in a population (Hedrick & Miller, 1994). In contrast, if rare variants are deleterious, selective retention of such alleles could reduce the population's fitness (Hedrick *et al.*, 1986; Lacy, 2000). Additionally, strategies to retain specific rare alleles might result in loss of genetic variation across the remainder of the genome (Haig, Ballou & Derrickson, 1990; Vrijenhoek & Leberg, 1991; Miller, 1995). Rare allele retention strategies have been shown to be ineffective at retaining overall genetic variation when selecting breeding pairs for captive population management (Haig *et al.*, 1990; Miller, 1995). Nevertheless, such strategies could be effective in a population-based management approach in which individuals with rare alleles are preferentially retained in the population, but may or may not actually produce offspring because breeding is not managed.

The most effective genetic management strategies for captive population management have been those that consider genome-wide variation, rather than variation at a suite of target loci. Specifically, a management strategy that minimizes the average kinship (i.e. coancestry) in a population is an effective way to retain genetic diversity and limit the accumulation of inbreeding (Ballou & Lacy, 1995; Fernández & Toro, 1999; Sonesson & Meuwissen, 2001). A population's average kinship can be managed through breeding genetically valuable individuals (i.e. those with few relatives in the population and low mean kinships (MKs); Ballou & Lacy, 1995). However, it is impossible to dictate breeding pairs in free-ranging populations. In order to minimize average kinship in wild or semi-wild populations in which breeding pair selection is not possible, individuals with high MKs could be removed from populations. For example, removing individuals with high MK values and replacing them with unrelated individuals is outlined in the conservation plan for island populations of the endangered takahe in New Zealand (Grueber *et al.*, 2010). The concept has also been evaluated as a possible option for controlling the population size of wild horses on Assateague Island, while still maintaining genetic variation (Eggert *et al.*, 2010). Although MK-based strategies require genetic data for individual animals and established pedigrees, they could offer a distinct advantage for conserving genetic variation in intensively managed wild populations when such data are available.

Just 200 years ago, plains bison *Bison bison bison* numbered 30–50 million in herds of up to 10 000 animals (Redford & Fearn, 2007). By the late 1800s, massive overhunting and land use change reduced the population to roughly 1000 individuals, <1% of the historical population size. Efforts to establish managed herds led to an increase in the number of bison to over 500 000 individuals in North America (WCS, 2015). The successful recovery of bison is limited by the fact that the majority of extant herds are descendants of fewer than 100 bison from five private herds and a remnant population from the Yellowstone National Park (Coder, 1975). Additionally, <4% of the contemporary North American bison population (~19 000 animals) is currently maintained in conservation herds; the rest are maintained in privately owned or commercial herds. These 19 000 bison are divided into 54 conservation herds, where they are independently managed to maintain the long-term viability of the species (Gates & Aune, 2008).

Though bison have made a remarkable demographic recovery, a number of obstacles remain to ensure genetic viability over the long term. First, conservation herds were established with small numbers of individuals that remained after the severe bottleneck (Halbert, 2003; Halbert & Derr, 2008). Surplus animals from these conservation herds were often used to establish new herds, potentially exacerbating the loss of genetic variation. Second, gene flow between herds has been sporadic during the past century, often limited by concerns about disease introduction (Williams & Barker, 2001). Third, conservation herds are typically maintained at small population sizes to avoid permanent habitat damage and accommodate multiple-use goals on small, isolated reserves (Boyd, 2003; Boyd *et al.*, 2010). To maintain consistent population sizes, individuals are typically removed from populations each year. These obstacles make it critical that management of conservation herds focuses on retaining as much existing variation as possible. The annual removal of individuals is a key stage at which management actions could be designed to maximize the retention of genetic variation over time.

In this study, we provide an assessment of alternative culling strategies for maintenance of genetic variation in managed wildlife populations. Our primary goal was to compare the long-term retention of genetic variation and accumulation of inbreeding among three types of culling strategies, including one that considered genetic variation directly by measuring variation at a suite of variable loci, one that used genome-wide measures of variation and one that relied solely on demographic information (sex and age). To achieve this goal, we built an individual-based simulation, parameterized in accordance with bison biology, to project levels of genetic

variation and inbreeding over time by each of the three management strategies. Such individual-based, forward-in-time models are useful to predict the long-term genetic impact of management actions in small, potentially vulnerable populations (Haig *et al.*, 1990; Bruford *et al.*, 2010; Hoban, Bertorelle & Gaggiotti, 2012) or in populations of long-lived species (Tracy *et al.*, 2011) for which it would take many decades to observe effects of management actions.

Materials and methods

An individual-based computer simulation was constructed using the Visual Studio development environment (v10.0) to test the genetic impacts of three alternative culling strategies for wildlife management (Supporting Information Fig. S1). All culling strategies maintained balanced sex ratios in the population and preferentially culled yearlings.

Overview of culling strategies

MAF strategy

The MAF culling strategy was intended to maximize the retention of genetic variation by using a target set of microsatellite loci to guide culling decisions. Alleles at each target locus were ranked in priority based on their frequencies, and then individuals were selected for cull based on an absence of rare alleles. The overall rarity of an individual's alleles was quantified as MAF, calculated as the frequency of an individual's alleles averaged across all target loci:

$$\mathrm{MAF} = \frac{\sum_{n=1}^{N}(P_{n1} + P_{n2})}{2N},$$

where N represented the number of loci and P_{n1} and P_{n2} represented the population frequencies of the first and second alleles at the nth locus in a given individual. An individual's MAF ranged from a value >0.0 to 1.0, with lower values representing individuals with more rare alleles. Yearlings with the highest MAF values were iteratively selected for cull one at a time until the cull quota was reached, with MAF values being recalculated after each individual cull.

