

Conservation genomics: disequilibrium mapping of domestic cattle chromosomal segments in North American bison populations

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Abstract

Introgressive hybridization is one of the major threats to species conservation, and is often induced by human influence on the natural habitat of wildlife species. The ability to accurately identify introgression is critical to understanding its importance in evolution and effective conservation management of species. Hybridization between North American bison (*Bison bison*) and domestic cattle (*Bos taurus*) as a result of human activities has been recorded for over 100 years, and domestic cattle mitochondrial DNA was previously detected in bison populations. In this study, linked microsatellite markers were used to identify domestic cattle chromosomal segments in 14 genomic regions from 14 bison populations. Cattle nuclear introgression was identified in five populations, with an average frequency per population ranging from 0.56% to 1.80%. This study represents the first use of linked molecular markers to examine introgression between mammalian species and the first demonstration of domestic cattle nuclear introgression in bison. To date, six public bison populations have been identified with no evidence of mitochondrial or nuclear domestic cattle introgression, providing information critical to the future management of bison genetic resources. The ability to identify even low levels of introgression resulting from historic hybridization events suggests that the use of linked molecular markers to identify introgression is a significant development in the study of introgressive hybridization across a broad range of taxa.

Keywords: bison, domestic cattle, hybridization, microsatellites, nuclear introgression, species conservation

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Introduction

Natural interspecies hybridization, with or without introgression of genetic material, is known within all biological kingdoms and is considered an important evolutionary process (Dowling & Secor 1997; Barton 2001). In some cases, natural hybrids may successfully compete with and replace parental taxa distributions or invade new ecological niches, leading to new adaptive complexes and eventually

new species (Lewontin & Birch 1966; Arnold & Hodges 1995). Human-influenced hybridization of wildlife species is generally discouraged (Simberloff 1996), however, so as to minimize human impact on the evolution of natural species and prevent population or species extinction (Rhymer & Simberloff 1996; Allendorf *et al.* 2001). As the impact of humans on wildlife species has become better understood and molecular biology techniques have advanced, anthropogenic-induced interspecies hybridization has become an ecologically and politically important research area.

Bison (*Bison bison*) are endemic to North America, having first entered the continent via the Bering land bridge approximately 500 000–250 000 BP (Guthrie 1970; McDonald

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1981). In contrast, the first domestic cattle (*Bos taurus*) on the continent arrived in the early 1500s (Rouse 1973). The two species do not readily produce hybrids and will preferentially mate with their own species if given opportunity (Jones 1907; Boyd 1908, 1914; Goodnight 1914). Although the two genera are estimated to have diverged between 1.0 and 1.5 million years ago (Ma) (Hartl *et al.* 1988; Wall *et al.* 1992; Ritz *et al.* 2000), they still share the same number of chromosomes ($n = 30$), identical chromosome banding patterns (Basur & Moon 1967; Ying & Peden 1977), and highly similar autosomal gene content and order (Schnabel *et al.* 2003). At the apex of the decline of North American bison in the late 1800s, a small number of private ranchers effectively served to save the species from extinction through the establishment of small foundation herds (Coder 1975). Each of these herds were to some extent either used experimentally to create bison–domestic cattle hybrids or supplemented with bison from herds involved in such interspecies experiments (Garretson 1938; Coder 1975). These small herds were later used to stock protected US and Canadian federal and state bison populations, the eventual surplus of which have served to supply virtually all extant public and private bison herds (Coder 1975; Dary 1989).

Controlled breeding of male bison to female domestic cattle has been recorded extensively, although the birth rate of first generation (F_1) offspring is very low (Boyd 1908; Steklenev & Yasinetskaya 1982). Evidence of domestic cattle maternal introgression from historic hybridization has been identified in several public bison populations through analysis of mitochondrial DNA (mtDNA; Polziehn *et al.* 1995; Ward *et al.* 1999). In the most extensive mtDNA study to date, 5.2% of the bison tested (30/572) were found with domestic cattle mtDNA, representing 40% (6/15) of the examined US and Canadian bison populations (Ward *et al.* 1999). Conversely, no evidence of male-mediated domestic cattle introgression in North American bison has been found (Ward *et al.* 2001; Verkaar *et al.* 2003), as would be expected given the observed difficulties in producing viable male offspring from bison–domestic cattle crosses (Boyd 1914; Goodnight 1914; Steklenev & Yasinetskaya 1982; Steklenev *et al.* 1986).

Due to the uniparental inheritance of the mitochondrial genome, it is possible for a bison herd with a history of domestic cattle hybridization to contain no mtDNA evidence of introgression. Therefore, it is necessary to evaluate levels of domestic cattle nuclear introgression in bison to accurately assess the significance of introgressive hybridization and potential impact of domestic cattle introgression on the conservation of the bison species. In this study, we chose to evaluate nuclear introgression using microsatellite markers, which have a relatively high mutation rate (Weber & Wong 1993; Ellegren 2000) and have been used to assess cross-species introgression across a range of

mammals (e.g. Goodman *et al.* 1999; Miller *et al.* 2003; Vilá *et al.* 2003). Furthermore, microsatellite markers have proven to be adaptable from domestic cattle to bison (Schnabel *et al.* 2000, 2003) and several microsatellites have been preliminarily identified with bison-specific alleles in other studies (Penedo 1996; Mommens *et al.* 1998; Wilson & Strobeck 1999). One of the major disadvantages in using microsatellite markers for detecting alien alleles is the inability to differentiate electromorphs acquired through introgression from those derived through symplesiomorphy or convergence. However, the utilization of linked markers allows for more accurate identification of introgressed genomic regions, as nonrandom association of alleles at closely linked loci may persist for many generations following hybridization (Barton & Gale 1993; Rieseberg *et al.* 1995; Allendorf *et al.* 2001) and symplesiomorphy or convergence of alien-type alleles at multiple linked loci of a potential introgressant is highly unlikely (Estoup *et al.* 1999, 2000). As such, suspect alien alleles at potentially diagnostic loci were validated in this study using closely linked confirming microsatellite markers.

Materials and methods

Initial microsatellite screening

A total of 100 microsatellite markers representing regions from 29 of the 30 bison chromosomes and the X chromosome were chosen from available domestic cattle genome map databases (<http://www.marc.usda.gov/> and <http://locus.jouy.inra.fr/>). A complete marker list is available upon request from the authors. Representative bison and domestic cattle samples were screened to detect potential species-specific alleles, following protocols similar to those presented below. Allelic distributions for bison were determined from representatives (Table 1) of the only two continuously free-ranging bison populations in the world (Coder 1975): Yellowstone National Park (YNP; *Bison bison bison*) and Wood Buffalo National Park (WBNP; *Bison bison athabasca*). These populations presumably represent distinct subspecies based on morphological variation (Van Zyll de Jong *et al.* 1995), although other lines of evidence challenge the subspecific differentiation of wood and plains bison (Peden & Kraay 1979; Geist 1991; Ward *et al.* 1999; Wilson & Strobeck 1999). Neither recorded historic (Coder 1975) nor genetic (Polziehn *et al.* 1995; Ward *et al.* 1999) evidence of hybridization with domestic cattle exists for the YNP or WBNP bison populations. Furthermore, these populations are expected to adequately represent native allelic variation in North American bison (Ward *et al.* 1999; Wilson & Strobeck 1999; Schnabel *et al.* 2000). Allelic distributions for domestic cattle were determined from five domestic cattle breeds (Table 1) based on their prominence in North

