

Genetic Variation and Differentiation of Bison (*Bison bison*) Subspecies and Cattle (*Bos taurus*) Breeds and Subspecies

MATTHEW A. CRONIN, MICHAEL D. MACNEIL, NINH VU, VICKI LEESBURG, HARVEY D. BLACKBURN, AND JAMES N. DERR

From the University of Alaska Fairbanks, School of Natural Resources and Agricultural Sciences, Matanuska Experiment Farm, 1509 South Georgeson Drive, Palmer, AK 99645 (Cronin and Vu); Delta G, 145 Ice Cave Road, Miles City, MT 59301 (MacNeil); USDA Agricultural Research Service, Fort Keogh Livestock and Range Research Laboratory, Miles City, MT 59301 (Leesburg); USDA Agricultural Research Service, National Animal Germplasm Program, 1111 South Mason St, Fort Collins, CO 80523 (Blackburn); and Department of Veterinary Pathobiology, Texas A&M University, College Station, TX 77845-4467 (Derr).

Address correspondence to Matthew A. Cronin at the address above, or e-mail: macronin@alaska.edu.

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Abstract

The genetic relationship of American plains bison (*Bison bison bison*) and wood bison (*Bison bison athabascae*) was quantified and compared with that among breeds and subspecies of cattle. Plains bison from 9 herds ($N = 136$), wood bison from 3 herds ($N = 65$), taurine cattle (*Bos taurus taurus*) from 14 breeds ($N = 244$), and indicine cattle (*Bos taurus indicus*) from 2 breeds ($N = 53$) were genotyped for 29 polymorphic microsatellite loci. Bayesian cluster analyses indicate 3 groups, 2 of which are plains bison and 1 of which is wood bison with some admixture, and genetic distances do not show plains bison and wood bison as distinct groups. Differentiation of wood bison and plains bison is also significantly less than that of cattle breeds and subspecies. These and other genetic data and historical interbreeding of bison do not support recognition of extant plains bison and wood bison as phylogenetically distinct subspecies.

Key words: cattle breeds, genetic variation, microsatellite DNA, plains bison, subspecies, wood bison

Introduction

Two subspecies of bison (*Bison bison*) have been recognized in North America. Plains bison (*B. bison bison*) range was historically across much of the United States and southwestern Canada, wood bison (*B. bison athabascae*) occurred in northwestern Canada, and their original ranges were contiguous (Potter et al. 2010). The subspecies designations are based on morphology (i.e., skull, horn, and body proportions and size, hair patterns), but there is not a consensus on their validity (McDonald 1981; Reynolds et al. 1982; van Zyll de Jong 1986, 1993; Geist 1991; van Zyll de Jong et al. 1995; Boyd et al. 2010a), and genetic studies have not supported plains bison and wood bison as subspecies (Stormont et al. 1961; Ying and Peden 1977; Peden and Kraay 1979; Bork et al. 1991; Cronin 1993; Cronin and Cockett 1993; Polziehn et al. 1996; Halbert et al. 2005; Douglas et al. 2011). There

are microsatellite allele frequency differences between some herds of wood bison and plains bison, but all extant wood bison herds contain genetic material from plains bison after the introduction of plains bison into Wood Buffalo National Park in 1925–1928 (Geist 1991; Wilson and Strobeck 1999). Despite the uncertainty of the designation of subspecies, wood bison are considered a threatened subspecies under US and Canadian endangered species laws (Aune and Wallen 2010, Federal Register 2012), so their taxonomic status is relevant to conservation and management (Boyd et al. 2010a, 2010b).

Bison and cattle (*Bos taurus*) are closely related, and bison are sometimes classified as *Bos bison* (Boyd et al. 2010a), so relative levels of genetic differentiation of bison and cattle may be informative regarding intraspecies taxonomy. Cattle subspecies include the taurine cattle (*Bos taurus taurus*) and indicine cattle (*B. t. indicus*), which are genetically

differentiated, adapted to either tropical (*indicus*) or temperate (*taurus*) environments, and readily interbreed with the hybrids exhibiting heterosis (Loftus et al. 1994; MacHugh 1996; MacHugh et al. 1997, 1998; Fries and Ruvinsky 1999; McKay et al. 2008; Chan et al. 2010). Modern domestic breeds within each subspecies are groups with separate genealogy and selection history (e.g., Cleveland et al. 2005) and provide a standard of genetic divergence comparable with populations with little or no gene flow between them.

Previous studies with microsatellites have compared genetic variation in bison and cattle and identified interspecies introgression (MacHugh et al. 1997; Ritz et al. 2000; Schnabel et al. 2000; Halbert et al. 2005; Hedrick 2009). However, comparative assessment of bison and cattle subspecies has not been done. Our objective is to quantify the microsatellite variation within and between the putative bison subspecies, and compare levels of genetic divergence with those of cattle breeds and subspecies.