Pedigree-based strategy

The goal of the pedigree-based strategy (MK) was to minimize kinship across the population, thereby maximizing the retention of genome-wide variation. For this strategy, yearlings were chosen for cull based on how well represented their genomes were in the rest of the population. Animals with high representation (i.e. those with many relatives) were chosen for culling, while those with low representation (i.e. those with few relatives) were retained. The kinship (f) of a pair of individuals is the probability that two alleles at a given locus, one randomly drawn from each individual, are identical by descent from a common ancestor (Falconer, 1981). An individual's MK is then the average of pairwise fs between that individual and all living individuals in the population, including itself (Ballou & Lacy, 1995). MKs range from 0.0 to 1.0 and provide a measure of the representation of an individual's genome within a population; individuals with lower MKs have fewer relatives and, on average, carry rarer alleles than individuals with higher MKs. Yearlings with the highest MK values were iteratively selected for cull, one at a time, until the cull quota was reached, with MK values being recalculated after each individual cull.

Random removal strategy

As its name suggests, the random removal culling strategy (RANDOM) randomly removed yearlings from the population until the target size, with an even sex ratio, was reached. The strategy represented an important comparison to the two previously described, data-driven strategies because a random removal represented the least costly culling strategy to implement, both financially and with regard to required personnel, as it would require no genetic or demographic data other than sex and age.

Bison parameters

The simulation was parameterized in accordance with bison biology, using genetic and demographic information from the bison herd managed by the US Fish and Wildlife Service at the Fort Niobrara National Wildlife Refuge (FTN) in north-central Nebraska (Table 1). The FTN herd is managed annually with a population objective of 350 bison to remain in balance with the habitat and needs of other species. Extensive demographic data, complete genotypes for 55 microsatellite loci, and a nearly complete pedigree exist for this herd as of 2004. Data from 2004 to 2010 were used to parameterize the simulation.

Based on an annual 'adult' mortality rate of 3% for females and 5% for males and an annual 'juvenile' mortality rate of 5% for both sexes (Meagher, 1986), a mortality function was defined to accurately reflect age-specific mortality (see General Simulation Overview, for additional details) (Supporting Information Fig. S2). A maximum age of 24 years was specified to model a realistic lifespan (Meagher, 1986). We used the FTN pedigree to generate age-specific fecundity values (M_x; the number of same-sex offspring produced by an individual during an age class) with the PM_x software program (Fig. 1; Ballou, Lacy & Pollak, 2011). From the M_x distributions, we derived that reproduction generally occurs between ages 4 and 16 for males and between ages 3 and 21 for females. Although calving rates vary considerably between bison herds (Shaw & Carter, 1989), in concordance with recent research (Borgreen, 2010) we specified that 82% of breeding-age female bison in the FTN herd annually produce offspring. The yearly percentage of sexually mature males assigned as breeders was set at 46% based on the average proportion of males in a given year that sired offspring within the FTN herd (data from 2004 to 2010). Male breeders were further categorized as either subordinate or dominant. Using the number of offspring annually sired

Table 1 Parameters used for the model, based on bison biology and data from the bison herd at the Fort Niobrara National Wildlife Refuge

Input file parameters	
Founder total	259
Target microsat total	55
Non-target microsat total	55
Loop parameters	
Target size (*T*)	350
Number of years to run	100, 200, and 500
Iterations	1,000
Breeding parameters	
Age range females will breed	2–21
Age range males will breed	4–16
Offspring produced by each breeding female	1
Proportion of males that will breed	0.46
Proportion of females that will breed	0.82
Dominant male breeding parameters	
Proportion of breeders that are dominant	0.21
Age range dominant males breed	8–12
Proportion of offspring produced by dominant males	0.40
Number of years males are dominant	1
Mortality parameters	
Female adult mortality	0.03×1.15^{AGE}
Male adult mortality	0.05×1.16^{AGE}

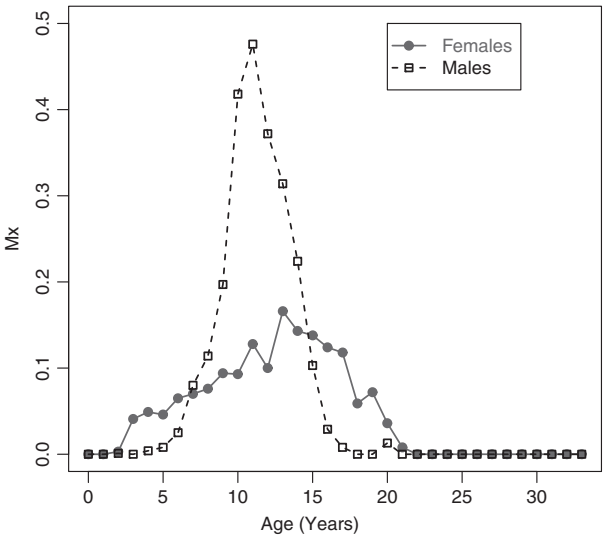

Figure 1 M_x values for males and females from the Fort Niobrara National Wildlife Refuge bison herd generated from known pedigree. M_x = the number of same-sex offspring produced by an individual during an age class.

by each male in the FTN herd, we defined dominant males as those for which the number of sired offspring exceeded the third quartile (>3 offspring) and found that these dominant males fell between 8 and 12 years of age (Fig. 1). The yearly percentage of dominant males was then specified as 21% of all breeding males, which was the average yearly percent of dominant males calculated for the FTN herd. Finally, we specified that 40% of offspring were sired by dominant males, which was the average for the FTN herd (Fig. 1).

We used the breeders from the 2004 FTN bison herd (100 male, 159 female) as the initial starting population for our simulation. Year of birth, sex and microsatellite genotypes at 55 loci were specified from available records. For individuals lacking genotype data for a particular locus, a custom R script (R Development Core Team, 2014) was used to generate missing data by randomly drawing alleles based on their frequencies within the starting population. Because the full FTN pedigree started in 2004, all individuals within the starting population were assumed to be equally related to establish a baseline from which to measure future loss of gene diversity (*GD*) and inbreeding (all pairwise kinships assumed to be 0.0; kinship to self assumed to be 0.5). The target size of the simulated population was specified as 350 bison, in concordance with the population objective for the FTN herd.

