Conserving Genetic Diversity in Yellowstone Bison

The effects of population fluctuations and variance in male reproductive success in age structured populations

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Conserving genetic variability in large mammals: Effect of population fluctuations and variance in male reproductive success on genetic variation in Yellowstone bison

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Abstract

Loss of genetic variation through genetic drift in isolated populations can reduce population viability. However, relatively little is known about loss of variation caused by fluctuating population size and realistic variance in reproductive success in age structured populations. We built an individual-based simulation model to examine how actual management strategies influence the effective population size (N_e) and allelic diversity in realistic population scenarios from Yellowstone bison. Simulation scenarios included a population size (Nc) ranging from 250 to >3,000 bison, a wide range of variance in male reproductive success, loci with 2, 5, or 20 alleles, and several realistic population culling strategies causing fluctuations in Nc. The resulting effective population size ranged from 746 to 1,176 with high to moderate variance in male reproductive success, respectively, for a stable population size of 2,000 bison. The N_e/Nc ratio remained stable around 0.33-0.38 for high variance in male reproductive success irrespective of the census size or the culling scenarios, but dropped to only 0.04 with extreme variance in male reproductive success. Heterozygosity was maintained at >95% over 200 years (~28 bison generations) for all simulation scenarios with Nc >500 and non-extreme variance in male reproductive success. The conservation of allelic diversity depended more on average Nc than N_e in fluctuating populations. Simulations suggest that 95% of allelic diversity will be maintained over 100 years if the Nc remains above 2,000 - 3,000 bison. However, less than 90% of alleles will be maintained at loci with more than five alleles (e.g. at immune system loci). Variance in male reproductive success had little effect on allelic diversity except under unrealistically-extreme variance in male reproductive success. These simulations are among the most realistic and extensive to date for a large mammal and should be informative in other species of large mammals with similar demography and mating systems.

Introduction

Isolated populations often suffer reduced viability due to loss of genetic variation (Allendorf & Ryman 2002). The effective population size (N_e) is an important parameter for assessing genetic variability because N_e is a function of the rate of loss of genetic variation (Charlesworth 2009) and influences a population's respond to selection (Leberg 2005). We consider here the inbreeding effective size (N_{eI}), which is computed from the loss of heterozygosity (H_e) (Leberg 2005) usually with the assumption of a stable population without age structure (Felsenstein 1971).

Little is known about N_e, the N_e/N_c (census size) ratio, and rates of loss of allelic diversity (AD) in natural populations with overlapping generations, age structure, and substantial fluctuations in Nc (Ryman *et al.* 1981; Jorde & Ryman 1995; Waples &Yakota 2007). Knowing the N_e/N_c ratio would be useful for estimating the effective size of a population from census data (or vice-versa) if the ratio were stable (Kalinowski & Waples 2002; Luikart *et al*. 2010). Few studies have quantified the effects of age structure, mating system variation, or fluctuations in Nc (e.g., from culling) on N_e (Harris & Allendorf 1989; Rieman & Allendorf 2001). Nonetheless, conservation geneticists have suggested that a reasonable management goal for maintenance of genetic variation is to retain 90-95% of H_e over 100-200 years (Soulé *et al.* 1986; Allendorf & Ryman 2002).

Few studies have quantified the maintenance of AD, probably because AD is strongly influenced by the number and frequency of alleles at a locus and, therefore, is more difficult to assess than H_e. AD is generally lost more rapidly than H_e and is more sensitive for measuring loss of variation (Allendorf 1986; Leberg 1992). Allelic diversity can range widely from two to more than 20 alleles per locus. Single nucleotide polymorphism (SNPs) loci typically have only two alleles per locus, whereas microsatellites and immune system loci typically have five to 20 alleles per locus in large mammals (Morin et al. 2004, Mikko *et al.* 1997).

The Yellowstone bison herd is important for conservation because it is the only large (>2,000 individuals) and wild population of plains bison (*Bison bison*). It also is one of only three herds to exhibit no evidence of hybridization with cattle (Freese *et al.* 2007; Halbert & Derr 2007; Sanderson *et al.* 2008). However, the population is geographically isolated and likely has moderate to high variance in reproductive success due to a polygamous mating system in which a few males could dominant most breeding, and which could lead to relatively rapid loss of genetic variation. Also, the population has been repeatedly culled during the past two decades to reduce migratory movements to winter-ranges outside the park where bacterial disease brucellosis could potentially spread from bison to cattle (Rhyan *et al*. 2009; Plumb *et al.* 2009).

Sporadic culls of more than 1,000 bison (~30% of the herd), combined with intervening periods of exponential population growth, have resulted in substantial fluctuations in bison population size (Fuller *et al.* 2007) and concerns about possible reductions in genetic variation (Halbert 2003; Animal Welfare Institute 2008; Hedrick 2009). While fluctuations in population size can affect N_e, several factors (e.g., sex ratio, overlapping generations, and variation among individual contributions to the gene pool) complicate the estimation of N_e (Allendorf & Luikart 2007). Thus, genetic studies and modelling are needed to quantify the loss of variation and evaluate potential effects of alternate culling strategies.

Gross *et al*. (2006) showed that maintaining approximately 2,000 individuals should retain 95% of H_e and allelic diversity (at microsatellite loci) in Yellowstone bison over the next 100 years. However, while the initial model of Gross *et al*. (2006) was informative, it did not consider the effects of variance in male reproductive success (VMRS) or annual variation in population size due to culling. In fact, no studies we know of have modelled the effects of fluctuating population size and realistic VMRS (e.g. caused by a dominance hierarchy) in age structured populations although it could be a relevant factor to maintain genetic diversity.

We used computer simulations of an individual-based model of bison populations to assess the potential effects of VMRS, amount of initial genetic variation, and fluctuating population size on N_e, loss of H_e, and loss of AD over a 100-200 year period. To parameterize the simulation models, we used input values representative of Yellowstone bison (e.g., microsatellite data from 48 loci, Halbert 2003) and existing age-specific birth and death rates, and population age structure estimated for Yellowstone bison (Fuller *et al.* 2007, Brodie 2008, Geremia *et al*. 2009, Yellowstone National Park unpublished data).

We addressed four questions:

1) What is the N_e and N_e/Nc ratio of Yellowstone bison under current circumstances?

2) Do population fluctuations caused by culling lead to reduced heterozygosity and allelic diversity under realistic age structure and culling scenarios?

3) How does variance in male reproductive success caused by the presence of dominant males affect the loss of allelic diversity?

4) How is loss of allelic diversity influenced by the initial number of alleles per locus (e.g., at SNPs, microsatellites, and highly-variable immune system loci)?

Methods

Computer Simulations

We simulated populations of bison using the computer program NewAge (N_e with age structure; T. Antao *et al*. unpublished; Fig. 1), a new program in Python using the simuPOP simulation environment (Peng & Kimmel 2005; Peng & Amos 2008). This software simulates individual-based age-structured populations using demographic parameters (age- and sex-specific birth and death rates) and Mendelian inheritance at neutral genetic loci defined by the user. Selection and mutation were not included in the model. Some cases were also simulated using PEDAGOG (Coombs *et al*. 2010) for comparative and verification purposes (e.g. effect of VMRS and culling on N_e and AD).