Materials and Methods

Animals from 9 herds of plains bison and 3 herds of wood bison including ancestor–descendant herds, and cattle from 14 taurine breeds and 1 herd of unknown ancestry from U.S. populations and 2 indicine breeds were sampled (Table 1). DNA was extracted from bison tissues with organic extractions (Cronin and Cockett 1993) and the Qiagen (Valencia, CA) DNeasy Tissue Kit, except the Alaska wood bison for which DNA was extracted from blood (MacNeil et al. 2007). Taurine cattle DNA samples were provided by the National Animal Germplasm Program (Blackburn 2009). Indicine cattle blood samples were obtained from EMBRAPA of Brazil and the US National Animal Germplasm Program and were collected on FTA™ Elute Micro Card (GE Life Science, Pittsburgh, PA).

Genotypes were obtained for bison and cattle for 34 microsatellite loci that are not linked in the cattle genome (see Supplementary Table 1 online). The taurine cattle genotypes previously reported by MacNeil et al. (2006, 2007) were generated on a Licor 4300 DNA Analyzer (Li-Cor, Lincoln, NE). We obtained genotypes for all bison and indicine cattle using an Applied Biosystems 3100 Genetic Analyzer system (Life Technologies, Carlsbad, CA) using 4 fluorescently labeled M13(-29) primers (6FAM, VIC, PET, and NED). We used Bionline MyTaq HS DNA Polymerase Kit and the manufacturer's recommended conditions for rapid amplification (Bionline, Tauton, MA). Polymerase chain reactions (PCRs) for all loci consisted of 2 μ L 5 \times MyTaq Reaction Buffer, 1 μ M each of forward, reverse, and labeled M13(-29) primers, 0.1 Unit MyTaq DNA Polymerase, 50 ng/ μ L DNA template and water for a 10 μ L reaction. For the indicine cattle, 1–2 mm² of FTA™ paper with blood was washed with the manufacturer's suggested short tandem repeat (STR) protocol with an extra wash including 0.1 mAU concentration of proteinase-K and used directly in 15 μ L PCRs. Thermoprofiles were the same for all loci, with the exception of annealing temperature (see Supplementary Table 1 online), and consisted of 1 denaturation step at 95 °C for 1 min, followed by 35 cycles

under the following conditions (95 °C for 15 s; respective T_a for 15 s, 72 °C for 10 s), and a final extension step at 72 °C for 2 min. Genotype scoring was done automatically with GeneMapper (v.3.7) and manually inspected for accuracy. We standardized the allele sizes for the Li-Cor and ABI systems by genotyping 22 taurine cattle on both systems.

For data analysis, we did not use the loci that were monomorphic in bison so the measures of genetic variation for bison were not biased downward relative to cattle. We calculated the mean number of alleles per locus (*A*), observed heterozygosity (*H*_o), and expected heterozygosity (*H*_e) with the Microsatellite Toolkit program (Park 2001) and identified private alleles for each species and potential subspecies. Allelic richness (AR) was calculated with the HP-Rare program version (6 June 2006) (Kalinowski 2005). Pairwise *F*_{st} between herds and breeds (Weir and Cockerham 1984), inbreeding coefficient *F*_{is}, analyses of Hardy–Weinberg equilibrium and linkage disequilibrium were calculated with the Genepop program Ver.3.3 (Raymond and Rousset 1995). We calculated genetic distances (*D*_s; Nei 1972) for each pair of herds and breeds with the program Populations 1.2.32 (Langella 1999).

We calculated *D*_s and *F*_{st} between each pair of herds and breeds, and compared these measures between bison herds, cattle breeds, and bison and cattle subspecies, considering all of the bison herds and only herds with more than or equal to 10 samples (i.e., excluding the Copper River, Chitna, Farewell Alaska, Miner Institute, and Nielson plains bison herds), with a 2-tailed \bar{z} test of the means of inter-herd distances for each group (plains bison, wood bison, taurine cattle, and indicine cattle) considering a significance level of *P* < 0.05. *D*_s and *F*_{st} were used in cluster analysis with the neighbor-joining method (NJ; Saitou and Nei 1987) in MEGA version 5 (Tamura et al. 2011) to generate dendrograms. We also calculated *D*_s and *F*_{st} with samples combined for each of the 4 potential subspecies (plains bison, wood bison, taurine cattle, and indicine cattle).

We used the Bayesian clustering method with no a priori assignment of individuals to populations with the STRUCTURE program (Pritchard et al. 2000; Falush et al. 2003). We ran STRUCTURE for *K* = 1 to *K* = 29 (bison and cattle) and for *K* = 1 to *K* = 12 (bison only), where *K* is number of assumed populations. We used a 20 000 sample burn-in period and 100 000 Markov chain Monte Carlo sample repetitions with the admixture model. We did 10 replicates of this procedure for each *K* value. The log probability of data Ln*P*(*D*) and the statistic ΔK (Evanno et al. 2005) were estimated for each value of *K*. ΔK quantifies the rate of change of Ln*P*(*D*) between successive *K* values, and the highest ΔK is the most likely *K* in situations when *K* is not clearly indicated by Ln*P*(*D*) values (Evanno et al. 2005; Pritchard et al. 2010).

Results

Microsatellite genotypes were obtained for 201 bison and 321 cattle. Alleles for all of the loci are dinucleotide repeats. Five loci also had some alleles that differed by 1 nucleotide (BMS1315, BMS2614, BMS468, BMS510, and ILSTS028).

