



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

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ARTICLE INFO

Article history:

Received 7 March 2011

Received in revised form 24 February 2012

Accepted 27 February 2012

Available online 8 May 2012

Keywords:

Allelic diversity
Effective population size
Simulation modelling
Age structure
Population growth rate
Population viability
Genomic diversity
Conservation genetics
Yellowstone bison

ABSTRACT

Loss of genetic variation through genetic drift can reduce population viability. However, relatively little is known about loss of variation caused by the combination of fluctuating population size and variance in reproductive success in age structured populations. We built an individual-based computer simulation model to examine how actual culling and hunting strategies influence the effective population size (N_e) and allelic diversity in Yellowstone bison over 200 years (~28 generations). The N_e for simulated populations ranged from 746 in stable populations of size 2000 up to 1165 in fluctuating populations whose census size fluctuates between 3000 and 3500 individuals. Simulations suggested that ~93% of allelic diversity, for loci with five alleles will be maintained over 200 years if the population census size remains well above ~2000 bison (and if variance in male reproductive success is high). However for loci with 20 alleles, only 83% of allelic diversity will be maintained over 200 years. Removal of only juveniles (calves and yearlings) resulted in longer generation intervals which led to higher maintenance of allelic diversity (96%) after 200 years compared to the culling of adults (94%) when the mean census size was 3250 (for loci with five alleles). These simulations suggest that fluctuations in population census size do not necessarily accelerate the loss of genetic variation, at least for the relatively large census size and growing populations such as in Yellowstone bison. They also suggest that the conservation of high allelic diversity (>95%) at loci with many alleles (e.g., ≥ 5) will require maintenance of a populations size greater than approximately 3250 and removal of mainly or only juveniles.

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1. Introduction

Isolated populations often suffer reduced viability due to loss of genetic variation (Allendorf and 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

response to selection (Leberg, 2005). We considered the inbreeding effective size (N_{ei}), which is computed from the loss of expected heterozygosity (H_e) (Leberg, 2005), usually with the assumption of a stable population without age structure (Felsenstein, 1971).

Relatively little is known about N_e and rates of loss of allelic diversity (AD) resulting from the combined effects of age structure, mating system variation (e.g., high variance in male reproductive success resulting from a dominance hierarchy), and fluctuations in population census size (N_c) caused by culling or hunting (Ryman et al., 1981; Harris and Allendorf, 1989; Frankham, 1995; Jorde and Ryman, 1995; Waples and Yokota, 2007). Nonetheless, conservation geneticists have suggested that a reasonable management goal for maintenance of genetic variation is to retain approximately 95%

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of H_e over 100–200 years (Soulé et al., 1986; Allendorf and Ryman, 2002).

Few studies have quantified the dynamics of AD (i.e. the probability of loss of alleles), probably because it is very dependent on the frequencies of alleles and the census size which limits the number of alleles transmitted and, therefore, is more difficult to quantify than H_e . However, AD is important because it could influence long term adaptive potential and disease resistance, and is generally lost more rapidly than H_e (Allendorf, 1986; Leberg, 1992; Luikart et al., 1998). Allelic diversity at polymorphic loci can range widely from two to more than 20 alleles per locus across the genome. Single nucleotide polymorphisms (SNPs) and allozyme loci typically have only two alleles per locus, whereas microsatellites and immune system loci often 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 (>2000 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 and Derr, 2007; Sanderson et al., 2008). However, the population is geographically isolated and likely has moderate or high variance in reproductive success, as in many ungulates (Hogg et al., 2006; Ortego et al., 2011) due to a polygamous mating system and a dominance hierarchy in which a limited proportion of males breed most of the females 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 there is limited acceptance for wild bison due to the bacterial disease brucellosis, which cattle ranchers fear could spread from the bison to cattle (Plumb et al., 2009; Rhyen et al., 2009).

Sporadic culls of more than 1000 bison (~30% of the herd), combined with intervening periods of exponential population growth, have resulted in substantial fluctuations in bison population size (Geremia et al., 2009; White et al., 2011) and concerns about possible reductions in genetic variation (Halbert, 2003; Animal Welfare Institute, 2008; Hedrick, 2009). Fluctuations in population size might affect N_e and AD differentially and interact with age structure and variation in individual reproductive success to complicate the estimation of N_e (Allendorf and Luikart, 2007). Thus, genetic studies and modelling are needed to quantify the loss of variation and evaluate potential effects of population fluctuations and alternate culling strategies. This is especially urgent as several plaintiffs recently filed complaints in the US District Court purporting that the National Park Service and US Forest Service have taken management actions (e.g., culls) that threaten the genetic viability of Yellowstone bison (e.g., *Western Watersheds Project et al. versus Salazar et al.*, 2011).

