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RH: Population substructure in Yellowstone bison • Wallen et al.

Population substructure in Yellowstone bison assessed using genetic and demographic approaches

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ABSTRACT Population substructure and gene flow can affect overall genetic diversity, which is necessary for wildlife to adapt to environmental change. Yellowstone bison are an important genetic resource for restoration of the species (*Bison bison*) because they exhibit high diversity and have no evidence of hybridization with cattle. We used fecal DNA collected during breeding seasons in 2005-2008 to evaluate genetic diversity and substructure among apparent breeding groups. Mitochondrial DNA analyses revealed two haplotypes, with higher frequency of haplotype 8 in the northern breeding herd, and significant genetic differentiation between the two breeding areas: the northern and central herds ($F_{ST} = 0.40$). Microsatellite analyses revealed allele frequencies with low levels of subdivision between the central and northern breeding herds ($F_{ST} = 0.02$ in 2006 and 0.01 in 2008). The population has two genetically similar breeding groups distinguishable by mtDNA caused by strong female philopatry and relatively high male-mediated gene flow. Radio-marked adult females provided evidence of female fidelity, but emigration between breeding groups was substantial during 2007-2011. We recommend long-term monitoring of microsatellite allele and mitochondrial haplotype frequencies to track genetic diversity and population substructure. We expect F_{ST} values to fluctuate as the population responds to the bison density in the two breeding herds, management actions (e.g., culling), and natural selection.

KEY WORDS bison, conservation, genetic diversity, gene flow, female philopatry, population connectivity subdivision, telemetry, management, Yellowstone.

Maintaining genetic diversity is necessary for wildlife species to avoid inbreeding depression and adapt to changing environments (Frankham 1996, Allendorf and Luikart 2007). The rate of adaptation in response to environmental variables such as climate, diseases, and anthropogenic influences is generally correlated with the amount of genetic diversity among individuals in a population (Mills 2007). Harvesting wildlife populations may increase the rate of loss of genetic diversity and result in reduced evolutionary potential (Allendorf et al. 2008). Conversely, harvesting may not result in a significant loss of genetic diversity if movements occur among subpopulations, population abundance is high, and harvests are random (Allendorf et al. 2008).

During the 19th century, tens of millions of plains bison (*Bison bison*) ranged across North America from the Arctic Circle to Mexico and from Oregon to New Jersey (Hall and Kelson 1959, Dary 1974, Isenberg 2000). The species is an essential component of native grassland system ecology and North American tribal cultures (McHugh 1972). However, >95% of contemporary bison are managed like livestock on private ranches and conservation preserves. About 5% are managed as wildlife, but only 2% are managed as free-ranging populations subject to many natural evolutionary processes (Freese et al. 2007, Hedrick 2009, Gates et al. 2010).

Yellowstone bison occupy the headwaters of the Yellowstone and Madison River watersheds (USDI and USDA 2000, Plumb et al. 2009). Their distribution is constrained by a court-mediated agreement to a conservation area that includes Yellowstone National Park and limited public lands outside the park in Montana (Figure 1). Bison that migrate to lower elevations during winter gain access to food resources that are more readily available due to lower snow depths (Bjornlie and Garrott 2001, Gates et al. 2005). However, the Yellowstone bison population has been chronically exposed to the disease brucellosis (*Brucella abortus*) and

70 management operations to prevent co-mingling of bison with cattle outside the park
71 disproportionately remove migratory individuals (Plumb et al. 2009).
72 Yellowstone bison are a valuable conservation population because they represent the only
73 population to continuously occupy portions of their current distribution, are one of a few bison
74 populations that interact with the full suite of native ungulates and predators in a harsh
75 wilderness environment, and have shown no evidence of hybridization with cattle (Meagher
76 1973, Halbert and Derr 2007, Plumb et al. 2009). The population dwindled to 25-50 individuals
77 during 1850-1902. Subsequently, the population was restored through husbandry, protection, and
78 translocation and, today, more than 3,500 bison in two breeding herds (central, northern) are an
79 integral part of the Yellowstone ecosystem (Gates et al. 2005, White et al. 2011a).
80 The conservation of Yellowstone bison is controversial because they are chronically infected
81 with brucellosis (*Brucella abortus*) and management actions to limit the risk of transmission to
82 cattle outside Yellowstone National Park in Montana have resulted in the killing of >6,000 bison
83 since 1985 (Keiter 1997, Cheville et al. 1998, Bienen and Tabor 2006, White et al. 2011a).
84 Culling the population in large numbers could affect the substructure of the population if the
85 removal is non random among and within the breeding groups. While conservation
86 constituencies argue for greater tolerance of Yellowstone bison in Montana, agricultural interests
87 argue for constraining their distribution within the boundary of the park until brucellosis is
88 eliminated (Plumb et al. 2009). There is also litigation regarding whether the Yellowstone bison
89 population has significant genetic substructure that warrants separate management for different
90 breeding herds (Figure 1; Animal Welfare Institute 2008, Western Watershed Institute et al. vs.
91 Salazar et al. 2010).

92 Yellowstone bison have high genetic variation relative to other North American bison
93 populations (Wilson and Strobeck 1999, Halbert and Derr 2008) and currently subdivide into two
94 apparent breeding herds during the rut. The northern herd congregates in the Lamar Valley and
95 on adjacent plateaus for the breeding season during July 15 through August 15 (Figure 1).
96 During the remainder of the year, these bison use habitats in the Yellowstone River drainage,
97 which extends 80 kilometers between the upper Lamar and the Paradise Valley north of
98 Gardiner, Montana. The central herd occupies the central plateau of Yellowstone National Park,
99 extending from the Pelican and Hayden valleys in the east to the lower elevation and thermally
100 influenced Firehole Valley and Madison headwaters area in the west. Bison from the central
101 herd congregate in the Hayden Valley for breeding. Most of these bison move between the
102 Madison, Firehole, Hayden, and Pelican valleys during the rest of the year. However, some
103 bison travel to the northern portion of the park and mix with the northern herd before returning to
104 the Hayden Valley for the subsequent breeding season (Geremia et al. 2011).
105 Halbert et al. (2012) reported evidence of genetic population substructure (2 genetically
106 distinct groups exhibiting F_{ST} = 0.03) in Yellowstone bison from DNA collected
107 opportunistically when bison were culled on winter ranges outside the boundaries of
108 Yellowstone National Park in Montana during 1999 through 2003.They surmised this
109 substructure was a result of genetic drift evolving naturally over eight bison generations (1936 to
110 2000). However, their samples were not collected on the breeding ranges where reproduction
111 occurs. Sampling on the breeding range is difficult and risky because no capture facilities are
112 located in or near the breeding ranges and chemical immobilization for sample collection is risky
113 when groups of bison are congregated in large numbers during the breeding season. Thus, a non-