Table 1 Bison and cattle herds measures of genetic variation for 29 polymorphic microsatellite loci

Subspecies/breed/location/year collected	Ancestral herd ^a	Sample size	H_e	H_o	Average alleles per locus	Allelic richness	F_{is}
Bison							
Plains bison							
Alaska Copper River 2011	National Bison Range	7	0.46	0.41	2.6	2.6	0.12
Alaska Chitna River 2011	National Bison Range	2	0.48	0.45	1.9	1.9	0.11
Alaska Farewell Lake 2008	National Bison Range	2	0.48	0.48	2.0	2.0	-0.02
Alaska Delta 2011	National Bison Range	31	0.47	0.47	3.5	3.2	0.01
New York Miner Agriculture Institute ^{b,c} 1984	Unknown	4	0.50	0.50	2.7	2.7	0.01
Canada Nielson private herd ^f ~1992	Unknown	9	0.49	0.45	3.5	3.5	0.10
Montana National Bison Range ^c ~1985	Mixed	24	0.49	0.46	3.7	3.5	0.07
Montana/Wyoming Yellowstone NP ~1985	Native and mixed	28	0.52	0.52	3.8	3.5	-0.01
Utah Henry Mts ^c 1984	Yellowstone National Park	29	0.47	0.45	3.1	2.9	0.40
Wood bison							
Canada MacKenzie Bison Sanctuary ^c ~1985	Wood Buffalo National Park	10	0.48	0.46	3.0	3.0	0.03
Canada Wood Buffalo NP ^c ~1985	Native and National Bison Range	30	0.50	0.48	4.1	3.6	0.04
Alaska Wildlife Conservation Center 2010	Wood Buffalo National Park	25	0.44	0.38	3.2	3.0	0.12
Bison average		201	0.48	0.46	3.1	2.9	0.08
Cattle							
Taurine cattle breeds							
Alaska Chirikof Island mixed breeds ^d 2005	Mixed American breeds possibly Russia	24	0.58	0.60	4.3	4.0	-0.04
Simmental ^d	Switzerland	19	0.56	0.56	4.7	4.5	0.01
Scotch Highland ^d	Scotland	18	0.55	0.56	4.0	3.9	-0.03
Saler ^d	France	21	0.63	0.61	5.2	4.9	0.04
Hereford ^d	England	21	0.58	0.56	4.6	4.3	0.03
Limousin ^d	France	17	0.62	0.59	4.8	4.7	0.05
Charolais ^d	France	18	0.63	0.64	5.4	5.2	-0.01
Red Angus ^d	Scotland	18	0.62	0.60	4.6	4.4	0.02
Tarantaise ^d	France	9	0.64	0.67	4.1	4.1	-0.05
Shorthorn ^d	England	15	0.56	0.54	4.1	4.1	0.04
Texas Longhorn ^d	Texas	13	0.65	0.66	4.9	4.9	-0.02
Pineywoods ^d	Gulf Coast United States	18	0.67	0.59	5.2	5.0	0.13
Florida Cracker ^d	Florida	5	0.63	0.62	3.4	3.5	0.02
Criollo ^d	Spain	30	0.68	0.66	6.6	5.6	0.03
Angus ^d	Scotland	22	0.61	0.59	4.7	4.4	0.03
Indicine cattle breeds							
Nellore Brazil	Brazil from India	21	0.62	0.56	5.0	4.8	0.09
Gyr Brazil	Brazil from India	32	0.68	0.59	6.3	5.5	0.13
Cattle average		321	0.62	0.60	4.8	4.6	0.03

^aHerd history references: [Coder \(1975\)](#); [Polzella et al. \(1996\)](#); [Wilson and Strobeck \(1999\)](#); [Potter et al. \(2010\)](#).^bExtirpated herd, established prior to 1908 at Heart's Delight Farm Chazy, NY, we assumed to be plains bison.^cIncludes samples from [Cronin and Cockett \(1993\)](#).^dSamples from [MacNeil et al. \(2006\)](#), (2007).

All 34 of the loci were polymorphic in cattle. Four loci (BMS836, CSSM036, CSSM038, and BMS1316) were monomorphic in bison and 1 locus (ILSTS059) did not amplify reliably in bison. These 5 loci were excluded, leaving 29 loci in the analysis (see [Supplementary Table 1](#) online).

Variation at the 29 loci was higher in cattle than in bison ([Table 1](#)). There are 12 cases in which genotypes are not in Hardy–Weinberg equilibrium considering a significance level of $P < 0.000065$ after Bonferroni adjustment ($\alpha = 0.05$ with 772 tests), including 11 cases with heterozygote deficiency and 1 case with heterozygote excess (see [Supplementary Table 1](#) online). These deviations may be due to small sample sizes, nonrandom mating, selection, or other violations of Hardy–Weinberg assumptions in the herds sampled, and we included these loci and herds in our analysis.

