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CONSERVATION GENETIC ANALYSIS OF THE TEXAS STATE BISON HERD

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The Texas State Bison Herd is directly descended from the herd assembled from 5 wild-caught bison by Charles Goodnight in the 1880s. In 1997, 36 bison were used to establish a herd at Caprock Canyons State Park. To aid in the development of a long-term genetic conservation plan for this population, we examined and analyzed allelic variation at 54 microsatellite loci representing each of the nuclear chromosomes in the bison genome. The current Texas State Bison Herd population exhibits low genetic diversity and heterozygosity levels compared with bison at Yellowstone National Park and Theodore Roosevelt National Park. Parentage analysis indicates that relatively few adults have contributed offspring in the last 5 years, leading to low effective population size estimations and a rapid increase in the average age of animals in the herd. The very limited number of original founders, multiple population bottlenecks over the last 120 years, and chronically small population size, coupled with genetic drift and inbreeding, have resulted in dangerously low levels of genetic diversity. This, in turn, has likely triggered demographic problems such as low recruitment and high calf mortality rates. Population viability analysis based on current population demography reveals that there is a 99% chance of extinction of the herd within the next 41 years. Based on these findings, the continued existence of this historically important bison population appears doubtful without the introduction of new genetic variation from another plains bison herd.

Key words: bison, Charles Goodnight, conservation genetics, microsatellites

Charles Goodnight was one of a few private ranchers who served to rescue the American bison species (*Bison bison*) from near extinction in the late 1800s (Coder 1975; Dary 1989). At the apex of the species decline in the mid-1880s, 5 wild-caught bison were used to found the Goodnight herd (Coder 1975). By 1887, the herd contained 13 bison and by 1910 the number had increased to 125 bison (Dary 1989). The Goodnight herd continued to grow, apparently reaching proportions of between 200 and 250 bison for several years in the 1920s (Haley 1949). Following Goodnight's death in 1929, the herd changed ownership several times and reliable population size estimates are unavailable. By the 1970s the population was estimated at 40 to 100 bison (Swepston 2001). In 1997, the remaining 36 bison were donated to Texas Parks and Wildlife and moved to Caprock Canyons State Park in the Texas panhandle, forming the Texas State Bison Herd. Over the last 120 years this population has remained reproductively isolated, therefore representing the only extant bison population directly descended from the original Charles Goodnight herd.

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Currently, there are 2 recognized subspecies of bison, wood bison (*Bison bison athabascae*) and plains bison (*Bison bison bison*), based on physical size and pelage characteristics (Hall 1981; McDonald 1981), although the division is challenged by evidence of nongenetic (environmental) causes of phenotypic variation (Geist 1991) and the absence of measurable genetic differences (Peden and Kraay 1979; Polziehn et al. 1996; Ward et al. 1999; Wilson and Strobeck 1999). Krumbiegel and Sehm (1989) used analysis of pelage, physical size, and horn characteristics from pre-1900 illustrations to further split the plains bison into 2 subspecies: southern plains bison (*Bison bison bison*) and northern plains bison (*Bison bison montanae*). Charles Goodnight himself observed phenotypic differences between the northern and southern plains herds (Haley 1949). Variation in size and pelage characteristics in bison are largely influenced by environment and nutrition (Geist 1991), which might explain the observed differences between the northern and southern plains bison. Even if such subspecies at 1 time did exist, they have been undoubtedly crossbred in the past 100 years (Coder 1975; Dary 1989; McHugh 1972), so that the only known true remnant of the southern plains bison is contained in the Texas State Bison Herd.

Charles Goodnight was internationally famous for breeding bison to Angus domestic cattle (*Bos taurus*) in an effort to produce a more robust and hardy beef breed (Goodnight 1914;

Haley 1949). Evidence of introgression is still present in the descendants of Goodnight's original experiments, as 6 of the original 36 members of the Texas State Bison Herd contained domestic cattle-type mitochondrial DNA (population abbreviation JA—Ward 2000; Ward et al. 1999). Subsequent genetic testing demonstrated both a unique bison mitochondrial type and distribution of nuclear alleles in the herd when compared with various wood and plains bison herds (Ward 2000).