Table 1 Domestic cattle breeds and bison populations sampled

Species	Breed/population	Location	Abbreviation	Sample size
<i>Bos taurus</i> (domestic cattle)	Angus		AN	10
	Hereford		HE	16
	Holstein		HO	13
	Shorthorn		SH	12
	Texas Longhorn		TLH	13
<i>Bison bison athabasca</i> (wood bison)	Elk Island National Park	Alberta, Canada	EIW	25
	Mackenzie Bison Sanctuary	NWT*, Canada	MBS	35
	Wood Buffalo National Park	Alberta/NWT*, Canada	WBNP	24
<i>Bison bison bison</i> (plains bison)	Antelope Island State Park	Utah, USA	AI	32
	Custer State Park	South Dakota, USA	CSP	39
	Clayton Williams Ranch†	Texas, USA	CW	11
	Elk Island National Park	Alberta, Canada	EIP	25
	Finney State Game Refuge	Kansas, USA	GC	32
	Fort Niobrara National Wildlife Refuge	Nebraska, USA	FN	27
	Henry Mountains State Park	Utah, USA	HM	21
	Maxwell State Game Refuge	Kansas, USA	MGR	40
	National Bison Range	Montana, USA	NBR	38
	Texas State Bison Herd	Texas, USA	TSBH	35
	Yellowstone National Park	Wyoming‡, USA	YNP	28

*Northwest Territories; †private ranch; samples obtained with owner permission; ‡parts of Yellowstone National Park are also in Montana and Idaho.

America during the late 1800s (J. O. Sanders, personal communication) when hybridization between the two species primarily occurred (Coder 1975).

DNA extraction and marker amplification

Blood or hair samples were collected from 328 plains bison (11 populations), 84 wood bison (3 populations), and 64 domestic cattle (5 breeds) as outlined in Table 1. Total genomic DNA was isolated from whole blood using the Super Quik-Gene protocol (AGTC) and standard phenol-chloroform-isoamyl alcohol extraction (Sambrook *et al.* 1989) or hair follicles following the protocol by Schnabel *et al.* (2000). Fluorescent labels (TET, 6-FAM, or HEX) were added to each forward microsatellite primer. Amplification was performed in 5- μ L reactions, multiplexed when possible, on a GeneAmp® PCR System 9700 thermal cycler (Applied Biosystems) using the thermal profiles in Table 2. Fragments were separated, sized, and genotyped on an ABI377 Automated DNA Sequencer or ABI310 Genetic Analyser (Applied Biosystems). GS500 (Applied Biosystems) or Mapmarker LOW (Bioventures) was used as an internal size standard and Genotyper 3.6 (Applied Biosystems) was used for allele identification and comparison. Replicate samples were included as necessary to standardize allele size calling between instruments and size standards.

Analysis of potentially diagnostic microsatellites

Due to the lack of a priori evidence of the actual occurrence of domestic cattle nuclear DNA in YNP and WBNP, it was necessary to assume that domestic cattle nuclear introgression may be present in either or both populations. The probability of alien alleles of identical size at a given nuclear marker in both the WBNP and YNP bison populations is considered low since there is no direct historical connection between the populations (Coder 1975; Wilson & Strobeck 1999). Therefore, microsatellite markers with no alleles shared in common between domestic cattle and either the YNP or WBNP populations were considered to be potentially diagnostic for identifying domestic cattle introgression in North American bison. In this way, alien (non-bison) alleles would not be misclassified as native (bison) alleles unless they were present in both bison populations, which is unlikely based on the history of the populations.

A total of 14 markers separated by at least 20 cM and representing 10 nuclear autosomes were identified as potentially diagnostic for detecting domestic cattle nuclear introgression in bison based on nonoverlapping allele size ranges (Table 2). One of these markers, PIT17B7, has a single 143-bp (base pair) allele shared between Hereford cattle (HE, frequency 3.1%) and bison from YNP (frequency 1.8%), but not found in bison from WBNP (Appendix).

Table 2 Microsatellite loci and amplification protocols

Region	Locus	Type	Chromosome	Position*	Reference	Amplification protocol¶	<i>Bos taurus</i> range**	<i>Bison bison</i> range††
1A	AGLA17	Confirming	1	0.0	Kappes <i>et al.</i> (1997)	g	214–219	215
	IFNAR15-2	Diagnostic	1	0.7	S. Davis, pers. comm.†	e	159–161	167
	BM6438	Confirming	1	1.6	Bishop <i>et al.</i> (1994)	c	257–268	253–270
	TGLA49	Confirming	1	1.9	Crawford <i>et al.</i> (1995)	c	108–124	110
	INRA117	Confirming	1	8.4	Vaiman <i>et al.</i> (1994)	a	92–104	102–108
1B	PIT1 7B7	Diagnostic	1	34.0	S. Davis, pers. comm.‡	h	128–143	143–159
	BMS4017	Confirming	1	34.8	Kappes <i>et al.</i> (1997)	a	148–158	145–165
	BM4307	Confirming	1	35.2	Bishop <i>et al.</i> (1994)	i	183–199	185–187
1C	INRA119	Confirming	1	68.7	Vaiman <i>et al.</i> (1994)	g	130–138	122–128
	BM7145	Diagnostic	1	69.2	Kappes <i>et al.</i> (1997)	c	116–118	108–110
	BMS4008	Confirming	1	71.7	Kappes <i>et al.</i> (1997)	d	152–179	158–164
1D	BMS4040	Diagnostic	1	98.8	Kappes <i>et al.</i> (1997)	e	85–99	75
	BMS4019	Confirming	1	98.8	Kappes <i>et al.</i> (1997)	a	197–201	191–206
2	CSSM42	Diagnostic	2	34.4	Moore <i>et al.</i> (1994)	c	173–217	167–171
5A	BL23	Diagnostic	5	28.6	Bishop <i>et al.</i> (1994)	f	242–256	234–236
	AGLA293	Confirming	5	32.0	Crawford <i>et al.</i> (1995)	b	218–239	218
	BMS1315	Confirming	5	32.5	Stone <i>et al.</i> (1995)	c	135–147	134–146
5B	RM500	Diagnostic	5	55.6	Barendse <i>et al.</i> (1994)	b	125–135	123
10A	SPS113	Diagnostic	10	29.2	Moore & Byrne (1992)	c	135–154	128–132
14	BM4513	Diagnostic	14	62.5	Bishop <i>et al.</i> (1994)	c	139–166	132–134
18	TGLA227	Diagnostic	18	84.7	Kappes <i>et al.</i> (1997)	b	79–106	73
23	PRL	Diagnostic	23	43.2	Creighton <i>et al.</i> (1992)	g	162–164	Null
	PRL2	Confirming	23	43.2	this study§	e	242–248	246
	RM185	Confirming	23	45.1	Barendse <i>et al.</i> (1994)	e	90–108	92
	BM7233	Confirming	23	49.1	Stone <i>et al.</i> (1995)	a	100–124	103–118
24	BMS2270	Diagnostic	24	21.2	Kappes <i>et al.</i> (1997)	d	80–98	66–70
	ILSTS065	Confirming	24	25.2	Kemp <i>et al.</i> (1995)	a	131–143	Null
26	HEL11	Diagnostic	26	20.7	Kaukinen & Varvio (1993)	f	179–203	142–175
	BM1314	Confirming	26	24.8	Bishop <i>et al.</i> (1994)	g	143–167	137
27	CSSM36	Diagnostic	27	39.8	Moore <i>et al.</i> (1994)	a	162–185	158