Previous computer simulation studies have examined the effects of different demographic parameters on N_e in ungulate populations with stable population size (Ryman et al., 1981; Shull and Tipton, 1987), but they did not consider fluctuating population sizes or variance in reproductive success. Kaeuffer et al. (2007a) modelled the effects of fluctuating population size, life span, and variance in reproductive success in an age structured population of mouflon (*Ovis orientalis*) to help infer N_e and the degree of polygyny in a natural population. However they did not focus on the maintenance of heterozygosity and allelic diversity or the effect of dominance hierarchy.

We used computer simulations of an individual-based model of age structured bison populations to assess the potential effects of variance in male reproductive success (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. We addressed four questions: (1) What is the N_e of Yellowstone bison under current circumstances? (2) Will population fluctuations caused by culling

likely reduce H_e and AD by more than 5% in 200 years? (3) How rapidly is AD reduced by VMRS caused by a male dominance hierarchy? (4) How is loss of AD influenced by the initial number of alleles per locus which is variable across the genome (e.g., at SNPs, microsatellites, and highly-variable immune system loci)?

2. Methods

2.1. Computer simulations

We simulated populations of bison using the computer program NewAge (N_e with age structure; Fig. S1 in Online Supporting information; T. Antao et al. unpublished), a new program in Python using the simuPOP simulation environment (Peng and Kimmel, 2005; Peng and 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.

We used input values representative of Yellowstone bison to parameterize the simulation models (Appendix S1 in Online Supporting information), 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 demographic scenario, we ran 50 independent replicate simulations. We also considered Yellowstone bison to be one deme, which is consistent with recent genetic and field data (Fuller et al., 2007; Bruggeman et al., 2009; R. Wallen, unpublished data). For example, $F_{ST} \approx 0.005$ between the central and northern breeding groups in 2008 for 15 microsatellites; F. Gardipee and G. Luikart, unpublished data).

We varied the initial number of alleles per locus (allelic richness), fluctuating and stable population size (N_e), VMRS (i.e., variance in offspring production), and different culling strategies to evaluate the effects on genetic variation. Each simulation replicate began with the initialization of demography and genotypes. Alleles for 48 independent microsatellite loci were randomly assigned to individuals proportional to allele frequencies described for Yellowstone bison (AD : average = 5.1, range = 3–10; H_e : average = 0.65, range = 0.39–0.82; see Halbert, 2003) so the initial population is in Hardy–Weinberg proportions.

Alternate scenarios with different starting allelic richness of 2 (e.g., SNP scenario) and 20 (highly polymorphic neutral loci) alleles-per-locus across the genome were initialized following an uniform Dirichlet distribution (e.g., Tallmon et al., 2004), using the number of alleles-per-locus as alpha parameter, 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 100–200 years. Simulations were also run using 100 loci with 99 alleles per locus (a limit for the software we used) to reduce variance in estimates of loss of H_e and increase precision of N_e estimates. Note that here we are concerned with the effect of mating and culling on effective size, not on its estimation, and this is why we used the whole population with many highly variable loci (as in Harris and Allendorf, 1989). The number of alleles was large enough to have good precision on the estimation of N_e and H_e , so this scenario is a good framework for further comparisons.

2.2. Vital rates and age distribution

Demographic vital rates and age-structure were generated following a Leslie matrix model (Moran, 1962; Crow and Kimura, 1970; Waples and 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 reaching age $x + 1$) and fecundity (average number of progeny) rates (Appendix S1). Scenarios with stable population size ($\lambda = 1.00$) were obtained using these survival rates but randomly removing newborns above a fixed number, thus simulating high rates of neo-natal mortality. In these scenarios, fecundity values represent the natural probability of females reproducing.

The maximum attainable age for bison was set at 15 or 20 years (Brodie, 2008). Simulations with the maximum life span set at 15 years old yielded results similar to the default value of 20 years. Thus, we present only scenario results using a maximum age of 20 years. 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 (burn in) to achieve a stable population size, $N_c = 2000$, with a stable age distribution (Fig. S1) obtaining the base population. We then allowed the population to either remain stable in size or grow depending on the simulation (e.g., Waples and Yokota, 2007) during 200 years. 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., H_e , 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.

2.3. Culling

At the beginning of each annual cycle, N_c was computed to determine if culling should be performed depending on the scenario (Appendix S2 in Online Supporting information). Culling was conducted whenever population size exceeded a threshold value (4500 or 3500 depending on the scenario). We compared the N_e and genetic variation in the two fluctuating population scenarios with a stable population. To make fair comparisons, we simulated the same global census size during the whole period of study. Thus, the stable population had a $N_c = 3250$ that is the arithmetic mean between the extreme sizes of large (2500–4000) and small (3000–3500) fluctuating scenarios.