We used input values representative of Yellowstone bison to parameterize the simulation models, including microsatellite data from 48 loci, age-specific birth and death rates, and population age structure (Halbert 2003; Fuller *et al*. 2007; Brodie 2008; Geremia *et al*. 2009; National Park Service, unpublished data). For each scenario, we ran 50 independent replicate simulations. We also considered Yellowstone bison to be one deme, which is consistent with both genetic and field data (Fuller et al. 2007, Bruggeman et al. 2009; R.Wallen and F. Gardipee unpublished data).

We simulated scenarios to evaluate fluctuating and stable population size (Nc), variability in male reproductive success (i.e., offspring production), initial number of alleles per locus, and the effects of different culling strategies. Each simulation replicate began with the initialization of demography and genotypes. Genotypes for 48 independent microsatellites were randomly assigned to individuals proportional to allele frequencies described for Yellowstone bison (AD = 5.1, $H_e = 0.65$ from a sample of more than 200 bison; Halbert 2003). Alternate scenarios with different starting allelic richness of 2 (e.g., SNP scenario) and 20 (immune system locus scenario) alleles-per-locus were initialized following a Dirichlet distribution (e.g., Tallmon *et al*. 2004), which yielded an initial H_e of 0.33 and 0.90, respectively. These scenarios were included to evaluate the influence that initial allelic richness has on the conservation of genetic variation over 100-to-200 years. A few simulations were also run using 100 loci with 99 alleles per locus to reduce variance in estimates of loss of H_e and increase precision of N_e estimates.

Demographic vital rates and age-structure were generated following a Leslie matrix model (Moran 1962; Crow & Kimura 1970; Waples & Yokota 2007) that allows for population growth at a constant rate and stable age distribution. Demographic vital rates were defined as age and sex specific survival (probability of an individual of age *x* to reach age *x+1*) and fecundity (average number of progeny) rates (Table 1). Scenarios with stable size ($\lambda = 1.00$) were obtained using these survival rates by removing newborns above a fixed number, thus simulating high rates of neo-natal mortality.

The maximum attainable age for bison was set at 15 or 20 years (Brodie 2008). An extra scenario, using a Wright-Fisher population (discrete generations, random mating) with separate sexes, was conducted as a control to assess the effects of overlapping generations and mating system.

After initialization, we allowed each replicate to run for 20 years to achieve a stable population size, Nc = 2,000, with a stable age distribution (Figure 1). We then allowed the population to either remain stable in size or grow depending on the simulation (e.g., Waples & Yokota 2007). Each annual cycle started with the evaluation of population size and then culling, if necessary, followed by the computation of summary statistics for the population (e.g., heterozygosity, number of individuals for each sex and age class). Age was increased by 1 year and newborns were assigned to age 0 at the end of each annual cycle.

Culling

Culling scenarios were simulated to evaluate the effects of removing selected demographic groups (all ages, bison <3 years old, and bison >3 years old) and management for population census size (stable population size, fluctuation between 2,500 and 4,000-large, and fluctuation between 3,000 and 3,500). Culling was random with respect to gender (i.e. proportional to the observed sex ratio and age structure. At the beginning of the year, Nc was computed to determine if culling should be performed depending on the scenarios (Table 2). We compared the N_e and genetic variation in the two fluctuating population scenarios to a stable population of size Nc = 3,250, which is the mean of 2,500 and 4,000, and of 3,000 and 3,500. In stable population scenarios, the total number of newborns each year is constant and the birth rates represent the natural probability of females reproducing but with high neo-natal mortality. Culling was random among the demographic groups considered.

The feasibility of these scenarios is reasonable because bison can be aged reliably up to 4 years of age from tooth eruption patterns (Olson 2005). Culling was conducted whenever population size exceeded a threshold value (4,500 or 3,500 depending on the scenario). Suitable individuals (e.g., random across target age classes) were culled until the target population size (2,000 or 3,000) was reached. There were always enough individuals (of target ages) to accommodate each culling scenario. Thus a representative level of culling (i.e. reduction in Nc) was conducted in all scenarios.

Mating

Our model simulates the polygamous nature of bison (Reynolds *et al*. 2003, Brodie 2008). Females were only allowed to have one offspring each season (intra-year monogamy) by choosing females randomly without replacement to be mated. Each female has a probability of being mated depending on her age and age-specific birth rate (Table 1).

Little is known about male reproductive success in bison. Lott (1979) reported that 33% of males produce 66% of offspring. Berger and Cunningham (1994) reported that 10% of males produce 50% of offspring. Halbert *et al*. (2004) reported that 10% of males produce 40% of offspring. However these studies are largely based on behavioural observations rather than paternity analysis.

We modelled four different scenarios regarding VMRS: 1) all adult males had the same probability of mating (Random VMRS) within a given year; 2) 10% of the dominant males aged 6 to 14 years produced 50% of the offspring each year (ModerateVMRS); 3) 10% of the dominant males (age 6-14) produced all of the offspring each year (high VMRS); and 4) 1% of

the dominant males produced all the offspring each year (extreme VMRS). The fourth scenario represents rather extreme VMRS. All dominant males remained dominant for 5 years unless they died after 1-4 years of being dominant. If the number of dominant males decreased due to death, a new random (non-dominant) male between the ages of 6 and 10 years became dominant and kept that status for 5 years or until he died. Gametes from chosen parents were joined to produce newborn individuals. After mating, natural deaths were simulated by random removal of individuals proportionally to the inverse of the survival parameter for each age class (Table 1).

Computation

At each year, the whole population was sampled (for all simulated loci) to calculate number of alleles and H_e. Allelic diversity remaining at time *t* relative to time 0 (zero) was calculated as $AD_t = (A_t-1)/(A_0-1)$ (Allendorf 1986). Generation length (*g*), the average interval separating the births of one generation from the births of the next (Carey 1993), was also calculated each year as the average age of all individuals producing offspring.

Effective population size (N_{eI}) obtained from loss of H_e ($N_e[H]$) across 200 years was calculated for each simulation replicate following Harris & Allendorf (1989) as

$$N_e[H] = \frac{1}{-2\exp\left[\frac{\ln[H]_{200} - \ln[H]_0}{\frac{200}{T}}\right]2} \quad (1)$$

where H_0 and H_{200} are the expected heterozygosis at time 0 and 200 respectively, and *T* is the observed generation time. This estimate behaved similarly to Felsenstein's (1971) estimator for overlapping generations in stable population size scenarios.

N_e/Nc after 200 years was obtained as harmonic mean for 50 replicates of N_e divided by the arithmetic mean of Nc across 200 years of evolution (Kalinowski & Waples 2002). To reduce noise due to the low number of alleles, and given that N_e depends only on the demography, we ran the same scenarios but using 100 microsatellites with 99 alleles initialized by a Dirichlet distribution. N_e and N_e/Nc were calculated using those scenarios with 100 loci.

Validation

The quantitative values of AD from both computer simulation programs (NewAge and PEDAGOG, Coombs *et al.* 2010 were nearly identical for all scenarios compared (see Table S1 in Supplementary Information). Thus we present results only from NewAge.

Results

   We simulated more than 60 different scenarios. Simulations with the maximum age set at 15 yielded resulted very similar to the default value of 20 years. Thus, we present only scenario results using a maximum age of 20 years.