To determine the effects of genetic-based culling strategies on non-target microsatellite loci, we created an additional panel of 55 loci that was tracked and evaluated, but not used to guide culling. At the beginning of our simulation, population-level allele frequencies for each non-target locus were determined by randomly selecting a frequency distribution from among the target set of microsatellite loci. Two alleles for each non-target locus were then assigned to each individual based on that locus' selected frequency distribution. This resulted in two different sets of loci with similar starting measures of heterozygosity and allelic richness. Data from both sets of loci were summarized for all tested culling strategies, but only the initial set of empirically generated data was used to inform culling for the MAF strategy.

General simulation overview

1 An initial starting population was loaded into the simulation. The following information was specified for each individual: sex, birth year, and the two panels of microsatellite genotypes (target and non-target). Pairwise kinships also were specified between all starting individuals.
2 Breeding individuals were identified. Potential breeders were first identified as those individuals that fell within specified reproductive age ranges. The specified percentage of potential breeders was then randomly selected to produce offspring. To allow for a polygynous mating system, a specified percentage of males selected to produce offspring was randomly flagged as dominant breeders.
3 Offspring were produced. Each breeding female produced one offspring, which was randomly assigned, with equal probabilities, a sex of male or female. The sire of each offspring was determined to be a dominant or subordinate breeding male based on the specified probability. After the dominant or subordinate designation was selected, the specific sire was randomly selected from among those two breeding groups. Mendelian inheritance was used to gener-

ate the multi-locus genotypes of each offspring by randomly assigning one sire and one dam allele to each locus. Pairwise kinships (f) between each newly created offspring and all other individuals living in the population were calculated as $f_{xy} = 0.5(f_{xs} + f_{xd})$, where subscripts s and d referred to the sire and dam of each y offspring (Falconer, 1981).

4 Mortality occurred for all ages based on sex specific mortality functions. Male ($0.05 \times 1.16^{\text{Age}}$) and female ($0.03 \times 1.15^{\text{Age}}$) mortality functions included a starting mortality value and a multiplier raised by the age of an individual. The multiplier was used to ensure 100% mortality was observed at a biologically realistic age (20 for males, 25 for females).

5 Culling of yearlings was completed. The number of male and female yearlings to cull was calculated by subtracting the target number of individuals for each sex (half of total target population) from the total number of individuals of each sex. Individuals were iteratively culled through one of the strategies being tested until the number of culls calculated in the previous step was completed. If the number of males and females to be culled was unequal, individuals of the sex requiring the greater number of culls were first removed until the sex ratio to be culled was equalized. At that point, individuals of alternating sex were culled, starting with a male, with MAF or MK values being recalculated between each individual cull.

6 All individuals were aged 1 year. Steps 2–6 were repeated for 100, 200 or 500 years. Summary statistics for genetic variation and inbreeding were calculated on a yearly basis, immediately following Step 6. Summary statistics included allelic richness (A) measured as the mean number of alleles per locus, observed heterozygosity (H) calculated directly for each locus across all individuals and then averaged across loci (Hartl & Clark, 1997), proportional GD (expected heterozygosity) calculated as $1 - \overline{MK}$ (where $\overline{MK}$ is the average MK in the population; Ballou & Lacy, 1995), and average inbreeding in the population (F), equal to the kinship between an individual's sire and dam averaged across all individuals ($\overline{F}$; Falconer, 1981). Measures of allelic richness and observed heterozygosity were calculated separately for target and non-target loci.

Evaluation of culling strategies

Culling strategies were evaluated through a variety of genetic variation and inbreeding measures (A, H_o, GD and $\overline{F}$), which were averaged across 1000 simulation iterations. Summary statistics were reported at 100, 200 and 500 year intervals. The coefficient of variation (CV) was used to characterize summary statistic variability across iterations in relation to the mean.

Sensitivity analysis

The sensitivity of the simulation to input parameters was evaluated by analyzing the response of the genetic outputs to variations in target population size, mortality and proportion of breeding males. We tested three alternative target population sizes (200, 500 and 1000 individuals) and three alternative levels of mortality [200%, 300% and 400% of the starting mortality values (0.05 for males and 0.03 for females)]. We also tested three alternative percentages of total breeding males (25%, 50% and 100%); to observe only the effect of the proportion of breeding males, no males were categorized as dominant.

Results

Founding population summary statistics

The founding population had a mean allelic richness of 4.418 for the target set of loci (used by the MAF strategy) and 4.397 for the non-target set of loci. Average observed heterozygosity was 0.585 for target loci and for non-target loci. Since all founding individuals were assumed to be unrelated, GD started at 0.998 and the average inbreeding coefficient was 0.000 (Table 2).

Evaluation of model output

As predicted for any population of finite size, we observed a reduction in allelic richness and GD, and an increase in inbreeding, for all strategies. Heterozygosity increased or decreased depending on the strategy employed. All strategies succeeded in maintaining the target population size and a balanced sex ratio. Differences among strategies in the amount of genetic variation retained and the extent of inbreeding were evident at the 100-year time step and became more pronounced over time. Differences in the pattern of genetic variation loss were also detected between the target and non-target microsatellite loci for some culling strategies.

Of the three culling strategies, the RANDOM strategy preserved the least variation, as measured by allelic richness and observed heterozygosity (Fig. 2; Table 2). This strategy also yielded the lowest GD and highest average inbreeding coefficient across all years of the model (Fig. 2; Table 2). Outcomes of the RANDOM strategy were similar for the target and non-target sets of microsatellite loci; after 500 years, both sets of loci exhibited comparable decreases in allelic richness (44.3% and 44.8% reductions) and heterozygosity (34.9% and 36.5% reductions). GD decreased by 36.4% and inbreeding increased to 0.360 (Fig. 2). The RANDOM strategy exhibited the largest variation across simulation iterations, yielding among the highest CV values for all genetic diversity measures (Table 2).