*As mapped in the domestic cattle genome; †forward primer (5'-CCTCCTGTTTACCTCTGAC-3'); reverse primer (5'-AAATAAGCCAGCAAAACACA-3'); ‡forward primer (5'-AGCAGATATACAGCCTTTGG-3'); reverse primer (5'-AATGATTCTGTCCCTTTCACT-3'); §forward primer (5'-GGCTTGAGGTCAGAGAATTAAAGC-3'); reverse primer (5'-DGTTCATACAACTCTAAGT-3') designed from EMBL accession X16641 using MACVECTOR 5.0 (International Biotechnologies). ¶(a) 94 °C 4 min; 5 cycles of 94 °C 30 s, 58 °C 15 s, 72 °C 5 s; 35 cycles of 94 °C 15 s, 56 °C 15 s, 72 °C 2 s; 72 °C 20 min. (b) 94 °C 3 min; 3 cycles of 94 °C 30 s, 54 °C 20 s, 72 °C 5 s; 37 cycles of 94 °C 15 s, 53 °C 10 s, 72 °C 3 s; 72 °C 20 min. (c) 94 °C 4 min; 6 cycles of 94 °C 30 s, 58 °C (–0.5 °C/cycle) 15 s, 72 °C 5 s; 27 cycles of 94 °C 30 s, 54 °C 15 s, 72 °C 2 s (+1 s/cycle); 72 °C; 20 min. (d) 94 °C 2 min; 6 cycles of 94 °C 30 s, 58 °C (–0.5 °C/cycle) 15 s, 72 °C 5 s; 29 cycles of 94 °C 15 s, 54 °C 15 s, 72 °C 5 s; 72 °C 20 min. (e) 94 °C 3 min; 6 cycles of 94 °C 30 s, 59 °C (–0.5 °C/cycle) 15 s, 72 °C 5 s; 25 cycles of 94 °C 15 s, 56 °C 15 s, 72 °C 5 s; 72 °C 20 min. (f) 94 °C 3 min; 5 cycles of 94 °C 30 s, 56 °C 15 s, 72 °C 5 s; 30 cycles of 94 °C 20 s, 52 °C 15 s, 72 °C 5 s; 72 °C 20 min. (g) 94 °C 3 min; 5 cycles of 94 °C 30 s, 58 °C 20 s, 72 °C 5 s; 30 cycles of 94 °C 20 s, 54 °C 15 s, 72 °C 5 s; 72 °C 20 min. (h) 94 °C 2 min; 5 cycles of 94 °C 20 s, 51 °C 20 s, 72 °C 30 s; 32 cycles of 94 °C 20 s, 49 °C 20 s, 72 °C 30 s; 72 °C 14 min. (i) 94 °C 4 min; 3 cycles of 94 °C 30 s, 55 °C 20 s, 72 °C 5 s; 35 cycles of 92 °C 15 s, 54 °C 15 s, 72 °C 2 s; 72 °C 20 min; **domestic cattle allele range (bp) as determined in AN, HE, HO, SH, and TLH breeds (see Table 1); ††bison allele range (bp) as determined from YNP and WBNP populations (see Table 1).

Consequently, this allele was classified as potentially alien. Screening with these 14 markers on the remaining 360 wood and plains bison samples revealed eight markers in nine populations with potentially alien alleles as follows

(Appendix): IFNAR15-2 (CSP), PIT17B7 (AI, CSP, FN, GC, HM, JA, MGR, NBR, YNP), BM7145 (CSP, GC, MGR, NBR), BMS4040 (CSP), BL23 (GC, MGR), PRL (CSP), BMS2270 (CSP), HEL11 (CSP). In addition, from the originally

examined 100 microsatellites, other potentially diagnostic markers were identified (Ward 2000; T. J. Ward & J. N. Derr, unpublished), though evidence of domestic cattle introgression has not been detected for any of these additional markers.

For the eight identified chromosomal locations, at least one closely linked (0–7.7 cM) confirming microsatellite marker (Table 2) was scored for all domestic cattle and bison samples as described above. At minimum, 98% of the samples were scored for each marker. Allele sizes and frequencies for all domestic cattle breeds and bison populations are presented in the Appendix for each marker (Table 2). The 143-bp allele for PIT17B7 found in the AI, CSP, FN, GC, HM, JA, MGR, NBR, and YNP populations was considered a native bison allele based on genotypes at the linked locus BMS4017, where alleles of common size with domestic cattle were not found in the same individuals (Appendix). The 139-bp allele for PIT17B7 found in the CSP and FN populations, however, was considered a confirmed alien allele based on shared domestic cattle-length alleles at the linked loci BMS4017 and BM4307 in the same individuals. The potentially alien 95-bp allele for BMS4040 in the CSP population was also considered a native bison allele, as domestic cattle-length alleles for the closely linked marker BMS4019 were not detected in CSP bison carrying the 95-bp BMS4040 allele.

Null alleles in domestic cattle could lead to an underestimate of domestic cattle introgression in bison. To test for this possibility, exact tests of Hardy–Weinberg equilibrium (HWE) were conducted using the program GENEPOP 3.1d (Raymond & Rousset 1995). The complete enumeration method was performed when less than five alleles were present in a given population. Otherwise, the Markov chain method following Guo & Thompson (1992) was used to produce an unbiased estimate of the exact *P* value. Additionally, pairwise genotypic disequilibrium was evaluated for 28 loci and all bison collectively, excluding PRL and ILSTS065 which have null alleles in bison (Table 2), using Fishers exact test in GENEPOP. Individual population tests of disequilibrium were not possible due to small sample sizes. In all analyses, *P* values of less than 0.05 were considered significant and the following Markov chain parameters were employed: 10 000 dememorizations, 200 batches, and 10 000 iterations/batch.

Statistical analysis of power to detect introgression

The bison populations examined here were established 25 to 35 generations ago (generation time of 3 years; Berger & Cunningham 1994) with a small number of bison, from which a portion may have been first- or second-generation hybrids (Garretson 1938; Coder 1975; Dary 1989). Within a few generations, any introgressed domestic cattle segments would have been distributed throughout a given closed

population and persisted to present through random mating. The known histories of these bison herds do not fit current models for estimating the probability of detection of nuclear introgression, which are designed to detect first- and second-generation crosses (Nason & Ellstrand 1993; Epifanio & Philipp 1997; Miller 2000) or backcross (BC) individuals formed through continuous crossing to a single parental species (Floate *et al.* 1994; Boecklen & Howard 1997). Therefore, we developed a more appropriate statistical model for the detection of persistent, diffuse alien segments in closed populations to calculate the power of detection of nuclear introgression as follows.