Effective population size and N_e/Nc ratios

The N_e ranged from 746 in stable populations of size 2,000 up to 1,165 in fluctuating populations of size 3,000 to 3,500 with High VMRS. All stable population census size simulations resulted in N_e/Nc ratios of 0.37 with high variance in male reproductive success (VMRS), and 0.58-0.59 with Moderate VMRS. Population census size fluctuations caused a reduction in N_e by as much as 14% relative to stable populations whose size (Nc =3250) equalled the mean of the fluctuating populations (Supplementary Information, Table S2). While N_e/Nc ratios vary little regardless of population growth rate or method of culling (young vs. old) (Table 3.B), it appears that mild fluctuations give higher N_e values than strong fluctuations, as expected (Table S2). The more variance among males contributing to reproduction, the greater the reduction in effective population size, as expected (Table 3.A).

Heterozygosity and allelic diversity

Greater than 95% of heterozygosity was maintained over 200 years for all scenarios considered with at least 1,000 bison (at microsatellite loci with 5 alleles per locus) (Table 4). Population fluctuation under all of the culling strategies we simulated also preserved >95% of initial heterozygosity for this time interval. Extreme VMRS (i.e., 1% of dominant males producing all the offspring) was the only male mating scenario in which H_e decreased below 95% (Table 4).

Greater than 95% of AD was maintained over 200 years only if the census size was large (Nc >2,000) with 5 or fewer alleles per locus and High or Moderate VMRS (Table 5.A; Fig. 4). Fluctuations in the Nc, especially culling only older individuals, reduced the probability of conserving AD relative to a stable population size (Table 5.B and Figure 2). Slower population growth rates led to slightly greater losses of AD.

Variation in male reproductive success

Only minor differences were observed between the scenarios with random and moderate VMRS (Table 6). The percentage of males siring at least one offspring in their lifetime was 39.4 vs. 36.6 in a stable population of 2,000 bison. Note that these values are given for all living males, not just those reaching reproductive age. There was much greater difference between moderate and high VMRS than between random and moderate VMRS.

High VMRS over 200 simulated years reduced AD at microsatellite loci to 94.0% if Nc=2,000, but AD was 95.2 % when Nc= 3,000 bison (Figure 3). Under the less extreme VMRS simulations, at least 95% of AD was retained if Nc ≥ 2,000 bison after 100 years or Nc ≥ 3,000 bison after 200 years.

Initial number of alleles per locus

With only 2 alleles per locus (e.g., SNPs), AD was maintained at >95% of initial values for 200 years under the high VMRS simulation (Figure 4). The maintenance of allelic diversity was substantially reduced in populations with a greater number of alleles per locus (Figure 4). For example, with 20 alleles per locus, 95% of AD was maintained only approximately 50 years.

Heterozygosity was maintained at between 97.9% and 98.5% under all different initial numbers of alleles per locus.

Culling

Culling strategies leading to population fluctuations resulted in different before- vs. after-cull age structure. Random culls (Cull.RND) did not affect subsequent age distribution or reproductive potential (Figure 5). However, culling only young animals (Cull.Juv) resulted in a gap in the age structure as the proportion of females entering the prime breeding age was reduced. Thus, a few years of slow population growth occurred before the simulated rate of increase resumed. Culling of older animals (Cull.Adult) resulted in some cases where population size decreased below 2,500 for one or more years after the culling event (except for the highest lambda, $\lambda=1.12$). This decrease was caused by the elimination of a high proportion of prime age reproductive individuals. While the gap in age structure was less noticeable under scenarios using higher growth rates, the genetic consequences were similar.

Generation length

Culling of juvenile individuals (compared to random culling) resulted in substantially longer generation intervals, increasing them from 7.9 to 8.7 years; whereas culling adults reduced generation interval to only 6.7 years (Table 7, Figure S1). High VMRS resulted in longer generation times (7.2 years) than moderateVMRS (6.3 years). Differences in the population size and the magnitude of population fluctuation had little influence in generation length (Table 7).

Discussion

Wildlife managers need to understand the relationship between N_e and the conservation of genetic variation in natural populations, and to monitor populations to evaluate how well predictive models perform (Schwartz *et al*. 2007). Managers must consider AD along with N_e because N_e estimators (e.g., inbreeding and variance N_e) might not be good predictors of the loss of AD over the short term or in fluctuating populations. Our study illustrates how the loss of AD depends on the initial number of alleles per locus and their frequency distribution within a population (Crow & Dennison 1988; Allendorf & Luikart 2007). Our results are informative regarding the N_e resulting from VMRS and fluctuating Nc that are common among ungulates but that are significantly different from the ideal or simplified population models that are often used in conservation genetics.

Effective size and N_e/Nc

The N_e for Yellowstone bison is likely >750, given our simulations including scenarios such as high VMRS, Nc ≥2,000 bison and $\lambda=1.08$ with random or targeted culling of either young or old individuals. This N_e is higher than the common recommendation of maintaining N_e >500 individuals to maintain genetic variation for adaptation to environmental change (Franklin 1980; Allendorf & Ryman 2002). Most of our N_e estimates, over a range of demographic parameters, varied from N_e ~750 bison for high VMRS to N_e ~1,200 bison for ModerateVMRS. While the population size of Yellowstone bison has been relatively large (generally >2,000) since 1980

(Fuller *et al.* 2007), extreme VMRS could severely reduce N_e (e.g., to <500 bison) and contribute to poor conservation of both H_e and AD.

The N_e/Nc ratios we estimated are generally in agreement with estimates (0.30 - 0.45) for bison at Badlands National Park in South Dakota, USA (Berger & Cunningham 1994). In our simulations, the N_e/Nc ratio for Yellowstone bison varied between 0.30 and 0.60 with realistic VMRS and population fluctuations. This stability of N_e/Nc is important because it allows managers to approximate N_e given Nc. Only extreme VMRS reduced the N_e/Nc ratio to <10%, but this extreme might onlyl happen in small populations where a few males could dominate the breeding of most females. The N_e, N_e/Nc ratio, and genetic variation could decrease more rapidly in continuously declining populations compared to stable or moderately-fluctuating populations we considered.

Heterozygosity

Our results quantify the benefit of large Nc and N_e (e.g., N_e > 500) for the maintenance of genetic variation in wildlife populations. The H_e retained over 200 years remained >95% for all scenarios except for the unrealistically extreme VMRS. In all scenarios of fluctuating population census size, the H_e retained after 200 years was >98%. The reduction of H_e could be accompanied by fixation of deleterious recessive alleles and thus fixation of inbreeding depression, as well as a reduction of heterozyogote advantage (overdominance), which could occur for certain loci such as those involved in disease resistance (e.g., major histocompatibility complex; MHC)

Allelic diversity

Conservation of 95% of current AD is likely during the first 100 years under most scenarios we considered including moderate-to-high VMRS, Nc > 2,000 bison, and approximately five alleles per locus, regardless of the culling strategy. However, over 200 years, a stable Nc of approximately 2,000 bison is not likely to maintain 95% of initial AD, even with only moderate VMRS. Nonetheless, maintenance of 95% of AD will likely be achieved with a fluctuating population size that increases to Nc >3,500 bison.