Culling was random among all age classes or random within the age groups culled (e.g., among juveniles less than 3 years old or adults greater than 3 years old). Individuals were culled until the target population size (2500 or 3000) was reached. These scenarios are feasible because bison can be aged reliably up to 4 years of age from tooth eruption patterns (Olson, 2005). There were always enough individuals of target ages to accommodate each culling scenario. Thus, a realistic level of culling (i.e., reduction in N_c) was conducted in all scenarios.

2.4. Mating

Our model simulates the polygamous nature of bison (Reynolds et al., 2003; Brodie, 2008). Females were allowed to have only one offspring each season 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 (Appendix S1).

Little is known about male reproductive success in bison. Lott (1979) suggested 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 were largely based on behavioural observations rather than DNA-base

paternity analysis which gives the true paternity and variance in male reproductive success.

We modelled four different scenarios regarding variance in male reproductive success: (1) all adult males had the same probability of mating within a given year (random VMRS); (2) 10% of the males were dominant and produced 50% of the offspring each year; the remaining offspring were produced by randomly chosen, non-dominant males (moderate VMRS); (3) 10% of the males were dominant and produced all of the offspring each year (high VMRS); and (4) 1% of the males were dominant and produced all the offspring each year (extreme VMRS). Dominant males were randomly chosen from males aged 6–10 years, and remained dominant during 5 subsequent years provided that they survived. Thus, the maximum age of a dominant male was 14 years. If a dominant male died, a non-dominant male aged 6–10 years was randomly chosen to become dominant.

2.5. Computation

Each year, the whole population was sampled (for all simulated loci) to calculate the parametric number of alleles and H_e . Allelic diversity remaining at time t (A_t) relative to time 0 (A_0) was calculated as $AD_t = (A_t - 1)/(A_0 - 1)$ (Allendorf, 1986). The generation interval (G) was also approximated each year as the average age of all the individuals producing offspring (which, to be accurate, should include only those offspring becoming parents).

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

$$N_e[H] = \frac{1}{-2\exp\left(\frac{\ln(H_{200}) - \ln(H_0)}{\frac{200}{G}}\right) + 2} \quad (1)$$

where H_0 and H_{200} are the expected heterozygosity at time 0 and 200 respectively, and G is the mean generation interval across 200 years. We used this estimate to focus on the loss of genetic variation and it behaved similarly to Felsenstein's (1971) demographic estimator for overlapping generations in stable population size scenarios, as expected.

To increase precision of the N_e estimates, and given that N_e depends only on the demography, we ran the same simulation scenarios but used 100 microsatellites with 99 alleles-per-locus initialized by a Dirichlet distribution. N_e and N_e/N_c were calculated using those scenarios with 100 loci.

3. Results

We simulated more than 60 different population scenarios to assess effects on genetic variation of different population sizes, population growth rates (λ), variance in male reproductive success, initial numbers of alleles per locus, as well as age-targeted culling which caused fluctuations in age structured populations. We also performed a comparative verification of the performance of our NewAge program using an independent computer simulation program PEDAGOG (Coombs et al., 2010) and obtained similar results from the two programs (Appendix S3 in Supporting information).

3.1. Effective population size

The N_e for simulated populations ranged from 746 in stable populations of size 2000 (Appendix S3) up to 1165 in fluctuating populations of size changing from 3000 to 3500 (Table 1). Population size fluctuations caused a reduction in N_e by as much as 14% relative to a stable population whose size (N_c) equalled the

Table 1
Average^a N_e computed from the loss of heterozygosity in stable populations (no fluctuation) and for fluctuating populations for each of three culling strategies (random = RND, juveniles = Juv, and adult) and with high variance in male reproductive success in which only 10% of the prime-age males produce all offspring each year (i.e., males of 6–14 years are of prime age and can potentially reproduce).

Growth rate	No fluctuation $N_e = 3250$	High fluctuation ($N_e = 2000$ –4500)			Low fluctuation ($N_e = 3000$ –3500)		
	High & RND neonate mortality ^b	RND ^c	Juv ^d	Adult ^e	RND ^c	Juv ^d	Adult ^e
$\lambda = 1.02$	1202	1106	1114	1039	1143	1165	1120
$\lambda = 1.08$	1070	1054	1158	1002	1083	1066	1022
$\lambda = 1.12$	1066	1051	1130	997	1080	1060	1015

^a All standard errors <15.

^b To maintain a stable population, the probability of neonate survival was reduced by 25%, 19% and 18% for $\lambda = 1.02$, 1.08, and 1.12 respectively (compared to the fluctuating population scenarios).

^c Random culling among all age classes.

^d Random culling among juvenile (age < 3) age classes.

^e Random culling among adult (age > 3) age classes.

arithmetic mean of the fluctuating populations (mean $N_e = 3250$; Table S2.2).

