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Genetic Population Substructure in Bison at Yellowstone National Park

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Abstract

The Yellowstone National Park bison herd is 1 of only 2 populations known to have continually persisted on their current landscape since pre-Columbian times. Over the last century, the census size of this herd has fluctuated from around 100 individuals to over 3000 animals. Previous studies involving radiotelemetry, tooth wear, and parturition timing provide evidence of at least 2 distinct groups of bison within Yellowstone National Park. To better understand the biology of Yellowstone bison, we investigated the potential for limited gene flow across this population using multilocus Bayesian clustering analysis. Two genetically distinct and clearly defined subpopulations were identified based on both genotypic diversity and allelic distributions. Genetic cluster assignments were highly correlated with sampling locations for a subgroup of live capture individuals. Furthermore, a comparison of the cluster assignments to the 2 principle winter cull sites revealed critical differences in migration patterns across years. The 2 Yellowstone subpopulations display levels of differentiation that are only slightly less than that between populations which have been geographically and reproductively isolated for over 40 years. The identification of cryptic population subdivision and genetic differentiation of this magnitude highlights the importance of this biological phenomenon in the management of wildlife species.

Key words: *bison, conservation, population structure, wildlife management*

Genetic isolation among subpopulations affects many demographic and evolutionary processes. For example, reduced gene flow can lead to the accumulation of genetic differences between subpopulations from genetic drift, mutation, and/or selection. Genetic substructure does not always coincide with obvious morphological or geographical differences between subpopulations, and cryptic substructure has only been revealed in recent decades through analysis of molecular genetic data (e.g., Proctor et al. 2005; Yoshio et al. 2009). The recognition of population substructure is fundamental to the identification of management units and an important consideration for wildlife conservation.

Range contraction and landscape fragmentation have led to the restriction of many terrestrial mammals to isolated populations, thereby creating artificial population substructure. For example, the largest extant land mammal in North America, American bison (*Bison bison*), ranged across the continent in large intermixing herds of thousands of individuals in the 19th century (McHugh 1972) but now exists in isolated populations generally of less than 1000 individuals (Gates et al. 2010). The artificial isolation of these populations has led to detectable levels of genetic differentiation (Wilson and Strobeck 1999; Halbert and Derr 2008). Populations of more than 5000 bison are currently supported on only 2 landscapes: the Greater Yellowstone Area in Wyoming/Montana/Idaho, USA (including Yellowstone and Grand Teton National Parks) and the Greater Wood Buffalo Area in Alberta/Northwest Territories, Canada (Gates et al. 2010). The large number of bison on these complex landscapes provides opportunity for natural population substructure, which could have important implications for the long-term evolution of these populations.

In this study, we investigate genetic substructure within the Yellowstone National Park bison population, which is among the most critical to bison conservation. The Yellowstone population is one of only a few "occupying extensive native landscapes where human influence is minimal and a full suite of natural limiting factors is present" (Gates et al. 2010), although large numbers have been culled along the park boundaries intermittently since the 1980s (Cheville et al. 1998; US Department of Interior and US Department of Agriculture 2000). Additionally, the population is a valuable genetic resource. Unlike most populations examined to date, no evidence of domestic cattle genetic introgression has been identified in Yellowstone bison (Ward et al. 1999; Halbert et al. 2005; Halbert and Derr 2007). Furthermore, this population has high levels of genetic diversity and heterozygosity compared with other populations (Wilson and