The MAF strategy retained the highest allelic richness (decrease of 16.3%) and increased the observed heterozygosity (increase of 17.1%) relative to the founder population after 500 years, but only at the target microsatellite loci used to inform culls (Table 2; Fig. 2). At the non-target set of loci, genetic variation was lost at a rate comparable to the RANDOM strategy (allelic richness decreased by 44.0% and heterozygosity decreased by 33.5%). However, genome-wide measures of variation indicated better retention of diversity (GD decreased by 33.6%) and lower accumulation of

Table 2 Measures of genetic variation for the founding population under each culling strategy at each time step (100, 200, 500 years)

Measures of genetic variation	RANDOM				MAF				MK			
	Founding population	100 years	200 years	500 years	Founding population	100 years	200 years	500 years	Founding population	100 years	200 years	500 years
Target *A*	4.418	3.688 (0.022)	3.250 (0.031)	2.459 (0.052)	4.418	3.922 (0.017)	3.809 (0.024)	3.696 (0.047)	4.418	3.824 (0.018)	3.480 (0.023)	2.787 (0.035)
Non-target *A*	4.373	3.626 (0.055)	3.186 (0.051)	2.413 (0.060)	4.491	3.702 (0.024)	3.258 (0.032)	2.516 (0.044)	4.327	3.684 (0.025)	3.351 (0.025)	2.707 (0.035)
Target *H*	0.585	0.541 (0.026)	0.494 (0.038)	0.381 (0.076)	0.585	0.669 (0.036)	0.687 (0.039)	0.685 (0.039)	0.585	0.557 (0.022)	0.524 (0.031)	0.437 (0.050)
Non-target *H*	0.586	0.529 (0.045)	0.484 (0.056)	0.372 (0.086)	0.591	0.538 (0.026)	0.497 (0.038)	0.393 (0.069)	0.578	0.543 (0.022)	0.512 (0.033)	0.426 (0.054)
GD	0.998	0.903 (0.008)	0.825 (0.016)	0.635 (0.041)	0.998	0.907 (0.007)	0.836 (0.012)	0.663 (0.026)	0.998	0.931 (0.003)	0.876 (0.005)	0.732 (0.010)
F	0.000	0.089 (0.079)	0.168 (0.077)	0.360 (0.072)	0.000	0.086 (0.070)	0.157 (0.064)	0.332 (0.051)	0.000	0.061 (0.049)	0.116 (0.034)	0.262 (0.027)

Values for simulations were averaged over 1000 iterations with the coefficient of variation provided in parentheses.
MAF, mean allele frequency; MK, mean kinship; *A*, allelic richness; *H*, heterozygosity; *GD*, gene diversity.

inbreeding (0.332) over 500 years under the MAF strategy compared to RANDOM (Table 2). The MAF strategy yielded very different patterns of variation among iterations for the target and non-target sets of loci. The strategy was very consistent among iterations for allelic richness and heterozygosity for target loci, as well as for *GD*. However, the CV was on par with the RANDOM strategy for non-target loci (Table 2).

The MK strategy resulted in the highest retention of allelic richness and heterozygosity for the non-target set of loci (Fig. 2; Table 2). Target and non-target loci exhibited similar reductions in allelic richness (36.9% and 37.7%) and heterozygosity (25.3% and 26.3%; Table 2). The MK strategy also resulted in the highest *GD* (decrease of 26.3%) and accumulated the least inbreeding (0.262) than all other strategies at the 500-year time step (Fig. 2). The MK strategy had the lowest variation among iterations for inbreeding and *GD*, and the lowest CV values for allelic richness and heterozygosity for non-target loci (Table 2). Variation among iterations was similar for target and non-target loci.

Sensitivity analysis

The three culling strategies proved to be robust to changes in target population size, proportion of successful breeding males, and mortality for each age and sex class. As the target population size increased above the original value of 350 individuals, allelic richness, heterozygosity, and *GD* were higher and inbreeding ($\overline{F}$) was lower, but the overall pattern for each strategy remained the same (Supporting Information Table S1a). Generally, more variation among culling strategies was observed as the proportion of males that successfully bred was reduced (Supporting Information Table S1b). Overall, decreasing the sex- and age-specific mortality values resulted in less variation among culling strategies (Supporting Information Table S1c).

Discussion

Wildlife management strategies are often designed to control a population's size and demography, but such strategies also can inadvertently impact a population's genetic variation. For species like bison, where management includes the regular removal of individuals to maintain small population sizes on restricted landscapes, management actions can be tailored to address genetic diversity retention. Our research evaluated three alternative wildlife culling strategies to determine which strategy would provide the greatest advantage for conserving a population's genetic variation while maintaining a particular target size. Our simulations demonstrated that the information used to select individuals for removal notably influence the rate at which a population loses various measures of genetic variation (Fig. 2). Furthermore, our results indicated that incorporating genetic data into culling decisions, rather than relying solely on demographic parameters, generally improves the retention of genetic variation and reduces the accumulation of inbreeding over time.