Assume two categories of founders for a given bison population: hybrid founders and purebred founders. Let *p* be the expected proportion of haploid domestic cattle (DC) genome represented in the hybrid founders such that an *F*₁ hybrid as a founder would represent the entire DC genome (*p* = 1) and a BC₁ (first-generation backcross) hybrid as a founder would represent half the DC genome (*p* = 0.5). If *f* backcross individuals are part of the founders then *p* = 1 – 0.5^{*f*}. Assume then that the hybrid founders are merged with a group of purebred bison and allowed to random mate for a sufficient number of generations such that each bison within the population has some proportion, *m*, of nuclear DC introgression. Given *t* independent, selectively neutral, unlinked diagnostic markers used to detect introgression in a randomly sampled section of *n* individuals, we will call a marker informative for detecting introgression if it falls into the region of the genome for which DC DNA was present in the hybrid founders. The probability of detecting DC introgression within a population is then represented by:

$$\begin{aligned}
 P(p, m, n, t) &= 1 - P(0 \text{ DC alleles detected in } n \text{ animals for } 1 \text{ marker})^t \\
 &= 1 - [P(0 \text{ DC in } 1 \text{ animal} \mid \text{marker informative})^n \\
 &\quad \times P(\text{marker informative}) + P(0 \text{ DC in } 1 \text{ animal} \mid \text{marker} \\
 &\quad \text{uninformative})^n \times P(\text{marker uninformative})]^t \\
 &= 1 - [(1 - m/p)^n p + 1^n (1 - p)]^t \quad \text{for } m \leq p \\
 &= 1 - [p(1 - m/p)^n + (1 - p)]^t \quad \text{for } m > p \quad (\text{eqn 1})
 \end{aligned}$$

When the entire DC genome is represented in the hybrid founder group, *p* = 1 and equation 1 reduces to:

$$P(m, n, t) = 1 - (1 - m)^{nt} \quad (\text{eqn2})$$

Equations (1) and (2) are based on the assumption of uniform representation of DC DNA in the hybrid founders group. Portions of the DC genome will likely be over-represented, and therefore violate this assumption, in cases where the hybrid founders are related or when more than one backcross generation individual was part of the hybrid founder group.

Results

A total of seven genomic regions in five bison populations were identified with evidence of domestic cattle introgression as follows (Table 3): CSP, six regions; FN, one region; GC, two regions; MGR, two regions; NBR, one region. The maximum detected alien allele frequencies shown in Table 3 were averaged across the 14 regions, producing the maximum-likelihood estimate of migration rate assuming HWE and unlinked marker loci for each population as follows: CSP, 0.0152; FN, 0.0159; GC, 0.0180; MGR, 0.0107;

Table 3 Summary of alien (*Bos taurus*) alleles identified in bison populations

Region	Locus	Alien allele	Population	Frequency
1A	AGLA17	219	CSP	0.0128
	IFNAR15-2	161	CSP	0.0128
	BM6438	257	CSP	0.0132
	TGLA49	112	CSP	0.0256
	INRA117	96	CSP	0.0395
1B	PIT1 7B7	139	CSP	0.0256
		139	FN	0.2222
	BMS4017	148	CSP	0.0270
		154	FN	0.2037
	BM4307	189	CSP	0.0256
		197	FN	0.2037
1C	INRA119	136	CSP	0.0256
		132	GC	0.1406
		132	MGR	0.0125
		132	NBR	0.0658
	BM7145	116	CSP	0.0128
		116	GC	0.1724
		116	MGR	0.0125
		116	NBR	0.0658
	BMS4008	166	GC	0.1875
		166	MGR	0.0125
		166	NBR	0.0789
5A	BL23	246	GC	0.0625
		246	MGR	0.1282
	AGLA293	228	GC	0.0625
		228	MGR	0.1375
	BMS1315	135	GC	0.0645
		135	MGR	0.1375
23	PRL	158	CSP	0.0385
	PRL2	242	CSP	0.0405
	RM185	100	CSP	0.0385
	BM7233	113	CSP	0.0263
24	BMS2270	90	CSP	0.0256
	ILSTS065	131	CSP	0.0260
26	HEL11	187	CSP	0.0541
	BM1314	157	CSP	0.0405

See **Table 1** for population abbreviations. Boldface type indicates the highest detected frequency of *B. taurus* introgression within a region for a particular population.

NBR, 0.0056. While the six identified bison regions with domestic cattle introgression in CSP were fairly consistent in frequencies of alien alleles (2.56% for region 1C to 5.41% for region 26), those for GC (region 1C 18.75% to region 5A 6.45%) and MGR (region 5A 13.75% to region 1C 1.25%) were highly variable (Table 3). Overall, the population-level frequency of introgression per locus ranged from 1.25% for region 1C in MGR bison to 22.22% for region 1B in FN bison (Table 3). In total, 12.9% (53/412) of the bison analysed had domestic cattle alleles in one or two genomic regions, divided by population as follows: 12 CSP (30.8%); 10 FN (37.0%); 13 GC (40.6%); 12 MGR (30.0%); 6 NBR (15.8%). Five bison (4 CSP, 1 GC) were identified with domestic cattle alleles in two genomic regions. No bison from CSP were identified with more than two introgressed genomic regions. Three bison were homozygous for domestic cattle alleles at one or more loci: FN (ID#4), PIT17B7, BMS4017, and BM4307; FN (ID#10), PIT17B7; GC (ID#7), BM7145.

To examine the possibility of null alleles, all marker-population combinations with two or more alleles were tested for HWE (351 tests). A total of 18 significant ($P < 0.05$) deviations were detected, which is within the range expected by random chance. No more than two significant deviations were detected for the same marker with the exception of HEL11, where six of 19 tests (five bison populations, one domestic cattle breed) showed significant deviation from HWE. The score test (Rousset & Raymond 1995) was utilized to test for HEL11 heterozygote deficiency using the GENEPOP parameters previously described. Of the 14 bison populations and five domestic cattle breeds examined, three bison populations had a statistically significant ($P < 0.05$) deficiency of HEL11 heterozygotes. Extreme heterogeneity in the amplification intensity of different alleles in bison was noted for HEL11, most likely accounting for these results. No significant deviations from HWE were detected for the closely linked marker, BM1314. It is unlikely that genotyping error or null alleles in HEL11 would lead to an underestimate of introgression in this region, as domestic cattle chromosomal segments would likely have been detected at BM1314 (Table 2; Appendix). However, if recombination between HEL11 and BM1314 has substantially reduced the linkage disequilibrium between these markers, introgression may be underestimated in this region.

Nonrandom associations among unlinked loci can be indicative of population admixture, and would be expected if observed domestic cattle introgression were the product of relatively recent hybridization. To test this hypothesis, genotypic disequilibrium was evaluated in a pairwise manner across all loci (Table 2). Although 378 comparisons were possible, only 236 valid comparisons were obtained due to the high number of fixed loci and low frequency alleles. Of the within-region (separated by ≤ 8.4 cM)

comparisons, 70.0% (14/20) were in significant disequilibrium ($P < 0.05$), while only 5.1% (11/216) of the among-region (nonsynthetic or separated by > 20 cM if syntenic) comparisons were significant.

