**Conserving Genetic Diversity in Yellowstone Bison**

*Draft* Bison genetics research outline (not for distribution or citation)

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**Introduction:**

There is increasing concern that bison in Yellowstone National Park could lose genetic variation more rapidly than is acceptable to maintain current and long term fitness and adaptive potential. A major concern is that a large number of bison, 5 to 35%, are culled in most years, and this could rapidly reduce genetic variation. The culling will continue in the future in order to prevent spread of brucellosis to cattle, which could happen when bison migrate out of Yellowstone Park near cattle grazing areas (Interagency Bison Management Plan 2000). Genetic studies are needed to quantify rates of loss off variation, guide culling strategies, and set population size management goals in Yellowstone Park.

Evidence suggests that maintaining 1000 to 2000 bison in each of the two breeding groups (central and northern groups) should retain 95% of heterozygosity and allelic richness in Yellowstone bison over the next 200 years (Gross and Wang 2006). However, there is uncertainty in these numbers due to the possible existence of strong polygamy (dominant males), population genetic subdivision (i.e., distinct breeding groups), and inter-annual variation in population size due to recurrent, large-scale culls to reduce the risk of brucellosis transmission to cattle. Gross and Wang (2006) did not consider effects of polygamy, subdivision or extensive size fluctuations.

Evidence of genetic subdivision among Yellowstone bison was detected by Halbert (2003) and Gardipee (2007). Preliminary microsatellite data detected minor genetic differentiation between the two Yellowstone breeding areas, Hayden Valley and Lamar Valley ($F_{ST}$ = 0.01; Gardipee et al., unpublished data). This suggests there is relatively high gene flow occurring between the breeding areas in Yellowstone.

We will use individual-based computer simulations of bison populations to assess the potential effects of polygamy and brucellosis risk management culling on rates of loss of heterozygosity and allelic variation over the next 200 years. Individual-based computer simulations can be used to evaluate rates of loss of heterozygosity and to estimate effective population size in age structured populations (e.g., Harris and Allendorf 1989). To parameterize the simulation models, we will use Yellowstone bison age-specific birth and death rates where data exist, and other bison population data (Table 1) where necessary (Brodie 2008, Geremia et al. 2009, and Yellowstone National Park unpublished data).

The effective population size ($N_e$) is important in wildlife management because it influences the rate of loss of genetic variation, inbreeding (mating between relatives), fixation of deleterious alleles, and ability of a population to respond to selection (Leberg 2005). $N_e$ estimators also can provide the most sensitive molecular genetic metric for detecting genetic bottlenecks and sudden increased rates of loss of genetic variation (e.g. Luikart et al. 1999).

$N_e$ is usually much less than the population census size ($N_C$). $N_e$ is reduced below the $N_C$ by phenomena such as unequal sex ratio, variation in reproductive success among individuals, and fluctuations in $N_C$ through time. Most estimates of $N_e$ suggest that it is only about 10-40% of $N_C$ (Frankham 1995). Given the polygamous mating system of bison (in which few males mate with many females), the $N_e$ could be at the lower end of this range. For example, a few males could dominate breeding over a few years and greatly reduce the $N_e$, even if the $N_C$ remains large. Little is known about the degree of polygamy in bison, but computer simulations allow testing a range of polygamy to bracket degree of polygamy (and variance in male reproductive success) likely to occur.

To quantify our ability to monitor $N_e$ and to detect a potential reduction of $N_e$, we will use computer simulations of Yellowstone bison. We will quantify the bias and precision of $N_e$ estimators that use microsatellite DNA data, and will use allele frequencies similar to those in Yellowstone bison. Genetic marker-based Ne estimators generally provide the best estimates of effective population size and rates of loss of variation (Frankhan 1995, Luikart et al. 1999; Allendorf and Ryman 2002; Schwartz et al. 2007). Several computational methods have been recently developed to estimate $N_e$. from genetic marker data. However, they have not been thoroughly evaluated in age structured populations. We will evaluate the usefulness of recent estimators of $N_e$ that are based on linkage (gametic) disequilibrium (Waples and Do 2008, Tallmon et al. 2008). We also will attempt to evaluate the usefulness of an $N_e$ estimator based on temporal change in allele frequencies (Waples and Yokota 2007). These two methods (linkage disequilibrium and temporal variance method) are the two most promising methods for estimating $N_e$ and detecting reductions of $N_e$ in natural populations

**Research Questions:**
We have two main research questions. One reflects our need predict if Yellowstone bison are likely to lose more than 10% of genetic variation in the next 200 years. The second reflects our need to use DNA-based estimators of $N_e$ to detect an unacceptable reduction of effective population size (e.g., $N_e$ < 100 or 200), if such a reduction occurs. Our specific questions are as follows:

**1)** What percentage of the current heterozygosity & allelic diversity will be retained in Yellowstone bison over the next 200 years (~20 generations) given that fluctuations in population size due to culling will continue; Culling will likely lead to removals of 5% to 30% of individuals depending in population abundance and winter weather.

a. Do age structured, polygamy, population fluctuations, and subdivision (e.g., $F_{ST}$= 0.01) cause bison populations lose heterozygosity and allelic variation more rapidly than 10% in 200 years?