The 29 polymorphic loci are not physically linked in the cattle genome ([MacNeil et al. 2007](#)). Of all pairwise comparisons of the 29 loci, 5 pairs of loci showed significant linkage disequilibrium for the combined bison and cattle genotypes considering a significance level of 0.00012 after Bonferroni adjustment ($\alpha = 0.05$ with 406 tests): AGLA227 and BMS1247; BL1029 and BMS510; BMS2614 and BMS510; BMS468 and BMS574, and BMS510 and ILSTS028. However, the loci in each pair are on different chromosomes in the cattle genome (see [Supplementary Table 1](#) online), and cattle and bison have the same chromosome number ([Bhambhani and Kuspira 1969](#)), but the chromosomal location of these loci in bison is not known.

There are 389 alleles at the 29 polymorphic loci in bison and cattle. Three hundred forty alleles occur in the taurine and indicine cattle breeds, 7 additional alleles occur in only the Chirikof Island cattle herd, and 42 occur in only bison. Of the 340 alleles in cattle breeds, 140 (0.41) are in taurine cattle only (108 with frequency ≥ 0.05 in at least 1 breed), 55 (0.16) are in indicine cattle only (37 with frequency ≥ 0.05 in at least 1 breed), and 145 (0.43) are shared by the taurine and indicine cattle subspecies. One hundred sixty of the 389 alleles occur in bison, including 39 of 160 alleles (0.24) in plains bison only (27 with frequency ≥ 0.05 in at least 1 herd), 13 of 160 alleles (0.08) in wood bison only (11 with frequency ≥ 0.05 in at least 1 herd), and 108 of 160 alleles (0.68) shared by plains bison and wood bison (see [Supplementary Table 2](#) online).

The interspecies D_s values between the 12 bison herds and 16 cattle breeds are greater than the values within either species. Ranges of D_s values within and between plains bison and wood bison herds overlap ([Table 2](#) and see [Supplementary Table 3](#) online). The mean D_s between the 3 wood bison herds and 9 plains bison herds is not significantly different than the mean D_s between the plains bison herds ($P = 0.34$) or between the taurine cattle breeds ($P = 0.09$), but is significantly less than the mean D_s between the taurine and indicine subspecies ($P < 0.01$; see [Supplementary Table 3](#) online). Considering only the bison herds with $N \geq 10$, the mean D_s between the wood bison herds and plains bison herds is not significantly different than the mean D_s between plains bison herds ($P = 0.15$), and the D_s between the taurine

cattle breeds ($P < 0.01$) and between the taurine and indicine subspecies ($P < 0.01$) are significantly greater than the D_s between wood bison and plains bison herds.

Like D_s , the interspecies F_{st} is greater than the intraspecies values, and F_{st} values overlap among the plains and wood bison herds ([Table 2](#) and see [Supplementary Table 3](#) online). The mean F_{st} between plains bison herds and wood bison herds is significantly greater than between plains bison herds ($P < 0.01$), but significantly less than the mean F_{st} between taurine cattle breeds ($P < 0.01$) and the taurine and indicine subspecies ($P < 0.01$). Considering only the bison herds with $N \geq 10$, the mean F_{st} between wood bison herds and plains bison herds is not significantly different than between plains bison herds ($P = 0.18$), and is significantly less than the mean F_{st} between the taurine cattle breeds ($P < 0.01$) and between the taurine and indicine subspecies ($P < 0.01$).

When the samples are combined into 4 potential subspecies (plains bison, wood bison, taurine cattle, and indicine cattle), distances are considerably greater between the taurine and indicine subspecies ($D_s = 0.75$, $F_{st} = 0.19$) than between plains bison and wood bison ($D_s = 0.11$, $F_{st} = 0.09$) as for the inter-herd means, although the absolute values are less for the combined samples. The interspecies distances between the 16 cattle breeds and 12 bison herds are also smaller for the combined samples ($D_s = 1.18$, $F_{st} = 0.29$) than for the inter-herd means. The D_s and F_{st} values are smaller in the analysis of combined data because the inter-herd means give equal weight to each inter-herd distance regardless of the number of samples in them, whereas the pooled samples weigh contributions from each population in proportion to the number of samples in each.

The NJ analyses show bison, taurine cattle, and indicine cattle differentiated in separate clusters, whereas the plains bison and wood bison are not in separate clusters ([Figure 1](#)). In the D_s -derived NJ dendrogram, the 3 wood bison herds (including the ancestor–descendant Wood Buffalo National Park and Alaska wood bison herds) occur in a cluster with the Miner Institute plains bison, within a larger cluster containing the Neilson plains bison and the ancestor–descendant Yellowstone National Park and Henry Mountains plains bison. This cluster is separate from a cluster with the ancestor–descendant National Bison Range and the 4 Alaska plains bison herds ([Figure 1a](#)). In the F_{st} -derived NJ dendrogram, wood bison occur in a cluster within a larger cluster including the Yellowstone and Henry Mountains herds and 3 other plains bison herds. The National Bison Range and 3 of the 4 Alaska plains bison herds occur in a separate cluster ([Figure 1b](#)). The taurine and indicine cattle occur in separate clusters in both dendrograms, whereas the cattle breeds' relationships vary between the D_s and F_{st} dendrograms.