114 invasive sampling approach is necessary to evaluate genetic differences among the breeding
115 groups.
116 The objectives of this study were to (1) develop a non-invasive fecal DNA sampling
117 approach for wild bison, (2) quantify the magnitude of genetic population substructure between
118 the two Yellowstone bison breeding groups, and (3) compare genetic data with observational
119 data (via radio-marked individuals) to assess connectivity between subpopulations (i.e. breeding
120 hers).
121
122 **METHODS**
123 **Sample collection and DNA preparation**
124 We collected 59 fecal samples from bison in the central breeding herd during July 2005 and
125 152 fecal samples from bison in both the central and northern breeding herds during August and
126 September in 2006 and 2008. Samples (~4 grams) were collected within 30 minutes of
127 defecation, mixed with 20 ml of 95% ethanol, and stored in coolers for up to 8 hours before
128 being stored at -20º C for up to 1 year prior to DNA extraction. Each sample was assigned a
129 relative age class determined through field observations of bison horn length and width, body
130 size, and body condition. We sampled different sexes and ages, but limited our sampling to less
131 than 20% of individuals within each observed grouping of bison and moved among
132 congregations within each breeding range to avoid re-sampling individuals.
133 We extracted DNA in a laboratory designated for non-invasive studies located in a separate
134 building from where PCR was conducted. We used the QIAamp© Stool Mini Kit (QIAGEN)
135 according to manufacturer's protocol with the following modifications. We thoroughly mixed
136 fecal material from each sample to ensure homogeneous distribution of cellular material. We

transferred ~1.5 ml of fecal suspension into 2 ml microtubes which were centrifuged at 16,100 relative centrifugal force for 5 minutes. As much ethanol as possible was drawn off, leaving behind approximately 200 µl of feces, which was then used in the QIAamp© Stool Mini Kit extraction protocol until the final step, which was repeated to harvest as much DNA as possible (Gardipee 2007).

Mitochondrial DNA

Previous mtDNA sequencing studies revealed eight distinct haplotypes from samples collected across the species distribution of *Bison bison* (Ward et al. 1999, Vogel et al. 2007). We used primers BISCR-16348F 5'-CTACAGTCTCACCGTCAACCC-3' and BISCR-16990R 5'-GATGAGATGGCCCTGAAGAA-3' (Shapiro et al. 2004, Vogel et al. 2007) to amplify a 470 bp segment of the bison mtDNA control region. PCR was carried out in 25 µl volumes containing 8.95 µl sterile HPLC H_2O, 2.5 µl Invitrogen©10X PCR buffer, 1 µl dNTP's, 0.5 µl of each primer, 2.5 µl BSA (2 ng/µl), 1.25 µl MgCl (50 mM), 0.3 µl Invitrogen© Platinum *Taq* Polymerase (5 units/µl), and 7.5 µl of the DNA extract. Amplification was performed in an MJ Research PTC-200 Peltier Thermal Cycler using the following touchdown protocol: 94 C for 5 min, followed by one cycle of 94 C for 30 s, 60 C for 30 s, and 72 C for 30 s. For the subsequent 10 cycles, all conditions remained the same except that the annealing temperature decreased by 0.5 degrees per cycle. This was followed by 25 cycles of 94 C for 30 s, 55 C for 30 s, and 72 C for 30 s. The profile concluded with a single extension of 72 C for 5 min.

Prior to sequencing, we purified PCR products using QIAquick© purification columns according to manufacturers' instructions with 1 exception; the final elution was carried out with 20 µl of Buffer EB instead of the recommended 30-50 µl to compensate for potential low

quantity template DNA. Negative extraction and PCR controls were used to monitor for possible contamination. No evidence of contamination was detected. We analyzed 70 samples with restriction enzyme analysis of PCR products using *Ssp1*, which cuts haplotype 8 into two fragments (372 bp and 98 bp), but does not cut haplotype 6. Restriction digests were conducted following manufacturer's instructions (Invitrogen). Digested products were run out on 2% agarose gels for two hours, stained with ethidium bromide solution, and visualized using a Hitachi FMBIOII scanner. The combined results of sequencing and RFLP analysis were used to determine the frequency of haplotypes among bison sampled on different breeding ranges.

Microsatellites

Waples and Gaggiotti (2006) suggest that genetic structure may be detected even when migration rates are high if an adequate number of highly variable markers are used (e.g. 10-20 microsatellite loci). We genotyped 17 microsatellite loci, which represent a subset of the 54 markers previously used by Halbert (2003). We chose loci with relatively short allele lengths ($\leq$200 base pairs), high variability in Yellowstone bison, and high amplification success in multiplex PCR using fecal DNA samples.

We amplified the 17 microsatellite loci within five different multiplex PCR profiles. Fragment analysis was carried out on an ABI 3130*xl* using the GS-600 LIZ size standard and GeneMapper v3.7 software was used to size fragments and identify alleles for each locus. Peaks less than 50 relative fluorescent units were not scored. Genotypes for each locus from all electropherograms were independently scored by two individuals. Each sample was repeat genotyped a minimum of four to eight times for all loci. Consensus genotypes for each sample were determined for each locus using the following criteria: a heterozygote genotype had to be

observed at least twice to be accepted as a heteryzygote, and a homozygote had to be observed at least three times among replicate PCR's.