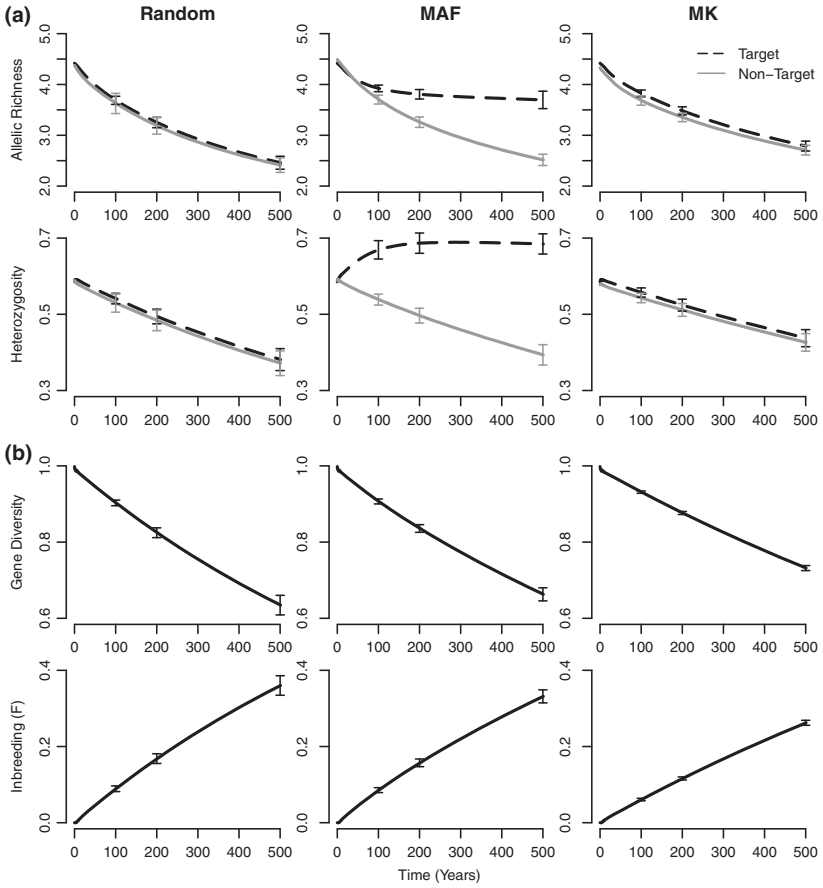

Figure 2 Average allelic richness and observed heterozygosity for both target and non-target loci (a) and genomic measures of variation (gene diversity and inbreeding) (b). Error bars represent one standard deviation at 100, 200, and 500 years.

Loss of alleles and a reduction in genome-wide heterozygosity in small populations result in loss of overall genetic variation. Since loss of genetic variation can be partially mitigated by increasing population size (e.g. Supporting Information Table S1a), wildlife managers often attempt to maximize the population size to minimize the effects of genetic drift (Epps *et al.*, 2005; Dixo *et al.*, 2009) and the related accumulation of inbreeding (Soulé & Mills, 1998). As population size decreases, maintaining stable demography and retaining genetic variation become increasingly important to prevent local extinction (Lande, 1988). In our study, the differences in genetic variation became more profound as population size decreased, demonstrating that the choice of management strategy becomes increasingly important as population size decreases (Supporting Information Table S1a). For range-restricted species such as bison, where habitat is limited and populations must be maintained at particular target sizes, management has historically focused on removal strategies based on demographic parameters to select individuals for cull. The advantage of such strategies is that they require only limited data and resources to implement. Our RANDOM culling strategy relied solely on demographic data (an individual's age and sex) to inform culls. At the end of 500 years, the RANDOM strategy yielded the lowest allelic richness, observed heterozygosity and *GD*, as well as the highest average inbreeding of the three tested culling strategies (Table 2). Further, the RANDOM, as well as the MAF, culling strategies exhibited high variance in measures of genetic variation across iterations, indicating less predictability in the outcome of these strategies and potentially important impacts on population persistence. These results indicate that although demographically based removal strategies can be easy to implement and effective at maintaining sex and age ratios, incorporating genetic data into culling decisions improves a population's long-term retention of genetic variation and thus, its adaptive potential.

We tested two alternative culling strategies (MAF and MK) that utilized genetic data. Although such strategies require additional resources and can be challenging to implement when compared to a demographically based removal strategy, both our MAF and MK strategies generally performed better at retaining genetic variation and limiting inbreeding than our RANDOM strategy. The MAF strategy was designed to maximize the retention of genetic variation by conserving as many different alleles as possible within a target set of loci used to inform culls. A perceived advantage

of this strategy was that it did not designate particular alleles as important or 'conservation-worthy', but rather aimed to conserve as many alleles as possible at as equal frequencies as possible. In theory, such an allele conservation strategy could produce a population with a higher heterozygosity than was present prior to management actions by creating more equal allele frequencies than existed in the founder population. This result was in fact observed, with the MAF strategy consistently retaining both the highest heterozygosity and allelic diversity at the suite of target loci used to inform culls (Table 2; Fig. 2). A potential drawback of this culling technique was that it aimed to maximize genetic variation at specific target loci with no regard for how the rest of the genome might be affected. In fact, although the MAF strategy effectively maximized genetic variation at a suite of target loci, it was ineffective in maintaining genetic variation at non-target loci and thus genome-wide variation. In contrast, the MK culling strategy performed the best at maximizing the retention of genome-wide variation (Table 2; Fig. 2). Therefore, of the three culling strategies tested, we found the MK strategy to be the superior method of culling intensively managed wildlife populations with respect to genome-wide measures of variation and inbreeding.

Our MK culling strategy is similar in concept to the pedigree-based strategies used by captive breeding programs that utilize MK for selecting breeding pairs (Ivy & Lacy, 2012). Pedigree-based breeding strategies that minimize the overall kinship in a population have been shown by both computer simulations (Ballou & Lacy, 1995; Fernández & Toro, 1999; Sonesson & Meuwissen, 2001) and empirical data (Montgomery *et al.*, 1997) to be the best strategies for retaining genetic variation, while limiting inbreeding, in conservation breeding programs. Our data extend these findings to demonstrate that our MK strategy outperformed both alternative strategies at limiting inbreeding and retaining genome-wide variation (as measured by pedigree-based measures and empirically calculated heterozygosity at a suite of non-target loci; Table 2). Although previous evaluations of pedigree-based culling strategies for wildlife management are rare (but see Eggert *et al.*, 2010), it is perhaps not surprising that our data support the utility of pedigree-based approaches to directly manage genetic variation in wildlife populations. An individual's MK is a measure of its genetic distinctiveness in a population; individuals with low MKs have few relatives and rare alleles, while individuals with high MKs have many relatives and common alleles. Thus, by preferentially selecting individuals with low MKs to breed, conservation breeding programs equalize, to the extent possible, the genetic representations of a population's founders and thereby maximize the retention of genetic variation over time. Our simulations indicate that MKs also are useful for selecting individuals to cull because such a strategy similarly equalizes founder genome representations by preferentially removing individuals whose genomes are over-represented in the population as a whole.