The Texas State Bison Herd is maintained on approximately 320 acres of Indian grass (*Chrysopogon nutans*) and sideoats gramma (*Bouteloua curtipendula*), and is provided ample supplemental native hay and water as necessary. The herd receives yearly vaccinations and almost daily visual inspections by state biologists and is not known to suffer from any common ungulate disease (Swepston 2001). Nevertheless, the population exhibits low natality and high calf mortality rates compared to other captive bison herds, and over the past 6 years has only increased from 36 to 40 bison (Halbert et al. 2004). Furthermore, the average age of the population has risen by 2.6 years over this same period (Halbert et al. 2004). In 2000, 8 mature (>3 years old) bulls were fertility tested using electroejaculation (Genetic Resources International, Navasota, Texas). Of these, 4 exhibited normal sperm motility and morphology, but the remainder had abnormalities outside acceptable baseline ranges including low motility, bent tails, and detached heads (D. A. Swepston, pers. comm.). Although some abnormal readings are expected from a single collection on bulls never before worked for fertility testing, the semen characteristics are suggestive of male fertility problems in the Texas State Bison Herd. In December 2001, all 18 adult female bison were pregnancy tested using the pregnancy specific binding protein test (Texas Veterinary Medical Diagnostic Laboratory, College Station, Texas). Results indicated that 15 bison (~83%) were pregnant. From these apparent pregnancies, 5 calves were born and only 1 survived into 2003 (Swepston et al. 2004), confirming the trend of poor recruitment in this herd. As such, it is probable that male infertility and the inability of females to carry pregnancies to term are negatively affecting the recruitment and population growth rates observed in the herd over the past 6 years.

Previous work has established the usefulness of cattle genetic markers in bison (Mommens et al. 1998; Schnabel et al. 2000; Wilson and Strobeck 1999). A set of 15 standard bison parentage microsatellites failed to resolve parentage issues within the Texas State Bison Herd (Schnabel et al. 2000). As such, we sought to develop an expanded microsatellite panel, including markers located on all 29 bison autosomes and both sex chromosomes, to assess the genetic variation present in this population, resolve parentage issues, and develop a long-term genetic conservation plan for the herd. We compared the genetic diversity present in this population to bison from Yellowstone National Park (Wyoming, Montana, and Idaho) and Theodore Roosevelt National Park (North Dakota). Additionally, we karyotyped adults from the Texas State Bison Herd to investigate potential chromosomal aberrations, which if present might also explain the low natality rates reported.

Materials and Methods

Sample collections.—Blood and tail hair samples were collected from each of the 19 male and 21 female bison in the Texas State Bison Herd in December 2001. Approximately 1 ml of whole blood was applied to FTA cards (Whatman, Newton Center, Massachusetts). Additionally, pokeweed-stimulated (Invitrogen, Carlsbad, California) short term lymphocyte cultures (72 h) were started from peripheral blood of 31 bison (14 males, 17 females). Metaphase chromosome spreads were obtained according to standard colcemid and hypotonic fixation technique (Chowdhary et al. 1994). Chromosomes were stained with 5% Giemsa (Sigma, St. Louis, Missouri) in 0.07 M phosphate buffer (pH 6.8). Diploid chromosome number and chromosome morphology were analyzed using Cytovision 2.7 software (Applied Imaging, Santa Clara, California).

Blood samples were collected from 100 bison from Theodore Roosevelt National Park south unit in 2000. DNA was isolated following the Super Quik-Gene protocol (Analytical Genetic Testing Center, Denver, Colorado) and standard phenol-chloroform-isoamyl alcohol (PCI) extraction (Sambrook et al. 1989). Additionally, 100 liver samples were collected from deceased bison that migrated beyond Yellowstone National Park boundaries from 1996 to 2001. Approximately 0.5 g frozen liver was pulverized in liquid nitrogen and tissue lysis buffer consisting of 1× STE (100 mM NaCl, 10 mM Tris, 1 mM ethylenediaminetetraacetic acid [EDTA], 2% sodium dodecyl sulphate (SDS), and 4 mg/ml Proteinase K was added. Following overnight incubation at 55°C, the tissue was treated with 20 μg RNAse and standard PCI extraction.

Marker choice and multiplexing.—Fifty-four unlinked bovine microsatellite markers were selected from the U. S. Department of Agriculture gene mapping database (www.sol.marc.usda.gov) such that there was a minimum of 1 marker per nuclear chromosome and at least 40 cM between syntenic markers. Fifteen of the markers were used following the protocols from Schnabel et al. (2000) as designed for bison parentage testing, with minor changes in the fluorescent dyes and polymerase chain reaction (PCR) protocols used. Markers were multiplexed based on non-overlapping allele size ranges and dye types (Table 1).