Data analysis

Amplification success for each locus was calculated by the proportion of PCR amplifications that resulted in a scoreable genotype. Allelic dropout was inferred when a homozygous genotype was scored for a locus that was heterozygous according to the consensus genotype produced. Allelic dropout rate was computed as the proportion of all genotypes among loci and individuals with a dropout. False alleles were inferred when a distinct third allele was observed within a PCR compared to other replicate PCR's for each sample. GenAlEx 6.3 (Peakall and Smouse 2006) was used to estimate allele frequencies, number of alleles, observed heterozygosity, expected heterozygosity, F-statistics, and number of emigrants between breeding herds.

Dispersal (or emigration) is defined as movement from one spatial unit to another, without return (at least in the short term; Stenseth and Lidicker 1992). We used ARLEQUIN v.3.1 (Excoffier et al. 2005) and GENEPOP v. 1.2 (Raymond and Rousset 1995, Rousset 2008) to estimate F_{ST} between Yellowstone bison in the central and northern breeding herds, test for Hardy-Weinberg proportions and gametic disequilibrium. Allelic richness, a measure of allelic diversity that accounts for sample size, was estimated using FSTAT (Goudet 2002). We used the Wilcoxon signed ranks test and the sign test in SPSS v. 16.0 to test for significant differences in allelic richness between our results and Halbert (2003).

Population assignment tests were conducted using GenAlEx 6.3 (Peakall and Smouse 2006) and Arlequin v.3.1 (Excoffier et al. 2005). GenAlEx uses the frequency-based assignment test

206 based on methods from Paetkau et al. (1995 and 2004). The expected genotype frequency at
207 each locus is calculated for each sample, assuming random mating within each population,
208 multiplied across loci and log-transformed to give a log likelihood value (Peakall and Smouse
209 2006). For each individual multilocus genotype, a log likelihood of each individual originating
210 in each population is calculated using the allele frequencies of the respective population.
211 Arlequin determines the log-likelihood of each individual multi-locus genotype in each
212 population sample, while assuming that the individual originated from that population (Excoffier
213 et al. 2005). The allele frequencies estimated in each sample from the original composition of
214 the samples are used for computing the likelihood. All loci are assumed to be independent, such
215 that the global individual likelihood is obtained as the product of the likelihood at each locus
216 (Excoffier et al. 2005). We used GenAlEx to compute the log likelihood of each individual
217 originating from each subpopulation using the leave one out procedure in which each individual
218 is removed when computing allele frequencies and log likelihoods.
219
220 **Radio collars**
221 During 2002-2010, 108 adult female bison were fit with telemetry collars as a part of
222 ongoing monitoring actions to track population demographic and disease prevalence rates
223 (Bruggeman et al. 2007, Geremia et al. 2009). Bison were chemically immobilized during the
224 autumn and early winter months following standard capture procedures and released (Kreeger et
225 al. 2002, Gannon et al. 2007). We captured and collared bison primarily during October-
226 December of each year, but a limited number of bison were fitted with collars during January-
227 May (Wallen and Blanton 2007, White et al. 2011b). Bison were generally relocated monthly,
228 but at least once every three months using telemetry homing techniques (White and Garrott

1990). To assess movement and connectivity between breeding groups, we recorded annual movement patterns of individual bison.

RESULTS

Mitochondrial DNA

The mtDNA analyses revealed two haplotypes among the 151 bison tested during 2005 and 2006. These haplotypes differed by four nucleotide sites and matched published haplotypes 6 and 8 (Ward et al. 1999, Gardipee 2007). Haplotype 6 was the most common, while haplotype 8 was found only in 34 bison, most of which were from the northern breeding herd (Table 1). Haplotype frequencies in the central breeding herd appeared stable and F_{ST} was not significantly different from zero between 2005 and 2006 (Fst = xx; $P > 0.1$?). Therefore, we pooled both years of samples from the central herd for comparisons of F_{ST} with the northern herd (Table 1). Strong and highly significant genetic differentiation was evident between the northern and central breeding herds due to the disproportionate frequency of haplotype 8 in the northern breeding herd ($F_{ST} = 0.402$, $P < 0.001$).

Microsatellites

Amplification success for each locus was high (range = 0.93-1.00) and averaged 0.95 for samples collected in 2006 and 0.99 for 2008 samples (Appendix A). Allelic dropout was the main source of genotyping error, while few false alleles were observed. The average allelic dropout for 2006 samples (0.11) was higher than observed in 2008 samples (0.04). The error rate for false alleles ranged from 0.00-0.02 for both years. Fifty-three samples with the highest genotyping error rates were excluded from further analysis, to help ensure high quality data.

No pair on the X chromosome and thus we dropped if from some analyses. Three private alleles were observed in bison from the northern herd, and no private alleles were observed in the central herd (Appendix C). The calculated number of migrants per generation between the two breeding herds ranged from 11.1 in 2006 to 20.6 in 2008.

Mean and range of heterozygosity among loci was xxx, and xxx, respectively.

There was no significant difference in the expected heterozygosity between years for the central herd (0.60 vs. 0.65) or the northern herd (0.61 vs. 0.62).

We documented relatively weak population subdivision in our nuclear microsatellite data. F_{ST} between breeding herds ranged from 0.012 (2008) to 0.022 (2006) using all 17 markers. We identified four loci with relatively high inbreeding coefficient (F_{IS}) (Appendix B). Removal of these four loci individually from estimates of F_{ST} reduced our genetic differentiation results by less than one percent (Table 2). These data suggest a significant deviation from a panmictic population (P <0.01 in all estimates) and distinguishable differences between the two breeding groups.

Analysis of microsatellite loci revealed significant, but much lower, population subdivision between bison sampled from the central and northern breeding herds. All 17 loci were polymorphic within each breeding herd (range = 2-6 alleles per locusPopulation assignment tests provided evidence that the two breeding groups are genetically distinguishable and that dispersal is apparent. Arlequin assigned five(?)samples as dispersers (not originating from the range of collection. GenAlEx assigned the same five (?) individuals (P< 0.01 for each assignment). Seven to 22 percent of northern range samples assigned to central range populations (P < 0.01 for each assignment) while 0 to 15 percent of central range samples assigned to the northern range (Table 3).

