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Detection of mitochondrial DNA from domestic cattle in bison on Santa Catalina Island

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Summary

In 1924, 14 American bison (*Bison bison*) were introduced to Santa Catalina Island, California and sporadically supplemented thereafter with additional animals. To reduce the herd and its impact on native vegetation, over 2000 animals have been exported during the past four decades. Today, the herd is estimated to contain around 250 individuals. Genetic analysis was performed on 98 animals removed from the island in 2004. Forty-four samples (45%) had domestic cattle mitochondrial DNA (mtDNA), 12 (12%) had previously reported bison haplotypes and 42 (43%) had a new haplotype differing by one base pair from a previously reported bison haplotype. A complement of five restriction enzymes was found to be useful in identifying bison with domestic cattle mtDNA.

Keywords American bison, *Bison*, Bovidae, genetics, introgression, mtDNA.

The census size of American Bison (*Bison bison*) dropped from millions of individuals in 1800 to <1000 by the late 1800s (Isenberg 2000). Intensive conservation averted extinction and re-established bison in reserves throughout North America. Many populations face potential genetic problems from founder effects, small effective population sizes and restricted gene flow (Berger & Cunningham 1994). Bison are also affected by the genetic introgression from past efforts to hybridize male bison with female cattle to create hardier beef breeds (Coder 1975). Remnants of these hybridizations are detectable in bison mitochondrial (Ward *et al.* 1999) and nuclear genomes (Halbert & Derr 2007). Hybrid bison populations have unresolved conservation issues (Minard 2003), including the loss of genetic integrity and co-adapted gene complexes (Ward *et al.* 1999).

The Catalina Island Conservancy (CIC) manages a bison herd outside its native range on Santa Catalina Island, California. In 1924, 14 bison were introduced for a film (Gingrich 1974). Although poorly documented, the source of these bison was likely the Sherwin Ranch in Colorado, whose herd originated in turn from the Goodnight Ranch in Texas (Propst 1995). The CIC herd has been periodically augmented and in 1987 grew to as many as 524 individuals (Sweitzer *et al.* 2005). To reduce the impact on native vegetation, over 2000 animals have been exported to the mainland since 1969. The population currently has a census size of 250–350 animals (Sweitzer *et al.* 2005). The CIC

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Table 1 Diagnostic restriction enzyme digests of bison and cattle mtDNA.

	Bison bison		*Bos taurus*	
Restriction enzyme	Cut site[1]	Fragment size (bp)	Cut site	Fragment size (bp)
BstNI	–	1082	16 261	534
				567
StyI	16 108	408	–	1101
		674		
ApoI	15 839	11	15 839	32
	15 850	59	15 871	60
	229	145	16 105	145
	289	168	16 252	147
		699	229	168
			289	234
				315
BsrGI	16 949	121	15 965	120
	16 078	259	16 085	271
		702		710
ScrFI	16 219	229	16 219	42
	110	346	16 261	187
		507	110	347
				525

[1]Position of restriction enzyme cut, according to the Anderson *et al.* (1982) domestic cattle sequence (V00654).