Results for the STRUCTURE analysis with 12 bison herds and 17 cattle breeds resulted in the greatest ΔK (451.4) indicating the best support for $K = 3$ groups. For $K = 4$, $\Delta K = 47.0$ and all other ΔK values are less than 1.6. The analysis with $K = 3$ separated the samples into 3 groups: 1 group with plains bison and wood bison combined with proportion of membership of 0.98–0.99, a second group with all taurine cattle with proportion of membership of 0.98–0.99,

Table 2 Summary of genetic distances (D_s and F_{st}) between bison and cattle herds for 29 microsatellite loci

Herds compared	All herds, D_s			Herds with $N > 10, D_s$			All herds, F_{st}			Herds with $N > 10, F_{st}$		
	Mean (SE) ^a	Range	95% CL ^b	Mean (SE) ^a	Range	95% CL ^b	Mean (SE) ^a	Range	95% CL ^b	Mean (SE) ^a	Range	95% CL ^b
Bison												
Plains bison versus plains bison herds	0.212 (0.015)	0.069–0.491	0.031	0.152 (0.025)	–	–	0.106 (0.010)	–	–	0.114 (0.020)	–	–
Wood bison versus wood bison herds	0.075 (0.014)	0.048–0.096	0.061	0.075 (0.014)	–	–	0.051 (0.018)	–	–	0.051 (0.018)	–	–
Plains bison versus wood bison herds	0.234 (0.017)	0.099–0.471	0.035	0.196 (0.017)	–	–	0.149 (0.011)	–	–	0.146 (0.014)	–	–
All bison herds versus all bison herds	0.215 (0.011)	0.048–0.491	0.023	0.166 (0.015)	–	–	0.121 (0.008)	–	–	0.124 (0.012)	–	–
Bison ancestor/descendent herds												
Yellowstone versus Henry Mountains plains bison	0.100 (NA)	N/A	N/A	0.100 (NA)	–	–	0.076 (NA)	–	–	0.076 (NA)	–	–
National, Bison Range versus Alaska plains bison	0.139 (0.026)	0.069–0.189	0.081	0.069 (NA)	–	–	0.055 (0.011)	–	–	0.051 (NA)	–	–
Canada wood bison versus Alaska wood bison	0.088 (0.008)	0.080–0.096	0.101	0.088 (0.008)	–	–	0.068 (0.003)	–	–	0.068 (0.003)	–	–
Alaska plains bison versus Alaska plains bison	0.184 (0.030)	0.103–0.279	0.078	N/A	–	–	0.049 (0.018)	–	–	0.068 (0.003)	–	–
Cattle												
Between 14 taurus breeds	0.265 (0.007)	0.105–0.432	0.015	–	–	–	0.106 (0.004)	–	–	0.106 (0.004)	–	–
Angus versus Red Angus breeds	0.105 (NA)	N/A	N/A	–	–	–	0.038 (N/A)	–	–	0.038 (N/A)	–	–
Chirikof Island Alaska versus 14 taurus breeds	0.407 (0.018)	0.306–0.583	0.039	–	–	–	0.171 (0.006)	–	–	0.171 (0.006)	–	–
Between 2 indicus breeds	0.223 (NA)	N/A	N/A	–	–	–	0.08 (N/A)	–	–	0.08 (N/A)	–	–
14 taurus breeds versus 2 indicus breeds	0.952 (0.030)	0.635–1.353	0.061	–	–	–	0.253 (0.008)	–	–	0.253 (0.008)	–	–
Cattle and Bison												
Between 16 cattle breeds and 12 bison herds ^c	1.356 (0.012)	1.035–2.023	0.024	–	–	–	0.364 (0.003)	–	–	0.364 (0.003)	–	–
Between 14 taurus breeds and 12 bison herds	1.366 (0.014)	1.035–2.023	0.027	–	–	–	0.368 (0.003)	–	–	0.368 (0.003)	–	–
Between 2 indicus breeds and 12 bison herds	1.287 (0.020)	1.149–1.500	0.041	–	–	–	0.336 (0.007)	–	–	0.336 (0.007)	–	–

^aMean (and standard error) values of pairwise genetic distances D_s and F_{st} (see [Supplementary Table 3](#) online) of the herds identified in [Table 1](#).^b95% Confidence level of the mean.^cIncludes mean of pairwise genetic distances of 12 bison herds with 14 taurus cattle breeds and 2 indicus breeds ([Table 1](#)).