Radio collars

During 2002-2011, 108 radio-collared females were monitored to track which ranges they occupied during the breeding season and where they migrated during winter. Since 2007, 11 of these bison dispersed from the central breeding herd to the northern breeding herd, while four

bison dispersed from the northern herd to the central herd. Of the 15 dispersing females, four moved back and forth between breeding ranges among years. Thirteen of the dispersing bison produced calves on their new range (e.g., northern) that were conceived on the range they left (e.g., central), while 25 calves were produced by these dispersing bison through mating and calving on their new range. Ninety-three percent of these migrants successfully reared at least one calf with the breeding herd to which they dispersed (i.e., representing effective gene flow). One female produced four calves, while two females produced three calves. Eleven of these emigrants were still alive in 2012.

DISCUSSION

The history of restoring Yellowstone bison to a wild, free-roaming population is complex. After Congress passed the Lacy Act in 1896 to provide the Army with legal measures to protect the remaining two dozen wild bison in the central portion of Yellowstone National Park, a long and intensive restoration program was initiated. The introduction of 21 bison from two populations of unrelated breeding descent in Montana and Texas brought together divergent genetic stock at the turn of the 20th century in northern Yellowstone (Cahalane 1944). Also, several individuals were gathered from the endemic herd in the Pelican Valley of central Yellowstone and introduced into the northern herd during the early population growth period. After 12 years of restoration efforts and many males escaping from the introduced herd, it was thought that the two groups began to mingle and interbreed (Cahalane 1944).

Until the mid-1960s, park management practices limited natural restoration of a wild bison population exhibiting their ecological role in the system (Meagher 1973). Through 1938, park staff annually rounded up the northern herd at the Lamar Buffalo Ranch to cull and feed the

306 bison during winter. In 1936, 71 bison from the northern herd were re-located to vacant ranges
307 in the Firehole and Hayden valleys of central Yellowstone (Cahalane 1944). Subsequently, the
308 northern herd was gradually released from traditional animal husbandry practices and allowed to
309 evolve some natural patterns of distribution and abundance by 1952 (Meagher 1973). However,
310 periodic culling of bison in the Lamar, Firehole, and Pelican valleys was conducted to manage
311 population abundance and eliminate brucellosis reactors until 1967 (Meagher 1973). In 1968, a
312 policy shift towards the preservation of ecosystem processes and the natural regulation of
313 ungulates released the bison population from artificial culling, and the restoration of grizzly
314 bears and wolves in the system provided opportunity for Yellowstone bison to further restore
315 themselves as a wild free-roaming population subject to the evolutionary processes of
316 competition, predation, weather, and social interaction (Cole 1971). Total population abundance
317 increased to approximately 5,000 bison in 2005 and has fluctuated between 2,000 and 5,000 for
318 the past 30 years (Plumb et al. 2009). Thus, the population subdivision and genetic
319 differentiation exhibited by Yellowstone bison was likely initially created in large part by an
320 active restoration program and intensive management of the population.
321 Halbert et al. (2012) suggested that genetic differences between the central and northern
322 herds was due to the combined effects of maintained population substructure, genetic drift, and
323 effective population size, and that non-random culling of the population could lead to reduced
324 genetic diversity and distinctiveness among the two groups. While genetic drift may have
325 affected the genetic diversity observed between the two breeding groups, intensive management
326 practices may have significantly affected the differentiation currently observed through the
327 deliberate establishment of a second bison breeding group (northern herd) from animals that
328 were translocated from other herds in Montana and Texas with different genetic lineagesBison

that wintered in the Pelican and Hayden valleys began intermingling by the 1950s (Gates et al. 2005), which most likely influenced the genetic diversity of the central herd. Thus, the current population structure of Yellowstone bison is likely a result of the differential influence the endemic gene pool provided to the introduced gene pool on two separate breeding areas. Today, bison breeding activities are concentrated in the Hayden (central) and Lamar (northern) valleys, with migration to lower elevation ranges as winter progresses (Geremia et al. 2011). Thus, the relatively low differentiation (F_{ST} <0.04) reported by Halbert et al. (2012) and in our study, has changed slightly in the one generation between our respective sampling, and represents small differences in allele frequencies.

mtDNA

Our mtDNA results suggest genetic differences between the central and northern breeding herds of Yellowstone bison are most likely due to female philopatry. Philopatric behavior by females is common in many species of wildlife. Waterfowl including trumpeter swans (*Cygnus buccinators*), Canada geese (*Branta canadensis*), wood ducks (*Aix sponsa)*, common eider (*Somateria mollissima*), canvasbacks (*Aythyava lisineria*), mallards (*Anasplaty rhynchos*), and harlequin ducks (*Histrionicus histrionicus*) tend to migrate back to the natal breeding grounds of the female (Hepp et al. 1987, Anderson et al. 1992). Mammals, including ungulates, also exhibit a high degree of female philopatry especially among reindeer (*Rangifer tarandus*), moose (*Alces alces*), pronghorn (*Antilocapra americana*), and bighorn (*Ovis canadensis*) (Ryman et al. 1980, Côté et al. 2002, Coltman et al. 2003, White et al. 2007). Theory predicts that if females grow up to be philopatric mothers, males should disperse more to avoid inbreeding with their mothers and any other philopatric female relatives. Wolff (1994) noted no evidence that juvenile

dispersal resulted from resource competition or parental aggression. In contrast, he observed that juvenile dispersal appeared to be voluntary and associated with the presence of the opposite-sex parent in the natal area. Consequently, our results support that these ecological processes (female philopatry and male dispersal) occur in Yellowstone bison and benefit the population by protecting genetic diversity and limiting the potential for breeding among related individuals.