The power to detect DC introgression ($m = 0.10\%$) in a population is dependent upon the level of introgression in a population (m), the number of individuals sampled (n), and the number of markers utilized (t ; Equation 1; Fig. 1). Figure 1a illustrates values of m encompassing the range of average detected nuclear introgression from this study (0.56% to 1.80%) assuming the entire domestic cattle genome was represented in the initial hybrid founder group ($p = 1.00$), and indicates a 95% power of detecting introgression if at least 45 individuals are sampled from a closed population. At lower levels of introgression, similar probabilities of detection are obtained only when more than 100 individuals are sampled per population (Fig. 1a). The difference in detection power between a population in which $p = 1.00$ and $p = 0.25$ is minimal compared with differences due to the distribution of domestic cattle segments in a population (m), as illustrated in Fig. 1b. Even when only one-fourth of the domestic cattle genome is represented in the initial hybrid founder group ($p = 0.25$), such as when a single BC₂ individual is introduced into a population of purebred bison, and at relatively low levels of introgression per individual such as $m = 0.50\%$, a 95% probability of detection of introgression is obtained when a minimum of 75 individuals are sampled (Fig. 1b). When few individuals are sampled ($n \leq 20$), however, additional markers (t) are necessary to provide adequate power to detect introgression (Fig. 1c). As more individuals are sampled from a population, the addition of more markers ($t > 10$) provides diminishing returns on the probability of detection (Fig. 1c).

Discussion

Molecular techniques have been used to detect nuclear introgression secondary to interspecies hybridization between a number of bovine species (MacHugh *et al.* 1997; Giovambattista *et al.* 2000; Nijman *et al.* 2003; Verkaar *et al.* 2003). Several factors influence the ability to detect genetic introgression between two taxa, including the marker

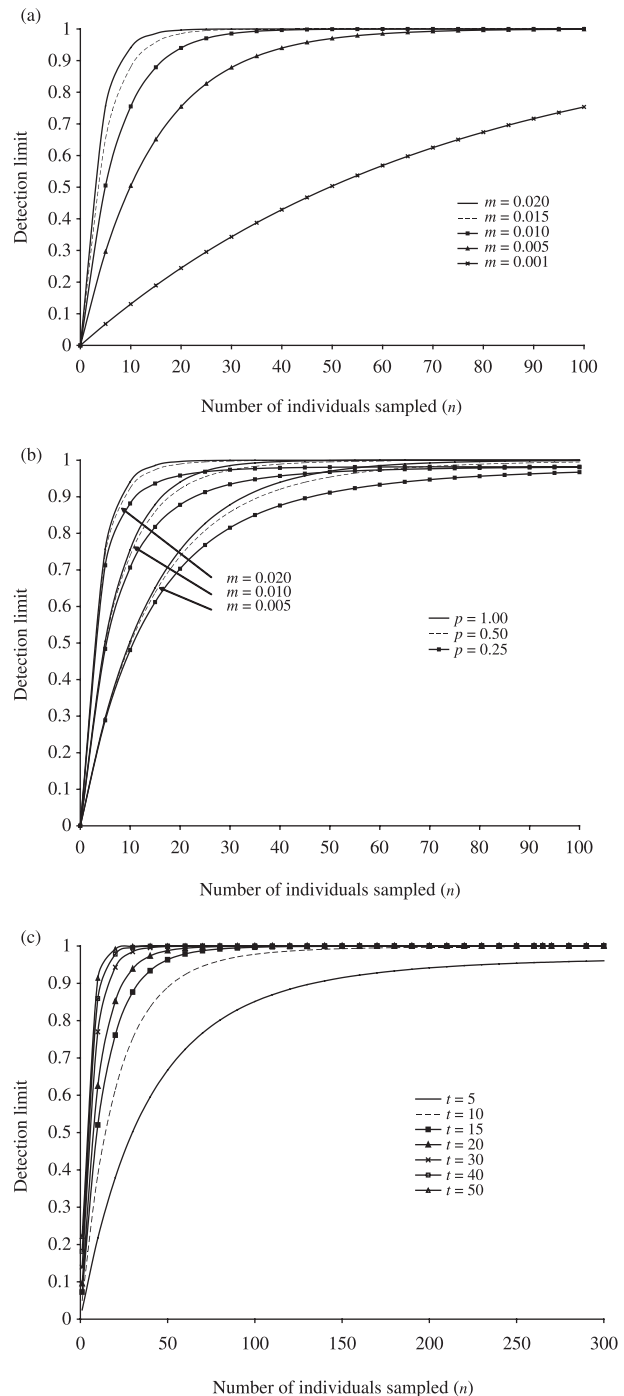


Fig. 1 Power of detection of introgressive hybridization across a range of individuals sampled (n) from a population using 14 nuclear diagnostic loci (t), according to Equation (1). (a) The effect of the level of introgression, m , when the entire domestic cattle haploid genome is represented in the hybrid founders ($p = 1.00$). Range of m encompasses that detected in bison populations in this study. When $m \geq 0.05$, the power of detection is more than 95% when at least 45 individuals are sampled. (b) As p decreases, it is necessary to screen more individuals from a given population to ensure a more than 95% probability of detecting introgression. When one-fourth of the domestic cattle haploid genome is represented in the hybrid founders ($p = 0.25$) and the level of introgression in the extant population is 0.5%, the power of detection is more than 95% when at least 75 individuals are sampled. (c) Effect of the number of markers utilized (t) on detection limit, given conservatively low values of $p = 0.5$ and $m = 0.005$. The power of detection is more than 95% when at least 60 individuals are sampled even when $t = 10$; with more markers, it is necessary to sample fewer individuals to obtain the same power of detection. As this graph illustrates, however, when $t > 10$ the addition of more markers provides diminishing returns on the limit of detection of introgression given a sufficient number of sampled individuals per population.

system, length of time since hybridization, type of hybrid (e.g. F_1 vs. BC), and genetic distinctness of parental species.

While others have used differences in microsatellite allele frequencies to detect introgression between related species (e.g. Goodman *et al.* 1999; Miller *et al.* 2003; Vilá *et al.* 2003), these methods have several disadvantages including the necessity of complex mathematical models (assignment methods) to sort hybrid from parental types and the innate tendency to misclassify individuals due to shared alleles and size homoplasy (Cornuet *et al.* 1999; Estoup *et al.* 1999). Size homoplasy remains a potentially confounding issue even given nonoverlapping allele size ranges (Angers *et al.* 2000; Estoup *et al.* 2002), although the use of linked confirming markers greatly reduces the probability of wrongly classifying an individual as hybrid (Estoup *et al.* 1999). Herein, we chose a rigorous experimental approach including the use of 14 diagnostic loci with virtually no shared alleles between bison and domestic cattle (with the exception of the 143-bp PIT17B7 allele; Appendix) and closely linked confirming markers to minimize the obstacles in detecting hybridization between closely related species. However, the physical distances between markers within a tested region and variations in introgressed region size throughout the genome could potentially lead to the misclassification of alien alleles at native alleles. That is, confirmation of true introgression at a diagnostic marker might not be obtained through a chosen confirming marker if the marker happens to fall outside the region of introgression (e.g. possibly BMS4040 in CSP). The misclassification of these alleles would lead to an underestimation of the level of introgression in a population. Further evaluation is necessary to more accurately describe and evaluate the potential occurrence of this type of error.