For multiplexes 3, 80 through 83, 85, 86, and URB011, PCR conditions in 5 μL total volume were as follows: 50 ng template DNA or 1 FTA punch (1.2 mm, prepared according to manufacturer recommendations); 0.05 to 0.4 μM each primer; 1× MasterAmp PCR Enhancer (Epicentre, Madison, Wisconsin); 500 μM deoxynucleotide triphosphates 3.0 mM $MgCl_2$; 1× reaction buffer; 0.5 units *Taq* DNA polymerase (Promega, Madison, Wisconsin). Conditions for multiplexes 1 and 84 were as above with the exception of 1.5× reaction buffer and 3.5 mM $MgCl_2$. Conditions for multiplex 2 were as above with the exception of 1.2× reaction buffer and 3.25 mM $MgCl_2$. All reactions were run on a GeneAmp PCR System 9700 thermal-cycler (PE Biosystems, Foster City, California) under the parameters given in Table 1. PCR products were separated on an ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, California) using an internal size standard (Mapmarker LOW, Bioventures, Inc., Murfreesboro, Tennessee). Genotyper 3.6 software (Applied Biosystems) was used for allele identification and comparison.

Data analysis.—Likelihood-based parentage testing was performed with the software program CERVUS 2.0 (Marshall et al. 1998) using the following analysis parameters: 10,000 cycles, 1% genotyping error rate, 80% relaxed confidence, and 95% strict confidence. GENEPOP software (version 3.1d—Raymond and Rousset 1995) was used to evaluate Hardy-Weinberg equilibrium, heterozygosity, and the number of alleles/locus for the 54 loci among the populations tested with the

TABLE 1.—Summary information for 54 nuclear microsatellite loci used in this study: range of alleles in base pairs (R_A), number of alleles observed (N_A), and observed heterozygosity (H_O) at Yellowstone National Park (YNP), Theodore Roosevelt National Park (TRNP), and the Texas State Bison Herd (TSBH).

Marker	Label[a]	Multiplex[b]	Chromosome (position)[c]	R_A	$N_{A\text{-YNP}}$	$N_{A\text{-TRNP}}$	$N_{A\text{-TSBH}}$	$H_{O\text{-YNP}}$	$H_{O\text{-TRNP}}$	$H_{O\text{-TSBH}}$
AGLA232	NED	83	13 (79.5)	155–173	5	5	2	0.65	0.60	0.35
BL1036	NED	85	14 (78.7)	177–193	4	4	2	0.55	0.64	0.45
BM1225	NED	2	20 (8.0)	239–271	5	6	3	0.73	0.73	0.68
BM1706	6-FAM	2	16 (80.6)	232–254	5	3	3	0.51	0.20	0.40
BM17132	6-FAM	1	19 (58.6)	85–95	6	4	3	0.70	0.63	0.13
BM1824	6-FAM	84	1 (108.6)	178–198	6	5	2	0.73	0.57	0.35
BM1862	6-FAM	80	17 (86.3)	201–215	5	6	3	0.70	0.84	0.43
BM188	HEX	84	26 (40.4)	99–123	7	4	4	0.95	0.05[d]	0.85
BM1905	NED	2	23 (64.3)	172–184	3	3	3	0.36	0.47	0.68