Microsatellites

Need to discuss briefly the error rate (why are we sure it is low enough to have no effect on our results? Need to discuss the low Fst. Why is it so far lower than mtDNA Fst?

Few movement studies have been conducted to describe the differences in dispersal or migratory movements between bison genders. However, it has been observed that adult bull bison move about the system more widely and will pioneer new habitats prior to female led groups occupying these same locations (Meagher 1989, Taper et al. 2000, Gates et al. 2005). This kind of dispersal behavior allows adult males the opportunity to find suitable unrelated mates and facilitates access to new resources when population densities limit the acquisition of food. This behavior has been reported for other ungulates such as white-tailed deer (*Odocoileus virginianus*) and moose (Ryman et al. 1980, Nelson and Mech 1984). Taper et al. (2000) described how wide-ranging movements by males led to range expansion by Yellowstone bison when individuals began using habitats along the west boundary of the park in the 1990s. This type of exploratory behavior can lead to more regular migration and ultimately emigration if individuals decide that resources are more available in new areas.

Radio-collars

Movements by radio-collared females revealed that, on average, 28 percent of females from the central herd migrated to northern Yellowstone during winter (Geremia et al. 2011). Several of these individuals began simply by migrating to the north during winter and eventually stayed after a number of years exhibiting migratory tendencies. Our low microsatellite F_{ST} values suggest high gene flow between the two breeding herds has occurred over many generations and is likely facilitated by males moving between herds. However, our observations of radio-marked female bison exhibit clear evidence that in the most recent generation female groups are emigrating between the ranges in substantial numbers and contributing to changes in allele frequencies such that differentiation between the breeding groups is likely decreasing. Fuller et al. (2009) suggested that a significant amount of emigration has likely occurred between the two breeding ranges since 1981. Demographic studies revealed that the northern herd grew in abundance during 1981-2000 while sustaining a rate of culling that equaled the growth rate of the group. With emigration between the two breeding groups clearly evident in the population, a low genetic differentiation value should be expected and our microsatellite analysis supports this process.

Most species and populations are not genetically uniform, but have genetic differentiation and spatial substructure of the population (Manel et al. 2003). A population with an intermediate level of subdivision in which individuals have a reasonable probability of migration among all subpopulations is likely the best opportunity for conserving genetic diversity and minimizing the probability of extinction. This type of scenario provides for local adaptation where a progression toward panmixia would not be expected.

Brucellosis

399 Brucellosis risk management actions resulted in the removal of nearly 3,700 Yellowstone
400 bison from the population during 2001-2009 (Government Accountability Office 2008, White et
401 al. 2011). The culling of wildlife populations can lead to three types of genetic change:
402 alteration of population subdivision; loss of genetic diversity; and selective genetic changes
403 (Allendorf et al. 2008). Our results characterize the level of population subdivision in
404 Yellowstone bison and genetic diversity indices for the two breeding herds. Although strong
405 culling occurred just before our 2008 sampling, there was very little change in differenation over
406 the previous generation. Not surprisingly, allelic diversity and heterozygosity are similar to
407 those computed approximately one generation ago from pooled data of 500 bison sampled on
408 winter ranges beyond the boundary of Yellowstone National Park (Halbert et al. 2012; Figure 2,
409 Appendices D and E). This is expected as the total population size remained relatively large.
410 The National Park Service conservation mandate for wildlife populations includes allowing
411 them to exert their natural function and role on ecosystem processes (National Park Service
412 2006). Yellowstone bison provide disturbance that enhances and sustains native plant
413 communities through wallowing, seed dispersal, grazing, nitrogen deposition, and rubbing on
414 trees. Yellowstone bison are also a food resource for predators, scavengers, and decomposers.
415 Bison survival is dependent upon locating forage, water, and refugia in a diverse landscape with
416 extreme climatic conditions (Barmore 2003, Garrott et al. 2009). Thus, allowing Yellowstone
417 bison to migrate freely is an important evolutionary process and a long-term monitoring effort
418 will provide information to evaluate fluctuations in genetic diversity (including population
419 substructure).
420
421 **MANAGEMENT IMPLICATIONS**

422 The identification of management units is fundamental to the conservation of wildlife
423 populations and represents crucial baseline information for interpreting long-term monitoring
424 results that characterize management effects (Palsbøll et al. 2006). Our results support that
425 Yellowstone bison can be characterized as a single population with genetically similar, yet
426 distinguishable, maternal breeding groups (matri-lines or lineages) on the northern and central
427 ranges. When harvesting, managing, or conserving wildlife populations, evolutionarily
428 enlightened management occurs when both the ecological and evolutionary consequences of
429 management decisions are considered (Ashley et al. 2003).
430 Our genetic analyses suggesting extensive dispersal between the two breeding groups closely
431 agrees with estimates of dispersal by radio-collared, adult females during 2006-2011 (10
432 percent). Each radio-collared bison likely represents more than one individual dispersing
433 because their gregarious nature generally keeps them in groups of 25 or more as they move about
434 the ecosystem (McHugh 1972, Berger and Cunningham1994). Thus, we recommend managers
435 implement measures to (1) facilitate population sex ratios that do not deviate more than 10%
436 from equal proportions, and (2) preserve subpopulation distribution during the breeding season
437 that protects near equal proportions, or at least greater than 1,500 bison on the northern and
438 central ranges. Additionally, the park should monitor genetic diversity in the Yellowstone bison
439 population at least once every one to two generations. Our results show that collecting DNA
440 from fecal samples on the breeding range can be effectively conducted without capture of
441 individual bison and data analyses from these samples can be conducted to monitor allele
442 frequencies.
443

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LITERATURE CITED

Allendorf, F., and G. Luikart. 2007. Conservation and the genetics of populations. Blackwell Publishing, Malden, Massachusetts, USA.

Allendorf, F., P. England, G. Luikart, P. Ritchie, and N. Ryman. 2008. Genetic effects of harvest on wild animal populations. Trends in Ecology and Evolution 23:327-337.