To our knowledge, this study represents the first use of linked microsatellite markers to examine introgression between two mammalian species. Furthermore, this study represents an important step in understanding the genetic composition of public bison populations in that the detection of domestic cattle introgression using maternal (Ward *et al.* 1999), paternal (Ward *et al.* 2001), and biparental markers can now be compared and contrasted with known population histories. Of the 14 bison populations examined in this study, we detected domestic cattle nuclear introgression in five populations: CSP, FN, GC, MGR, and NBR. In contrast, seven of these populations were previously identified with domestic cattle mtDNA haplotypes: AI, CSP, CW, GC, MGR, NBR, and TSBH (Ward *et al.* 1999; Ward 2000; TSBH abbreviation JA). Therefore, mtDNA introgression without evidence of nuclear introgression has been identified in the AI, CW, and TSBH populations. Cytoplasmic introgression in the absence of nuclear introgression has been reported in other species (e.g. Rieseberg & Wendel 1993; Avise 1994; Arnold 1997), although it is

unclear whether these discrepancies are due to reticulate phylogenetic events, drift, recombination, insufficient power of nuclear detection, or differential selection for cytoplasmic and nuclear genes. Given the number of individuals sampled for these populations (Table 1), the probability of detecting nuclear introgression using these 14 markers is < 90% if the level of introgression (m) is < 0.5% and $p = 1.0$ (Fig. 1a), and even lower if $p < 1.0$ (Fig. 1b). Therefore, it is possible that sampling additional markers or individuals per population would provide sufficient additional power to detect nuclear introgression. The histories of these bison populations, however, provide further insight into potential causes of detected cytoplasmic introgression in the absence of nuclear introgression. For instance, the TSBH was originally founded from five bison in the late 1800s (Coder 1975) and maintained with low census sizes for much of the next century (Haley 1949; Dary 1989). Bison from the TSBH were then used as founders for the CW private ranch population (D. Swepston, personal communication). Therefore, drift or founder effects may have lead to the absence of detected nuclear introgression in these populations. Only one out of the 95 bison from AI examined by Ward *et al.* (1999) had domestic cattle-type mtDNA; this individual was not screened in the present study. As the mtDNA domestic cattle introgression present in the AI population is most likely due to recent transfers of bison from MGR (Ward *et al.* 1999), it is possible that additional sampling of bison from AI would reveal nuclear introgression in the same regions as detected for MGR (Table 3).

Cytoplasmic introgression has not been previously detected in the FN population (Polziehn *et al.* 1995; $n = 20$; Ward *et al.* 1999; $n = 34$), which we identified as the population with the highest level of introgression in any nuclear region (1B; Table 3). The FN bison population was founded in 1913 with six bison of unknown sex from a private ranch and two bulls from YNP, supplemented in 1935 and 1937 with eight bulls from CSP total, and again supplemented in 1952 with five bulls from NBR (Garretson 1938; Coder 1975; R. Huber, personal communication). As no introgression has been detected within region 1B in YNP or NBR bison and the domestic cattle alleles detected in CSP bison in region 1B are not of the same size as those from FN bison (Appendix), the most likely source of the detected FN nuclear introgression is one or more of the original six founding bison.

To date, evidence of both mitochondrial (Polziehn *et al.* 1995; Ward *et al.* 1999) and nuclear domestic cattle introgression has been established for the CSP, GC, MGR, and NBR bison populations. Domestic cattle mtDNA of at least two haplotypes was previously identified in 20.6% (7/34) of CSP bison (Ward *et al.* 1999), while in the current study 30.8% (12/39) of CSP bison were identified with nuclear introgression, including six genomic regions. The CSP population was founded in 1914 with 36 bison originating

from a private rancher, Pete Dupree (Coder 1975; Dary 1989), and was later supplemented with bison either directly or derived from Wind Cave National Park and YNP (Garretson 1938; Dowling 1990). Given the results presented here for YNP and the presence of exclusively bison mtDNA in both YNP and Wind Cave National Park bison examined to date (Polziehn *et al.* 1995; Ward *et al.* 1999), it is most likely that multiple hybrid founders from the original Dupree herd produced the relatively high levels of detected nuclear and mitochondrial introgression in the CSP bison population. Additionally, the results presented here support the finding of Ward *et al.* (1999) of the likely origin of domestic cattle introgression in the NBR bison population through recent transfers of bison from MGR in 1982 (D. Wiseman, personal communication), as the two populations share a domestic cattle mtDNA haplotype and similarly sized nuclear alleles at three linked loci (INRA119, BM7145, BMS4008; Appendix) in region 1C (Table 3).

Hybrid swarm can occur rapidly in closed populations several generations beyond an initial introgressive hybridization event. Once a population has reached this level of dispersed introgression, it is no longer possible to recapitulate parental taxon germplasm (i.e. eliminate introgression; Allendorf *et al.* 2001). From this study, three lines of evidence indicate that introgression detected in these bison populations is several generations removed from the initial hybridization event. First, the lack of genotypic disequilibrium among loci from different genomic regions is indicative of hybrid swarms persisting several generations after introgressive hybridization (Allendorf *et al.* 2001). Second, the low levels of detected introgression and low number of individuals with more than one detected region of introgression indicate that, at minimum, several generations of random mating have served to recombine genomic regions of alien origin. Finally, the well-documented histories of these bison populations, including timing and origin of migrants, suggest historic hybridization as the only explanation for the findings in this and previous studies (Polziehn *et al.* 1995; Ward *et al.* 1999).

Considering the results presented here and in Ward *et al.* (1999), four likely epicentres of domestic cattle genetic introgression are evident among populations examined to date: CSP, FN, MGR/GC, and TSBH. Bison from each of these populations have been used in the establishment and/or supplementation of other populations, and the transfer of domestic cattle introgression from one population to another has been corroborated with genetic data in several cases (e.g. MGR to NBR). These findings underscore the importance of considering population histories and genetic background in reintroduction and transfer programs to prevent the introduction of hybrid individuals into otherwise genetically pure populations.

Selection for or against domestic cattle alleles in genes near the microsatellite loci examined here might serve to

drive detected allele frequencies to unexpectedly high (or low) levels. Two regions were identified in this study with relatively high levels of introgression: region 1B in FN (22.22%) and region 1C in GC (18.75%). However, there is no direct evidence that selection has acted to promote the persistence of domestic cattle alleles in these regions, as neither demonstrates significant deviations from HWE. The comparatively high frequencies of domestic cattle alleles in these regions are more likely the result of related hybrid founders with introgression in the same genomic region or random drift during the establishment of these populations when effective population sizes were very low.