The conservation of AD was influenced more by the mean Nc in a fluctuating population (Figure S2, Supporting information) than by the lowest Nc reached for the culling levels we simulated (i.e., removing up to 37% of the population). This is unlike the N_e, which is strongly affected by the minimum Nc reached during fluctuations, because the average N_e is computed from the harmonic mean (not arithmetic mean), which is strongly influenced by small N_e values. The AD maintained was lowest when only adults years were culled (Cull.>3). This was likely caused by the reduction in generation length and reduction in the average Nc caused by removal of a large proportion of reproductive individuals.

Our results regarding the conservation of AD support the contention of Gross *et al*. (2006) that control strategies that increase generation time (e.g., culling young animals) can enhance retention of genetic variation in small populations (<500 bison) (see also Ryman et al. 1981). Our results suggest that such enhancement would occur in larger herds as well (> 2,000 animals) and is more pronounced for conservation of AD than for H_e. Yellowstone is the only

remaining wild herd of plains bison that currently meets the objective of maintaining a large population size with greater than 2000 individuals (Gates *et al*. 2010).

Influence of molecular marker type

Microsatellites in Yellowstone bison have approximately five alleles per locus (Halbert 2003; F. Gardipee, unpublished data). Our simulations show that loci with higher initial AD (e.g., 20 alleles) have a higher probability of loss of alleles over time (85% after 200 years) because most alleles are found at low frequency when many alleles exist per locus. Halbert (2003) noted that some populations had as many as 11 alleles at the loci she studied. Mikko et al. (1997) reported 9 alleles at an MHC locus from a small sample of 20 bison from 3 herds, suggesting AD at MHC is likely higher than reported at neutral microsatellite loci. However, there were questions about cattle admixture among the bison Mikko sampled. Additional research on MHC and other genes is needed to understand rates of loss of variation at important functional loci.

Influence of variance in male reproductive success

A polygamous mating system can limit or eliminate the genetic contribution of many males and thereby increase VMRS which can rapidly reduce genetic variation in a population. Due to the uncertainty of this demographic parameter in a large population of wild bison, we tested a broad range of VMRS. The most likely male mating scenario for bison among those we considered is the Moderateor moderate parameter used for simulating VMRS. However, we used 'high' VMRS as a default input for simulations that evaluated fluctuating populations because it likely represents a worst case scenario. Thus, if scenarios using high VMRS result in conservation of AD and H_e then the Yellowstone bison population should more likely to meet conservation goals, or at least not be limited by reproductive strategy exhibited by the male gender. Maintenance of dominance status for five years (unless death occurs) could also be conservative (overestimate) because the cost of maintaining dominance is energetically high and often involves fighting to exhibit dominance while limiting foraging during the rut (Lott 1979).

Influence of population fluctuations

Under most scenarios, fluctuating population census size had only a small effect on N_e and AD when the population size was larg. Both the N_e (and N_e/N_c ratios) were similar among culling strategies and ranged between 997 and 1,208 for N_e and 0.33 and 0.37 for N_e/N_c.

Small fluctuations in Nc where only young animals were removed (cull < 3) resulted in the largest mean Nc and the longest generation times, suggesting that this strategy would be relatively effective for maintaining genetic variation. While the effects of culling on AD and H_e varied little among the simulations of fluctuating Nc, both AD and H_e remain consistently higher when mean Nc is maintained at >3,000 bison.

The conservation of genetic variation in fluctuating populations is most influenced by population growth rate and the average Nc. Thus, culling strategies that result in a higher average population size (e.g. allow growth to Nc >4,000) will preserve more genetic diversity and a higher N_e.

Culling (or harvest) of wildlife populations is conducted to meet a variety of population objectives. Population growth rate appears to have a stronger influence on maintenance of genetic diversity than different culling strategies. Nonetheless, our simulation results indicate that managers should focus culling on the younger age cohorts while preserving the older age cohorts where possible to increase generation time and slow the loss of variation over time. These results agree with those from Ryman et al. (1981) where computer simulations showed that hunting pressure on younger individuals increased the generation time and thus the H_e maintained over time for moose. They estimate N_e for females from the mean and variance of individual lifetime production of offspring surviving to reproductive age and including heritability of fertility, ignoring males and VMRS. They also simulated stable population size and age structure.

The results suggest that culling to maintain population census size goals will seldom accelerate loss of genetic variation when population size remains larger than 2,000 to 3,000 individuals. In general, our results support the contention of Gross *et al.* (2006) that control strategies can have large effects on the retention of genetic variation if population sizes become lower than 2,000 bison. This agrees with the concept presented by Plumb *et al.* (2009) that maintenance of a population census size that fluctuates between 2,500 and 4,500 bison would conserve genetic diversity, especially if large population fluctuations are infrequent and average population census size is maintained above 3,000 bison.

Conclusions

Stochastic simulation modelling is a flexible but under-appreciated approach for estimating N_e and loss of genetic variation for a range of management scenarios and for populations with realistic age structure and complex demography (Harris & Allendorf 1989). We showed how simulation modeling could help assess N_e and loss of genetic variation, given observed and relatively detailed demographic and molecular data for the Yellowstone bison population. Conservation strategies to preserve genetic variation in Yellowstone bison would benefit from including our results as a predictive hypothesis while using an adaptive management approach and genetic monitoring to test the accuracy of our estimates (Ludwig and Walters 2002; Luikart et al. 2010).

In our simulations, VMRS was the factor with the strongest influence on N_e and the loss of variation. Thus future research could improve upon predictions of loss of variation if more field work yielded estimates of male reproductive success. Paternity assignment methodologies, including recently improved molecular and statistical methods, may be insightful in acquiring this type of empirical data. We did not consider high variance in female reproductive success or heritability of fitness, both of which could increase the rate of loss of variation by perhaps 10 - 20% (Ryman et al. 1981).

The number of alleles per locus also had a strong influence on loss of AD, as expected (Fig. 4). Future research should collect empirical data from functional and high-diversity loci (e.g., MHC) to improve understanding of the distribution of AD genome-wide and thus to improve estimates of loss of genomic AD.

Future genetic modeling to facilitate population conservation could be improved by including natural selection. Natural selection (e.g. balancing selection) can provide beneficial effects that increase the probability of maintaining AD and H_e. Alternatively, directional selection might reduce AD and H_e compared to our models here. Our model was unable to account for selection, thus our estimates of genetic variation maintained could be different for certain genome regions, e.g. MHC.

Our simulations are amont the most realistic and extensive to date for a large mammal and should be useful for assessing N_e and the loss of AD for other species with similar demography and mating systems (e.g. large mammals, especially ungulates). Our simulations suggest that considering both N_e and Nc is important for conserving AD and H_e, and to better understand the N_e/Nc ratio. They also show that fluctuations in population size around an established census size goal do not imply increased loss of genetic variation, at least for the relatively large Nc considered here. Computer simulations clearly have increasing power to help wildlife managers investigate strategies to conserve genetic variation for many population scenarios and species.