Anderson, M. J., J. M. Rymer, and F. C. Rohwer. 1992. Philopatry, dispersal and the genetic structure of waterfowl populations. Pages 365-395 *in* B. D. J. Batt, A. D. Afton, M. G. Anderson, C. D. Ankney, D. H. Johnson, J. A. Kadlec, and G. L. Krapu, editors. Ecology and management of breeding waterfowl. University of Minnesota Press, Minneapolis, Minnesota.

Animal Welfare Institute. 2008. Emergency rulemaking petition to protect the genetic diversity and viability of the bison of Yellowstone National Park. http://www.awionline.org/ht/a/GetDocumentAction/i/6716. Accessed May 21, 2011.

Ashley, M., M. Willson, O. Pergams, D. O'Dowd, S. Gende, and J. Brown. 2003. Evolutionarily enlightened management. Biological Conservation 111:115-123

Barmore, W. J. Jr. 2003. Ecology of ungulates and their winter range in northern Yellowstone National Park: Research and synthesis, 1962-1970. National Park Service, Yellowstone National Park, Mammoth Hot Springs, Wyoming, USA.

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

Bienen, L., and G. Tabor. 2006. Applying an ecosystem approach to brucellosis control: Can an old conflict between wildlife and agriculture be successfully managed? Frontiers in Ecology and Environment 4:319-327.

Bjornlie, D. D., and R. A. Garrott. 2001. Effects of winter road grooming on bison in Yellowstone National Park. Journal of Wildlife Management 65:423-435.

Bruggeman, J. E., R. A. Garrott, P. J. White, F. G. R. Watson, and R. L. Wallen. 2007. Covariates affecting spatial variability in bison travel behavior in Yellowstone National Park. Ecological Applications 17:1411-1423.

Cahalane, V. H. 1944. Restoration of wild bison. Transactions North American Wildlife and Natural Resources Conference 9:135-143.

Chesser, R. K. 1991. Gene diversity and female philopatry. Genetics 127:437-447

Cheville, N. F., D. R. McCullough, and L. R. Paulson. 1998. Brucellosis in the greater Yellowstone area. National Academy Press, Washington, D.C., USA.

488 Cole, G. F. 1971. An ecological rationale for the natural or artificial regulation of native
489 ungulates in parks. Transactions of the North American Wildlife and Natural Resources
490 Conference 36:417-425.
491 Coltman, D. W., J. G. Pilkington, and J. M. Pemberton. 2003. Fine-scale genetic structure in a
492 free living ungulate population. Molecular Ecology 12:733-742.
493 Côté, S. D., F. Dallas, F. Marshall, R. J. Irvine, R. Langvatn, and S. D. Albon. 2002.
494 Microsatellite DNA evidence for genetic drift and philopatry in Svalbard reindeer. Molecular
495 Ecology 11:1923-1930
496 Dary, D. A. 1974. The buffalo book: The full saga of the American animal. Swallow Press,
497 Chicago, Illinois, USA.
498 Estes-Zumpf, W. J. Rachlow, L. Waits, and K. Warheit. 2010. Dispersal, gene flow, and
499 population genetic structure in the pygmy rabbit (*Brachylagus idahoensis*). Journal of
500 Mammalogy 91:208-219
501 Excoffier, L., G. Laval, and S. Schneider. 2005. ARLEQUIN ver. 3.0: An integrated software
502 package for population genetics data analysis. Evolutionary Bioinformatics Online 1:47-50.
503 Frankham, R. 1996. Relationship of genetic variation to population size in wildlife.
504 Conservation Biology10:1500-1508.
505 Freese, C. H., K. E. Aune, D. P. Boyd, J. N. Derr, S. C. Forrest, C. C. Gates, P. J. P. Gogan, S.
506 M. Grassel, N. D. Halbert, K. Kunkel, and K. H. Redford. 2007. Second chance for the
507 plains bison. Biological Conservation 136:175-184.
508 Fuller, J. A., R. A. Garrott, and P. J. White. 2009. Emigration and density dependence in
509 Yellowstone bison. Pages 237-253 *in* R. A. Garrott, P. J. White, and F. G. R. Watson, editors.

510 The ecology of large mammals in central Yellowstone: Sixteen years of integrated field
511 studies. Elsevier, San Diego, California, USA.
512 Gardipee, F. M. 2007. Development of fecal DNA sampling methods to assess genetic
513 population structure of greater Yellowstone bison. Thesis, University of Montana, Missoula,
514 Montana, USA.
515 Gannon, W. L., R. S. Sikes, and the Animal Care and Use Committee of the American Society of
516 Mammalogists.2007. Guidelines of the American Society of Mammalogists for the use of
517 wild mammals in research. Journal of Mammalogy 88:809-823.
518 Garrott, R. A., P. J. White, and F. G. R. Watson, editors. 2009. The ecology of large mammals
519 in central Yellowstone: Sixteen years of integrated field studies. Elsevier, San Diego,
520 California, USA.
521 Gates, C., B. Stelfox, T. Muhly, T. Chowns, and R. Hudson. 2005. The ecology of bison
522 movements and distribution in and beyond Yellowstone National Park: A critical review with
523 implications for winter use and transboundary population management. University of
524 Calgary, Alberta, Canada.
525 Gates, C. C., C. H. Freese, P. J. P. Gogan, and M. Kotzman, editors. 2010. American bison:
526 Status survey and conservation guidelines 2010. IUCN, Gland, Switzerland.
527 Geremia, C., P. J. White, R. A. Garrott, R. Wallen, K. E. Aune, J. Treanor, and J. A. Fuller.
528 2009. Demography of central Yellowstone bison: effects of climate, density and disease.
529 Pages 255-279 *in* R. A. Garrott, P. J. White, and F. G. R. Watson, editors. The ecology of
530 large mammals in central Yellowstone: Sixteen years of integrated field studies. Elsevier,
531 San Diego, California, USA.
532 Geremia, C., P. J. White, R. L. Wallen, F. G. R. Watson, J. Treanor, J. Borkowski,