3.2. Heterozygosity and allelic diversity

Greater than 95% of H_e was maintained over 200 years for all scenarios considered with at least 1000 bison (Fig. 1). Population fluctuation for all of the culling strategies also preserved >95% of initial H_e over 200 years. Extreme VMRS (i.e., 1% of males producing all the offspring) was the only male mating scenario in which H_e decreased below 95%.

Greater than 95% of AD was maintained over 200 years only if population size was large ($N_e > 2000$) with only 5 or fewer alleles per locus and moderate VMRS (Table 2A; Fig. 2). Interestingly, the AD maintained with age structure and high VMRS was far lower (83% AD maintained over 200 years) than that in a simulation of a simple Wright–Fisher population (93%, W–F in Fig. 2) with no age structure and random VMRS.

3.3. Variation in male reproductive success

High VMRS over 200 simulated years reduced AD at microsatellite loci to 94.0% of its original value in the stable base population if

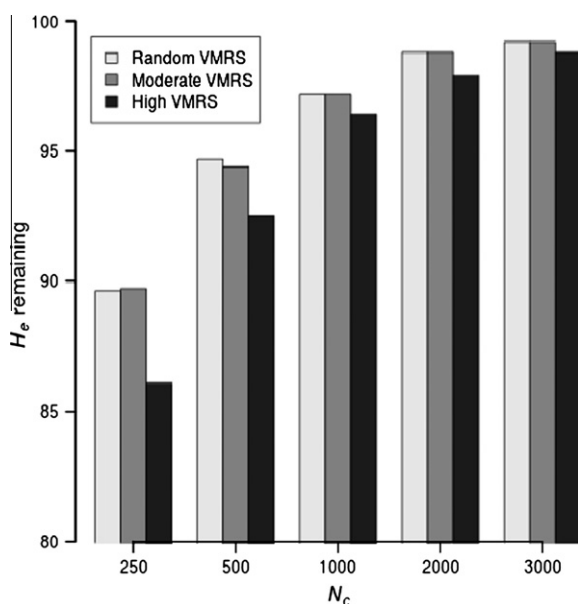


Fig. 1. Average H_e remaining (% of initial H_e from year 0) after 200 years in a stable population ($\lambda = 1.0$) with each of three different values of variance in male reproductive success (VMRS) and a stable population ($N_e = 2000$). All standard errors <0.40 (not presented).

Table 2
Average^a AD (% of AD remaining from year 0) after 200 years in (A) stable populations ($\lambda = 1.0$) of five different sizes, and in (B) fluctuating populations with high variation in male reproductive success, i.e., only 10% of males produce all offspring and only males of 6–14 years old are allowed to reproduce. All simulations were initiated with microsatellite data with approximately five alleles per locus.

A		Variation in male reproductive success		
Population size (N_e)		Random	Moderate	High
250		75.4	75.4	68.8
500		85.9	85.3	81.3
1000		91.6	91.1	88.9
2000		94.8	94.7	94.0
3000		96.3	96.3	95.2

B		High fluctuation ($N_e = 2000$ –4500)			Low fluctuation ($N_e = 3000$ –3500)		
Growth rate	No fluctuation ($N_e = 3250$)	High & RND neonate mortality ^a			High & RND neonate mortality ^a		
	High & RND neonate mortality ^a	RND ^b	Juv ^c	Adult ^d	RND ^b	Juv ^c	Adult ^d
$\lambda = 1.02$	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

^a Stable population size maintained by high and random neonate mortality.

^b Random culling among age classes.

^c Random culling among juvenile (age < 3) age classes.

^d Random culling among adult (age > 3) age classes.

$N_e = 2000$. With high VMRS, 95.2% of AD was retained for a stable population size (N_e) of 3000 bison (Figs. 3 and 4).

The percentage of males siring at least one offspring in their lifetime was 36.6 for moderate VMRS and only 12.8 for high VMRS, in a stable population of 2000 bison (Appendix S4 in Online Supporting information). Note that these values include all living males, not just those reaching reproductive age (e.g., males that die before age 3).

3.4. Initial number of alleles per locus

With 20 alleles per locus, 95% of AD was maintained for only approximately 50 years. With only 2 alleles per locus (e.g., for SNP loci), AD was maintained at >95% of initial values for 200 years under the high VMRS simulation (Fig. 2). Heterozygosity was maintained at between 97.9% and 98.5% under all initial numbers of alleles per locus.

3.5. Culling, fluctuations, N_e , and AD

The N_e ranged from 997 to 1165 among culling strategies (Table 1).