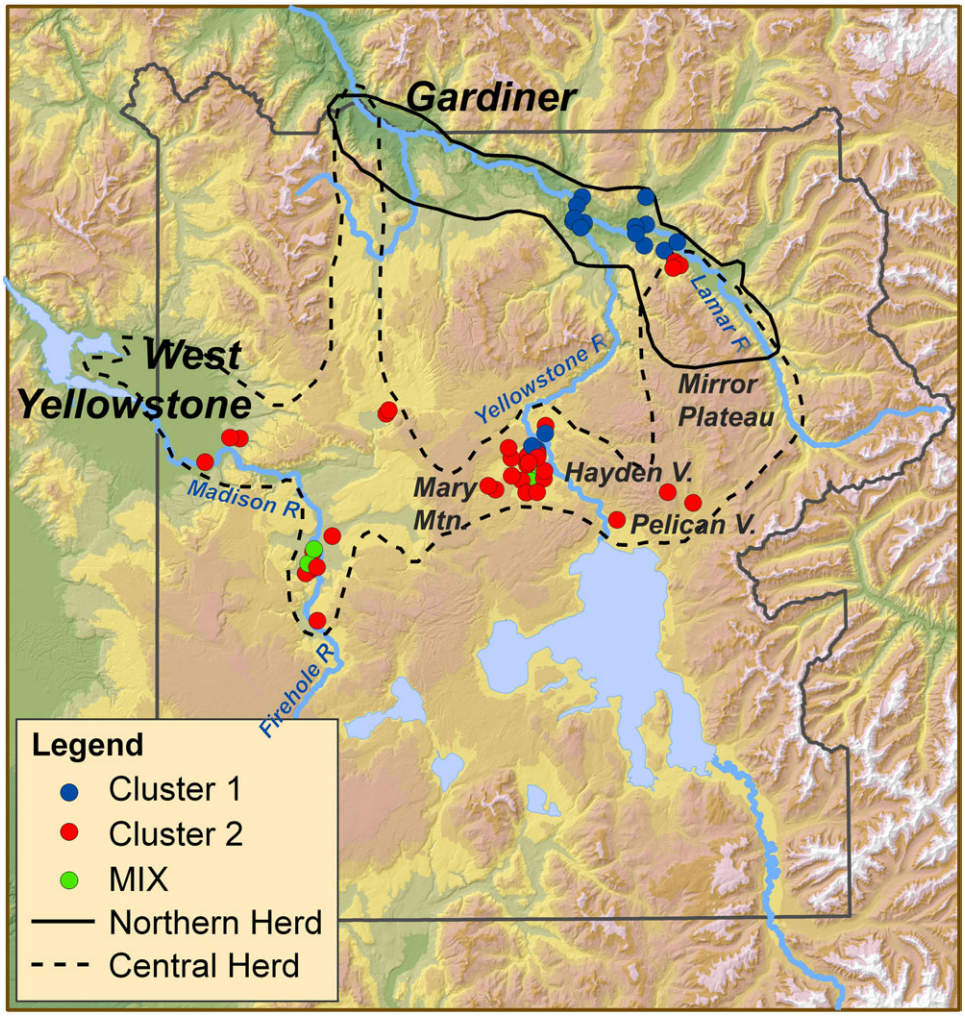

Figure 1. Map of Yellowstone National Park indicating the locations and genetic types of bison live captured between February 2000 and October 2003. Bison with at least 70% assignment to one of the clusters are indicated by blue (cluster 1) or red (cluster 2) circles, and green circles indicate bison with less than 70% assignment to a single cluster (mixed). The dashed and solid lines represent the maximum annual distribution of central and northern herd Yellowstone bison, respectively. Abbreviations: R, river; Mtn, mountain; and V, valley.

Strobeck 1999; Halbert and Derr 2008), underscoring the significance of Yellowstone bison in species conservation.

Historically, 3 bison herds within Yellowstone were documented based on winter distributions (Mary Mountain, Pelican Valley, and Lamar Valley), although none of the herds remained isolated year-round (Meagher 1973). As the total number of bison increased to more than 3000 in the mid1990s, 2 herds have been generally recognized: the central herd consists of the former Mary Mountain and Pelican Valley herds and the northern herd consists of the former Lamar Valley herd (Olexa and Gogan 2007). The smaller northern herd moves northwestward during the winter toward Gardiner, Montana, and the central herd moves westward toward West Yellowstone, Montana (Figure 1). In some winters, segments of the central herd have been observed moving northward toward Gardiner, Montana, and the herds may intermingle during these times (Gates et al. 2005). Radiotelemetry data indicate that the herds remain isolated during the summer breeding season (Olexa and Gogan 2007). However, the question of whether the recognized herds represent genetically distinct units remains unanswered. In this study, we use nuclear microsatellite markers and a multilocus Bayesian clustering method to evaluate the possibility of genetic substructure among Yellowstone bison and assess patterns of genetic variation among subpopulations.

Materials and Methods

Genetic Data Collection

Hair, blood, or liver tissue samples were taken from live-captured bison processed under protocols approved by a USGS Animal Care and Use Committee and from bison

Table 1 Collection details for Yellowstone bison samples included in this study

Location type	Location name	1997	1998	1999	2000	2001	2002	2003	Location total
Live capture	Central				18			21	39
	Northern				14	4			18
Winter cull	Gardiner	166						131	297
	West Yellowstone	63	1	67		13	141	14	299
Unknown	Unknown				7	1			8
	Year total	229	1	67	39	18	141	166	661

slaughtered under the authority of the Montana Department of Livestock. Methods for extracting DNA, marker selection, and multiplexed PCR assays were previously described (Schnabel et al. 2000; Halbert et al. 2004). A total of 46 autosomal microsatellite markers were used in this study (Supplementary Table S1). Separation of PCR products and genotype analysis were performed as previously described (Halbert and Derr 2008). A Microsoft Excel add-in (Park 2001) was used to identify duplicates and prepare data for downstream analysis.

Data Analysis

Each sample was determined to belong to a unique bison based on genotypes. To minimize bias, the offspring of any parent–offspring pairs detected in a previous study (Halbert 2003) were excluded from this analysis. Evidence of subpopulation structure was assessed using all 46 autosomal microsatellite markers and the multilocus Bayesian clustering method in the STRUCTURE 2.3 program (Pritchard et al. 2000). This method minimizes the presence of Hardy–Weinberg and linkage disequilibrium within a collection of multilocus genotypes by probabilistic assignment of individuals into K populations. After initial testing (Pritchard et al. 2000), a burn-in period of 40 000 replicates and 80 000 Markov chain Monte Carlo replicates was utilized with either the independent or correlated allele frequency model (Falush et al. 2003) and accounting for the possibility of admixture (ALPHAPROPSD = 0.08). The default program values were used for all other settings.

The entire data set was evaluated across 20 iterations at each K from 1 to 6, and the assignments of individuals to various subpopulations by the clustering method were compared with and without collection site information as prior information in the model (i.e., a priori and a posteriori comparisons, respectively). The most likely number of clusters within the data set was determined by examining averages and standard deviations (SDs) at each K for ln $P(D)$ (Pritchard and Wen 2004) and using the ΔK method (Evanno et al. 2005). Clusters among different replicates were sorted and aligned using the program CLUMPP 1.0 (Jakobsson and Rosenberg 2007), and the resultant membership assignments were visualized using the program DISTRUCT (Rosenberg 2004).