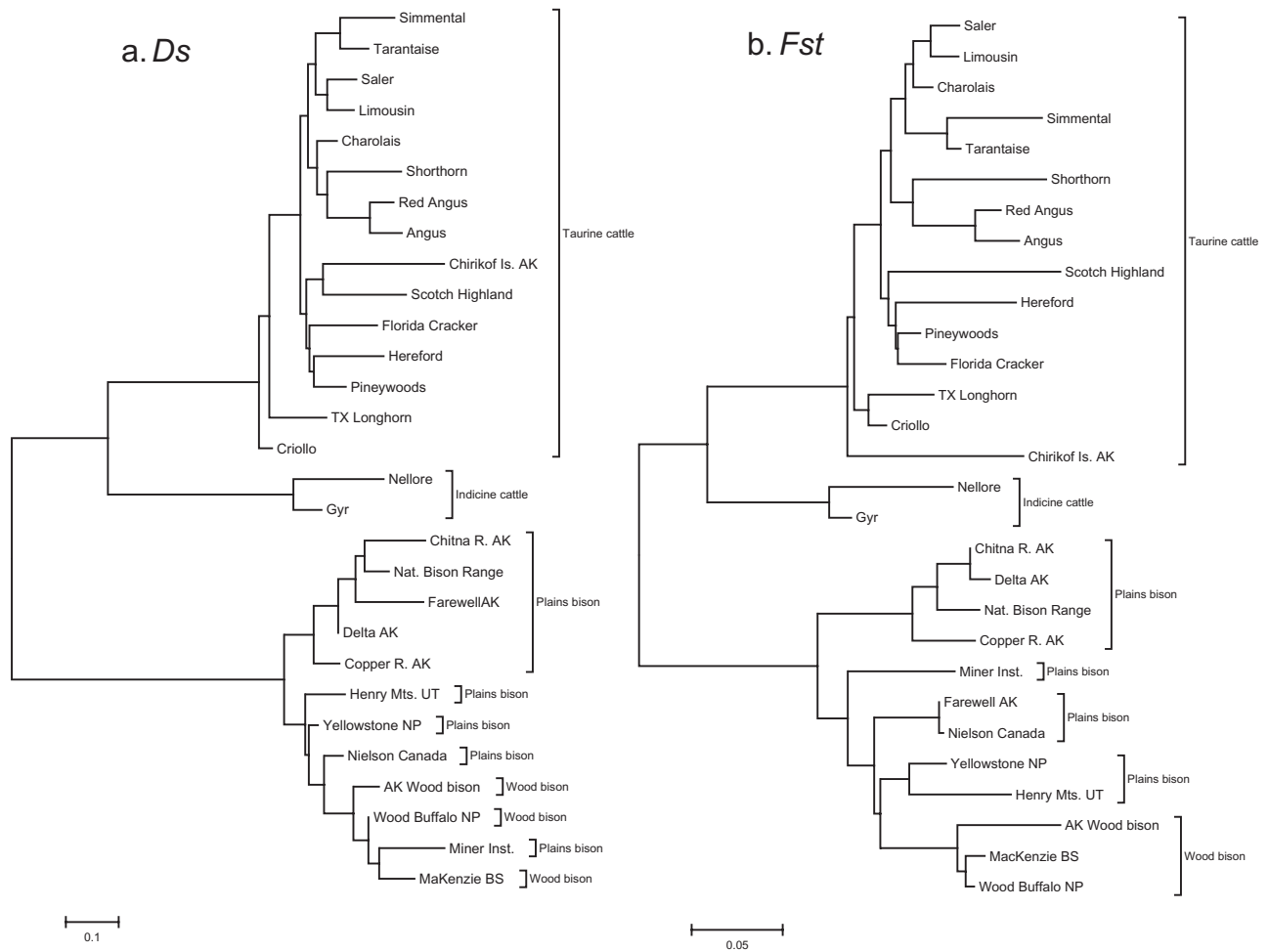


Figure 1. (a) NJ dendrogram of bison herds generated with D_s (Nei 1972) genetic distances. (b) NJ dendrogram of bison herds generated with F_{st} genetic distances.

and a third group with all indicine cattle with proportion of membership of 0.99 (Figure 2a and see Supplementary Table 4 online). In addition, when considering all a priori identified herds and breeds (i.e., $K = 29$), all plains bison and wood bison were still assigned to 1 group with proportion of membership of 0.94–0.98, whereas the cattle breeds and subspecies are highly differentiated (Figure 2b). With $K = 4$, bison are in 1 group with proportion of membership of 0.98–0.99, and indicine cattle are in a group with proportion of membership of 0.99, as with $K = 3$. However, the taurine cattle are in 2 groups, one consisting of the British-derived breeds (Angus, Red Angus, Shorthorn, Hereford, and Scotch Highland) with proportion of membership 0.87–0.98, and the other with the continental breeds (Simmental, Charolais, Limousin, Tarantaise, and Saller) and breeds of Iberian and admixed origin (Longhorn and Criollo) with proportion of membership of 0.95–0.99 (see Supplementary Table 4 online). These results show the British and continental cattle breeds (all in the *B. t. taurus* subspecies) that are more differentiated than are the putative bison subspecies.