b. If extreme polygamy occurs, could Yellowstone bison lose > 10% of heterozygosity or 10% of allelic variation in 200 years? For simulations, we will consider a wide range of male reproductive success, ranging from random mating (Fisher-Wright population), polygamy (10% of males father 50% of offspring), to extreme polygamy where only ~10% of adult males father all offspring (e.g., are dominant) each year for five consecutive years (low turnover of dominance).

c. If extreme population size fluctuations occur (e.g., from the combined effects of natural mortality and management removals) could Yellowstone bison lose > 10% of heterozygosity and allelic variation in 200 years? We will consider realistic population growth rates (lambda) ranging from 1.05 and 1.20. We will simulate the following fluctuations caused by culling:

- from ~4,000 animals to ~2000 animals culled each time the population size reaches 4,500 individuals
- from 4,000 to 3,000 culled every time the population size reaches 4,000 individuals
- to cull the population to 3,000 every year the population reaches >3,000 individuals
- to cull the population to 2,000 every year the population reaches >3,500 individuals

d. By how much can culling of only young individuals (0-to-3 year olds) slow the loss of variation (by maximizing the generation length interval, e.g. from approximately 10 years to12 or 14 years)?

e. Does targeted culling of one subpopulation influence rate of loss of variation (given Fst of 0.01 or 0.10)?

f. How much does the rate of loss of allelic diversity depend on the number of alleles per locus? Do bi-allelic loci lose variation significantly slower than loci with 5, or loci with 10 alleles per locus?

g. If the population unexpectedly declined to only 1,000 animals (and stayed at 1,000), would the population lose > 10% of heterozygosity or 10% of allelic variation in 200 years (e.g. if the decline occurred after generation 1 (at year 10)?

**2)** How can genetic monitoring be applied to provide for early detection of a severe reduction in $N_e$?

a. Can a reduction of $N_e$ to approximately $N_e$ = 100 be detected after approximately 10 years or 5 years following the reduction? (Note: An $N_e$ of 100 leads to approximately a loss of 5% of heterozygosity in 100 years, assuming a generation length of 10 years). We will mainly evaluate the power and accuracy of the LD-$N_e$ estimator of effective size, because its power is promising (Luikart et al., unpublished data) and its performance is poorly evaluated.

b. What is the effect of age structure on bias and precision of the LD-$N_e$ estimator of $N_e$?

c. Time permitting, we will compare the power and accuracy of the LD-$N_e$ method to the standard temporal variance (F-$N_e$) method (e.g., Waples 1989; Luikart et al. 1999), and the temporal variance methods developed for age-structured populations (Ryman & Jorde 2007).

**Methods (NEEDS REVISION)**

Both questions above will be addressed by simulating bison populations in SimuPop (Peng and Kimmal 2005) and in Pedigog (Combes et al. in press) followed by analyses of output in programs Genepop, LD-Ne and programs we are developing.
An initial description of how these programs work and what type of outputs they can provide to those making recommendations for conservation.

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***Question one: What percent of variation will be maintained***

We will simulate bison populations using available data on age structure (Table 1) and variance in male reproductive success. We will compute the Ne/Nc ratio and estimate rates of loss of heterozygosity and allelic diversity while simulating a stable bison population of abundance at 2,000 and at 3,500 individuals (these simulations require annual removal to maintain population abundance at a relatively static level). We will use these baseline a the genetic effects of more random removals that occur because migration to the low elevation winter ranges is correlated with population density and winter weather affects (Geremia et al 2009). We will also calculate Ne/Nc ratio and estimates of loss in heterozygosity and allelic diversity for some more realistic scenarios of removal (we will simulate removal of only 0 to 3 year olds vs. only adults, versus random removals to test the effects on generation length, Ne, and rates of loss of variation.

**Comment [W1]:** I think it would be more meaning ful to make the adult removal scenario simulate the removal of seropositive animals that would in effect be a non-random removal since a higher percentage of older animals test positive than would younger animals. I am sure it makes the code writing much more complicated but it would make the model much more informative for management / conservation purposes.

We will simulate male reproductive success with approximately 10% of males fathering 50% of the offspring, and with annual random turnover of dominance status. For extreme polygamy, we will have 10% of males fathering approximately 50% of the offspring but these males will remain dominant (and father 50% of offspring) for 5 consecutive years before losing their dominance status.

*H*e & AD (percent alleles remaining) will be monitored for the entire population at **100 loci** (50 msats [H≈0.60] and 50 SNPs [H≈0.30] ). *H*e will be converted into an Ne estimate using standard equations (as in Harris and Allendorf 1989). Annual outputs of age structure (Figure 1) and heterozygosity remaining, allelic diversity remaining, and the Ne-estimates will be plotted through time (Fig. 2).