Observed and expected heterozygosity (unbiased gene diversity; Nei 1987), average number of alleles per locus, allelic richness (El Mousadik and Petit 1996), and F statistics (Weir and Cockerham 1984) were calculated using the program FSTAT 2.9.3.2 (Goudet 1995, 2001). Allelic richness and expected heterozygosity are unbiased estimators of the observed number of alleles per locus and heterozygosity, respectively, which minimize differences due to sample size variances. Each population-marker combination was also tested for Hardy–Weinberg equilibrium (HWE) in FSTAT 2.9.3.2 using sequential Bonferroni correction for multiple tests. Genic and genotypic differentiation among populations were tested in the program GENEPOP 4 (Raymond and Rousset 1995) using the Markov chain method (Guo and Thompson 1992) with a 10 000 step dememorization, 150 batches, and 10 000 iterations per batch ($P < 0.05$ considered significant).

Comparison to Sampling Locations

Sampling locations were divided into 2 categories: live capture and winter cull. The live capture group included 57 bison sampled between February 2000 and October 2003 from the northern or central ranges (Table 1). None of the samples were collected during the summer breeding season (Gogan et al. 2005). This group is considered a control sample for the purposes of assigning individuals to genetic subpopulations.

An additional 596 samples were collected between 1997 and 2003 from bison processed along the park boundaries under an interagency management plan (US Department of Interior and US Department of Agriculture 2000). Most of these samples were collected between January 1 and April 30 (592 of 596; 99.3%). The winter cull group is further divided into approximate sampling location: Gardiner versus West Yellowstone. These sites represent the most frequently used winter exits for bison out of Yellowstone National Park and are separated by approximately 86 km following the most frequent routes of bison winter travel (Gates et al. 2005) or 52 km point-to-point.

Results

Description of Subpopulation Structure

Microsatellite genotypes were collected from 661 bison sampled between 1999 and 2003 (Table 1), and collection locations were recorded for 653 (98.8%) of these samples. Both the correlated and independent allele frequency models were used to evaluate clustering consistency and both gave similar results (Falush et al. 2003). Only the correlated allele frequency model is considered hereafter because migration between any subpopulations within Yellowstone National

Table 2 Genetic diversity comparison between genetic clusters identified in this study, including individuals with at least 70% membership assignment to the indicated cluster

Cluster	Range	n	H_O	H_E	A_R	Total alleles	Private alleles[a]
1	Northern	152	0.596	0.602	4.56	210	6
2	Central	453	0.621	0.623	4.63	218	14
All YNP		661	0.616	0.626	4.71	224	

Abbreviations: n, number of individuals; H_O, observed heterozygosity; H_E, expected heterozygosity; A_R, allelic richness; and YNP, Yellowstone National Park.

[a] Alleles identified in only 1 of the 2 clusters.

Park is biologically feasible. Evaluation of ln $P(D)$ (Pritchard and Wen 2004) and $\triangle K$ (Evanno et al. 2005) from multiple STRUCTURE simulations indicate the data set most likely represents at least 2 genetically defined clusters (Supplementary Figure S1). Although the ln $P(D)$ increases between $K = 2$ and $K = 3$ (Supplementary Figure S1A), indicating a possible third cluster, the $\triangle K$ values clearly indicate more support for $K = 2$ (Supplementary Figure S1B). These findings were consistent regardless of whether collection site information was considered a priori or a posteriori. As expected, when collection site information was included as prior information, the likelihood of the results (ln $P[D]$) increased (though only slightly). Furthermore, at K values above 2, the variability in the results increased substantially, indicting very poor support for 3 or more subpopulations. Therefore, we will hereafter consider only the conservative possibility of 2 clusters.

For all additional analyses, collection site information was not used as prior information in the model (a posteriori comparison only) based on the following: 1) This model is least likely to result in spurious assignments of individuals to 1 of the 2 subpopulations and 2) preliminary analysis indicated a lack of correlation in some years between collection site and subpopulation (see also below, Figure 3). Using the same parameters as above, lambda (λ) was estimated for $K = 2$ at 0.521. This estimate was used to run an additional 20 simulations at $K = 2$ to assign individuals to clusters because the lower λ value is expected to increase cluster assignments when many markers have rare alleles (see below). A total of 605 (91.5%) bison had an average assignment of at least 70% to a single cluster: 152 were assigned to cluster 1 and 453 were assigned to cluster 2 (Supplementary Figure S2). The remaining 56 individuals were not clearly assigned to a single cluster using these criteria; these bison did not appear to be F_1 offspring of any other samples included in this study (Halbert 2003), and no unique alleles were identified in these bison compared with the 2 clusters. To determine the impact of excluding the 56 individuals (8.5% of the total) of presumed "mixed" ancestry, F_{ST} was calculated based on the entire data set by using 0.5 as the cluster assignment cutoff. With these individuals included, F_{ST} is reduced to 0.0269, a 17% reduction compared with F_{ST} in the sample that excluded these individuals (0.0321, see below). Because the 2 clusters are strongly supported, we assume that the relatively small mixed group represents descendants of gene flow between the 2 subpopulations.

Based on the above, the data set including only the 605 bison with at least 70% probability of assignment to a single cluster was used to evaluate the genetic relationship between the clusters. Allele frequencies, allelic richness, and heterozygosity values for each group-marker combination are summarized in Table 2 (detailed data for individual loci provided in Supplementary Table S1). Each of the genetic diversity measures indicated slightly less diversity in cluster 1.