Better retention of genetic variation through direct genetic management has been demonstrated when selecting breeders to maintain captive populations (Ballou & Lacy, 1995; Ortega-Villaizan, Noguchi & Taniguchi, 2011) and choosing individuals for reintroductions (Haig *et al.*, 1990; Miller *et al.*, 2009; Jamieson, 2010; Tracy *et al.*, 2011). However, the stipulation that direct genetic management should focus on genomic measures of variation is important. Hedrick & Miller (1994) simulated captive breeding strategies that prioritized the retention of alleles at a suite of functional immune genes, the major histocompatibility complex (MHC), and observed the effect on variation across the rest of the genome. The authors characterized genome-wide reductions in genetic variation and fitness associated with selection for variation at the MHC and urged caution in the use of this genetic management technique due to its impact on variation at non-target portions of the genome. Although we did not model effects on functional loci, the decrease in genome-wide variation with the MAF strategy, and the associated increase in inbreeding, also could be expected to lead to detrimental declines in fitness (Charlesworth & Charlesworth, 1999). Furthermore, results similar to those reported by Hedrick & Miller (1994) are expected when selecting for variation at neutral loci, particularly if the effects of genetic hitchhiking are strong (Charlesworth & Guttman, 1996; Hey, 1999; Otto, 2000). Our results further support this assertion by demonstrating that our MAF strategy, which retained high allelic diversity and heterozygosity at targeted neutral loci, retained less variation at non-target loci than a strategy that utilized a genomic measure of variation for decisions (our MK strategy). If many more loci were used in the target panel, genetic diversity estimates would more closely approximate genome-wide variation (Miller *et al.*, 2014). This should yield convergent allelic richness and heterozygosity values for target and non-target loci as the MAF strategy was applied over time. Additional research would be necessary, however, to determine the degree of convergence between the overall results of the MAF and MK strategies when using increasing numbers of loci. There is a fundamental difference between culling individuals with common alleles (MAF) and culling those that are, on average, highly related to the population (MK). As an example, consider two full-siblings; while the MK strategy would treat those individuals as genetically identical and interchangeable, the MAF strategy would prioritize one over the other for cull based on which happened to receive more 'common' alleles through Mendelian inheritance. Thus, given these complexities, more research is warranted to determine the number of loci at which the MAF strategy is expected to converge with, or even possibly surpass, the MK strategy performance.

Our results suggest wildlife management strategies that incorporate goals for retaining genetic variation are better suited to preserving the evolutionary potential of wildlife populations than those that focus solely on a target size and demographic stability. Declines in genetic variation not only limit the evolutionary potential of a population, but can also have direct and immediate effects on factors such as the response to diseases and new pathogens (O'Brien & Evermann, 1988). For these reasons, bison are an exemplary example of a species in need of genetic management. Bison, as a species, underwent a severe bottleneck in the late

1800s, and were further bottlenecked as conservation herds were founded with few individuals. Thus, all contemporary bison populations can be assumed to have accumulated some level of inbreeding, with Hedrick (2009) estimating 0.367 inbreeding (equal to two generations of full sibling matings) in the Texas State Bison Herd. Although the direct effects of inbreeding in bison are unclear, even small amounts of inbreeding have been correlated with the susceptibility to bacterial disease in other wildlife populations (Acevedo-Whitehouse *et al.*, 2003). Historical erosion of genetic variation due to severe bottlenecks, serial founding events, and current levels of inbreeding make the preservation of remaining genetic variation through effective management strategies even more imperative to the persistence of bison.

Although the focus of our research was to evaluate culling strategies for wildlife populations managed *in situ*, our results also are applicable to captive population management. Although using euthanasia as a management tool is controversial in these settings (Penfold *et al.*, 2014), there are a number of challenges that culling could address. For example, management euthanasia of post-reproductive animals not critical to a population's social structure could be utilized to free 'space' that would allow for additional breeding in populations that are tightly maintained at carrying capacity (Lacy, 1991). A second application of management euthanasia, similar in concept to the culling scenario described for bison, is the removal of surplus offspring produced when specific breeding recommendations cannot be implemented. Species maintained in herds, flocks, schools or other similar groups (e.g. antelope, flamingos, bats, fish, frogs) can only be loosely managed by MK-based breeding strategies because specific breeding pairs cannot be dictated. Although the long-term genetic impacts of MK-based management euthanasia have not been tested against those of imperfect MK-based breeding strategies, we speculate that culling would provide greater long-term genetic benefits.

Finally, one of the more compelling reasons for captive breeding programs to consider management euthanasia is related to reproductive health. Penfold *et al.* (2014) summarized data for a set of taxonomically diverse species (including canids, felids, rhinoceros, bats, wildebeest and stingrays); their findings suggested that prolonged interruptions in breeding (such as produced with some forms of contraception), during which a female does not produce offspring, can jeopardize a female's future fertility and increase probabilities of uterine pathologies. To help ensure both female reproductive health and population viability, the authors suggested that captive breeding programs could adopt mixed management strategies that breed genetically valuable females at more regular intervals while judiciously using all available tools, including both culling and contraception, to manage the number of offspring produced (Penfold *et al.*, 2014). If such strategies are indeed to be adopted by captive breeding programs, our research suggests that modifying the pedigree-based breeding strategies already in use to cull genetically over-represented individuals would provide the greatest long-term genetic benefits.

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References

Acevedo-Whitehouse, K., Gulland, F., Greig, D. & Amos, W. (2003). Inbreeding: disease susceptibility in California sea lions. *Nature* **422**, 35.

Ballou, J.D. & Lacy, R.C. (1995). Identifying genetically important individuals for management of genetic variation in pedigreed populations. In *Population management for survival and recovery*: 76–111. Ballou, J.D., Gilpin, M. & Foose, T.J. (Eds). New York: Columbia University Press.

Ballou, J.D., Lacy, R.C. & Pollak, J.P. (2011). *PMx: software for demographic and genetic analysis and management of pedigreed populations, version 1.0*. Brookfield: Chicago Zoological Society.

Borgreen, M.J. (2010). *The reproductive performance of bison at the National Bison Range*. MS thesis, Montana State University.

Boyd, D. (2003). *Conservation of North American bison: status and recommendations*. MS thesis, University of Calgary.

Boyd, D. P., Willson, G. A., Derr, J. N. & Halbert, N. D. (2010). Genetics. In *American bison: status survey and conservation guidelines 2010. IUCN/SSC Bison Specialist Group*: 19–26. Gates, C.C., Freese, C. H., Gogan, P. J. P. & Kotzman, M. (Eds). Gland: IUCN.