Acknowledgments

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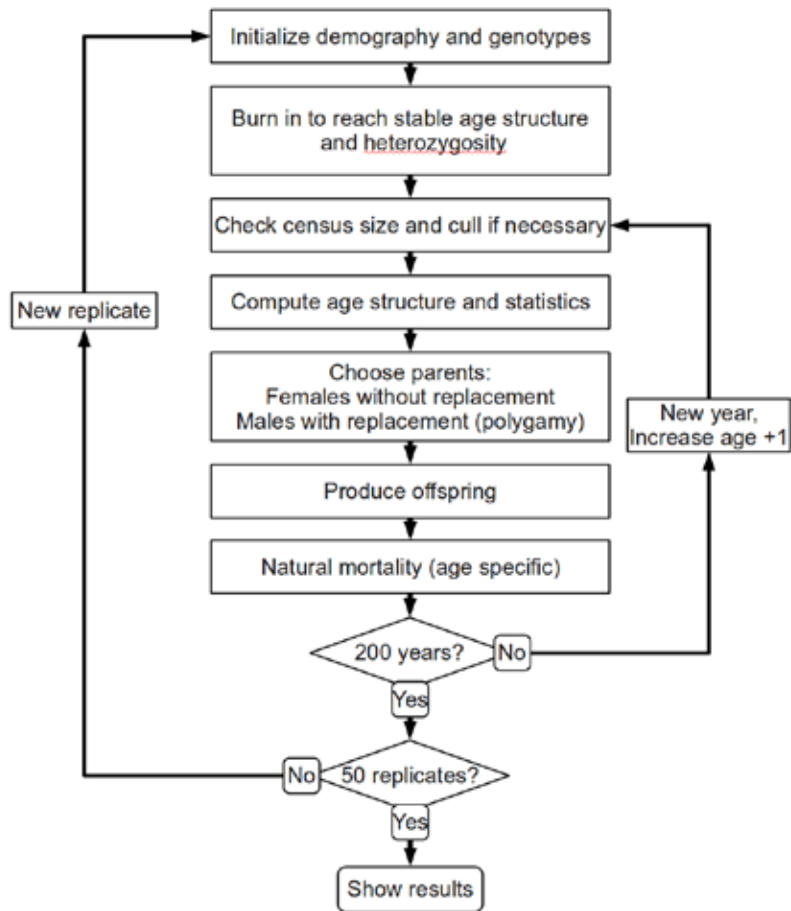
Figure 1. Procedure followed in simulations with the NewAge software.
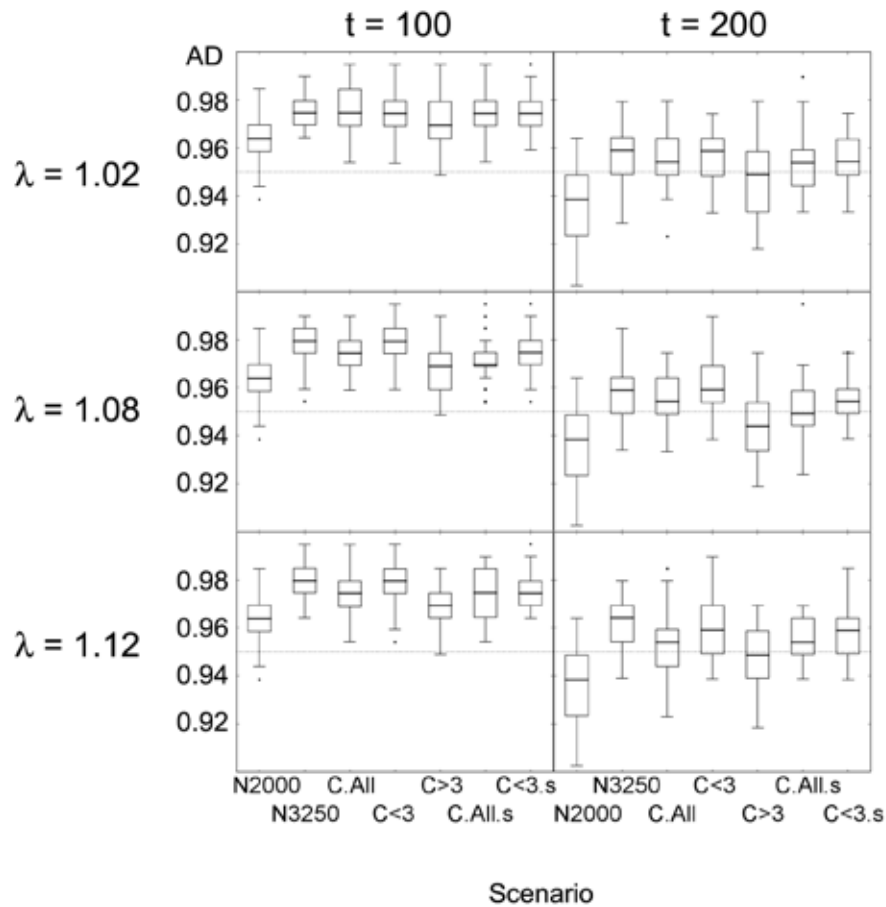
Figure 2. Effect of population growth rate (λ) and culling scenarios on proportion allelic diversity (AD) remaining for two time periods (t = 100 and 200 years). Scenarios: N2000, Nc=2000 with stable size using survival for λ=1.02; N3250, Nc=3250 using survival for the corresponding λ; C.All, Cull.RND; C<3, Cull.Juv; C>3, Cull.Adults; C.All.s, Cull.RND.LowFlux; C<3.s, Cull.Juv.LowFlux. Horizontal dotted line represents 95% threshold for AD remaining.
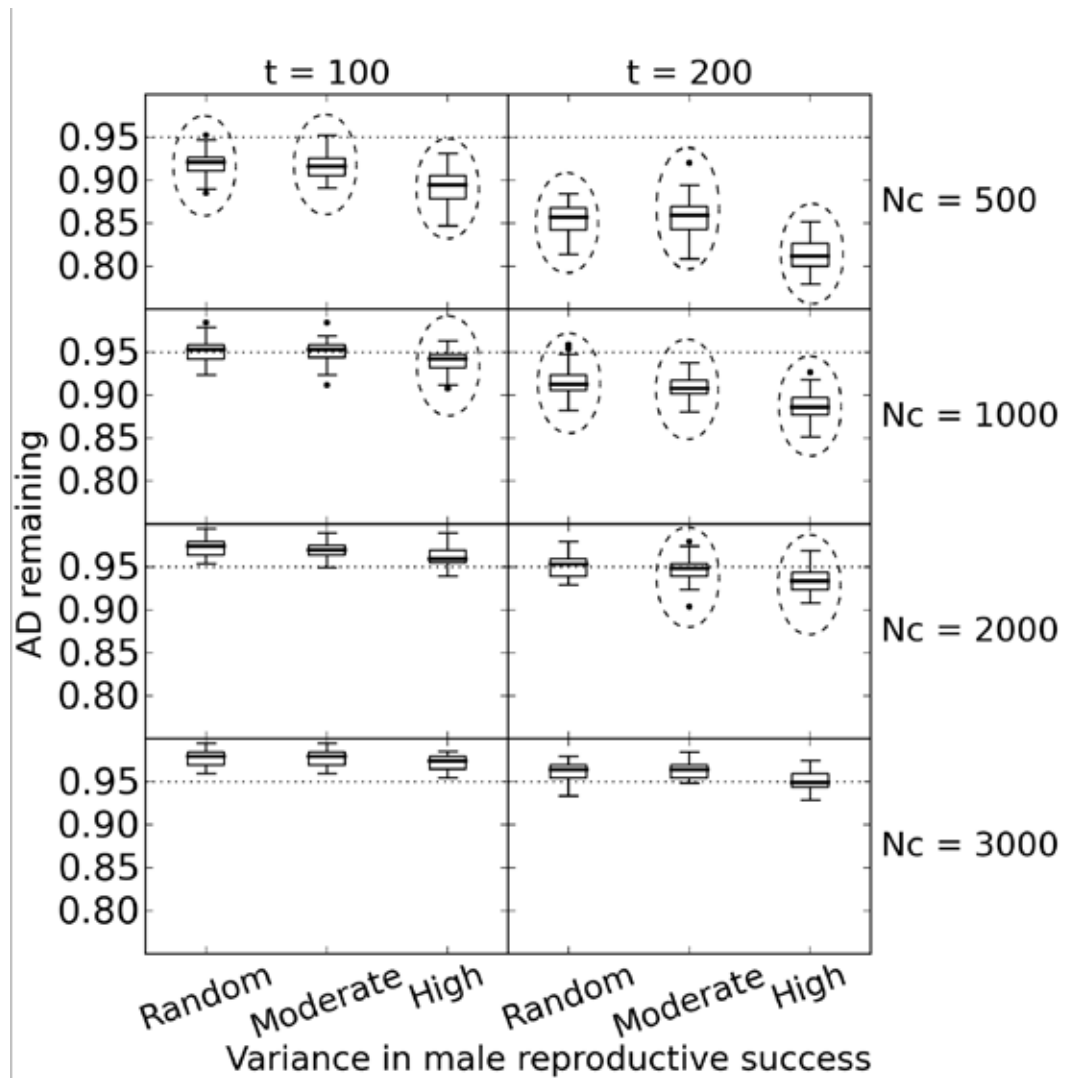
Figure 3. Effect of variance in male reproductive success (X-axis) on allelic diversity (AD) remaining (Y-axis), for population sizes of N_c = 500, 1000, 2000, and 3000 and for two time periods (t = 100 and 200 years), with 5 alleles per microsatellite locus. Box plot shows the median (middle line), 25th and 75th percentiles (box top and bottom edges), and outliers (single points) for 50 simulation