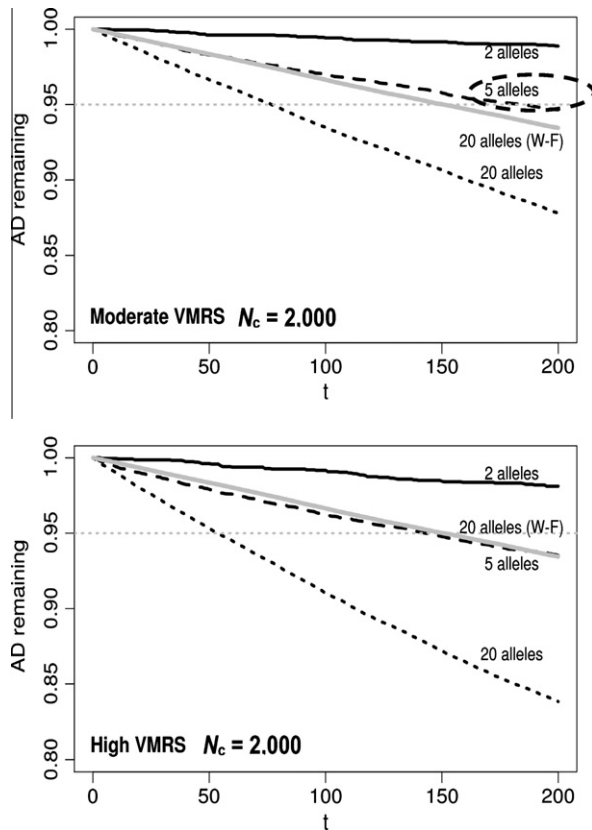


Fig. 2. Allelic diversity (AD) remaining for a stable population of size $N_c = 2000$ for three initial allelic richness values (two alleles per locus shown by solid lines; five alleles shown by dashed lines; and 20 alleles shown by dotted lines) and two scenarios for moderate and high VMRS. A Wright–Fisher “ideal” population with 20 alleles is represented by a grey solid line. Dashed oval indicates where 95% of AD remains (near 200 generations) for loci with five alleles per locus. Two scenarios of variance of male reproductive success are presented (moderate and high, see Table 2A), which likely bracket the true variance of male reproductive success in bison and other polygamous ungulate species. Horizontal grey dotted line represents the 95% of AD remaining.

Fluctuations in the N_c , especially when culling only adults reduced the probability of conserving AD compared to a stable population of size $N_c = 3250$ (Table 2B and Fig. 3). The same pattern of reduced maintenance of AD for adult culling occurred under relatively low and high population growth rates. The highest maintenance of AD (up to 96.1% over 200 years) occurred when culling only juveniles under moderate and high population growth rates: $\lambda = 1.08$ and 1.12 (Table 2, Fig. 3).

Culling strategies leading to population fluctuations resulted in different age structures before- versus after-cull (Fig. S2.1 in Appendix S2). Random culls (Cull.RND) did not affect the subsequent age distribution or reproductive potential. 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 initial simulated (equilibrium) rate of increase resumed. Culling of older animals (Cull.Adult) resulted in some cases where population size decreased below 2500 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-aged reproductive individuals.

3.6. Culling and generation interval

The generation interval (G) was strongly influenced by different culling scenarios (Table 3). For example, G was 6.7 versus 8.7 when

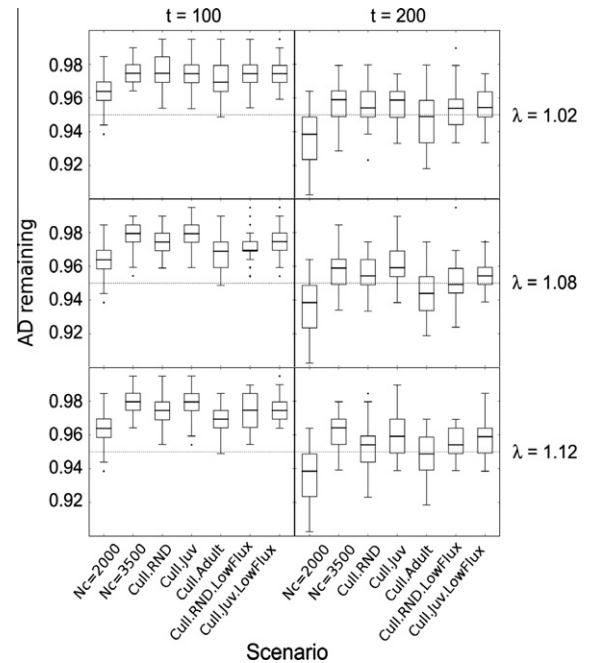


Fig. 3. Effect of population growth rate (λ) and culling scenarios on the allelic diversity (AD) remaining after each time period ($t = 100$ and 200 years). Box plot shows the median (middle line), 25th and 75th percentiles (box top and bottom edges), the lowest/highest datum still within 1.5 times the interquartile range of the lower/upper quartile (whiskers), and outliers (single points) for 50 independent simulation replicates. Horizontal dotted line represents the threshold at which only 95% of the initial AD remains. Scenarios $N_c = 2000$ and $N_c = 3250$ stand for a stable population of $N_c = 2000$ and $N_c = 3250$ respectively. The culling scenarios are described in Table 1 footnotes and Appendix S2 (in Online Supporting information).

