Founding Lineages and Genic Variability in Plains Bison (*Bison bison*) from Badlands National Park, South Dakota

LEROY R. MCCLENAGHAN, JR.*

Department of Biology
San Diego State University
San Diego, CA 92182-0057 U.S.A.

JOEL BERGER

Program in Ecology, Evolution, and Conservation Biology
University of Nevada
Reno, NV 89512 U.S.A.
and
Conservation and Research Center
Smithsonian Institution
Front Royal, VA 22630 U.S.A.

HAROLD D. TRUESDALE

Department of Biology
San Diego State University
San Diego, CA 92182-0057 U.S.A.

Abstract: *Starch-gel electrophoresis was used to screen 101 bison from Badlands National Park, South Dakota, for variation at 24 genetic loci. The population was descended from founder groups of about 6 and 3 individuals, separated geographically for a minimum of 64 years. The purpose of this study was (1) to estimate levels of genic variability in this bison population, (2) to assess the extent to which descendents of the two founder groups differ genetically, and (3) to compare the genetic characteristics of the Badlands population with other bison populations. The Badlands herd was found to be polymorphic for only a single locus (MDH-1). Descendents of the founder groups were homogeneous with respect to allelic and genotypic frequencies at this locus. The MDH-1 polymorphism has not been observed in other bison populations, while several polymorphisms reported in other bison populations were not detected in the Badlands herd. A*

Resumen: *La variación de 24 posiciones de genes fue analizado en 101 bisontes del Badlands National Park, South Dakota, mediante electroforesis en albumina. La población estudiada era descendiente de dos grupos fundadores, formados por alrededor de 6 y 3 individuos respectivamente, separados geográficamente por un mínimo de 64 años. El propósito de éste estudio fue (1) estimar los niveles de variabilidad genética presente en ésta población de bisontes, (2) evaluar el grado en que los descendientes de los dos grupos fundadores difieren genéticamente, y (3) comparar las características genéticas de la población de Badlands con otros poblaciones de bisontes. Se encontro que la manada de bisontes de Badlands es polimorfica para sólo una posición (MDH-1). Los descendientes de los grupos fundadores fueron homogeneos en respecto a frecuencias allelicas y genotipicas para esta posición. El polimorfismo MDH-1 no ha sido observado en otras poblaciones de bisontes, mientras que varios polimorfismos reportados en otras poblaciones de bisontes no fueron detectados en la manada de Badlands. Un promedio de heterozigosidad de 0.012 fue observado en la*

* *Correspondence and requests for reprints should be addressed to this author.*
Paper submitted 10/10/88; revised manuscript accepted 1/23/89.

mean heterozygosity of 0.012 was observed in the Badlands herd; this value is lower than that typically reported for mammals, though not as low as heterozygosities seen in other populations that have passed through severe bottlenecks in size. These results underscore the need for genetic data in planning breeding programs for species in captivity or managed in isolated reserves.

manada de Badlands; este valor es más bajo de lo que es típicamente reportado para mamíferos, aunque no es tan bajo como heterozigosidades observadas en otras poblaciones que han pasado por situaciones criticas en relación a su tamaño. Estos resultados subrayan la necesidad de contar con datos genéticos para planificar programas de crianza en cautividad o el manejo de especies en reservas aisladas.

Introduction

The amount of genetic variation in the gene pool of a given species is often used as a measure of its "evolutionary potential" (Allendorf & Leary 1986; Fisher 1930; Frankel & Soulé 1981), and has direct application to the field of conservation genetics, since rare and endangered species often display reduced levels of genetic variability (Bonnell & Selander 1974; O'Brien et al. 1983). Reductions in population size can result in diminished genetic diversity through drift; subsequent inbreeding may further reduce genetic variability within populations. Furthermore, gene flow among populations may be greatly reduced as suitable habitat becomes increasingly fragmented. A major thrust of conservation genetics is the development of management strategies to prevent further erosion of genetic diversity in endangered species (Chesser et al. 1980; Foose 1977).