A total of 20 "private" alleles were identified. Many of these alleles were found at very low frequencies (<0.01), and none had a frequency of more than 0.08. These alleles were not equally distributed between the clusters: 6 were identified in cluster 1 and 14 in cluster 2. However, the average private allele frequency per locus (Hedrick 1971) is similarly low in each cluster: 0.0037 for cluster 1 versus 0.0049 for cluster 2. The possibility that one or more of these private alleles are derived from domestic cattle was not directly assessed here (see methods of Halbert and Derr 2007). However, 15 of the 20 alleles are found in other US bison populations with no known direct relationship to Yellowstone National Park (Halbert and Derr 2008) and are therefore considered unlikely to be derived from domestic cattle. Additionally, a previous evaluation of these samples based on both mitochondrial and nuclear markers (14 microsatellites, different from those utilized here) failed to identify genetic evidence of prior domestic cattle hybridization (Halbert and Derr 2007).

Of the 46 markers evaluated, 40 (87.0%) have significantly different distributions of alleles (genic differentiation) and genotypes (genotypic differentiation) between the 2 clusters ($P < 0.05$). The genic and genotypic distributions for the remaining 6 markers are not significantly different between the 2 clusters: BL1036, BM4028, BM4440, BMS1074, BMS1117, and BMS1355. The F_{ST} (theta) estimate between the 2 clusters is 0.0321 (Weir and Cockerham 1984), and G''_{ST}, a G_{ST} measure standardized for both the average heterozygosity and the number of populations, is 0.0838 (Meirmans and Hedrick 2010). The F_{IS} and F_{IT} estimates are 0.0069 and 0.0232, respectively, which indicate that inbreeding does not occur at high levels within these subpopulations.

The null hypothesis of HWE across all 661 samples for all markers was rejected, and an overall heterozygote deficit was observed ($P < 0.0001$). Conversely, HWE was achieved within each of the clusters based on the STRUCTURE analysis; however, this result is expected given that the clustering algorithm of STRUCTURE seeks to minimize Hardy–Weinberg disequilibrium.

Genetic versus Geographic Clustering

Cluster assignments were compared with live capture data for 57 bison (Figures 1 and 2). Of the 39 bison sampled within the central range, 3 were assigned to cluster 1, 32 (82.1%) were assigned to cluster 2 (average membership 0.93 ± 0.06 SD), and 4 appeared to be of mixed origin (<70% assignment to a single cluster). Of the 18 bison sampled within the northern range, 14 (77.8%) were assigned to cluster 1 (average membership 0.96 ± 0.02 SD), 2 were assigned to cluster 2, and 2 appeared to be of mixed origin. From this analysis, there

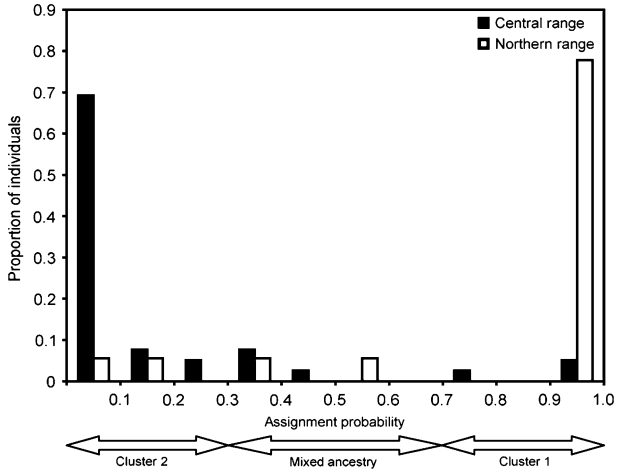

Figure 2. Frequency distribution chart of cluster assignments for bison live captured and sampled within the central ($n = 39$) or northern ($n = 18$) ranges, indicating a strong association between sampling location and cluster assignments. The proportion of individuals within each range for a given probability class (e.g., 0–0.1, 0.11–0.2, etc.) are indicated with vertical bars. The majority of bison captured in the central range (32/39 = 82.1%) were assigned to cluster 2 (assignment probability ≤ 0.3 to cluster 1), whereas the majority of bison captured in the northern range (14/18 = 77.8%) were assigned to cluster 1 (assignment probability ≥ 0.7 to cluster 1).

appears to be a strong association between the genetically defined clusters and sampling locations within Yellowstone National Park, with cluster 1 representing bison from the northern range and cluster 2 representing bison from the central range (Figure 2).

A similar comparison was performed for the 596 samples collected at the park boundaries as part of the winter cull protocol (Figure 3). Of the 297 bison sampled near Gardiner, Montana in 1997 and 2003, 121 (40.7%) were assigned to cluster 1, 153 (51.5%) to cluster 2, and 23 (7.7%) appear to be a mixture between the clusters. The proportion of individuals assigned to each cluster, however, varied greatly by year (Figure 3A). In 1997, most of the sampled bison were assigned to cluster 1 (113/166, 68.1%), whereas in 2003, most were assigned to cluster 2 (115/131, 87.8%).

Likewise, of the 299 bison sampled near West Yellowstone, Montana, from 1997 to 2003, 12 (4.0%) were assigned to cluster 1, 260 (87.0%) were assigned to cluster 2, and 27 (9.0%) appear to be a mixture between the clusters. The proportion of individuals assigned to each cluster was consistent in the West Yellowstone group, with between 84.3% (119/141 in 2002) and 92.3% (12/13 in 2001) in any given year assigned to cluster 2 (Figure 3B).