BM2113	6-FAM	2	2 (106.2)	127–153	4	7	2	0.63	0.64	0.23
BM2830	NED	86	5 (120.2)	142–166	10	6	4	0.79	0.81	0.63
BM4028	6-FAM	86	12 (79.7)	108–126	4	4	3	0.70	0.57	0.20
BM4107	HEX	85	20 (52.4)	165–185	5	5	3	0.68	0.72	0.48
BM4311	6-FAM	82	6 (89.7)	90–104	6	4	3	0.84	0.67	0.73
BM4440	NED	2	2 (55.0)	123–133	5	6	1	0.50	0.77	0.00
BM47	6-FAM	85	23 (9.1)	103–107	2	3	1	0.20	0.26	0.00
BM6017	HEX	82	X (4.7)	104–122	5	3	3	0.45[e]	0.49[e]	0.48[e]
BM711	6-FAM	82	8 (83.6)	161–175	4	2	2	0.56	0.17	0.38
BM720	VIC	2	13 (38.6)	203–235	7	4	3	0.85	0.66	0.43
BM757	HEX	83	9 (0.6)	190–200	6	4	2	0.56	0.23	0.40
BMC4214	HEX	84	3 (123.0)	175–187	5	4	1	0.71	0.67	0.00
BMS1001	NED	80	27 (5.1)	107–115	5	2	1	0.61	0.15	0.00
BMS1074	NED	80	4 (74.9)	154–160	4	4	3	0.54	0.63	0.75
BMS1117	HEX	3	21 (9.9)	89–99	3	4	2	0.59	0.66	0.18
BMS1172	6-FAM	3	4 (27.3)	86–104	6	5	4	0.61	0.58	0.48
BMS1315	HEX	85	5 (31.8)	135–149	4	3	2	0.64	0.53	0.20
BMS1355	NED	81	18 (2.8)	146–150	3	3	2	0.32	0.54	0.33
BMS1675	6-FAM	80	27 (64.1)	85–91	3	4	3	0.44	0.60	0.28
BMS1716	HEX	80	11 (47.7)	189–195	3	4	1	0.36	0.52	0.00
BMS1747	6-FAM	83	14 (4.2)	95–103	4	3	3	0.65	0.53	0.50
BMS1857	6-FAM	85	29 (0.9)	142–168	6	7	2	0.76	0.73	0.38
BMS1862	VIC	1	24 (32.8)	142–170	8	6	3	0.73	0.64	0.43
BMS2258	HEX	81	7 (75.0)	127–150	5	5	4	0.75	0.80	0.73
BMS2639	6-FAM	3	18 (57.0)	168–186	4	6	4	0.70	0.77	0.73
BMS410	NED	1	12 (0.0)	79–97	4	3	3	0.57	0.39	0.68
BMS510	VIC	1	28 (22.1)	91–95	4	4	2	0.74	0.66	0.33
BMS527	6-FAM	1	1 (55.9)	163–177	6	5	4	0.73	0.63	0.65
BMS528	6-FAM	83	10 (19.0)	140–152	5	4	3	0.82	0.67	0.30
BMS601	6-FAM	81	19 (99.5)	172–180	5	4	3	0.58	0.10	0.15
BMS812	NED	86	15 (68.8)	90–122	5	5	1	0.50	0.75	0.00
BMS911	HEX	81	X (136.2)	100–112	4	2	1	0.36[e]	0.53[e]	0.00[e]
BMS941	NED	83	17 (30.1)	81–83	2	2	2	0.46	0.13	0.25
HUJ246	NED	80	3 (67.9)	242–264	4	5	4	0.58	0.71	0.65
IL4	6-FAM	84	7 (30.5)	83–105	8	7	2	0.76	0.66	0.08
ILSTS102	NED	85	25 (6.5)	113–147	3	4	2	0.62	0.36	0.30
INRA037	6-FAM	81	10 (69.9)	120–132	4	4	3	0.64	0.73	0.35
INRA133	HEX	82	6 (8.2)	223–240	5	3	3	0.41	0.29	0.10
INRA189	NED	82	Y[f]	96	1	1	1	—	—	—
INRA194	HEX	86	22 (21.8)	144–160	4	3	3	0.73	0.43	0.45
RM372	VIC	1	8 (19.1)	118–136	5	5	3	0.68	0.81	0.40
TGLA122	NED	82	21 (67.3)	136–150	5	3	3	0.77	0.50	0.80
TGLA44	NED	84	2 (0.8)	149–159	6	3	2	0.70	0.57	0.03
TGLA53	6-FAM	86	16 (40.3)	134–140	3	3	3	0.50	0.64	0.55
URB011	6-FAM	URB011	29 (55.6)	143–153	6	6	2	0.78	0.74	0.43
Average					4.74	4.15	2.54	0.63	0.57	0.38
Standard deviation					1.58	1.38	0.91	0.15	0.20	0.24