Importantly, we have identified six public bison populations including four Canadian federal herds (EIP, EIW, MBS, WBNP), one US state herd (HM), and one US federal herd (YNP) with no evidence of either mitochondrial or nuclear domestic cattle introgression. The HM bison population is derived exclusively from YNP bison (Dowling 1990; J. Karpowitz, personal communication), while the MBS and EIW populations were founded with a presumed pure subpopulation of wood bison from WBNP (Banfield & Novakowski 1960; Geist 1991). Therefore, at present there exist at least two distinct lines of bison germplasm with no detectable levels of domestic cattle introgression. If the average level of introgression in these populations is less than 1.5% (m), the actual probability of detecting introgression in these six populations (n ranging from 21 to 35 individuals; Table 1) is < 95% (Fig. 1a, b). Since each of these populations are maintained at census sizes greater than 200, further evaluation of bison from these populations should add statistical power to the probability of detection if introgression does indeed exist at low levels (e.g. $m < 1.5\%$). Additionally, Wind Cave National Park and Wichita Mountains National Wildlife Refuge, representing at least one additional bison germplasm line (Coder 1975), have no evidence of mtDNA introgression (Ward *et al.* 1999) but remain to be examined for detectable levels of nuclear introgression.

As is the case for various other plant and animal species, herein we report an introduced species that has threatened the integrity of the germplasm, and therefore conservation, of a native wildlife species through introgression. The populations examined in this study represent an important cross-section of bison genetic diversity, as many of these populations have been used over the past 100 years to found and supplement public and private bison populations around the world. While historic hybridization has significantly impacted the integrity of extant bison germplasm, there remain closed bison populations with no evidence to date of domestic cattle genetic introgression. Therefore, it is critical that other known stock sources of bison germplasm are evaluated for both mitochondrial and nuclear evidence of introgression with domestic cattle, as the ability to circumvent further disruption of bison

germplasm through anthropogenic activities depends on the accurate identification and proper management of those populations with genetically unique and historically important lines of germplasm.

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Appendix

Allele frequencies for 30 microsatellites in 14 nuclear chromosomal regions in bison populations and domestic cattle breeds. Alien (domestic cattle) alleles detected in bison populations are listed in bold. See Table 1 for population abbreviations

AGLA17	AI	CSP	CW	EIP	EIW	FN	GC	HM	MBS	MGR	NBR	TSBH	WBNP	YNP	AN	HE	HO	SH	TLH
214															10.00	31.25	30.77	12.50	7.69
215	100.00	98.72	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00					7.69
216																			
219		1.28													90.00	68.75	69.23	87.50	84.62
IFNAR15-2	AI	CSP	CW	EIP	EIW	FN	GC	HM	MBS	MGR	NBR	JA	WBNP	YNP	AN	HE	HO	SH	TLH
159															10.00		3.85	25.00	3.85
161		1.28													90.00	100.00	96.15	75.00	96.15
167	100.00	98.72	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00					
BM6438	AI	CSP	CW	EIP	EIW	FN	GC	HM	MBS	MGR	NBR	TSBH	WBNP	YNP	AN	HE	HO	SH	TLH
253	25.00	63.16	81.82	54.00	20.00	56.52	57.81	66.67	44.29	69.23	67.11	42.86	31.25	64.29					
257		1.32													100.00	96.88	53.85	66.67	100.00
259																	23.08		
264	15.63	3.95					6.25												
266		3.95								5.13	11.84			1.79					
268	59.38	26.32	18.18	28.00	80.00	43.48	35.94	33.33	48.57	25.64	18.42	57.14	54.17	26.79		3.13	23.08	33.33	
270		1.32		18.00					7.14		2.63		14.58	7.14					
TGLA49	AI	CSP	CW	EIP	EIW	FN	GC	HM	MBS	MGR	NBR	TSBH	WBNP	YNP	AN	HE	HO	SH	TLH
108															15.00				3.85
110	100.00	97.44	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	5.00	43.75	3.85		
112		2.56													5.00		34.62	12.50	
115															75.00	56.25	30.77	87.50	57.69
117																			34.62
124																	30.77		3.85
INRA117	AI	CSP	CW	EIP	EIW	FN	GC	HM	MBS	MGR	NBR	TSBH	WBNP	YNP	AN	HE	HO	SH	TLH
92																			7.69
96		3.95													100.00	100.00	100.00	100.00	80.77
98																			3.85
100				14.00															
102	14.06			2.00	34.00		1.56	30.00	31.43		5.26		10.42	8.93					3.85
104	56.25	35.53	81.82	58.00	10.00	12.50	17.19	40.00	18.57	42.31	61.84	14.29	35.42	51.79					3.85
106	3.13	59.21	18.18	26.00	48.00	62.50	81.25	22.50	38.57	46.15	28.95	52.86	41.67	25.00					
108	26.56	1.32			8.00	25.00		7.50	11.43	11.54	3.95	32.86	12.50	14.29					

Appendix Continued

PIT17B7	AI	CSP	CW	EIP	EIW	FN	GC	HM	MBS	MGR	NBR	TSBH	WBNP	YNP	AN	HE	HO	SH	TLH
128															35.00	12.50	26.92	50.00	16.67
132																3.13			
133															5.00	9.38	7.69		5.56
135																9.38			
137															5.00		7.69	4.17	
139		2.56				22.22									50.00	62.50	57.69	41.67	77.78
141															5.00			4.17	
143	9.38	3.85				9.26	4.69	2.38		3.75	3.95	11.43		1.79		3.13			
145	81.25	53.85	72.73	50.00	32.00	33.33	25.00	61.90	22.86	28.75	27.63	41.43	25.00	37.50					
147		8.97		4.00															
150				4.00	26.00				30.00		5.26		27.08	17.86					
155	3.13	24.36	4.55	22.00	4.00	5.56	14.06	21.43	1.43	3.75	15.79		8.33	17.86					
157	1.56		22.73		18.00	1.85	3.13	7.14	15.71	12.50	39.47		16.67	25.00					
159	4.69	6.41		20.00	20.00	27.78	53.13	7.14	30.00	51.25	6.58	17.14	22.92						
161											1.32	30.00							
BMS4017	AI	CSP	CW	EIP	EIW	FN	GC	HM	MBS	MGR	NBR	TSBH	WBNP	YNP	AN	HE	HO	SH	TLH
145														14.29					
148		2.70													45.00	13.33	57.69	62.50	65.38
153		6.76	13.64	4.00				9.52	1.43	1.25			2.08						
154						20.37									50.00	20.00	11.54	25.00	23.08
155	1.56	51.35	50.00	32.00	8.00	50.00	89.06	16.67	61.43	56.25	55.26	62.86	47.92	42.86					
156																50.00	3.85	12.50	11.54
157					26.00				12.86		3.95	34.29	8.33						
158															5.00	16.67	26.92		
159	93.75	12.16	27.27		50.00	12.96		7.14	12.86	26.25	7.89		18.75	23.21					
161	1.56	12.16				1.85		4.76		10.00				1.79					
163	3.13	14.86	9.09	16.00	16.00	14.81	10.94	40.48	11.43	5.00	9.21	2.86	14.58	16.07					
165				48.00				21.43		1.25	23.68		8.33	1.79					
BM4307	AI	CSP	CW	EIP	EIW	FN	GC	HM	MBS	MGR	NBR	TSBH	WBNP	YNP	AN	HE	HO	SH	TLH
183															11.11				
185	98.44	75.64	54.55	95.83	60.00	77.78	84.38	92.86	71.43	60.00	85.53	47.14	70.83	100.00	22.22	12.50	16.67	9.09	
187	1.56	21.79	45.45	4.17	40.00	1.85	15.63	7.14	28.57	40.00	14.47	52.86	29.17			3.13			
189		2.56													11.11	37.50	54.17	54.55	19.23
191																6.25	4.17	9.09	15.38
197						20.37									55.56	40.63	25.00	22.73	38.46
199																		4.55	26.92