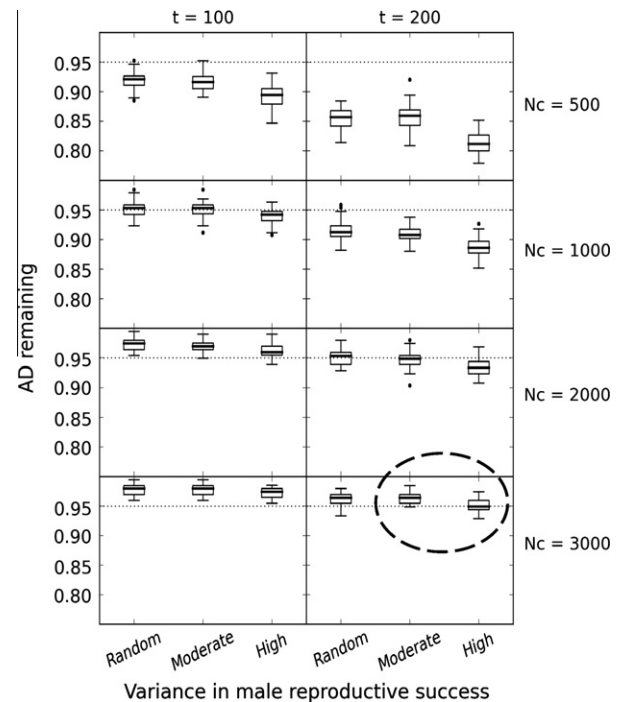


Fig. 4. Effect of variance in male reproductive success on allelic diversity (AD) remaining for different population sizes (N_c) and time periods (t in years) with five alleles per microsatellite locus. Box plot and horizontal dotted line as in Fig. 3. The dash line oval shows the two scenarios most likely to represent the current Yellowstone bison population (with N_c remaining above 3000).

Table 3

Mean^a generation interval (years) in (A) stable populations ($\lambda = 1.0$) with random, moderate and high variance in male reproductive success; and in (B) fluctuating populations with high VMRS.

A							
Population size (N_c)		Variation in male reproductive success					
		Random		Moderate		High	
250		5.6		6.2		7.1	
500		5.6		6.2		7.2	
1000		5.7		6.3		7.2	
2000		5.7		6.3		7.2	
3000		5.7		6.3		7.2	
B							
Growth rate	Average census size	High fluctuation (2000–4500)			Low fluctuation (3000–3500)		
		RND ^b	Juv ^c	Adult ^d	RND ^b	Juv ^c	Adult ^d
	$N_c = 3250$						
$\lambda = 1.02$	7.2	7.1	7.3	6.7	7.1	7.2	6.8
$\lambda = 1.08$	8.9	7.9	8.7	6.7	7.9	8.7	6.9
$\lambda = 1.12$	9.2	8.2	9.5	7.1	8.2	9.4	7.0

^a All standard errors <0.05.

^b Random culling among age classes.

^c Random culling among juvenile (age < 3) age classes.

^d Random culling among adult (age > 3) age classes.

culling only adults versus juveniles, respectively, and moderate population growth ($\lambda = 1.08$). Culling only juveniles similarly increased G compared to culling adults at all three population growth rates. The longest generation interval, $G = 9.5$, was achieved when culling juveniles at the highest population growth rate of $\lambda = 1.12$ (Table 3B).

4. Discussion

It is increasingly urgent for conservation biologists to precisely understand the relationship between N_c , N_e , and the conservation of genetic variation in natural populations. Biologists must consider AD along with N_e because N_e estimators (e.g., inbreeding and variance N_e) might not reliably predict the loss of AD over the short term or in fluctuating populations. Our study quantifies how the loss of AD changes with the initial number of alleles per locus in age structured and fluctuating populations; this quantification is difficult or impossible using analytical equations (Crow and Dennison, 1988; Luikart et al., 1998; Allendorf and Luikart, 2007).

Precise quantification of AD using realistic, stochastic population models is not possible without simulations because analytical equations do not exist for use in age structured populations with fluctuating size and a range of allele frequency distributions. Our results are informative regarding the N_e resulting from VMRS and fluctuating N_c that are common among ungulates. Yet the results are quite different from ideal or simplified population models, e.g., with no age structure, that are often used in conservation genetics (Fig. 2 – see the Wright–Fisher “W–F” model).