***Question two: Can genetic monitoring detect reduced Ne***

We will monitor He, A, and compute Ne using recent Ne-estimators, for the simulated populations above (in Questions 1) to evaluate potential power to detect changes in Ne using on the ground monitoring over the next 30 years. Sensitive genetic monitoring methods are badly needed for bison and other species to detect when a population is likely to lose excessive genetic variation. Ne estimators are likely to provide most sensitive indicator for detection of a genetic bottleneck of an increased rate of loss of variation (Luikart et al. 1999; Unpublished data).
Ne will be estimated from heterozygosity-loss, LD-Ne, the standard temporal method (Waples 1989) and perhaps the Ne-temporal method for age structured populations (Jorde & Ryman 2007).

The 'true Ne' will be computed from the total population sample of individuals and loci for each of 100 simulation replications for each scenario. The true Ne will be computed from the heterozygosity-loss method (inbreeding Ne), the standard temporal method (variance Ne), and the Felsenstein (1971) method accounting for age structure in populations.

To assess the effects of sample size on accuracy and precision of the LD-Ne estimator a random sample of 100, 50, and 25 individuals will be collected every year for the first 10 years, and then every 3 years (~ 0.3 bison generations). In addition, every 3 years, we will sample 25, 50, and 100 from calves and 1-year olds to evaluate the accuracy and precision of LD-Ne for estimating Nb and Ne. We could also use this cohort sampling to evaluate the temporal method for age structured pops of Jorde & Ryman (2007). This sampling will allow us to evaluate the power (and accuracy) of the different methods for detecting serious reduction of Ne.

To assess the effects of number of loci on accuracy and precision of the LD-Ne estimator, we will compare estimates from 10, 20, and 40 msats; and then 20, 40, 80 SNPs.

Project Summary:

1) We will produce a computer model that simulates an idealized population (i.e., no overlapping generations, no substructure, equal contributions to reproduction) and evaluates the potential effects of management removals (similar to those directed by the Interagency Bison Management Plan) on genetic diversity over 100-200 years.
2) We will produce an alternate computer modeling that simulates the Yellowstone bison population using the best available estimates of demography, genetic diversity, age structure, population substructure, and polygamy for Yellowstone bison breeding groups

and includes management removals similar to those experienced since 1985, to evaluate potential effects on genetic diversity over 200 years.

3) We will conduct a comparison of results from the idealized and realistic population models to test the effects of overlapping generations (age structure), population substructure, polygamy (unequal contribution of gender and individuals to the next generation of bison), fluctuating population abundance and management removal strategies on rates of loss of genetic diversity (heterozygosity and allelic diversity).
4) An objective assessment based on modeling and other information of the abundance per primary breeding herd needed to preserve 90 and 95% of current level of genetic diversity values. This assessment will assume continued fluctuations in breeding herd and population abundance.
5) Recommendations regarding genetic monitoring (e.g., Ne estimation & statistical methods, samples, timing, locations) to ensure Yellowstone would have the statistical power to detect a genetically significant change in diversity over time.

The questions identified above would be the focal subject of one or two separate publications (e.g., one pub for each of the two main questions above).

Table 1. Age structure of the Yellowstone bison population from summer ground classification counts (unpublished data).

| Year | Range | Adult Bull | 2/3 Bulls | Yearling Bulls | Adult Cow | 2/3 Cows | Yearling Cows | Calves | Unknown | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| 2004 | Central | 23 | 6.1 | 6 | 34.5 | 5.3 | 5.1 | 17.7 | 2.3 | 100 |
| 2005 | Central | 26.6 | 5.5 | 6.5 | 35 | 4.4 | 5.6 | 15.9 | 0.5 | 100 |
| 2006 | Central | 20.9 | 7.2 | 8.2 | 34.9 | 5.2 | 6.1 | 16 | 1.5 | 100 |
| 2007 | Central | 25.6 | 5.8 | 5.7 | 35.7 | 4.2 | 5.6 | 16.9 | 0.5 | 100 |
| 2008 | Central | 35.8 | 4.0 | 3.7 | 36.9 | 3.3 | 5.0 | 11.1 | 0.2 | 100.0 |
| | | | | | | | | | | |
| **Mean** | **Central** | **26.4** | **5.7** | **6.0** | **35.4** | **4.5** | **5.5** | **15.5** | **1.0** | **100.0** |
| | | | | | | | | | | |
| 2004 | Northern | 23.1 | 6.7 | 3.7 | 37.5 | 5 | 4.4 | 16.5 | 3.5 | 100.4 |
| 2005 | Northern | 24.2 | 7.5 | 5.9 | 36.4 | 4.4 | 4.7 | 13.3 | 3.7 | 100.1 |
| 2006 | Northern | 18.8 | 7.3 | 5.6 | 37 | 5.2 | 6.3 | 19.1 | 0.7 | 100 |
| 2007 | Northern | 15.8 | 5.9 | 7.1 | 38.5 | 4.8 | 5.5 | 21.7 | 0.6 | 99.9 |
| 2008 | Northern | 15.4 | 7.2 | 8.3 | 35.8 | 5.4 | 5.8 | 22.1 | 0.0 | 100.0 |
| | | | | | | | | | | |
| **Mean** | **Northern** | **19.5** | **6.9** | **6.1** | **37.0** | **5.0** | **5.3** | **18.5** | **1.7** | **100.1** |

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