A STRUCTURE analysis with only the 12 bison herds resulted in the highest ΔK (72.6) indicating the best $K = 3$. The ΔK for $K = 4$ is 46.1, and other ΔK values are less than 4.4. The analysis with $K = 3$ identified a group with proportion of membership of 0.94–0.98 for the Copper River, Chitna, Delta Alaska, and National Bison Range plains bison herds (green shading in Figure 2c), a group with proportion of membership of 0.87–0.98 for the Henry Mountains, and Yellowstone National Park plains bison herds (red shading in Figure 2c), and a group with proportion of membership of 0.88–0.99 including the 3 wood bison herds (blue shading in Figure 2c and see Supplementary Table 4 online). The Farewell Alaska, Miner Institute, and Nielson, plains bison herds had proportions of membership of 0.10–0.61 in the 2 groups of plains bison. The ancestor–descendant herds are in the same groups in this analysis including the Yellowstone and Henry Mountains Herds, the National Bison Range, and 3 of the Alaska plains bison herds, and the Wood Buffalo National Park and Alaska wood bison herds. In 2 cases, the descendant herds show relative homogeneity compared with

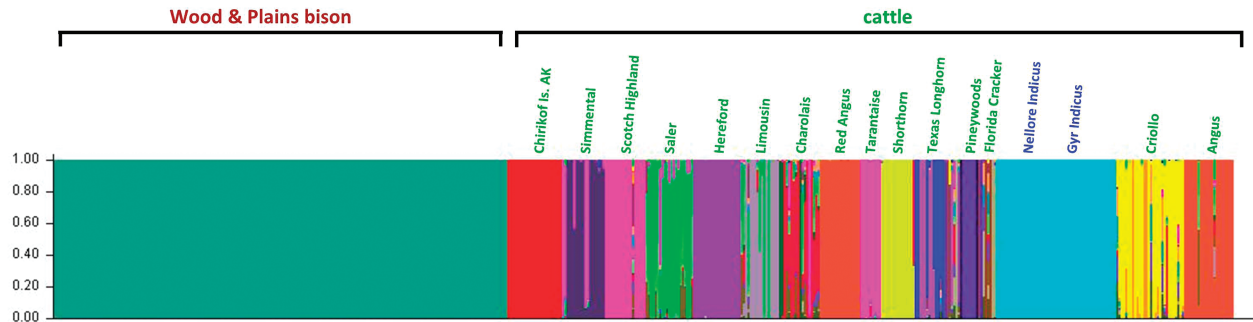
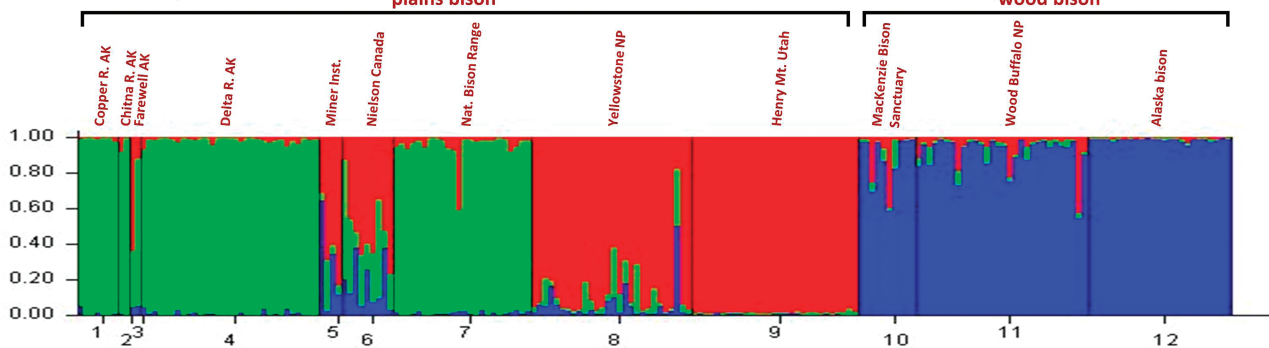
a. Bison and Cattle, K = 3**b. Bison and Cattle, K = 29****c. Bison only, K = 3**

Figure 2. Histograms of STRUCTURE results for bison and cattle with (a) $K = 3$ and (b) $K = 29$, and for bison only with (c) $K = 3$.

the ancestor herds. The ancestor Wood Buffalo National Park wood bison herd shows a degree of the green and red shading indicative of the 2 plains bison groups, whereas the descendant Alaska wood bison herd does not; and the ancestor Yellowstone National Park herd shows a degree of the green and blue shading indicative of the other plains bison and wood bison groups, whereas the descendant Henry Mountains herd does not (Figure 2c). This may reflect gene flow after the introduction of nonlocal bison to the native herds in Yellowstone and Wood Buffalo national parks, and the limited number of bison used to establish the descendant herds.

Discussion

Genetic distances, NJ cluster analysis, and STRUCTURE analysis indicate that several of the cattle breeds and the 2 cattle subspecies are differentiated, whereas plains bison and

wood bison are not. Other data support this result. First, analysis of 19 additional loci for our 12 bison herds, but with smaller sample sizes for the National Bison Range ($N = 4$), Yellowstone National Park ($N = 8$), and Henry Mountains ($N = 8$) herds combined with our 29 loci resulted in 48 loci with nonsignificantly different ($P = 0.26$) mean distances between plains bison herds ($D_s = 0.21$) and between plains bison herds and wood bison herds ($D_s = 0.23$; Cronin M, Derr J, unpublished data). Second, we calculated D_s with allele frequencies for 30 microsatellite loci for 3 wood bison herds and 11 plains bison herds reported by Halbert et al. (2005). The mean D_s between plains bison herds (0.08) is greater than the mean D_s between plains bison herds and wood bison herds (0.07), and the mean distances are not significantly different ($P = 0.39$). Third, analysis of 11 microsatellite loci (Wilson and Strobeck 1999) resulted in a greater mean $D_s = 0.23$ between 8 plains bison herds and 3 wood bison herds than a mean $D_s = 0.11$ between the 8 plains

bison herds, but the means are not significantly different ($P = 0.06$). Data from all of these studies are consistent and indicate that some herds of plains bison and wood bison are more genetically similar than some herds of plains bison are to other plains bison, and that plains bison and wood bison are not identifiable as distinct groups.