533 C. S. Potter, and R. L. Crabtree. 2011. Predicting bison migration out of Yellowstone National
534 Park using Bayesian Models. PLoS ONE 6:e16848.
535 Government Accountability Office. 2008. Yellowstone bison – Interagency plan and agencies'
536 management need improvement to better address bison-cattle brucellosis controversy.
537 Report GAO-08-291 to Congressional requesters. Washington, D.C., USA.
538 Halbert, N. D. 2003. The utilization of genetic markers to resolve modern management issues in
539 historic bison populations: Implications for species conservation. Dissertation, Texas A&M
540 University, Lubbock, Texas, USA.
541 Halbert, N., and J. Derr. 2007. A comprehensive evaluation of cattle introgression into US
542 Federal Bison Herds. Journal of Heredity 98:1-12.
543 Halbert, N. and J. Derr. 2008. Patterns of genetic variation in US federal bison herds. Molecular
544 Ecology 17:4963-4977.
545 Halbert, N., P. J. Gogan, P. W. Hedrick, J. M. Wahl and J. Derr. 2012. Genetic population
546 substructure in bison at Yellowstone National Park. Journal of Heredity 103:360-370.
547 Hall, E. R., and K. R. Kelson. 1959. Mammals of North America. Ronal Press, New York,
548 New York, USA.
549 Hedrick, P. W. 2009. Conservation genetics and North American bison (*Bison bison*). Journal
550 of Heredity 100:414-420.
551 Hepp, G. R., R. T. Hoppe, and R. A. Kennamer. 1987. Population parameters and philopatry of
552 breeding female wood ducks. Journal of Wildlife Management 51:401-404.
553 Isenberg, A. C. 2000. The destruction of the bison: An environmental history 1750-1920.
554 Cambridge University Press, Cambridge, UK.

555 Keiter, R. B. 1997. Greater Yellowstone's bison: Unraveling of an early American wildlife
556 conservation achievement. Journal of Wildlife Management 61:1-11.
557 Kreeger, T. J., J. M. Arnemo, and J. P. Raath. 2002. Handbook of wildlife chemical
558 immobilization. Wildlife Pharmaceuticals, Fort Collins, Colorado, USA.
559 Lacy, R. C. 1987. Loss of genetic diversity from managed populations: Interacting effects of
560 drift, mutation, immigration, selection, and population subdivision. Conservation Biology
561 1:143-158.
562 Larter, N. C., A. R. E. Sinclair, T. Ellsworth, J. Nishi, and C. C. Gates. 2000. Dynamics of
563 reintroduction in an indigenous large ungulate: the wood bison of Northern Canada. Animal
564 Conservation 4:299-309.
565 Manel, S., M. K. Schwartz, G. Luikart, and P. Taberlet. 2003. Landscape genetics: combining
566 landscape ecology and population genetics. Trends in Ecology and Evolution 18:189-197.
567 McHugh, T. 1972. The time of the buffalo. Alfred A. Knopf, New York, New York, USA.
568 Meagher, M. 1973. The bison of Yellowstone National Park. Science Monographs 1,
569 Government Printing Office, National Park Service, Washington, D.C., USA.
570 Meagher, M. 1989. Range expansion by bison of Yellowstone National Park. Journal of
571 Mammalogy 70:670-675.

572 Mills, L. S. 2007. Conservation of wildlife populations: Demography, genetics, and
573 management. Blackwell Publishing, Malden, Massachusetts, USA.
574 National Park Service. 2006. Management policies 2006. U.S. Department of the Interior,
575 Washington, D.C.
576 Nelson, M. E., and L. D. Mech. 1984.Home range formation and dispersal of deer in
577 northeastern Minnesota. Journal of Mammalogy 65:567-575.

578 Newman, D., and D. Pilson. 1997. Increased probability of extinction due to decreased genetic
579 effective size: experimental populations of *Clarkia pulchella*. Evolution 51:354–362.
580 Palsbøll, P. J., M. Bérubé, and F. W. Allendorf. 2006. Identification of management units using
581 population genetic data. Trends in Ecology and Evolution 22:11-16.
582 Paetkau D., W. Calvert, I. Stirling, and C. Strobeck. 1995. Microsatellite analysis of population
583 structure in Canadian polar bears. Molecular Ecology 4: 347-54.
584 Paetkau, D., R. Slade, M. Burden, and A. Estoup. 2004. Genetic assignment methods for the
585 direct, real-time estimation of migration rate: a simulation-based exploration of accuracy and
586 power. Molecular Ecology 13: 55-65.
587 Peakall, R. and P.E. Smouse. 2006. GENALEX 6: genetic analysis in Excel. Population genetic
588 software for teaching and research. Molecular Ecology Notes 6: 288-295.
589 Plumb, G. E., P. J. White, M. B. Coughenour, and R. L. Wallen. 2009. Carrying capacity,
590 migration, and dispersal in Yellowstone bison. Biological Conservation 142:2377-2387.
591 Raymond, M., and F. Rousset. 1995. GENEPOP (version 1.2): Population genetics software for
592 exact tests and ecumenicism. Journal of Heredity 86:248-249.
593 Reed, D., and R. Frankham. 2003. Correlation between fitness and genetic diversity.
594 Conservation Biology 17:230-237.
595 Rousset, F., 2008. Genepop'007: a complete reimplementation of the Genepop software for
596 Windows and Linux. Molecular Ecology Resources 8: 103-106.
597 Ryman N., C. Reuterwall, K. Nygrén, and T. Nygrén. 1980. Genetic variation and
598 differentiation in Scandinavian moose (*Alces alces*): are large mammals monomorphic?
599 Evolution 34:1037-1049.