[a] Fluorescent label used with forward primer (Applied Biosystems).

[b] Thermal parameters: 96°C 3 min; 4 cycles of 96°C 20 s, 58°C 30 s (−1°C/cycle), 65°C 90 s; 26 cycles of 96°C 20 s, 54°C 30 s, 65°C 90 s; 1 cycle of 96°C 60 s, 54°C 60 s, 65°C 20 min. For multiplex 84, the annealing temperature was increased to 56°C.

[c] As reported in the USDA cattle gene mapping database.

[d] Possible null allele present.

[e] Calculated on female population only.

[f] In non-pseudoautosomal region (Liu et al. 2002).

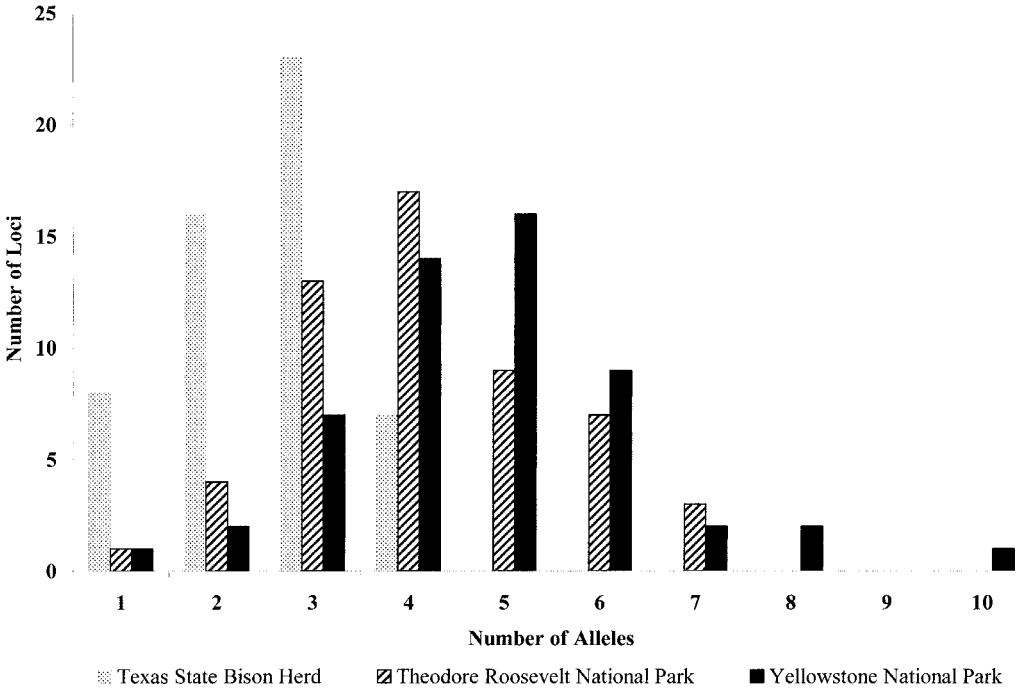

FIG. 1.—Allelic distribution for 54 nuclear microsatellite loci from 3 bison populations.

following Markov chain parameters: 10,000 step dememorization, 125 batches, and 40,000 iterations per batch. Additionally, heterozygosity and number of alleles per locus were compared between the Texas State Bison Herd calves born from 1998 to 2001 ($n = 15$) and extant adults ($n = 25$) for the 51 autosomal loci. All comparisons between populations of average heterozygosity and number of alleles were made using the standard unpaired *t*-test with $P < 0.0001$ considered significant. Genetic differentiation was investigated through F_{ST} (Weir and Cockerham 1984) evaluations using the program FSTAT (FSTAT, version 2.9.3.2. Lausanne, Switzerland: Institute of Ecology; www.unil.ch/izea/softwares/fstat.html—Goudet 2002).

Population viability analysis was performed using the program VORTEX version 8.42 (Lacy 1993) under the following conditions: simulation time 100 years, 10,000 iterations, breeding polygynous with 10% of adult males in breeding pool (based on parentage data below), no density dependence, and no correlation between probabilities of reproduction and survival due to environmental variation. Stable age distribution was used due to inadequate records of ages prior to 1997. Natality and mortality rates were calculated directly from Texas State Bison Herd records (Halbert et al. 2004), with an assumed standard deviation of 5%. In the 1st evaluation, inbreeding was included using the default parameters (3.14 lethal equivalents and 50% of genetic load due to lethal alleles) and 2 separate conservatively estimated stochastic events, each with a probability of 1% per year. The multiplicative effects on reproduction and survival were 0.6 and 0.8, respectively for the 1st stochastic event and 0.8 and 0.6, respectively for the 2nd stochastic event (scale: 0 = total loss, 1 = no effect). Simulations were completed with even less stringent stochastic event estimations, but the results were not significantly different. The 2nd evaluation was designed as above, but did not include inbreeding effects.

RESULTS

Metaphase chromosome preparations were obtained from 29 of the 31 Texas State Bison Herd cultures (12 males, 17 females). All 29 animals had normal karyotypes (2n = 60, XY male; and 60, XX female) with no detectable chromosome rearrangements.

All samples were completely genotyped to at least 99% completion for each of the 54 nuclear microsatellites used in this study. Eight monomorphic loci were detected in the Texas State Bison Herd compared with only 1 detected in each of the other populations (INRA189; Table 1). Of the 46 polymorphic autosomal loci in the Texas herd, 3 significantly deviated from Hardy-Weinberg expectations ($P < 0.05$: BM4028, BMS601, INRA133). Number of alleles per locus, size ranges, and heterozygosity values for all 3 populations compared in this study are found in Table 1. The distribution of alleles per locus is negatively shifted in the Texas herd when compared to Yellowstone National Park and Theodore Roosevelt National Park, as shown in Fig. 1. The average number of alleles per locus and heterozygosity in the Texas herd were significantly lower ($P < 0.0001$) when compared with either of the other populations.

The overall F_{ST} values across all 51 nuclear autosomal loci and 240 bison from 3 populations averaged 0.266 ± 0.017 *SE*. Pairwise F_{ST} values calculated by comparison of individual populations were as follows: Texas State Bison Herd compared to Theodore Roosevelt National Park, 0.3227; Texas State Bison Herd compared to Yellowstone National Park, 0.2513; Theodore Roosevelt National Park compared to Yellowstone National Park, 0.1583. Nine alleles present in the Texas herd were not found in the 200 tested bison from the other 2 populations (Appendix I).