replicates. Horizontal dotted line represents 95% threshold for AD remaining. Dashed ovals show cases with the mean <95% of AD maintained over 100 and 200 years (from 50 simulation replicates).
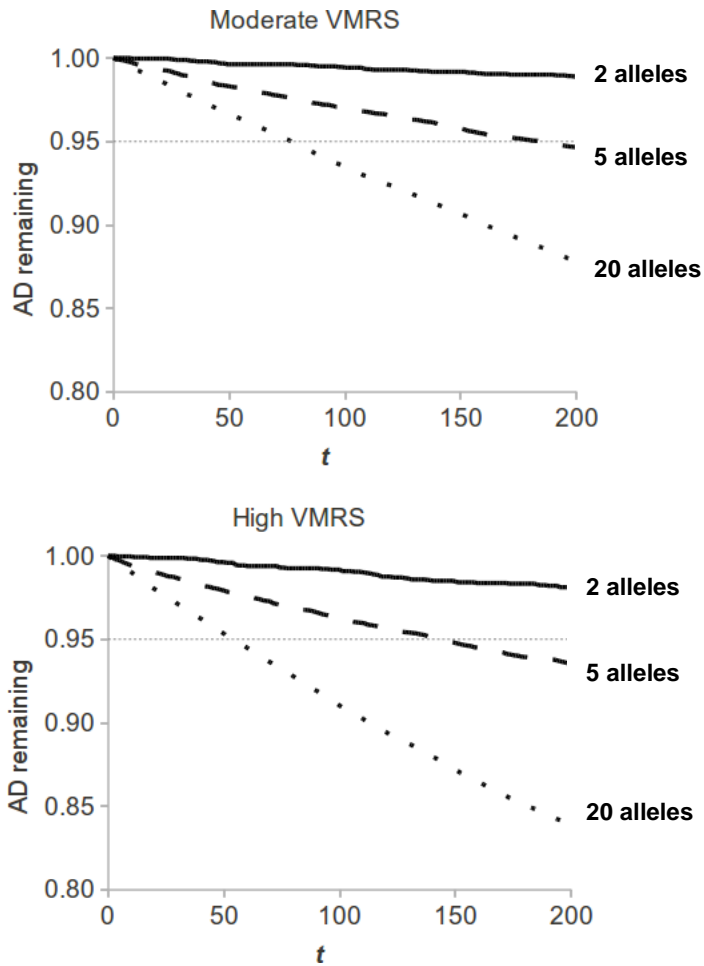
Figure 4. Allelic diversity (AD) remaining for a stable population of size Nc = 2,000 for three initial allelic richness values (2 alleles per locus shown by solid lines; 5 alleles, dashed lines; and 20 alleles shown by dotted lines at the beginning of each simulation replicate). Two scenarios of variance of male reproductive success (moderate and high, see Table 1). Horizontal grey dotted line represents 95% threshold.
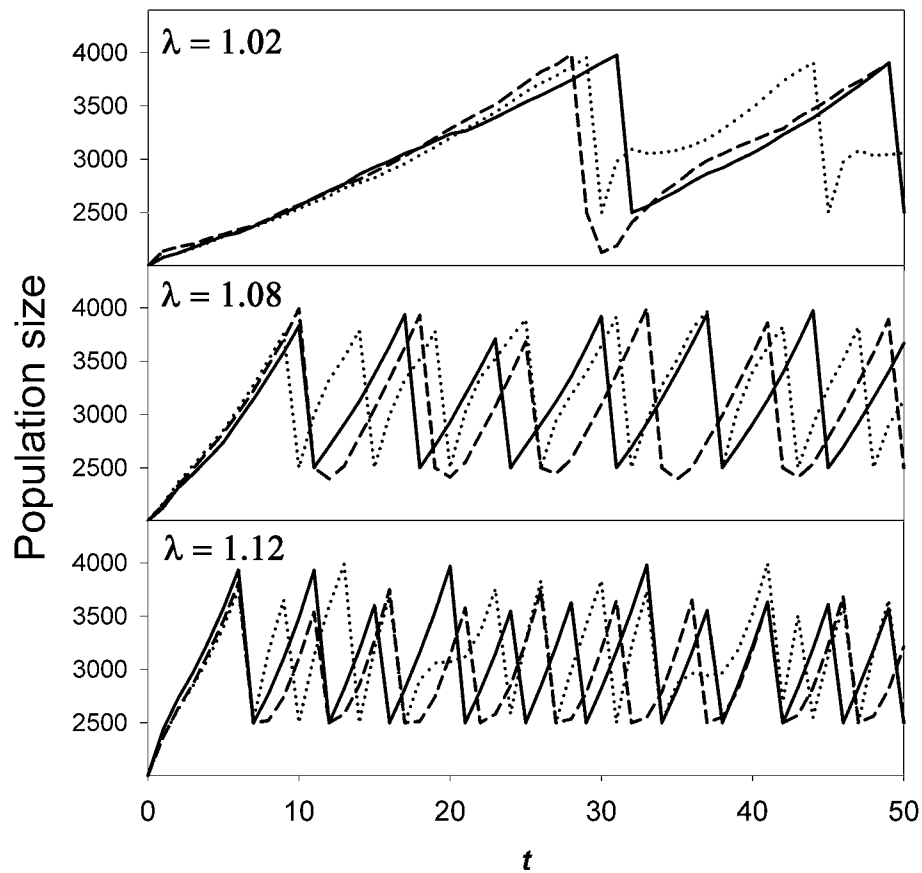
Figure 5. Population size during 50 years under different demographic parameters (λ) and different strategies of culling: Solid lines, Cull.RND; dashed lines, Cull.Juv; dotted lines, Cull.Adult (for fluctuations from Nc= 2,500 to 4,000). Population size at t = 0 is 2,000 and was maintained after 20 years of burn in. Horizontal dotted grey lines represent the objective size (2,500) to reach after culling to reduce Nc.