Species such as northern elephant seals (*Mirounga angustirostris*), cheetahs (*Acinoynx jubatus*), and Dall sheep (*Ovis dalli*), whose current populations have rebounded from small sizes caused by overharvest, recent habitat destruction, or natural events, show low levels of heterozygosity (Bonnell & Selander 1974; O'Brien et al. 1983; Sage & Wolff 1986). In the plains bison (*Bison bison*), populations were reduced from an estimated 30–60 million to 800–2,000 individuals in the last century (McHugh 1972; Roe 1970; Soper 1941), whereas extant European bison (*B. bonasus*) stem from a herd of 13 founders established in 1913 (Slatis 1960). While large populations of both bison species now exist, the genetic effects of past bottlenecks remain largely unknown (Shull & Tipton 1987), although recent evidence from European bison suggests that individuals with high inbreeding coefficients suffer greater juvenile mortality and longer interbirth intervals than less inbred individuals (Olech 1987).

The bison population of Badlands National Park, South Dakota, was founded in the 1960s by the introduction of 25 animals from Theodore Roosevelt National Park, North Dakota, and three from Fort Niobrara National Wildlife Refuge, Nebraska. The ancestors of both these lineages were from the original Fort Niobrara National Wildlife Refuge population, established in 1913. Some uncertainty exists about the source of the founding population at Fort Niobrara. In 1907, J. W. Gilbert of Friend, Nebraska, had about "forty head of elk, deer, and buffaloes" and in 1913 six bison were given to the National Refuge. In an August 6, 1965 letter, librarian R. Doner of the Gilbert Public Library stated, "In my search I finally found one man who said he remembered Mr. Gilbert got the first buffaloes out of a wild herd in northcentral Nebraska, and the others came from Valentine" (USFWS unpublished). In 1983, 20 animals were introduced into the Badlands population from the Colorado National Monument herd; all were descendants from two females and one male from the Denver area in 1925. Since 1983 all animals of both lineages have been sympatric in Badlands National Park and have experienced opportunities for interbreeding. In 1988 the Badlands population numbered about 700 and presented an excellent opportunity to examine several questions of interest to conservation geneticists. First, given the history of bison in North America, to what extent have levels of genetic variability been reduced within existing populations? Second, to what degree are the bison lineages at Badlands genetically differentiated? Answers to these questions are important because many populations of large mammals now occur in disjunct units and prudent management cannot progress unless assumptions about levels of genetic variability in relation to founding population size are examined.

Methods

One hundred and one blood samples were obtained from the bison population at Badlands National Park (BNP). Two groups were designated. We had anticipated that these would reflect founder population size and, hence, we designated them (erroneously, see below) as "outbred" — based on the 28 founders from Theodore Roosevelt National Park whose origins we did not know, and "inbred" — based on the three founders from Colorado. We later discovered that the founding sizes of both lineages were similar, and to avoid confusion here we simply designate these lineages as BNP/FN and BNP/C to identify their origins, Fort Niobrara and Colorado National Monument, respectively. All individuals in both lineages were identifiable by horn and coat

color characteristics, ear tags, or brands (Berger 1989), since the population had been studied for four years. Blood was collected from immobilized animals and those restrained in cattle chutes (Kock & Berger 1987; Berger & Peacock 1988) by puncture in the tail, ear, or throat. Blood was then placed on ice for 4–6 hours, centrifuged to separate plasma and red blood cell fractions, and then refrigerated until shipping in an insulated container 2 to 6 days later to LRM's laboratory. During sampling (1986 and 1987), BNP/FN bison comprised about 94 percent of the free-ranging population; hence our collection of blood from BNP/FN individuals (96 out of 101) was in proportion to their abundance in the population. None of the bison samples were hybrids from the two lineages.

Upon arrival in San Diego, all samples were inspected and then stored at -70° C until processed. Hemolysates were absorbed onto filter paper wicks, which were then placed into 11 percent starch gels with the following buffers: lithium hydroxide (pH 8.4, Selander et al. 1971), tris-citrate (pH 7.1; Selander et al. 1971), and JRP (pH 6.3; Ayala et al. 1972). Gels were subjected to an electric current (ca 60 ma) for about 7 hours for lithium hydroxide gels and (ca 30 ma) for about 16 hours for tris-citrate and JRP gels. Gels were sliced and stained for catalase (CAT), superoxidase dimutase (SOD), lactate dehydrogenase (LDH), peptidase (PEP), hemoglobin (HB), and hexokinase (HK) on lithium hydroxide gels; glutamate oxaloacetate transaminase (GOT), 6-phosphogluconate dehydrogenase (6-PGD), phosphoglucomutase (PGM), xanthine dehydrogense (XDH), and mannosephosphate isomerase (MPI) on tris-citrate gels; and acid phosphatase (ACP), malate dehydrogenase (MDH), malic enzyme (ME), and glucosephosphate isomerase (GPI) on JRP gels.