600 Stenseth, N. C., and W. Z. Lidicker, Jr., editors. 1992. Animal dispersal – Small mammals as a
601 model. Chapman and Hall, London, UK.
602 Taper, M., M. Meagher, and C. Jerde. 2000. The phenology of space: Spatial aspects of bison
603 density dependence in Yellowstone National Park. Project Report. Yellowstone National
604 Park, Mammoth, Wyoming. 263pp.
605 United States Department of the Interior, National Park Service (USDI) and United States
606 Department of Agriculture, Forest Service, Animal and Plant Health Inspection Service
607 (USDA). 2000. Record of decision for final environmental impact statement and bison
608 management plan for the State of Montana and Yellowstone National Park. Washington,
609 D.C., USA.
610 Vogel, A. B., K. Tenggardjaja, S. Edmands, N. D. Halbert, J. N. Derr, and D. Hedgecock. 2007.
611 Detection of mitochondrial DNA from domestic cattle in bison on Santa Catalina Island.
612 Animal Genetics 38:410-412.
613 Wallen, R., and D. Blanton. 2007. Bison capture and handling protocol for chemical
614 immobilization of bison in Yellowstone National Park. Yellowstone National Park,
615 Mammoth, Wyoming, USA.
616 Waples, R., and O. Gaggiotti. 2006. What is a population? An empirical evaluation of some
617 genetic methods for identifying the number of gene pools and their degree of connectivity.
618 Molecular Ecology 15:1419-1439.
619 Ward, T. J., J. P. Bielawski, S. K. Davis, J. W. Templeton, and J. N. Derr. 1999. Identification
620 of domestic cattle hybrids in wild cattle and bison species: a general approach using mtDNA
621 markers and the parametric bootstrap. Animal Conservation 2:51-57.

622 Western Watersheds Project et al. vs. Salazar et al. 2010. First amended complaint for
623 injunctive and declaratory relief. Case 9:09-cv-00159-CCL, Document 14-1. Filed May 17,
624 2010, United States District Court for the District of Montana, Missoula Division, Helena,
625 USA.
626 White, G. C., and R. A. Garrott. 1990. Analysis of wildlife radiotracking data. Academic Press,
627 San Diego, California, USA.
628 White, P. J., T. L. Davis, K. K. Barnowe-Meyer, R. L. Crabtree, and R. A. Garrott. 2007. Partial
629 migration and philopatry of Yellowstone pronghorn. Biological Conservation 135:518-526.
630 White, P.J., J. Treanor, and R. Wallen. 2011. Surveillance plan for Yellowstone bison:
631 Monitoring the effects and effectiveness of management actions. Yellowstone National Park,
632 Mammoth, Wyoming, USA.
633 White, P. J., R. L. Wallen, C. Geremia, J. J. Treanor, and D. W. Blanton. 2011. Management of
634 Yellowstone bison and brucellosis transmission risk – Implications for conservation and
635 restoration. Biological Conservation144:1322-1334.
636 Wilson, G. A., and C. M. Strobeck. 1999. Genetic variation within and relatedness among wood
637 and plains bison populations. Genome 42:483-496.
638 Wilson, G. A., and K. A. Zittlau. 2004. Management strategies for minimizing loss of genetic
639 diversity in wood and plains bison populations at Elk Island National Park. Parks Canada,
640 Edmonton, Alberta, Canada.
641 Wolff, J. O. 1994. More on juvenile dispersal in mammals. Oikos 71:349-352
642

643 **Figure 1.** Current and historic areas of the National Park used during breeding season by
644 Yellowstone bison and the intensive management zones at the boundary of the park where bison
645 migrate to during some winters. The IBMP management area indicates zones where bison are
646 tolerated in Montana during winter under certain constraints pursuant to the Interagency Bison
647 Management Plan (USDI and USDA 2000).
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651 **Figure 2.** Allelic richness (Ar) comparisons for samples collected from Yellowstone bison
652 during 1997-2002 and 2006-2008, using the same 17loci. Different ladders were used in each
653 laboratory, which may have resulted in a 1-4 base pair shift in allele calls. Therefore, there may
654 be some imprecision in these comparisons because of the difference in allele calls between the
655 two laboratories.
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657

Table 1. The number of Yellowstone bison sampled during 2005-2006 and the frequency of haplotype 6 in the mitochondrial DNA results.

	Sample Size			Proportion of Haplotype 6 (%)	
Breeding Group	2005	2006	Total	2005	2006
Central Range	59	35	94	89.8	100
Northern Range	0	57	57	0	50.8
Total Samples	59	92	151	-	-

662 **Must put Table 2 here reporting He, A (alleles per locus), and Fis We already have a table**
663 **with all this information in the supporting material. Are you suggesting we relocate that to**
664 **the main body of the manuscript?**

665

666 **Table 2.** Summary of microsatellite loci used to compute Fst using DNA extracted from fecal
667 samples collected on the breeding grounds of the Yellowstone bison. n refers to xxx.

668

Loci Used For F_{ST} Estimates	F_{ST} Estimate For 2006 Data	F_{ST} Estimate For 2008 Data
All 17 microsatellite markers	**0.022**	**0.012**
Without BMS 410 included (n = 16)	**0.015**	**0.008**
Without BMS 2258 included (n = 16)	**0.015**	**0.008**
Without TGLA 122 included (n = 16)	**0.014**	**0.007**

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672 **Table 3. Population assignment based on analyses of 17 microsatellite loci conducted in**
673 **GenAlEx 6.3 (GEN) and Arlequin 3.1 (ARL). The number of samples that were assigned**
674 **to their original range and year of collection are bolded (*N* = number of samples analyzed**
675 **from each range and year).**
676

Range and Year Collected	N	Software Program	Population assignment				Samples that assign to alternate range (%)
			Central 2006	Northern 2006	Central 2008	Northern 2008	
Central 2006	**21**	**Gen**	**16**	**0**	**5**	**0**	**0**
		Arl	**19**	**0**	**2**	**0**	**0**
Northern 2006	**23**	**Gen**	**3**	**17**	**2**	**1**	**22**
		Arl	**0**	**17**	**2**	**4**	**9**
Central 2008	**27**	**Gen**	**4**	**2**	**19**	**2**	**15**
		Arl	**3**	**2**	**21**	**2**	**11**
Northern 2008	**28**	**Gen**	**1**	**4**	**5**	**18**	**21**
		Arl	**0**	**3**	**2**	**23**	**7**

677