Migration between Subpopulations

To give a perspective on the amount of differentiation that we have documented, let us assume that genetic drift is the main factor causing the subpopulations to diverge while gene flow between them acts to reduce the extent of divergence. Let us use the "island model" of Wright (1940) so that the expected amount of differentiation in generation $t + 1$, $F_{ST(t+1)}$, is the result of the amount of genetic drift due to the finite effective population size N_e, the amount of gene flow (migration) m between the subpopulations, and the amount of differentiation in the previous generation (e.g., Hedrick 2011) or

$$F_{ST(t+1)} = (1 - m)^2\left[\frac{1}{2N_e} + \left(1 - \frac{1}{2N_e}\right)F_{ST(t)}\right]. \quad (1)$$

First, let us assume that the Yellowstone population was not subdivided before 1936 (Meagher 1973) so that $F_{ST} = 0$ at that point. Next, let us assume that on average our sample was taken around 64 years later (Table 1). Finally, let us assume that the generation length, the average age of a parent for their average offspring, is 8 years (Hedrick 2009), so that 64 years constitutes about 8 generations. Using the above expression, we can then find what combinations of m and N_e result in an F_{ST} after 8 generations equal to the observed 0.0321.

Figure 4 gives the expected change in F_{ST} for 3 combinations of m and N_e. First, if there is no gene flow ($m = 0$) between the populations, then $N_e = 122.6$ and $F_{ST} = 0.0321$ in generation 8. In this case, the theoretical value of F_{ST} continues to increase to 1 over time. Because gene flow has been documented between the groups, this example appears too extreme. In contrast, the highest rate of gene flow illustrated in Figure 4 occurs when $N_e = 50$ and $m = 0.107$. In this case, F_{ST} in generation 8 is only slightly lower than the eventual expected equilibrium value of 0.0378. However, this combination is unlikely because it requires a very high rate of gene flow (10.7%) each generation. The third combination given in Figure 4, where $N_e = 100$ and $m = 0.0230$ and intermediate between these 2 extremes, is more consistent with what is known about movements between the 2 groups, particularly during the rutting season (Olexa and Gogan 2007). In this case, F_{ST} continues to increase and asymptotes at 0.0950.

Let us assume that values of $N_e = 100$ and $m = 0.0230$ are reasonable working estimates of these parameters. First, this means that the number of migrants into and out of each subpopulation each generation is about 2 ($N_e m = 2.3$) or approximately 1 every fourth year. (Analysis with the software MIGRATE [Beerli and Palczewski 2010] gave an estimate of $N_e m$ out of the northern and central populations of 2.2 and 1.5, respectively.) Second, the level of differentiation, given these levels of genetic drift and gene flow, is expected to increase approximately 3-fold beyond that observed to a value of 0.095.

In this discussion, we have made a number of simplifying assumptions. First, we have assumed a constant level of genetic drift and gene flow each generation, though it is possible that these parameters vary over time. For example, the amount of genetic drift may have been larger in some generations, particularly early generations, because of low effective population size. Because the effective population size over generations is the harmonic mean of

