SHORT COMMUNICATION

Differential introgression of uniparentally inherited markers in bison populations with hybrid ancestries

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Summary

Historical hybridization between *Bison bison* (bison) and *Bos taurus* (cattle) has been well documented and resulted in cattle mitochondrial DNA (mtDNA) introgression, previously identified in six different bison populations. In order to examine Y chromosome introgression, a microsatellite marker (BYM-1) with non-overlapping allele size distributions in bison and cattle was isolated from a bacterial artificial chromosome (BAC) clone, and was physically assigned to the Y chromosome by fluorescence *in situ* hybridization. BYM-1 genotypes for a sample of 143 male bison from 10 populations, including all six populations where cattle mtDNA haplotypes were previously identified, indicated that cattle Y chromosome introgression had not occurred in these bison populations. The differential permeability of uniparentally inherited markers to introgression is consistent with observations of sterility among first generation hybrid males and a sexual asymmetry in the direction of hybridization favouring matings between male bison and female cattle.

Keywords bison, cattle, introgression, microsatellite, Y chromosome.

At the end of the 19th century, bison experienced a dramatic demographic decline in which they were reduced from several million to less than 1000 individuals. Efforts to save bison from extinction were independently undertaken by a small number of individuals who raised some of the last remaining bison in captivity. These efforts were ultimately successful, and there are currently more than 200 000 bison in the USA and Canada, most of which exist in production settings and should be considered domestic animals.

Many of the individuals involved in early efforts to save bison from extinction were interested in improving production characteristics in their cattle through hybridization with bison. Captive populations exposed to hybridization with cattle were used as the founding stock for the vast majority of current bison populations. Ward *et al.* (1999) identified cattle mtDNA haplotypes in six of 15 bison populations examined, demonstrating that historic hybridization events had resulted in the introgression of maternally inherited mitochondrial genomes. In order to determine if the paternally inherited Y chromosome had a similar pattern of introgression, variation at a microsatellite locus on the Y chromosome was examined.

Bacterial artificial chromosome (BAC) clones containing Y chromosome DNA were isolated by PCR screening a bovine BAC library (Cai *et al.* 1995) for clones containing Y chromosome specific BRY-1 sequences (Matthews & Reed 1991) using primers and PCR conditions as described by Puera *et al.* (1991). Bacterial artificial chromosome clones positive for BRY-1 were screened by blot hybridization for $(AC)_n$ microsatellite DNA using ^{32}P-labelled synthetic oligonucleotides. DNA from positive clones was partially digested with Sau3A and subcloned into the BamH1 site of pBluescript IISK+ (Stratagene Inc., La Jolla, CA, USA). Nucleotide sequence data (GenBank AF302050), obtained using an ABI PRISM 377 automated DNA sequencer (Applied Biosystems, Foster City, CA, USA), revealed the presence of a dinucleotide (AC) repeat, designated BYM-1, within a subclone from BAC clone 223 (BAC223).

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The assignment of BAC223 to the Y chromosome in cattle and bison was confirmed by fluorescence *in situ* hybridization (FISH) using cattle and bison chromosomes prepared from fibroblasts (Gallagher & Womack 1992). The FISH procedure followed standard protocols (Pinkel *et al.* 1986) with slight modifications (Gallagher *et al.* 1998). After hybridization, the biotinylated probe was detected with Cy3 conjugated to streptavidin resulting in red fluorescence signal. BAC223 hybridized intensively to the Y chromosomes of cattle and bison resulting in a painting effect, and there was also faint hybridization to the qter of the submetacentric X in both species, which is believed to contain the pseudoautosomal region of the X chromosome (Yeh *et al.* 1996; Gallagher *et al.* 1999).

Amplification primers (5′-CCTTGTTTGAGCTTGACCAGT-3′ and 5′-TTGCAGGCACAGAAACGGA-3′) flanking BYM-1 were designed using MacVector 5.0 software (Kodak Scientific Imaging Systems, Rochester, NY, USA). BYM-1 was genotyped in 143 male bison from 10 populations and 41 male cattle from four taurine breeds common in North America at the time hybridization occurred between cattle and bison (Table 1). This sampling represents a subset of individual bison that were previously tested for domestic cattle mtDNA haplotypes (Ward *et al.* 1999). Amplifications were performed in 5 μl reactions with 0.8 μM BYM1 primers, 2.5 mM $MgCl_2$, and 0.375 U of *Taq* polymerase (Promega), and consisted of an initial denaturation at 94° (4 min), then 35 cycles of 94° (20 s), 66° (20 s), and 72° (20 s), followed by a final extension at 72° (20 min). The forward amplification primer was labelled with 6-FAM fluorescent amidite (Applied Biosystems) and amplification products were sized on an ABI310 Gene Analyser using GENESCAN 3.1 software (Applied Biosystems). Bison were fixed for a 242-bp allele whilst cattle had alleles at 255 and 257 bp (Table 1).

The fact that no bison were found with a 255 or 257 bp allele indicates that none of the 143 male bison examined have cattle Y chromosomes from the four domestic cattle breeds examined. As these breeds probably represent the domestic cattle variation available to bison through historical hybridization events, it appears that Y chromosome introgression did not result from these hybridization events. Mitochondrial introgression was previously identified at frequencies as high as 100% in six of the bison populations examined here (Ward *et al.* 1999). The discordance in levels of introgression exhibited by uniparentally inherited markers can be explained in part by the observation that first generation (F_1) male hybrids between cattle and bison have very low viabilities and are generally sterile (Boyd 1908, 1914; Gray 1954). Reductions in fertility and viability are also found among female F_1 hybrids, although to a lesser extent than in male F_1 hybrids (Boyd 1908, 1914). However, fitness is quickly restored to both sexes in backcross generations (Boyd 1908, 1914).

Many of the populations examined were derived from small bison populations exposed to hybridization with cattle for several decades, so that Y chromosome introgression could have proceeded through crosses of male cattle and female F_1 or backcross generation hybrids. The fact that no cattle Y chromosomes were identified in the bison populations examined indicates that there may have been an additional barrier to Y chromosome introgression. Many accounts of human induced hybridization between bison and cattle indicate that male bison would readily breed female cattle, but the reverse cross was nearly impossible to achieve because male cattle were unwilling to breed female

Table 1 BYM-1 allele frequency data for bison and cattle.

Species	Population	Sample size	242 bp	255 bp	257 bp	f_{mi}
Bos taurus	Angus	10			1.00	
	Hereford	16			1.00	
	Holstein	5			1.00	
	Shorthorn	10		0.40	0.60	
Bison bison	Antelope Island State Park	10	1.00			0.01
	Custer State Park	19	1.00			0.21
	Elk Island National Park	21	1.00			
	Finney State Game Refuge	13	1.00			0.04
	Henry Mountains	7	1.00			
	Maxwell State Game Refuge	25	1.00			0.18
	Mackenzie Bison Sanctuary	14	1.00			
	National Bison Range	17	1.00			0.03
	Williams Ranch	3	1.00			1.00
	Wood Buffalo National Park	14	1.00			

f_{mi}: Frequency of domestic cattle mitochondrial introgression reported by Ward *et al*. (1999).

bison (Boyd 1908, 1914; Dary 1989). The complete absence of cattle Y chromosomes in bison populations with hybrid ancestries is consistent with the observation of a sexual asymmetry in the direction of hybridization favouring matings between male bison and female cattle, and suggests that male cattle may not have contributed in a significant way to the composition of bison populations with hybrid ancestries.

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