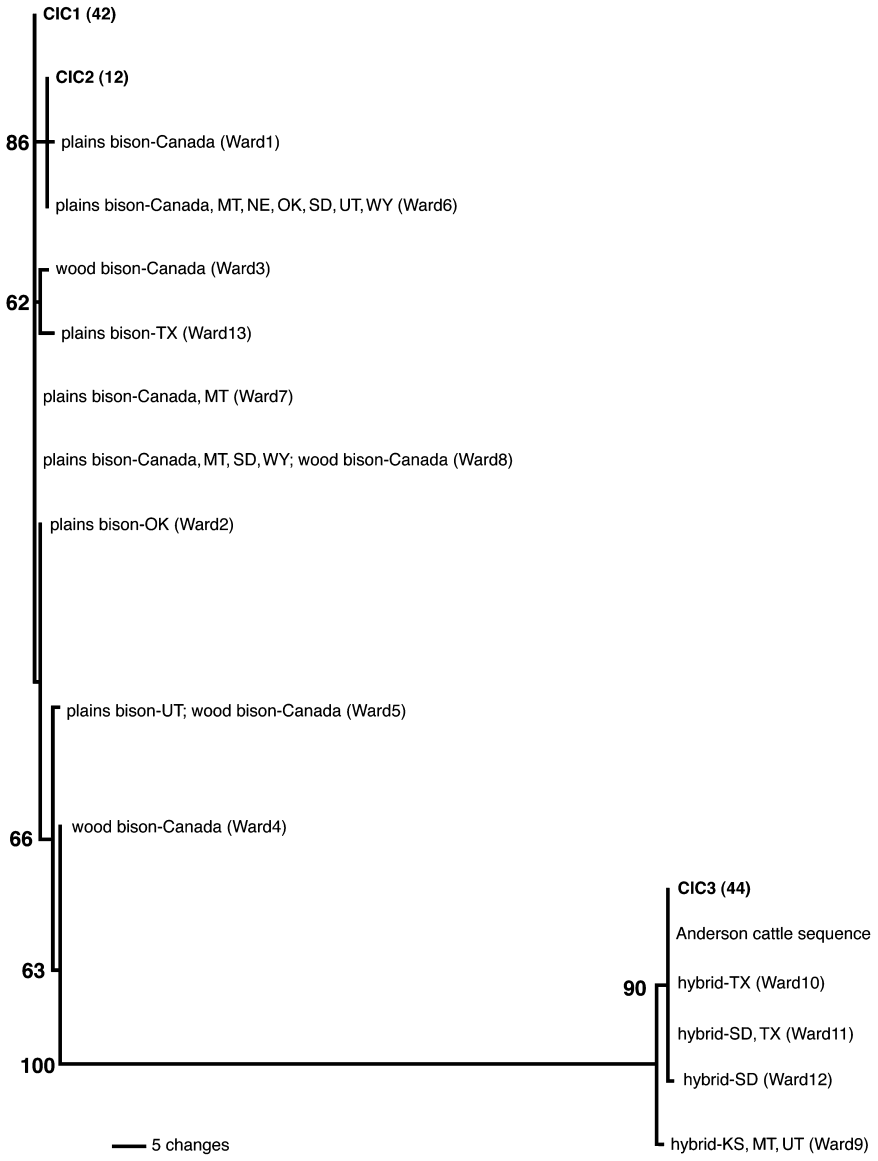

Figure 1 Aligned haplotypes (450 bp) from the mitochondrial DNA (mtDNA) control region of CIC bison; *Bos taurus* (Anderson *et al.* 1982; V00654, bases 15 854–16 281); and previously reported sequences (Ward *et al.* 1999) of *Bison bison bison* (plains bison), *Bison bison athabascae* (wood bison) and *Bison bison* with *Bos taurus* (domestic cattle) mtDNA (identified as hybrids). For each node, the number to the left indicates the percentage of 500 bootstrap replicates having that node. Numbers of individuals with a given haplotype are to the right of the CIC haplotypes. Sample locations are given for each of the Ward *et al.* (1999) sequences.

herd has never been subjected to genetic analysis; the objective of this analysis was to assess domestic cattle (*Bos taurus*) introgression.

Whole blood was taken from 98 bison culled in December 2004. Genomic DNA was extracted with a Gentra Puregene® Blood Kit (Qiagen); reference samples were stored on Whatman FTA® Cards (Alameda Chemical & Scientific). Approximately 640 bases of the mitochondrial control region were amplified by PCR using primers BISCR-16348F and BISCR-16990R (Shapiro *et al.* 2004; numbers refer to positions in V00654). Sequences were aligned and edited in SEQUENCHER 4.5 (Gene Codes Corporation). Phylogenetic trees were constructed with PAUP* 4.0b10 (Swofford 2003) by heuristic searches using maximum parsimony.

A restriction fragment length polymorphism (RFLP) assay was also developed to assist future analysis. The entire control region was amplified with primers 457 (5′-AGAGAAGGAGAACAACTAACCTCC-3′) and 15695 (5′-AACAGGAAGGCTGGGACC-3′) (Ward *et al.* 1999). Five enzymes were selected based on published sequences: BstNI, which cuts only domestic cattle sequences, StyI, which cuts only bison sequences, and ApoI, BsrGI and ScrFI, which have species-specific recognition sites (Table 1). DNA frag-

ments were visualized on 2% agarose gels with a DNA size-standard ladder and positive and negative controls.

Three haplotypes were found (DQ452026, DQ452027 and DQ452030) corresponding to CIC3, CIC2 and CIC1 respectively. Phylogenetic analysis found 150 equally parsimonious trees that were 114 steps long with a consistency index of 1.000 (one tree is shown in Fig. 1). The CIC3 sequence, which was 20.4% different than the CIC1 and CIC2 haplotypes, clustered with published cattle sequences (Fig. 1). Forty-four of the 98 samples (45%) had the CIC3 cattle haplotype, which is identical to the Ward 11 haplotype, a sequence in the Texas State bison herd descended from the Goodnight herd (Ward *et al.* 1999). The remaining 54 samples (55%) (CIC1: $n = 42$, CIC2: $n = 12$) clustered with previously reported bison haplotypes (Ward *et al.* 1999) (Fig. 1). Haplotype CIC2 is identical to Ward *et al.* (1999) sequences 1 and 6, while haplotype CIC1 is previously unreported and has a cytosine deletion at position 15 920 when compared with Ward *et al.* (1999) sequence 7.

The 98 samples were also scored as having bison vs. cattle mitochondrial haplotypes using both the RFLP protocol and the protocol of Ward *et al.* (1999). Results were completely congruent with each other and with the sequencing results.

The presence of domestic cattle mitochondrial DNA (mtDNA) in the CIC herd is consistent with reports of mixed ancestry in some bison herds (Ward *et al.* 1999; Halbert & Derr 2007). The similarity of the domestic cattle haplotype CIC3 to a sequence from a herd descended from the Goodnight herd (Ward *et al.* 1999) is also consistent with the known history of the CIC herd. The high percentage in the CIC herd of bison with domestic cattle mtDNA (45%) is surprising, compared with results from federal bison herds (no cattle mtDNA detected in 10 herds, 1.8% in the National Bison Range herd; Halbert & Derr 2007). This high rate of introgression into the CIC herd may have resulted from a founder effect (in either the initial introduction or subsequent supplementation), higher levels of cattle introgression in the private herds that furnished many founders compared with the federal herds, drift in the early years of the CIC herd or selection.

This study highlights new management concerns for the CIC. The Conservancy could remove hybrids based on mtDNA testing, although such culling will not remove dispersed nuclear introgression and could impact the effective population size of the CIC herd. Moreover, using culled animals from the CIC herd to supplement mainland bison herds that have no historical or genetic evidence of hybridization is ill-advised (Halbert & Derr 2007) and should be avoided.

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