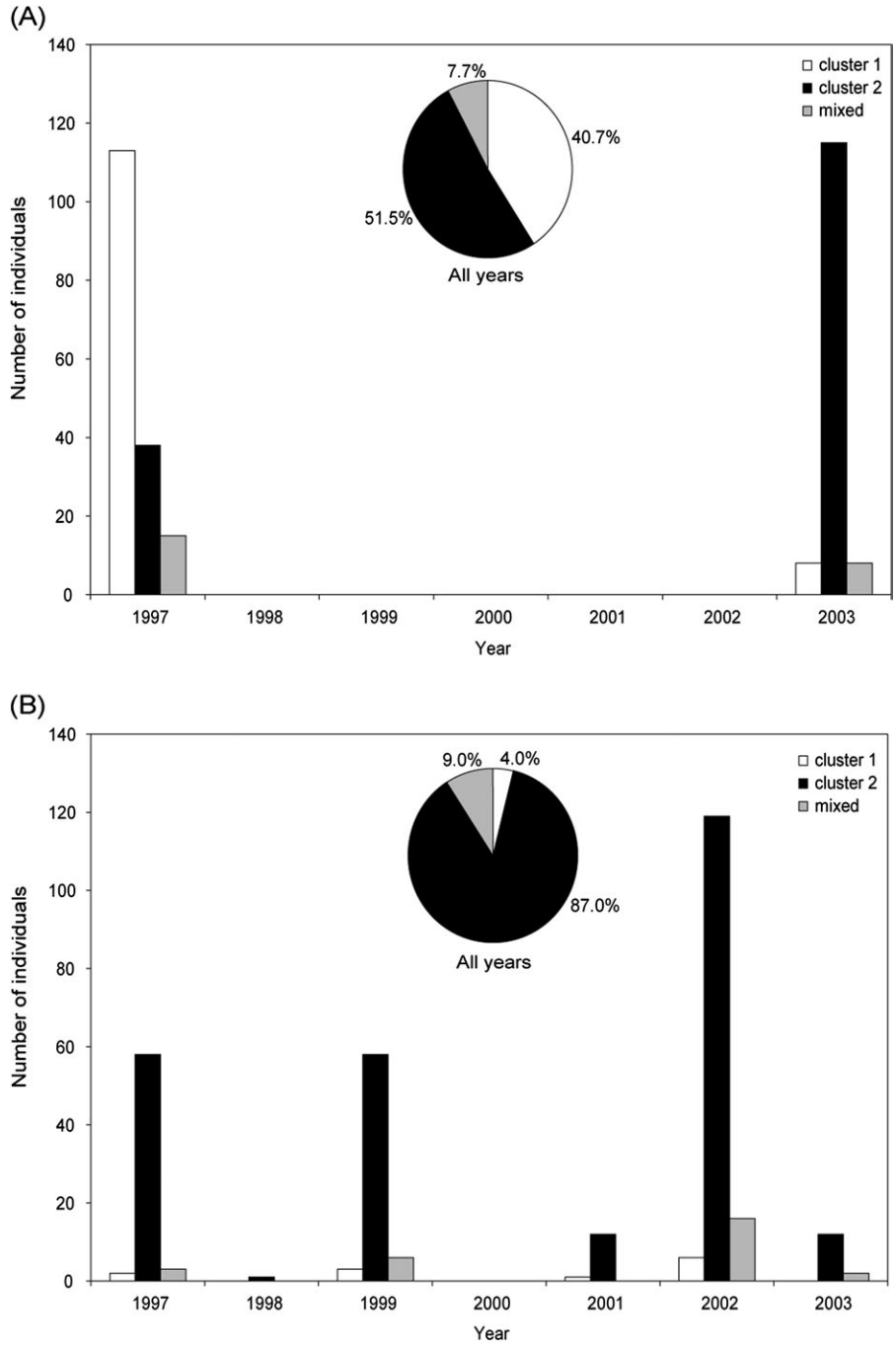

Figure 3. Cluster assignments of 596 bison sampled at the park boundaries as part of the winter cull protocol from 1997 to 2003. Panel (**A**) illustrates assignments for 297 bison sampled near Gardiner, Montana. Distinct differences in the distribution of cluster assignments were noted between 1997 and 2003. Panel (**B**) illustrates assignments for 299 bison sampled near West Yellowstone, Montana. The breakdown by year from this group is fairly consistent, with the majority of samples assigned to cluster 2 (87.0% overall).

the effective population size in a given generation (Hedrick 2011), early low numbers can greatly reduce the overall effective population size. Second, we have assumed that the observed value of F_{ST} is the true value of differentiation. This value, however, may be overestimated due to the exclusion of the 56 individuals that were not placed in 1 of the 2 clusters (see discussion above).

Although the observed value of differentiation is low compared with the F_{ST} value of 0.2 that is sometimes used to indicate strong differentiation (Mills and Allendorf 1996;

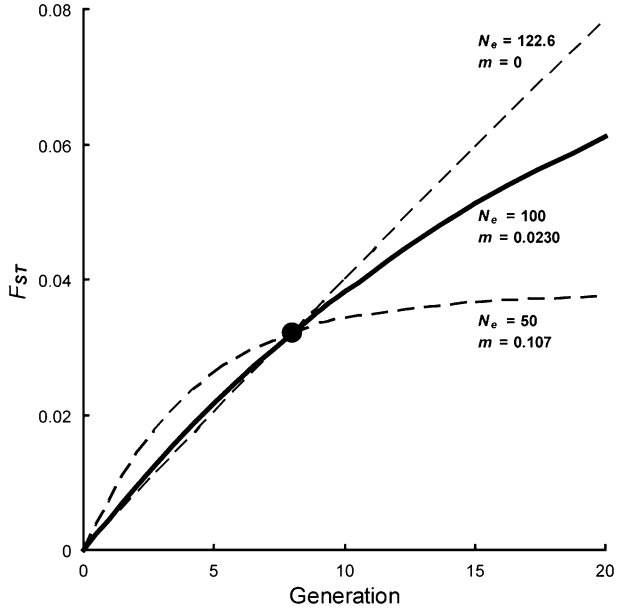

Figure 4. The level of F_{ST} expected over time for 3 combinations of genetic drift resulting from finite effective population size N_e and gene flow m that give the observed level of F_{ST} after 8 generations (solid circle).

Wang 2004), these 2 subpopulations have differentiated in a relatively short period of approximately 8 generations. Furthermore, F_{ST} for neutral microsatellite loci provides an indicator of the potential for differential adaptation. In other words, it appears that the isolation between these 2 groups is significant—8.4% of the maximum possible from the calculation of G''_{ST} above—and could lead to divergence of adaptively important genetic attributes given that their environments are significantly different (Christianson et al. 2005; Olexa and Gogan 2007).

Finally, the observed F_{ST} for the same microsatellite loci between Badlands National Park and Theodore Roosevelt National Park (South Unit) is 0.042, and the observed F_{ST} between Badlands National Park and Fort Niobrara National Wildlife Refuge is 0.045 (Halbert and Derr 2008). Both of these pairs of populations have been isolated from each other since 1963 (Halbert and Derr 2007), and the samples were taken in 2000–2002. In other words, over 5 generations of isolation, these pairs have accumulated a level of differentiation only slightly larger than we observed for the 2 Yellowstone subpopulations. If we assume that $m = 0$, then the expected differentiation is

$$F_{ST(t+1)} = 1 - e^{-t/(2N_e)} \quad (2a)$$

so that

$$N_e = \frac{-t}{2\ln(1 - F_{ST})}. \quad (2b)$$

Therefore, to explain the observed F_{ST}, N_e values of 58.3 and 54.3 over 5 generations would be sufficient. The present day census numbers for these populations are smaller than that for Yellowstone National Park (Halbert and Derr 2008), and these populations were founded with fewer bison (Halbert and Derr 2007). Given no gene flow and these estimated effective population sizes, this level of differentiation after 5 generations appears reasonable and is congruent with our Yellowstone subpopulation calculations.