Bruford, M.W., Ancrenaz, M., Chikhi, L., Lackman-Ancrenaz, I., Andau, M., Ambu, L. & Goossens, B. (2010). Projecting genetic diversity and population viability for the fragmented orang-utan population in the Kinabatangan floodplain, Sabah, Malaysia. *Endanger. Species Res.* **12**, 249–261.

Caballero, A. & Toro, M.A. (2000). Interrelations between effective population size and other pedigree tools for the management of conserved populations. *Genet. Res.* **75**, 331–343.

Charlesworth, B. & Charlesworth, D. (1999). The genetic basis of inbreeding depression. *Genet. Res.* **74**, 329–340.

Charlesworth, B. & Guttman, D.S. (1996). Reductions in genetic variation in Drosophila and *E. coli* caused by selection at linked sites. *J. Genet.* **75**, 49–61.

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

Crnokrak, P. & Roff, D.A. (1999). Inbreeding depression in the wild. *Heredity* **83**, 260–270.

Dixo, M., Metzger, J.P., Morgante, J.S. & Zamudio, K.R. (2009). Habitat fragmentation reduces genetic diversity and connectivity among toad populations in the Brazilian Atlantic Coastal Forest. *Biol. Conserv.* **8**, 1560–1569.

Eggert, L.S., Powell, D.M., Ballou, J.D., Malo, A.F., Turner, A., Kumer, J., Zimmerman, C., Fleischer, R.C. & Maldonado, J.E. (2010). Pedigrees and the study of the wild horse population on Assateague Island National Seashore. *J. Wildl. Mgmt.* **74**, 963–973.

Epps, C.W., Palsboll, P.J., Wehausen, J.D., Roderick, G.K., Ramey, R.R. II & McCullough, D.R. (2005). Highways block gene flow and cause a rapid decline in genetic diversity of desert bighorn sheep. *Ecol. Lett.* **8**, 1029–1038.

Falconer, D.S. (1981). *Introduction to quantitative genetics*. 2nd edn. New York: Longman Inc.

Fernández, J. & Toro, M.A. (1999). The use of mathematical programming to control inbreeding in selection schemes. *J. Anim. Breed. Genet.* **116**, 447–466.

Foose, T.J. & Ballou, J.D. (1988). Population management: theory and practice. *Int. Zoo. Yearb.* **27**, 26–41.

Gates, C. & Aune, K. (2008). *Bison bison*. In *The IUCN red list of threatened species, version 2014.3*. Available at: http:// www.iucnredlist.org.

Gilbert, F.F. & Dodds, D.G. (2001). *The philosophy and practice of wildlife management*. Malabar: Krieger Publishing Company.

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. *Biol. Conserv.* **96**, 319–330.

Gross, J.E. & Wang, G. (2005). *Effects of population control strategies on retention of genetic diversity in National Park Service Bison* (Bison bison) *herds*. Final report, Yellowstone Research Group USGS-BRD, US Geological Survey, Bozeman.

Grueber, C.E., Nakagawa, S., Laws, R.J. & Jamieson, I.G. (2010). Inbreeding depression accumulates across life-history stages of the endangered takahe. *Conserv. Biol.* **24**, 1617–1625.

Haig, S.M., Ballou, J.D. & Derrickson, S.R. (1990). Management options for preserving genetic diversity: reintroduction of Guam rails to the wild. *Conserv. Biol.* **4**, 290–300.

Hailer, F., Helander, B., Folkestad, A.O., Ganusevich, S.A., Garstad, S., Hauff, P., Koren, C., Nygard, T., Volke, V., Vila, C. & Ellegren, H. (2006). Bottlenecked but long-lived: high genetic diversity retained in white-tailed eagles upon recovery from population decline. *Biol. Lett.* **2**, 316–319.

Halbert, N.D. (2003). *The utilization of genetic markers to resolve modern management issues in historic bison populations: implications for species conservation*. PhD dissertation, Texas A&M University.

Halbert, N.D. & Derr, J.N. (2008). Patterns of genetic variation in US federal bison herds. *Mol. Ecol.* **17**, 4963–4977.

Harris, R.B., Wall, W.A. & Allendorf, F.W. (2002). Genetic consequences of hunting: what do we know and what should we do? *Wildl. Soc. Bull.* **30**, 634–643.

Hartl, D.L. & Clark, A.G. (1997). *Principles of population genetics*. Sunderland: MA Sinauer Associates.

Hedrick, P.W. (2009). Conservation genetics and North American bison (*Bison bison*). *J. Hered.* **100**, 414–420.

Hedrick, P.W. & Miller, P.S. (1994). Rare alleles, MHC, and captive breeding. In *Conservation genetics*: 187–204. Loeschcke, V., Jain, S.K. & Tomiuk, J. (Eds). Basel: Birkhaeuser Verlag.

Hedrick, P.W., Brussard, P.F., Allendorf, F.W., Beardmore, J.A. & Orzack, S. (1986). Protein variation, fitness, and captive propagation. *Zoo. Biol.* **5**, 91–99.

Hey, J. (1999). The neutralist, the fly and the selectionist. *Trends Ecol. Evol.* **14**, 35–38.

Hoban, S., Bertorelle, G. & Gaggiotti, O.E. (2012). Computer simulations: tools for population and evolutionary genetics. *Nat. Rev. Genet.* **13**, 110–122.

Ivy, J.A. & Lacy, R.C. (2012). A comparison of strategies for selecting breeding pairs to maximize genetic diversity retention in managed populations. *J. Hered.* **103**, 186–196.

Jamieson, I.G. (2010). Founder effects, inbreeding, and loss of genetic diversity in four avian reintroduction programs. *Conserv. Biol.* **25**, 115–123.

Keller, L.F. & Waller, D.M. (2002). Inbreeding effects in wild populations. *Trends Ecol. Evol.* **17**, 230–241.

Keller, L.F., Arcese, P., Smith, J.M., Hochachka, W.M. & Stearns, S. (1994). Selection against inbred song sparrows during a natural population bottleneck. *Nature* **372**, 356–357.

Komers, P.E. & Curman, G.P. (2000). The effect of demographic characteristics on the success of ungulate re-introductions. *Biol. Conserv.* **93**, 187–193.