Our results also show that D_s between cattle subspecies is significantly greater than D_s between plains bison and wood bison herds, the combined samples show 6.5 times less differentiation of wood bison and plains bison ($D_s = 0.114$) than between the cattle subspecies ($D_s = 0.748$), and there is a greater proportion of alleles shared by wood bison and plains bison (68%) than by the indicine and taurine subspecies (43%). The relatively smaller genetic distances between wood bison and plains bison are also apparent from specific comparisons of cattle breeds. The smallest distance among the cattle ($D_s = 0.11$) is between the Red Angus and Angus breeds (which are considered 1 breed in some countries with different alleles at a coat color locus), and between the Charolais, Saler, and Limousin breeds from south-central France. Comparable D_s values occur between the primary Wood Buffalo National Park wood bison herd and the Yellowstone National Park plains bison herd ($D_s = 0.098$). At a larger geographic scale, the D_s between the French breeds and the Scotch Highland breed were more than 0.30, which is greater than the mean D_s between the plains bison and wood bison herds (0.23). The domestication and selection histories of cattle breeds could influence patterns of genetic variation differently than wild populations, but we cannot assess this with our data for presumably selectively neutral microsatellites. However, genetic distances among cattle breeds with little or no gene flow provide a relevant comparative measure of population differentiation of bison with little or no gene flow since establishment of the extant herds.

We recognize that our analysis of allele-frequency-based genetic distances with clustering algorithms of populations with recent common ancestry and gene flow does not provide a strict phylogenetic comparison, but rather a population genetic assessment (Felsenstein 1982; MacHugh et al. 1997). However, our results are consistent with studies that show the taurine and indicine cattle subspecies meet the criterion of phylogenetic distinction (Loftus et al. 1994; MacHugh 1996; Chan et al. 2010) including markedly different microsatellite allele distributions (MacHugh et al. 1997, 1998), whereas plains bison and wood bison do not (Stormont et al. 1961; Ying and Peden 1977; Peden and Kraay 1979; Bork et al. 1991; Cronin 1993; Cronin and Cockett 1993; Polziehn et al. 1996; Douglas et al. 2011).

Regarding subspecies designations, it is necessary to recognize that subspecies is a formal taxonomic category, phylogeny is the basis of taxonomic classification (reviewed by Mayr 1982), and therefore phylogeny should be the basis of subspecies classifications (Avice and Ball 1990). However, because subspecies may have contiguous ranges and gene flow, designations are often uncertain and several authors have rejected the entire subspecies category as subjective (see Cronin 2006, 2007). This creates a paradox for biologists because subspecies can be designated by one author, rejected by another, and still others reject the entire subspecies ranking. This can result

in subspecies designations for which there is no scientific consensus. These factors make formal designation of bison subspecies a seemingly intractable taxonomic exercise. However, the bison subspecies are currently recognized by management agencies, so their taxonomy needs to be assessed. In this regard, the evidence discounting the putative bison subspecies can be summarized with 3 points:

1. Genetic data do not show phylogenetic distinction of extant wood bison and plains bison.
2. Wood bison and plains bison were mixed in Wood Buffalo National Park and there are no extant wood bison without some degree of plains bison ancestry.
3. Wood bison and plains bison show less divergence of microsatellite allele frequencies than cattle breeds and subspecies.

These results call into question the subspecies ranking of plains and wood bison and indicate that it is not supported on the basis of phylogenetic distinctiveness. In a classic critique of the subspecies concept, Wilson and Brown (1953) suggested that populations could be designated by geographic area without the subjectivity of subspecies designation. Thus, extant wood bison herds might be considered a northwestern (geographic) subpopulation of North American bison without the uncertainties of subspecies designation (Wilson and Strobeck 1999; Boyd et al. 2010a, 2010b).

These results have ramifications for policies concerning the management and conservation of bison populations. Both Canada and the United States maintain gene banks to conserve germplasm and tissue for agriculturally useful species including bison, and both countries have initiated collections of bison tissues. The results of this study will be used in further development of germplasm acquisition for bison and suggest that plains bison and wood bison should be considered geographic populations and not subspecies. Regarding management of wild bison populations, they could be managed to maintain phenotypic characteristics of interest, as is done with cattle breeds. That is, wood bison can be managed as a geographic subpopulation to maintain morphological characters (whether heritable or environmentally induced) or other potentially unique traits. However, the lack of subspecies-level differentiation suggests that managers of wild and domestic populations could interbreed wood bison and plains bison to maintain or enhance genetic variation and reduce the potential for genetic defects and reduced fitness resulting from inbreeding.

Supplementary Material

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

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