Staining of gels generally followed the methods of Selander et al. (1971), with the exceptions of CAT (Shaw & Prasad 1970); MPI (Nichols et al. 1973); and HK, PEP, and ACP (Harris & Hopkinson 1976). The most anodal form of each enzyme system was designated 1, with others numbered sequentially in order of decreasing anodal mobility. The most common allele at each locus was designated 100 with all other alleles designated according to the relative mobility of their products compared to that of the common allele. Observed genotypic frequencies were compared to expected Hardy-Weinburg frequencies by chi-square goodness-of-fit tests.

Levels of genic variability were estimated as the proportion of polymorphic loci (P), mean heterozygosity across loci by direct count (H_o), and mean heterozygosity expected from Hardy-Weinberg proportions (H_e). A locus was considered to be polymorphic if the frequency of the most common allele was ≤ 0.99 (Selander 1976).

Results

We were able to consistently resolve allozyme phenotypes at 24 presumptive genetic loci for all the bison sampled. We observed four forms of GPI; two forms of LDH, HK, ACP, PGM, HB, and MDH; and single forms of ME, GOT, 6-PGD, XDH, MPI, PEP, CAT, and SOD.

Neither the BNP/C nor BNP/FN group contained any unique alleles. Both groups were found to be polymorphic for only a single locus (MDH-1). One individual in the BNP/FN group was also observed to be heterozygous at the GOT-1 locus, but this locus was not considered to be polymorphic since the frequency of the most common allele at this locus (0.995) was >0.99.

Allele frequencies for MDH-1 in the BNP/FN and BNP/C groups (Table 1) were not significantly different ($X^2 = 0.09$; $P > 0.5$). However, observed genotypic frequencies at this locus in the BNP/FN group were significantly different from those expected on the basis of the Hardy-Weinberg equilibrium ($X^2 = 19.64$; $P < 0.005$); this deviation reflected a deficiency of heterozygotes. Observed genotypic frequencies for MDH-1 in the BNP/C group did not differ significantly from expected Hardy-Weinberg proportions ($X^2 = 0.32$; $P > 0.5$).

Since the Badlands bison population was only polymorphic for a single locus, estimates of genic variability were necessarily low (Table 1). The proportion of polymorphic loci was 0.042 in the BNP/C, BNP/FN, and pooled samples. Observed heterozygosities were 0.017 in the BNP/C group and 0.011 in the BNP/FN group (pooled $H_o = 0.012$). Expected heterozygosities in the BNP/FN and pooled samples ($H_e = 0.021$) were about twice as great as the observed heterozygosities in those groups, while the expected value in the BNP/C group ($H_e = 0.020$) was roughly equivalent to the observed value.

Table 1. Alleles and genotype frequencies at the Malate Dehydrogenase-1 (MDH-1) locus, proportions of polymorphic loci (*P*), and observed (H_o) and expected (H_e) heterozygosities for BNP/FN (N = 96), BNP/C (N = 5), and pooled (N = 101) samples of bison. Genotype frequencies expected from the Hardy-Weinberg equilibrium are given in parentheses; expected and observed frequencies for the BNP/FN and pooled samples were significantly different (P < 0.01).

	BNP/FN sample	*BNP/C sample*	*Pooled sample*
Mdh-1^{95}	0.448	0.400	0.446
Mdh-1^{100}	0.552	0.600	0.554
100/100	0.417 (0.305)	0.400 (0.360)	0.416 (0.307)
95/100	0.271 (0.495)	0.400 (0.480)	0.277 (0.494)
95/95	0.313 (0.201)	0.200 (0.160)	0.307 (0.199)
P	0.042	0.042	0.042
H_o	0.011	0.017	0.012
H_e	0.021	0.020	0.021

Discussion and Summary

Low levels of genic variability in bison were expected given the species' recent bottleneck in population numbers, the subsequent fragmentation into small populations, and concomitant prevention of gene flow between and inbreeding within isolated herds. Earlier studies based on limited numbers of genetic loci suggested that bison were largely monomorphic (Braend & Stormont 1963; Stormont 1982). Braend & Stormont (1963) found no detectable polymorphism in bison for transferrin and hemoglobin. Stormont (1982) reported that blood group variation in bison was extremely limited compared to that found in cattle. Our electrophoretic survey of 24 bison blood proteins found generally low levels of genic variability within the Badlands population. The observed value for the mean heterozygosity of 0.012 in the pooled Badlands sample (Table 1) is lower than both the mammalian class average of 0.039 reported by Wooten & Smith (1985) and an average of 0.029 for 10 species of large mammals reported by Baccus et al. (1983). However, this Badlands heterozygosity value is within the 95 percent confidence intervals of both these means and is therefore not statistically different from them.