Extant adults of the Texas State Bison Herd averaged 38.7% ± 1.4 *SD* heterozygosity and 2.59 ± 0.88 alleles/locus, whereas the calf group averaged 35.8 ± 1.7% heterozygosity

and 2.41 ± 0.85 alleles/locus (differences between groups not statistically significant). There is a 6.8% difference in the total number of alleles present in the current adult population (132 alleles) but absent from the calf population from 1998 and 2001 (123 alleles). Parentage was established with confidence for offspring born between 1998 and 2001, revealing that 5 bulls and 11 cows produced the 15 tested progeny. The bull producing the most progeny sired 6 offspring (40%), with an average for all 5 bulls of 3.00 ± 2.12 offspring/adult male whereas the most productive cow had 3 offspring (20%), with an average for all 11 cows of 1.36 ± 0.45 offspring/adult female. Semen samples from 4 of the 5 males that sired offspring displayed normal motility and morphology in 2000. The remaining sample demonstrated normal motility with limited morphological abnormalities.

Under the 1st population viability evaluation with conservative stochasticity and inbreeding, the probability of extinction of the Texas herd in 100 years was 0.9997 ± 0.0002 *SE*, with an average of 40.59 ± 0.13 years. Under the 2nd evaluation with conservative stochasticity and no inbreeding effects, the probability of extinction in 100 years was 0.9812 ± 0.0014, with an average of 45.71 ± 0.17 years.

Discussion

Of the 9 alleles found exclusively within the Texas State Bison Herd in this study, 8 have been previously reported as occurring in bison and/or domestic cattle (*Bos taurus*). Ward (2000) compared 64 domestic cattle including 5 breeds and 51 bison from 4 Canadian and 10 United States public herds and reported the BM4028 (126) and HUJ246 (242, 252) alleles solely in domestic cattle. Ward (2000) also found the BMS1857 (146) and INRA194 (160) alleles in bison (allele INRA194 [160] was reported as [159]). The BMS410 (95) and RM372 (128) alleles were detected in bison and domestic cattle by Schnabel et al. (2000) in a comparison of 903 bison from 4 Canadian and 10 United States public herds as well as 2 private herds and 107 domestic cattle which included 5 breeds. The TGLA53 (138) allele has also been observed in bison and domestic cattle (R. D. Schnabel, pers. comm.). However, the ILSTS102 (113) allele appears exclusive to the Texas herd. Though this allele alone does not substantiate subspecies status for these animals, it does demonstrate the unique genetic composition of this herd and corroborate previous genetic evaluations (Ward 2000).

A minimum effective population size (N_e) of 50 individuals is commonly used as a population management goal to minimize inbreeding for short-term population survival (Franklin 1980; Soulé 1980). If all adults from the Texas herd are considered (10 males, 15 females), then $N_e = 24$ (Caballero 1994; Wright 1931). The N_e formula of Lande and Barrowclough (1987) uses N_{em} and N_{ef} as the number of effective males and females (calculated here as 4.0 and 20.1, respectively) based on variance in offspring number, which in this case results in an effective population size of 13.3. This N_e estimate is lower than the previous calculation due to polygynous mating in bison, and indicates that genetic diversity will be lost at a rate equivalent to an idealized population of approximately 13 individuals. Regardless of how N_e is calculated, the effective population size of the Texas State Bison Herd is substantially lower than the recommended short-term minimum of 50 individuals.

Genetic drift is expected to decrease genetic diversity at a rate inversely proportional to population size (Lacy 1987). Loss of genetic diversity is demonstrated in the Texas State Bison Herd by a 6.8% difference in the total number of alleles present in the calf and adult populations. The rate of erosion of genetic diversity is estimated by this statistic, since the calves were sampled over a 4-year period, representing the approximate generation time in bison (Berger and Cunningham 1994). Clearly, genetic drift is currently causing a reduction in genetic diversity, and will continue to do so as long as the effective population size remains dangerously small.

The 3 populations examined in this study are genetically distinct, as measured through F_{ST} values, variation in allele sizes and frequencies at each locus, and differences in observed levels of heterozygosity. These results are consistent with the known histories of these herds in that each has been maintained in isolation for a minimum of 50 years (see below). Consequently, genetic drift has acted on the initial genetic makeup of each population, which might or might not have been similar, resulting in high levels of genetic differentiation. That more genetic distinction exists between the Texas State Bison Herd and either Yellowstone National Park or Theodore Roosevelt National Park than between the latter two was also anticipated due to the chronically small size of the Texas herd, which increases the effects of drift and further serves to differentiate populations genetically.