Tables

Table 1. Age-specific demographic parameters used in simulations for both the males and females. We considered three different population growth rates (λ), at equilibrium for age structure in the initial generation. Survival (s) is the percentage of individuals of a given age who lived until the next year. Birth rate (b) is the percentage of females of each age that gave birth to a calf. Scenarios with stable size ($\lambda = 1.00$) were obtained using these survival rates and then by limiting the number of births to a fixed number by 25, 18 and 18% of Nc for $\lambda=1.02$, 1.08 and 1.12 respectively (values needed to reach the age structure equilibrium).

λ	1.02[b]		1.08[a]		1.12[c]	
Age	s	b	s	b	s	b
0	55	0	76	0	77	0
1	76	0	84	0	88	0
2	89	70	92	46	99	28
3	95	70	92	46	99	40
4	89	95	92	70	99	52
5	86	93	92	70	99	62
6	91	93	92	70	99	70
7	87	93	92	70	99	74
8	76	93	92	70	99	78
9	82	92	92	70	99	80
10	77	92	92	70	99	80
11	54	92	92	70	99	80
12	57	92	92	70	99	80
13	55	92	92	70	99	76
14	64	80	92	70	98	72
15	57	80	92	70	98	34
16	75	57	92	70	50	28
17	75	46	92	70	50	24
18	75	34	92	70	50	16
19	75	22	92	70	50	12
20	0	13	0	70	0	6

[a] Modified from Fuller et al. (2007). Data from Yellowstone National Park, USA.
[b] Modified from Brodie (2008). Data for Wichita Mountains Wildlife Reserve, USA.
[c] From Brodie (2008). Data for a hypothetical rapidly growing bison herd, based on different populations.

Table 2. Culling strategies used to simulate fluctuating population size. Once population census size reached 4,000 (or 3,500), culling reduced Nc to 2,500 (or 3,000). The SmallNc scenario was included to assess the stability of the Ne/Nc ratio among different population census sizes, and to assess the effects of culling on rates of loss of genetic variation in small populations.

	Ages removed	Large population size threshold to start culling	Small population size to reach by culling
Cull.RND	0-20	4,000	2,500
Cull.Juv	0-2	4,000	2,500
Cull.Adult	4-20	4,000	2,500
Cull.RND.LowFlux	0-20	3,500	3,000
Cull.Juv.LowFlux	0-2	3,500	3,000
Cull.Adult.LowFlux	4-20	3,500	3,000

RND refers to random culling (proportional to abundance within each age)
Juv refers to juveniles culled (age < 3 years old)
Adult refers to adults only culled (age > 3 years old)
Culling was always random within each age class

Table 3. A) Average N_e/N_c ratio in stable populations ($\lambda = 1.0$) with a range in VMRS. "Random" VMRS means that the probability of siring offspring is equal among males within an age class. "Moderate" means that 10% of (dominant) males father 50% of offspring each year (see text). B) Fluctuating populations with High VMRS. RND refers to random culling among age classes. All standard errors <0.01.

A Population size	Variation in Male Reproductive Success			
	Random	Moderate	High	Extreme
250	0.65	0.59	0.37	0.04
500	0.64	0.58	0.37	0.04
1000	0.62	0.58	0.37	0.04
2000	0.61	0.59	0.37	0.04
3000	0.61	0.58	0.37	0.05

B	No Fluctuation	High Fluctuation (2,000 – 4,500)			Low Fluctuation (3,000-3,500)		
Growth rate	Nc = 3,250	RND	Juv	Adult	RND	Juv	Adult
$\lambda = 1.02$	0.37	0.35	0.34	0.34	0.36	0.36	0.37
$\lambda = 1.08$	0.33	0.34	0.35	0.33	0.34	0.34	0.36
$\lambda = 1.12$	0.33	0.34	0.36	0.34	0.34	0.34	0.36

Table 4. Average H_e maintained (% of initial H_e at year 0) after 200 years in a Stable Populations ($\lambda = 1.0$) with a range in VMRS. All standard errors were <0.4 except those cases with Extreme VMRS where standard errors were <1.0. Note: More than 98.4% of initial H_e was maintained in all Fluctuating populations scenarios considered, e.g. High VMRS, Nc = 2000-4,500 (data not shown).

Population size	Variation in Male Reproductive Success			
	Random	Moderate	High	Extreme
250	89.6	89.7	86.1	25.5
500	94.7	94.4	92.5	49.0
1000	97.2	97.2	96.4	73.7
2000	98.8	98.8	97.9	85.5
3000	99.1	99.2	98.8	89.8

Table 5. Average AD (% of AD remaining) after 200 years in A) Stable Populations ($\lambda = 1.0$), and B) Fluctuating populations. RND refers to random culling among age classes. All standard errors <0.05

A	Variation in Male Reproductive Success			
Population size	Random	Moderate	High	Extreme
250	75.4	75.4	68.8	14.3
500	85.9	85.3	81.3	29.8
1,000	91.6	91.1	88.9	52.7
2,000	94.8	94.7	94.0	68.6
3,000	96.3	96.3	95.2	76.7

B	Average census size	High Fluctuation (2,000 – 4,500)			Low Fluctuation (3,000-3,500)		
Growth rate	Nc = 3250	RND	Juv	Adult	RND	Juv	Adult
$\lambda = 1.02$	95.4	95.5	95.6	94.6	95.4	95.6	95.5
$\lambda = 1.08$	95.9	95.5	96.1	94.5	95.6	95.6	95.5
$\lambda = 1.12$	96.1	95.4	96.1	94.7	95.3	95.7	94.9

Table 6. Effect of variance in male reproductive success on the probability of siring offspring and on the mean number of off spring (all born or those living at least up to 7 years old) per siring male and a stable Nc of 2,000 bison. Random refers to random mating *within* a cohort (not across the entire male population). Under Random mating, only 39.4% of males sire offspring as more than 50% of males die before reproductive age. Random mating yielded the lowest variation in reproductive success. Moderate refers to moderate dominance of mating opportunities in a polygamous mating system in which 10% of males produce 50% of offspring each year (see Methods).

Variation in Male Reproductive Success	Percentage of all born males siring offspring in their lifetime	Mean number of offspring per male with offspring	Mean number of offspring living up to 7 years per male with offspring
Random	39.4	10.0	2.1
Moderate	36.6	14.9	3.1
High	12.8	35.2	6.9
Extreme	1.2	381.8	72.2

Table 7. Generation length (years) in A) Stable populations (λ = 1.0) and B) Fluctuating populations. All standard errors < 0.05. RND is random culling within each age class.