4.1. Effective size

The N_e for the contemporary Yellowstone bison population is likely greater than 750, given our simulations including stable population scenarios such as high VMRS with $N_c = 3250$ bison (Table 1) and with $N_c = 2000$ (Table S3.1), and fluctuating populations with culling of either random, juvenile, or adult individuals. This N_e is higher than some recommendations of maintaining $N_e > 500$ individuals to maintain genetic variation for adaptation to ongoing environmental change (Franklin, 1980; Allendorf and Ryman, 2002). Most of our N_e estimates varied from $N_e \approx 750$ bison for high VMRS to $N_e \approx 1200$ bison for moderate VMRS.

The N_e and genetic variation could potentially decrease more rapidly in continuously declining populations compared to stable or growing (positive λ) populations studied here. Thus, additional research is needed to assess N_e in declining populations that have a negative λ .

4.2. Allelic diversity

Over 200 years, a stable N_c of approximately 2000 bison is not likely to maintain 95% of initial AD , even with only moderate VMRS. However, maintenance of 95% of AD will likely be achieved over 200 years with a fluctuating population size that frequently increases to $N_c > 3500$ bison.

The conservation of AD was influenced more by the arithmetic mean of N_c in a fluctuating population (Fig. S2 in Online Supporting information) than by the lowest N_c reached for the culling levels we simulated (i.e., removing up to 37% of the population). This is unlike the case of N_e , which is strongly affected by the minimum N_c reached during fluctuations. This is 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 were culled (Cull. > 3; Table S3.1). This was likely caused by the reduction of the generation interval and reduction in the average N_c caused by removal of a large proportion of reproductive individuals (Table S3.1). It should be noticed that actual levels of AD could be even higher than those obtained in our simulations, given that we do not consider mutation. Furthermore, it was shown that selection could enhance genetic diversity in isolated ungulate populations (Kaeuffer et al., 2007b). The maintenance of AD is important from an adaptive perspective as, for example, the limit to selection response is determined by the initial number of alleles (Hill and Rasbash, 1986).

Yellowstone is the only remaining wild population of plains bison that currently meets the objective of maintaining a large population size with greater than 2000 individuals (White et al., 2011). Most North American populations have fewer than 400 bison because this species requires large conservation areas and modern society currently provides little space for wild bison outside nature preserves and national parks (Gates et al., 2010).

4.3. Influence of locus type

Microsatellites in Yellowstone bison have approximately five alleles per locus on average (Halbert, 2003; G. Luikart and F. Gardipee, unpublished data). Our simulations show that loci with higher initial AD (e.g., 20 alleles) have a much higher probability of loss of alleles over time (AD remaining is only 85% after 200 years). This is expected because many alleles occur at low frequency at such loci. Halbert (2003) noted that some bison populations had as many as 11 alleles at the microsatellite loci she studied. Immune system genes (e.g., MHC) often have 20 or more alleles per locus in many species.

4.4. Variance in male reproductive success

A polygamous mating system can reduce or eliminate the genetic contribution of many males and thereby increase VMRS which in turn can rapidly reduce genetic variation in a population (e.g., Kaeuffer et al., 2004). Due to the uncertainty of VMRS in a large population of wild bison, we tested a broad range of VMRS values. The most likely male mating scenario for Yellowstone bison is the moderate or high VMRS that we used for simulations. We used the high VMRS as a default input for simulations that evaluated fluctuating populations because it likely represents the worst case scenario for loss of variation. Thus, if scenarios using high VMRS result in satisfactory conservation of AD , then the

Yellowstone bison population should more likely meet conservation goals, or at least not be limited by the male reproductive strategy or dominance hierarchy.

Extreme VMRS could reduce N_e to <500 and contribute to poor conservation of H_e . However, extreme VMRS (e.g., where 1% of males father all offspring) seems unlikely to occur in large populations of bison where very few males are unlikely to dominate all breeding (Lott, 1979; Berger and Cunningham, 1994; Halbert et al., 2004). Extreme VMRS might only happen in small populations of some species where a few males could dominate the breeding of all females. The Texas State bison herd is a small population of 40–50 animals and 10% of males were estimated to produce 40% of the offspring (Halbert et al., 2004).

Maintenance of dominance status for 5 years could be an overestimate because the physical cost of maintaining dominance is energetically high and often involves fighting along with limited foraging during the rut (Lott, 1979). If dominance is actually maintained for less than 5 years the actual VMRS and the rate of loss of variation could be lower than reported here because it could reduce VMRS. Few or no studies have modelled a relatively realistic turnover of dominance status (e.g., up to 5 years) as we have done here.