Closed populations that have survived 1 or more population bottlenecks, especially when followed by consistently small census population sizes, will generally display an overall loss of genetic diversity (Nei et al. 1975). Empirical examples of populations with reduced genetic variability following historic bottleneck events are abundant, including the Alpine ibex (Maudet et al. 2002), black-footed ferret (Wisely et al. 2002), cheetah (O'Brien et al. 1983), elephant seal (Bonnell and Selander 1974; Hoelzel et al. 1993), Florida panther (Roelke et al. 1993), and greater prairie chicken (Bouzat et al. 1998). The survival of closed populations, however, is likely affected much less by initial population size than by maintenance population size due to the consequences of genetic drift in continuously small populations (Nei et al. 1975; Senner 1980). In fact, Senner (1980) reports that increasing the initial population size above 5 has little theoretical effect on long-term population survival, but that small increases in the maintenance population size have dramatic effects on the probability of long-term population survival. These theoretical results are congruent with historical data for the Texas State Bison Herd when compared to other extant bison herds. For example, Yellowstone National Park was founded with between 30 and 50 bison in 1902 (Coder 1975) and Theodore Roosevelt National Park with 29 bison in 1956 (M. Oehler, pers. comm.). Both of these populations grew quickly and neither experienced additional long-term population bottleneck events. Current census sizes for these populations are approximately 2,500 to 3,000 bison in Yellowstone

National Park and 750 bison in Theodore Roosevelt National Park.

Inbreeding increases at a rate inversely proportional to population size, thereby resulting in a single common lineage among all individuals of a closed population given ample generations (Senner 1980). Inbreeding is known to have widespread detrimental effects in naturally outbreeding mammalian species. Examples include low birth weight, decreased litter sizes, increased mortality, and increased sterility in Poland China swine (McPhee et al. 1931); cryptorchism, high levels of defective sperm, and heart defects in the Florida panther (Roelke et al. 1993); low sperm counts and high juvenile mortality rates in cheetahs (O'Brien et al. 1985); increased rates of juvenile mortality in several ungulate species (Ralls et al. 1979); and apparent vulnerability to infectious diseases in several mammalian species (O'Brien and Evermann 1988). The past 120 years of multiple population bottlenecks and chronically small population size have likely led to elevated levels of inbreeding in the Texas State Bison Herd. Several demographic features of the current population concur with documented examples of inbreeding depression, such as low natality rates, probable male infertility, and high calf mortality rates (Halbert et al. 2004). Although chromosomal aberrations present in even a few adults might have accounted for recruitment problems in this population, no such abnormalities were revealed through karyotyping, although small aberrations might be present but undetectable through these methods. Three of the tested males exhibiting sperm motility and morphology abnormalities did not sire any offspring in the 4-year test period, serving to decrease the effective population size and increase inbreeding in the Texas herd. Drift has likely compounded the issue of reduced fitness through the random loss of potentially important alleles and fixation of deleterious mutations (Lande 1994). This reduction in fitness further explains the nearly stagnant growth rate of this population compared with other closed bison populations.

Population viability analysis, including conservative stochasticity and inbreeding effects, reveals that the Texas State Bison Herd has a 99% chance (mean + 2.58 *SE*) of extinction within the next 41 years. Furthermore, simulation modeling of these data has shown that given the current mortality and natality rates, genetic diversity and heterozygosity are expected to continue to decline at significant rates (Halbert et al. 2004). There are very few examples of populations that have recovered in census size following bottleneck events despite apparent lack of genetic variation (e.g., elephant seal—Bonnell and Selander 1974; Hoelzel et al. 1993), and the long-term fitness effects on such populations are unknown. However, the Texas State Bison Herd has not shown any trend towards increased recruitment or decreased calf mortality rates in the past 6 years of intense management and care (Halbert et al. 2004). Although it is remotely possible that this population might survive the current inbreeding depression through purging of deleterious alleles, the end result would likely be a further reduction in genetic variation, increasing the probability of catastrophic demise by disease or natural disaster (Franklin 1980; Soulé 1980). Furthermore, the herd already exhibits low heterozygosity, which has been associated with an increased risk of extinction (Saccheri et al. 1998). The simulated addition of a few (up to 9) unrelated breeding males, under the assumption of increased fitness, results in steady and positive growth rates, increased heterozygosity, and slower disintegration of genetic diversity over the next 100 years (Halbert et al. 2004).