The relative amount of male to female gene flow can be estimated from nuclear (biparental) and mitochondrial DNA (mtDNA) (female) F_{ST} values, F_{ST} and $F_{ST(f)}$, respectively (González-Suárez et al. 2009). First, the amount of male differentiation, $F_{ST(m)}$ can be estimated as

$$F_{ST(m)} = \frac{F_{ST}F_{ST(f)}}{F_{ST(f)} - F_{ST} + 3F_{ST}F_{ST(f)}}. \quad (3a)$$

Then, if it is assumed that population is at or near equilibrium under the island model, the expected numbers of female and male migrants per generation for maternally inherited and paternally inherited genes are

$$Nm_f = \frac{1 - F_{ST(f)}}{2F_{ST(f)}}$$

and

$$Nm_m = \frac{1 - F_{ST(m)}}{2F_{ST(m)}}.$$

The ratio of these equations can then be solved for the ratio of the male and female gene flow rates as

$$\frac{m_m}{m_f} = \frac{F_{ST(f)}(1 - F_{ST(m)})}{F_{ST(m)}(1 - F_{ST(f)})}. \quad (3b)$$

Between the 2 populations of Yellowstone bison identified above, the estimate of F_{ST} for nuclear markers is 0.0321. Gardipee (2007) examined mtDNA variation in samples from the northern population from Lamar Valley (2006) and from the central populations from Hayden Valley (2005 and 2006). The 2 estimated $F_{ST(f)}$ values between these 2 locations were 0.218 (Hayden Valley 2005 and Lamar Valley 2006) and 0.367 (Hayden Valley 2006 and Lamar Valley 2006) for an average of 0.2925. Using the equation above and this mean estimate, the estimate of $F_{ST(m)}$ is 0.0325, nearly equal to the overall estimate of 0.0321, suggesting that nearly all of the gene flow is from males. The ratio of male to female gene flow was then estimated to be 12.31, also suggesting that most of the gene flow is from males. This difference in male and female gene flow is consistent with significantly higher female philopatry than male philopatry in bison, as discussed by Gardipee (2007).

Discussion

The genetic analyses presented support the hypothesis of 2 genetically distinct subpopulations of bison within Yellowstone National Park. As a metapopulation, the Yellowstone bison appear to be nonrandomly mating with fewer heterozygotes observed than predicted under HWE, which is expected if more than one distinctive breeding population

is sampled (Wahlund effect). To investigate this possibility, a Bayesian method was used to identify genetic clusters. The 2 clusters identified by this method are genetically distinct based on the distribution of both genotypes and alleles. In fact, a high number of private alleles were identified between the 2 clusters compared with that found for other federal herds (Halbert and Derr 2008), which may be the result of chance differences when the subpopulations were established or loss due to genetic drift.

Furthermore, the identified clusters appear to be biologically significant. When the individual cluster assignments were compared a posteriori with live capture sampling locations, a strong association was identified between the clusters and sampling location (Figures 1 and 2) despite the fact that none of the samples were collected during the summer breeding season when site fidelity is expected to be the strongest. Bison from the northern range were assigned primarily to cluster 1 and bison from the central range were assigned mostly to cluster 2 (hereafter Northern subpopulation and Central subpopulation, respectively). These differences are consistent with demographic comparisons of culled bison from each location. Analyses of both tooth wear patterns (Christianson et al. 2005) and parturition timing and synchrony (Gogan et al. 2005) have demonstrated significant differences between northern and central range bison, which are expected only when bison remain isolated for much of their lives.

Although the Northern subpopulation appears to have somewhat lower levels of genetic diversity (Table 2) and fewer private alleles compared with the Central subpopulation, it is not clear whether the observed differences are significant. For example, the larger number of private alleles in the Central subpopulation may reflect the larger sample size ($n = 453$) compared with the Northern subpopulation ($n = 152$).

A third distinctive herd in the Pelican Valley was previously proposed based on historical observations (Meagher 1973). Only 2 live capture samples from bison in the Pelican Valley were available for this study (Figure 1), and the possibility of a third genetic cluster cannot be completely discounted at this time. Direct sampling and genetic analysis of larger numbers of bison from the Pelican Valley would be useful in evaluating this possibility. However, radiotelemetry data indicate bison move continuously between the Pelican Valley and other portions of the central range during the winter (Olexa and Gogan 2007). Therefore, it is likely that bison from the Pelican Valley were included in the winter cull samples for this study, and comparatively low support for 3 clusters was identified in our analysis (Supplementary Figure S1). Furthermore, radiotelemetry data demonstrate constant movement of bison between the Pelican Valley and the Hayden Valley during the summer breeding season (Olexa and Gogan 2007), which suggests high levels of genetic exchange between these locations.

Although Yellowstone bison are subjected to a range of naturally limiting forces such as predation and forage competition, bison numbers are also reduced in many years through culling. Bison move to and beyond the park boundaries primarily from January through April when population numbers are high and weather conditions most severe (Gates et al. 2005). These bison are culled, sometimes in large numbers, along the park boundaries to reduce the probability of transmission of brucellosis to livestock on adjacent lands (Cheville et al. 1998; US Department of Interior and US Department of Agriculture 2000).

As with the live capture group, most of the winter cull bison were assigned to 1 of the 2 identified clusters (546/596 = 91.6%). Bison sampled near West Yellowstone appear to be nearly exclusively from the Central subpopulation (cluster 2). This observation was consistent across all 6 years in which samples were obtained (Figure 3B).

Winter cull samples were obtained from bison near Gardiner in only 2 years of the study (1997, 2003), and the distribution of cluster assignments was strikingly different between these years. Most of the bison sampled near Gardiner in 1997 appear to be from the Northern subpopulation (68.1% assigned to cluster 1) and those sampled in 2003 appear to be primarily from the Central subpopulation (87.8% assigned to cluster 2). These findings are consistent with observations that the entire northern herd moved toward the Gardiner boundary during the winter of 1996–1997 (Taper et al. 2000) but not 2002–2003 (Geremia et al. 2009).