4.5. Influence of culling on N_e

Unlike two previous simulation studies of moose (Ryman et al., 1981; Sæther et al., 2009), we found that the removal of only young bison did not reduce the N_e compared to the removal of only adults (Tables 1 and S2.2). This N_e difference between studies is not surprising given the difference between our simulations of bison and those for moose. First, the moose studies used more extreme age-specific culling than our study in that we culled both calves and juveniles whereas they culled only calves. Second, female moose with two calves had an increased survival rate because they were less likely to be culled (as hunters avoid killing females with calves), whereas our study did not alter adult survival based on fecundity. Third, moose obviously have different vital rates compared to bison (e.g., two calves per female is not possible in bison), and we showed that vital rate differences influence N_e (Table 1). Finally, for moose, N_e was computed assuming a constant size population, a projection matrix model (Sæther et al., 2009), and by using demographic information plugged into N_e -estimation equations, whereas our simulations computed N_e directly from the actual loss of heterozygosity over time (e.g., Harris and Allendorf, 1989).

4.6. Influence of culling on generation intervals

Culling of only juveniles gave the longest generation intervals (9.5 years), which translated into the highest maintenance of AD (up to 96.1%; Fig. 3) suggesting that juvenile removal would be relatively effective for maintaining genetic variation. The culling of only younger individuals preserves the older age cohorts and increases the generation interval which, in turn, slows the loss of variation over time. Our results suggest that such enhancement would occur in small as well as large herds (>2000 animals) and is more pronounced for conservation of AD than for H_e .

These results agree with those from Ryman et al. (1981) and Sæther et al. (2009) where computer simulations showed that hunting pressure on younger individuals increased the generation interval as well the maintenance of H_e over time.

5. Conclusions

Stochastic simulation modelling is an underappreciated approach to investigate strategies to conserve genetic variation for

nearly any species or population with age structure and complex demography, as illustrated here for bison. Conservation strategies to preserve genetic variation in Yellowstone bison will benefit from considering results from our simulations which included detailed demographic and molecular genetic data. Actual monitoring of genetic marker variation in Yellowstone bison (and other populations) would help test the accuracy of our estimated rates of loss of variation and N_e (Ludwig and Walters, 2002; Schwartz et al., 2007; Luikart et al., 2010). Some studies have commented on the importance of longitudinal genetic monitoring for genetic diversity in isolated populations of ungulates (Kaeuffer et al., 2007b; Ortego et al., 2011). The use of simulation modelling in these studies allowed an understanding of the mechanisms that regulate genetic diversity and provided a conservative estimate of the level of genetic diversity in the studied populations.

In our simulations, VMRS was the factor with the strongest influence on N_e and the loss of variation, when VMRS was high to extreme. Thus, future research could improve understanding of loss of variation by providing estimates of VMRS through paternity analyses in bison populations. 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 (heterozygosity) by perhaps 10–20% (Ryman et al., 1981).

The initial number of alleles per locus also had a strong influence on loss of AD (Fig. 2). This was expected (e.g., Allendorf, 1986) but never has been quantified for age structured and fluctuating populations with a realistic VMRS. The substantial difference in the AD maintained under simulation of an ideal Wright–Fisher population (93%) versus an age structured fluctuating population (83%) illustrates the importance of considering realistic simulation models. Future research also should collect empirical data from highly polymorphic loci (e.g., immune system genes) to improve understanding of the loss of AD at such loci and adaptive genes.

Our simulations are among 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., ungulates). Our simulations suggest that considering both N_e (the inbreeding effective size) and N_e are important for conserving H_e and AD , respectively. They also show that fluctuations in population size are unlikely to greatly accelerate the loss of genetic variation, at least for the relatively large N_e and positive population growth rates considered here. Finally, our simulations suggest that the conservation of high allelic diversity (>95%) at loci with many alleles (≥ 5) will require the maintenance of a populations size greater than ~ 3250 (mean N_e) and removal of mainly or only juveniles. This threshold value for population size could be even lower considering that the input of new alleles was not included in simulations (mutation and migration were neglected).

Acknowledgments

Funding for this work was provided by the National Park Service through the Rocky Mountain Cooperative Ecosystem Studies Unit at the University of Montana and by the Ministerio de Ciencia e Innovación (CGL2009-13278-C02), Xunta de Galicia and Fondos Feder (Grupos de Referencia Competitiva, 2010/80). A.P.-F. was supported by an Ángeles Alvariño fellowship from Xunta de Galicia (Spain). T.A. was supported by research grant SFRH/BD/30834/2006 from the Portuguese Science Foundation (FCT). G.L. was supported by grants from the Walton Family Foundation, CIBIO-UP, the US National Park Service, the Portuguese Science Foundation (FCT) grant PTDC/BIA-BDE/65625/2006, and the US. National Science Foundation Grants DEB 1067613 and DEB 074218. Helpful advice and comments were provided by A. Carvajal-Rodríguez, F. Gardipee, and three anonymous referees. We are especially

grateful for the helpful advice and extensive revisions suggested by Fred Allendorf, such as more a careful and extensive discussion of culling effects on N_e and G (generation interval).

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.biocon.2012.02.022.

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