Lacy, R.C. (1991). Zoos and the surplus problem: an alternative solution. *Zoo. Biol.* **10**, 293–297.

Lacy, R.C. (1997). Importance of genetic variation to the viability of mammalian populations. *J. Mammal.* **78**, 320–335.

Lacy, R.C. (2000). Should we select genetic alleles in our conservation breeding programs? *Zoo. Biol.* **19**, 279–282.

Lande, R. (1988). Genetics and demography in biological conservation. *Science* **241**, 1455–1460.

Leopold, A. (1933). *Game management*. New York: Charles Scribner's Sons.

Markert, J.A., Champlin, D.M., Gutjahr-Gobell, R., Grear, J.S., Kuhn, A., McGreevy, T., Roth, A., Bagley, M.J. & Nacci, D.E. (2010). Population genetic diversity and fitness in multiple environments. *BMC Evol. Biol.* **10**, 205.

McNeely, J.A., Miller, K.R., Reid, W.V., Mittermeier, R.A. & Werner, T.B. (1990). *Conserving the world's biological diversity*. Gland/Washington: IUCN, WRI, CI, WWF-US, The World Bank.

Meagher, M. (1986). *Bison bison*. *Mamm. Species* **266**, 1–8.

Miller, P.S. (1995). Selective breeding for rare alleles: examples from the Przewalski's Horse and California Condor pedigrees. *Conserv. Biol.* **9**, 1262–1273.

Miller, K.A., Nelson, N.J., Smith, H.G. & Moore, J.A. (2009). How do reproductive skew and founder group size affect genetic diversity in reintroduced populations? *Mol. Ecol.* **18**, 3792–3802.

Miller, J.M., Malenfant, R.M., David, P., Davis, C.S., Poissant, J., Hogg, J.T., Festa-Bianchet, M. & Coltman, D.W. (2014). Estimating genome-wide heterozygosity: effects of demographic history on marker type. *Heredity* **112**, 240–247.

Montgomery, M.E., Ballou, J.D., Nurthen, R.K., Briscoe, D.A. & Frankham, R. (1997). Minimizing kinship in captive breeding programs. *Zoo. Biol.* **16**, 377–389.

O'Brien, S.J. & Evermann, J.F. (1988). Interactive influence of infectious disease and genetic diversity in natural populations. *Trends Ecol. Evol.* **3**, 254–259.

Ortega- Villaizan, M., Noguchi, D. & Taniguchi, N. (2011). Minimization of genetic diversity loss of endangered fish species captive broodstocks by means of minimal kinship selective crossbreeding. *Aquaculture* **318**, 239–243.

Otto, S.P. (2000). Detecting the form of selection from DNA sequence data. *Trends Genet.* **16**, 526–529.

Peek, J.M., Boyce, M.S., Garton, E.O., Hard, J.J. & Mills, L.S. (2002). *Risks involved in current management of elk in Washington*. Olympia: Washington Department of Fish and Wildlife.

Penfold, L.M., Powell, D., Traylor-Holzer, K. & Asa, C.S. (2014). 'Use it or lose it': characterization, implications, and mitigation of female infertility in captive wildlife. *Zoo. Biol.* **3**, 20–28.

R Development Core Team. (2014). *R: a language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing. Available at: http://www.R-project.org.

Ralls, K., Brugger, K. & Ballou, J. (1979). Inbreeding and juvenile mortality in small populations of ungulates. *Science* **206**, 1101–1103.

Redford, K.H. & Fearn, E. (2007). *Ecological future of bison in North America: a report from a multi-stakeholder, transboundary meeting*. Bronx: Wildlife Conservation Society.

Shaw, J.H. & Carter, T.S. (1989). Calving patterns among American bison. *J. Wildl. Mgmt.* **53**, 896–898.

Sonesson, A.K. & Meuwissen, T.H.E. (2001). Minimazation of rate of inbreeding for small population with overlapping generations. *Genet. Res.* **77**, 285–292.

Soulé, M.E. & Mills, L.S. (1998). No need to isolate genetics. *Science* **282**, 1658–1659.

Tracy, L.N., Wallis, G.P., Efford, M.G. & Jamieson, I.G. (2011). Preserving genetic diversity in threatened species reintroductions: how many individuals should be released? *Anim. Conserv.* **14**, 439–446.

Vrijenhoek, R.C. & Leberg, P.L. (1991). Let's not throw the baby out with the bathwater: a comment on management for MHC diversity in captive populations. *Conserv. Biol.* **5**, 252–254.

Wayne, R.K., Forman, L., Newman, A.K., Simonson, J.M. & O'Brien, S.J. (1986). Genetic monitors of zoo populations: morphological and electrophoretic assays. *Zoo. Biol.* **5**, 215–232.

Wedekind, C. (2002). Manipulating sex ratios for conservation: short-term risks and long term benefits. *Anim. Conserv.* **5**, 13–20.

Wildlife Conservation Society (WCS). (2015). *The American Bison Society.* Available at: http://www.wcs.org/saving-wildlife/hoofed-mammals/bison/the-american-bison-society.aspx

Willi, Y., Van Buskirk, J. & Hoffmann, A.A. (2006). Limits to the adaptive potential of small populations. *Annu. Rev. Ecol. Evol. Syst.* **37**, 433–458.

Williams, E.S. & Barker, I.K. (2001). *Infectious diseases of wild mammals*. Ames: Iowa State University Press.

Wright, S. (1931). Evolution in Mendelian populations. *Genetics* **16**, 97–159.

Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Model flow for each simulation.
Figure S2. Mortality functions for males (solid red line) and females (dotted black line) were generated using known average adult mortality (0.05 for males; 0.03 for females), juvenile mortality (0.05), and age expectancy for the Fort Niobrara bison herd.
Table S1. Genetic variation measures averaged over 1000 iterations from the 500-year time step of the sensitivity analysis reflecting the genetic variation measures under each culling strategy with (a) different target population sizes (200, 500 and 1000), (b) with different proportions of the male population of breeding age able to breed (25%, 50% 100%), and (c) and alternative levels of mortality (200%, 300% and 400% of original).