Our estimates of genic variability in the Badlands herd can be directly compared to those of Baccus et al. (1983), who, as part of a study of genic variability in 10 species of artiodactyls, electrophoretically surveyed a small number of bison (N = 7) from the National Bison Range, Montana, for variation at 19 loci; they found a proportion of polymorphic loci of 0.053 and an observed mean heterozygosity of 0.023; these values are slightly higher than our estimates for levels of variability in the Badlands population (Table 1).

However, our results indicate that plains bison have higher levels of genic variability than several other mammalian species that have passed through significant bottlenecks in numbers. Bonnell & Selander (1974) reported total homozygosity at 24 loci in northern elephant seals, a species thought to have been reduced to as few as 20 individuals in the early 1890s. Similarly, O'Brien et al. (1983) assessed variation at 47 loci in cheetah populations from South Africa and found little or no heterozygosity.

Our results also indicate the need to adopt a conservative approach when inferring genetic structure based on historical information on the size of founding populations. It is apparent that the group we initially labeled "outbred" because we believed it was descended from a larger founder group, may have been less outbred than presumed. Genotypic frequencies at the MDH-1 locus were found to be significantly different from expected Hardy-Weinberg frequencies in the BNP/FN group; heterozygotes at this locus were observed to be underrepresented. While it is not possible to completely eliminate selection against heterozygotes as an explanation for this pattern, this would seem to be an unlikely explanation given the instability of the heterozygote disadvantage system (Hedrick 1983). Inbreeding also produces heterozygote deficiencies and is a more parsimonious hypothesis to explain the observed pattern at the MDH-1 locus in bison. While the 28 founders of the BNP population were descended from about 200 animals at Theodore Roosevelt National Park, that herd was, in turn, descended from an unknown but small number of individuals used to found the Fort Niobrara National Wildlife Refuge herd in 1913.

This study allowed us to examine whether two subpopulations of bison would be genetically differentiated given different historical effects. We found the gene pools of these subpopulations to be extremely similar. The two lineages were polymorphic for the same single locus (MDH-1), and allele frequencies at this locus were not significantly different between samples. These results suggest that drift has not led to any appreciable between-population differentiation for these two sympatric lineages. Alternatively, more proximate factors such as fluctuations in population size, differential reproductive success (Shull & Tipton 1987), or different responses of the two lineages after passing through the suspected bottleneck may reflect the current genetic structure of bison in BNP.

Irrespective of the mechanism, there is some evidence from the literature that the Badlands population differs genetically from other extant bison populations. Baccus et al. (1983) reported that the National Bison Range herd in Montana was monomorphic for MDH-1, while we found the Badlands animals to be highly polymorphic for this locus. Baccus et al. (1983) also reported that the GOT-1 locus was polymorphic with two alleles present in equal frequencies. Since we only observed a single heterozygote at this locus, we did not designate this locus as polymorphic. Also, we failed to find any evidence of the polymorphism at the 6-PGD locus that was described by Naik & Anderson (1970) for the bison population from the Wichita Mountains Wildlife Refuge, Oklahoma.

The observation that small bison populations may display varying levels of genetic differentiation has important management implications and underscores the need to employ techniques such as gel electrophoresis and DNA fingerprinting (Hill 1987) to describe population gene pools when planning breeding programs for species in captivity or managed in numerous isolated reserves. To be successful, efforts to increase population genetic diversity through the movement of individuals among populations (gene flow) must take into consideration the genetic composition and history of the founding populations.

Acknowledgments

We wish to thank C. Curry, C. Shaffer, and C. Waters for their assistance in the laboratory, C. Cunningham, M. and N. Kock, M. and M. Glass, and L. Kortge for help in the field, R. Ellis (USFWS at Fort Niobrara), two anonymous reviewers, and the National Park Service (at Badlands), the National Geographic Society, the Wildlife Preservation Trust, the Badlands Natural History Association, and the University of Nevada for support.

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