Although there is clear evidence for genetically distinct subpopulations among Yellowstone bison, the subpopulations do not appear to be completely isolated. Of the bison sampled in this study, 8.5% (56/661) were estimated to have less than 70% probability of being assigned to either cluster. Although it is possible that common genotypes prevented conclusive assignment probabilities, some of this fraction may also be due to admixture between the 2 subpopulations. Overall, the level of genetic differentiation observed between the 2 subpopulations is consistent with an average effective population size of around 100 within each subpopulation since they diverged about 64 years ago and a successful (breeding) migrant both into and out of each subpopulation every fourth year (this assumes that there is not too much variation over time in effective population size and gene flow). The level of differentiation between the 2 subpopulations is only slightly lower than between some of the other federal herds that have been completely isolated for over 40 years and have smaller population sizes. Given these estimates, the level of divergence is expected to continue to increase, and there is a potential for adaptive differentiation in the different environments inhabited by the Yellowstone subpopulations.

The effective population size of 100 suggested for each of the subpopulations is the average (ca. the harmonic mean, Hedrick 2011) over 8 generations and includes the years immediately following establishment and years with low numbers in one or both subpopulations. In a given generation, the effective population size may be around only 25% of the census population number (Nunney 1993), although it may be higher (Berger and Cunningham 1994) or lower (Wilson et al. 2005). Furthermore, the estimated effective population size of 100 is not related to what

effective population sizes should be recommended for the subpopulations in the future. To determine the appropriate effective population size for the long-term sustainability of the subpopulations, a thorough population viability analysis should be conducted.

Population subdivision theoretically leads to decreased genetic variation within individual subpopulations due to genetic drift, although overall variation is expected to increase due to differential drift of alleles and the establishment of new mutations within subpopulations (Lande and Barrowclough 1987). Therefore, the high levels of genetic variation observed among Yellowstone bison compared with other populations (Wilson and Strobeck 1999; Halbert and Derr 2008) may be explained by the maintenance of subpopulations and comparatively large effective size of the Yellowstone National Park population. Nonetheless, the identification of genetic subpopulations in this study raises serious concerns for the management and long-term conservation of Yellowstone bison.

Yellowstone bison have long been treated as a single metapopulation whereby the total number of bison is assumed to be the most important factor in determining appropriate winter cull levels (US Department of Interior and US Department of Agriculture 2000; Plumb et al. 2009). However, the unequal census sizes of the 2 subpopulations call this strategy into question: The Northern subpopulation ranges from 16% to 31% of the total population (US Department of Interior and US Department of Agriculture 2000; Gates et al. 2005). It is highly likely, therefore, that the 2 subpopulations have been disproportionately culled in some years. For example, approximately 735 bison were culled near Gardiner at the park's northern boundary during the 1996–1997 winter. Applying our estimate that around 68% of the bison culled near Gardiner that year originated from the Northern subpopulation (Figure 3A), we calculate that approximately 500 of the bison culled during the 1996–1997 winter were from the Northern subpopulation. Given the prewinter estimate for the Northern subpopulation of 877 bison (US Department of Interior and US Department of Agriculture 2000; Gates et al. 2005), the 500 culled bison represent approximately 57% of the entire subpopulation.

In contrast, combining the remaining 235 bison culled at the park's northern boundary with the 363 culled at the western boundary during the winter of 1996–1997 results in an estimated 600 bison culled from the Central subpopulation. These bison represent approximately 20% of the prewinter estimate of 2928 bison in the Central subpopulation (US Department of Interior and US Department of Agriculture 2000; Gates et al. 2005). Therefore, the rate of loss of genetic diversity may be quite different between the 2 subpopulations. Although the winter movement of bison from the central herd to the northern range may be density dependent (Fuller et al. 2009) and therefore somewhat predictable, it is not possible to separate bison at the northern boundary (Gardiner) based on subpopulation origin (Central vs. Northern) without invasive methods (e.g., permanent identification methods or on-site genetic analysis).

It is not clear at this point how the subpopulations may be changing over time or how the current bison management plan (US Department of Interior and US Department of Agriculture 2000) might influence the genetic integrity of the subpopulations. For example, when the total census size is less than 3000 bison, the current plan calls for holding 125 bison that test negative for brucellosis at a facility near Gardiner throughout the winter and then releasing the bison into the park in the spring. Such seronegative bison are most commonly calves (Rhyan et al. 2009). Being young, these bison may join the Northern subpopulation rather than return to the central range, which would erode the genetic distinctiveness between the 2 groups. Additional sampling and genetic analyses are needed to assess changes in genetic composition between the 2 subpopulations.

In conclusion, we have presented strong evidence for the existence of 2 genetically distinct subpopulations of bison within Yellowstone National Park. Our study has also revealed longitudinal differences in migration patterns among Yellowstone bison, as it appears that bison moving to the park boundary in the vicinity of West Yellowstone are consistently from the Central subpopulation, whereas those moving to the park boundary in the vicinity of Gardiner may originate from either the Central or Northern subpopulation. These observations warrant serious reconsideration of current management practices. The continued practice of culling bison without regard to possible subpopulation structure has the potentially negative long-term consequences of reducing genetic diversity and permanently changing the genetic constitution within subpopulations and across the Yellowstone metapopulation. Population subdivision is a critically important force for maintaining genetic diversity and yet has been assessed in only a handful of species to date. The identification of cryptic population subdivision of the magnitude identified in this study exemplifies the importance of genetic studies in the management of wildlife species.

Supplementary Material

Supplementary material can be found at http://www.jhered.oxfordjournals.org/.

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