A	Variation in Male Reproductive Success			
Population size Nc	Random	Low	High	Extreme
250	5.6	6.2	7.1	7.3
500	5.6	6.2	7.2	7.3
1,000	5.7	6.3	7.2	7.3
2,000	5.7	6.3	7.2	7.3
3,000	5.7	6.3	7.2	7.3

B	Average Census size	High Fluctuation (2,000 – 4,500)			Low Fluctuation (3,000-3,500)		
Growth rate	Nc = 3250	RND	Juv	Adult	RND	Juv	Adult
λ= 1.02	7.2	7.1	7.3	6.7	7.1	7.2	6.8
λ = 1.08	8.9	7.9	8.7	6.7	7.9	8.7	6.9
λ = 1.12	9.2	8.2	9.5	7.1	8.2	9.4	7.0

Supporting information.

Table S1. Comparison of AD and N_e obtained from two independent simulation programs, NewAge (50 replicates) and PEDAGOG (20 replicates) for six scenarios with a wide range of population growth (λ), variance in male reproductive success (VMRS), and culling (all individuals, young only or old only). Numbers in parentheses are standard errors (SE).

Scenario	AD		N_e	
	NewAge	PEDAGOG	NewAge	PEDAGOG
λ =1.00 Nc=2000 High VMRS	94.0 (0.0)	92.7 (0.4)	746 (4)	602 (7)
λ =1.00 Nc=2000 ModerateVMRS	94.7 (0.2)	93.7 (0.4)	1176 (7)	921 (6)
λ =1.00 Nc=2000 Ext. VMRS	68.6 (0.4)	66.5 (0.7)	89 (1)	81 (2)
λ =1.08 Cull.RND	95.5 (0.2)	95.3 (0.3)	1054 (7)	1007 (6)
λ =1.08 Cull.Juv	96.1 (0.2)	95.6 (0.3)	1158 (12)	941 (7)
λ =1.08 Cull.Adult	94.5 (0.3)	94.6 (0.2)	1002 (14)	1050 (7)

Table S2. Reduction of the N_e/Nc ratio due to fluctuating population size (relative to N_e/Nc for a stable population with the mean population size of $Nc = 3{,}250$). Numbers are the proportional reduction the N_e/Nc ratio. Culling only adult animals (age > 3) gave the greatest reduction in N_e (e.g., 14% for lambda 1.02).

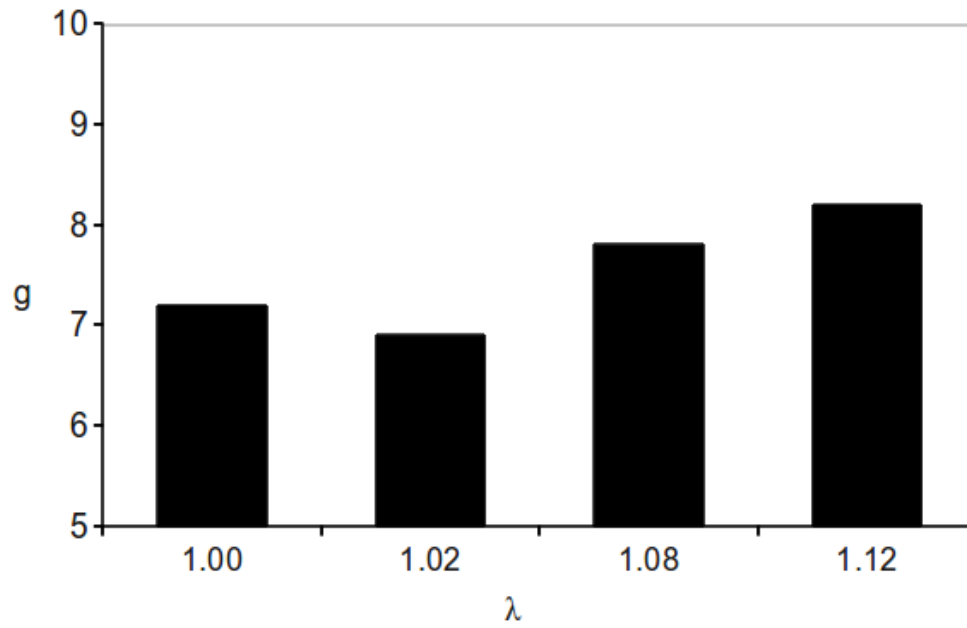| Growth rate	High Fluctuation (2,000 – 4,500)			Low Fluctuation (3,000-3,500)		
	All	Juv	Adult	All	Juv	Adult
$\lambda = 1.02$	0.09	0.08	0.14	0.05	0.04	0.06
$\lambda = 1.08$	0.03	0.00	0.08	0.00	0.02	0.06
$\lambda = 1.12$	0.03	0.00	0.08	0.00	0.02	0.06

Figure S1. Generation time (g) obtained for the different sets of demographic vital rates (λ) at age-structure equilibrium. Note that these values are fully dependent on the demographic parameters given in table S1 and not in the growth population rate (i.e. different vital rates could produce the same growth rate but different generation times).
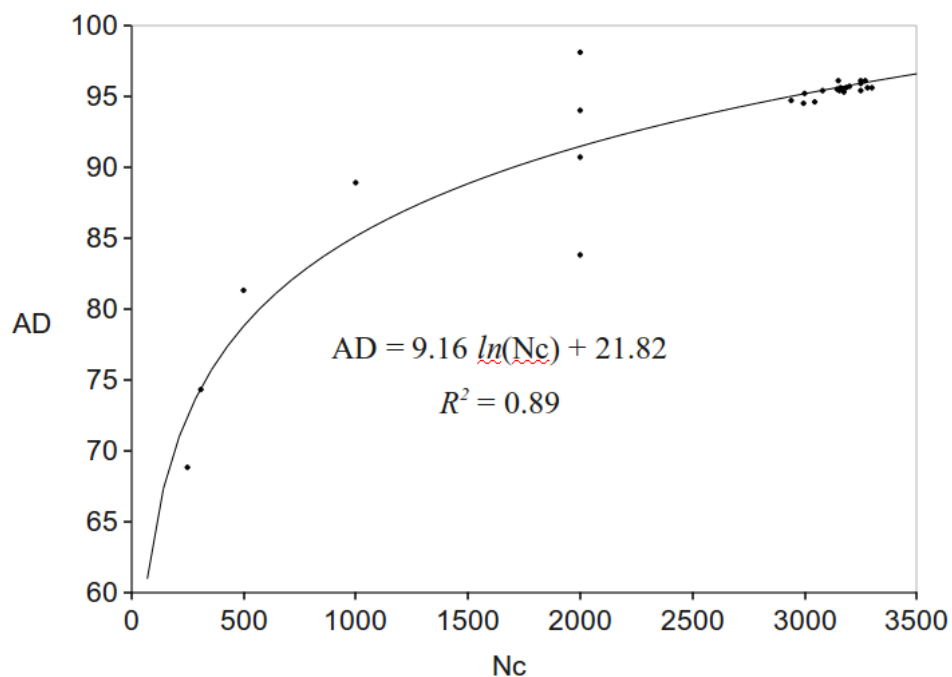
Figure S2. Allelic diversity remaining (AD) after 200 years plotted to averaged population census size (Nc) in all simulated scenarios with High VMRS and λ equal or higher than 1.0. The line represents the logarithmic regression, expressed by the equation. The four points for Nc =2000 are for loci with 2, 5, 10, and 20 alleles, from top down, respectively. All other points are for 5 alleles per locus.