It is our conclusion that the best remedy for the perilous demographic and genetic condition faced by the Texas State Bison Herd is the introduction of genetic variation through deliberate transplantation of bison into this population for the following reasons. First, although the allocation of this population to a separate bison subspecies is not warranted, the herd has a unique history and genetic constitution, making it an irreplaceable source of bison genetic variation. As such, the conservation and expansion of this population is of paramount importance. Second, the chronically small population size of the herd has led to low genetic variation and decreased heterozygosity as a compounded consequence of genetic drift and inbreeding, which in turn have negatively influenced the overall fitness and growth rate of this population. Third, the dangerously low effective population size suggests that even short-term survival of the herd is threatened. Finally, the addition of bison alleles from an outside source will enhance overall genetic diversity and most likely increase fitness and adaptive response in this population, providing the best chance for long-term survival (Couvet 2002; Lewontin and Birch 1966; Spielman and Frankham 1992).

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APPENDIX I

Texas State Bison Herd allelic frequencies for 54 microsatellite loci. For each locus, approximate called allele sizes (base pairs) and frequencies are given. Alleles present in the Texas State Bison Herd and not found in the other 2 tested populations are indicated by *.

Locus	Allele (frequency)			
AGLA232	161 (0.375)	165 (0.625)		
BL1036	191 (0.425)	193 (0.575)		
BM1225	241 (0.450)	253 (0.150)	269 (0.400)	
BM1706	232 (0.150)	238 (0.750)	250 (0.100)	
BM17132	85 (0.025)	87 (0.038)	91 (0.938)	
BM1824	180 (0.275)	198 (0.725)		
BM1862	205 (0.775)	207 (0.087)	215 (0.138)	
BM188	99 (0.250)	115 (0.062)	117 (0.400)	121 (0.287)
BM1905	172 (0.050)	176 (0.488)	184 (0.463)	
BM2113	143 (0.887)	145 (0.112)		
BM2830	148 (0.525)	152 (0.112)	158 (0.312)	164 (0.050)
BM4028	116 (0.075)	118 (0.188)	126 (0.738)*	
BM4107	165 (0.688)	179 (0.100)	183 (0.213)	
BM4311	90 (0.250)	98 (0.338)	104 (0.412)	
BM4440	125 (1.000)			
BM47	103 (1.000)			
BM6017	114 (0.213)	116 (0.672)	118 (0.115)	
BM711	161 (0.387)	167 (0.613)		
BM720	225 (0.400)	231 (0.038)	233 (0.562)	
BM757	194 (0.775)	200 (0.225)		
BMC4214	181 (0.988)	185 (0.013)		
BMS1001	113 (1.000)			
BMS1074	156 (0.225)	158 (0.325)	160 (0.450)	
BMS1117	89 (0.087)	91 (0.913)		
BMS1172	88 (0.025)	100 (0.013)	102 (0.350)	104 (0.613)
BMS1315	135 (0.125)	137 (0.875)		
BMS1355	146 (0.837)	150 (0.162)		
BMS1675	87 (0.013)	89 (0.800)	91 (0.188)	
BMS1716	191 (1.000)			
BMS1747	95 (0.675)	99 (0.262)	103 (0.062)	
BMS1857	146 (0.712)*	168 (0.287)		
BMS1862	160 (0.738)	164 (0.112)	170 (0.150)	
BMS2258	127 (0.463)	138 (0.112)	140 (0.188)	148 (0.237)
BMS2639	168 (0.475)	172 (0.050)	176 (0.062)	186 (0.412)
BMS410	83 (0.138)	89 (0.525)	95 (0.338)*	
BMS510	91 (0.213)	92 (0.788)		
BMS527	167 (0.412)	173 (0.050)	175 (0.500)	177 (0.038)
BMS528	146 (0.175)	148 (0.025)	150 (0.800)	
BMS601	172 (0.863)	174 (0.050)	180 (0.087)	
BMS812	90 (1.000)			
BMS911	104 (1.000)			
BMS941	81 (0.875)	83 (0.125)		
HUJ246	242 (0.525)*	252 (0.087)*	262 (0.112)	264 (0.275)
IL4	93 (0.038)	103 (0.962)		
ILSTS102	113 (0.150)*	143 (0.850)		
INRA037	122 (0.700)	124 (0.262)	126 (0.038)	
INRA133	223 (0.025)	238 (0.150)	240 (0.825)	
INRA189	96 (1.000)			
INRA194	154 (0.675)	156 (0.300)	160 (0.025)*	
RM372	128 (0.050)*	130 (0.188)	134 (0.762)	
TGLA122	140 (0.412)	142 (0.387)	148 (0.200)	
TGLA44	153 (0.013)	155 (0.988)		
TGLA53	134 (0.162)	136 (0.550)	138 (0.287)*	
URB011	147 (0.237)	149 (0.762)		

APPENDIX I.—Continued.