Appendix Continued

INRA119	AI	CSP	CW	EIP	EIW	FN	GC	HM	MBS	MGR	NBR	TSBH	WBNP	YNP	AN	HE	HO	SH	TLH
119		2.56																	
122		20.51	45.45	4.00		18.52	9.38	40.48	4.29	13.75		35.71	2.17	26.79					
124	4.69	55.13	45.45	42.00	88.00	77.78	53.13	52.38	72.86	62.50	50.00	57.14	78.26	37.50					
126			9.09	20.00	2.00		1.56		5.71	1.25	6.58		2.17	1.79					
128	95.31	19.23		34.00	6.00	3.70	21.88	7.14	14.29	21.25	36.84	7.14	17.39	33.93					
130					4.00				2.86						22.22	34.38	3.85	4.17	11.54
132							14.06			1.25	6.58				22.22	15.63	61.54	29.17	26.92
134															16.67	43.75		20.83	42.31
136		2.56													38.89	6.25	34.62	45.83	15.38
138																			3.85
BM7145	AI	CSP	CW	EIP	EIW	FN	GC	HM	MBS	MGR	NBR	TSBH	WBNP	YNP	AN	HE	HO	SH	TLH
108	95.31	71.79	100.00	70.83	78.00	88.89	82.76	78.57	75.71	77.50	93.42	100.00	81.25	78.57					
110	4.69	26.92		29.17	22.00	11.11		21.43	24.29	21.25			18.75	21.43					
116		1.28					17.24			1.25	6.58				90.00	96.88	65.38	95.83	88.46
118															10.00	3.13	34.62	4.17	11.54
BMS4008	AI	CSP	CW	EIP	EIW	FN	GC	HM	MBS	MGR	NBR	TSBH	WBNP	YNP	AN	HE	HO	SH	TLH
152																			15.38
156															44.44	18.75	23.08	20.83	42.31
158		1.28											2.17			28.13		12.50	
160	27.42	66.67	68.18	54.00	84.00	90.74	45.31	90.48	54.29	76.25	39.47	47.14	56.52	69.64		12.50		16.67	19.23
162	72.58	32.05	31.82	44.00	16.00	9.26	35.94	9.52	44.29	22.50	52.63	52.86	41.30	12.50					
164				2.00					1.43					17.86		3.13	7.69		
166							18.75			1.25	7.89				5.56	34.38	38.46	16.67	
168																	7.69		
172															5.56				
174															5.56				3.85
177																	19.23	16.67	7.69
179															38.89	3.13	3.85	16.67	11.54
BMS4040	AI	CSP	CW	EIP	EIW	FN	GC	HM	MBS	MGR	NBR	TSBH	WBNP	YNP	AN	HE	HO	SH	TLH
75	100.00	98.72	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00					
85																18.75	3.85	8.33	15.38
87															5.00				3.85
95		1.28																	
97															90.00	65.63	96.15	91.67	80.77
98															5.00				
99																15.63			

Appendix *Continued*

BMS4019	AI	CSP	CW	EIP	EIW	FN	GC	HM	MBS	MGR	NBR	TSBH	WBNP	YNP	AN	HE	HO	SH	TLH
191													4.17						
197										1.25					50.00	100.00	73.08	37.50	88.46
199		11.11		12.00			15.63	19.05	1.43	13.75			4.17	8.93	50.00		26.92	50.00	7.69
201						4.17				8.75								12.50	3.85
203	100.00	88.89	100.00	88.00	100.00	95.83	84.38	80.95	98.57	76.25	100.00	100.00	87.50	91.07					
206													4.17						
CSSM42	AI	CSP	CW	EIP	EIW	FN	GC	HM	MBS	MGR	NBR	TSBH	WBNP	YNP	AN	HE	HO	SH	TLH
167	50.00	63.51	95.45	74.00	64.58	80.00	70.31	71.43	91.18	60.26	56.58	67.14	80.43	58.93					
169	45.31	8.11	4.55	12.00	22.92			7.14	8.82	5.13	2.63		10.87	5.36					
171	4.69	28.38		14.00	12.50	20.00	29.69	21.43		34.62	40.79	32.86	8.70	35.71					
173																9.38		33.33	
175															15.00			4.17	
177																	3.85	4.17	3.85
179															30.00	12.50	23.08	37.50	34.62
181																3.13		4.17	
193																	7.69		
205																3.13	7.69	4.17	3.85
207																			3.85
209																		4.17	
211																3.13			3.85
213															55.00	68.75	15.38	8.33	46.15
217																	42.31		3.85
BL23	AI	CSP	CW	EIP	EIW	FN	GC	HM	MBS	MGR	NBR	TSBH	WBNP	YNP	AN	HE	HO	SH	TLH
234	100.00	100.00	100.00	100.00	100.00	100.00	93.75	100.00	94.29	87.18	100.00	100.00	93.48	100.00					
236									5.71				6.52						
242																9.38		8.33	
244															5.56	6.25	3.85		12.50
246							6.25			12.82					66.67	40.63	30.77	58.33	20.83
248															27.78	34.38	57.69	33.33	58.33
250																9.38	3.85		8.33
256																	3.85		

Appendix Continued

AGLA293	AI	CSP	CW	EIP	EIW	FN	GC	HM	MBS	MGR	NBR	TSBH	WBNP	YNP	AN	HE	HO	SH	TLH
218	100.00	96.15	100.00	96.00	100.00	100.00	93.75	100.00	100.00	83.75	98.68	100.00	100.00	100.00		40.63			
220		3.85		4.00						2.50	1.32								
222															25.00		3.85	4.17	
225																			33.33
226																			11.11
228							6.25			13.75					75.00	56.25	84.62	45.83	16.67
230																		29.17	16.67
232																		12.50	5.56
236																			11.11
239																3.13	7.69	8.33	5.56
BMS1315	AI	CSP	CW	EIP	EIW	FN	GC	HM	MBS	MGR	NBR	TSBH	WBNP	YNP	AN	HE	HO	SH	TLH
134	37.50	59.46	63.64	78.00	14.58	50.00	27.42	28.57	27.14	38.75	55.26	18.57	37.50	53.57					
135							6.45			13.75					60.00	33.33	61.54	75.00	30.77
136		8.11	36.36	6.00			32.26		2.86	10.00	14.47	81.43		19.64					
137															15.00	13.33		4.17	
139															25.00	26.67	23.08	8.33	15.38
140	51.56	10.81		8.00	58.33			19.05	22.86	13.75	10.53		20.83	19.64					
143																26.67	3.85	8.33	53.85
144					25.00				35.71				37.50						
146	10.94	21.62		8.00	2.08	38.89	24.19	52.38	11.43	21.25	19.74		4.17	7.14					
147																	11.54	4.17	
148						11.11	9.68			2.50									
RM500	AI	CSP	CW	EIP	EIW	FN	GC	HM	MBS	MGR	NBR	TSBH	WBNP	YNP	AN	HE	HO	SH	TLH
123	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00					
125																			11.11
127															10.00	34.38	15.38	12.50	38.89
129																	3.85		
131															20.00	6.25	19.23	12.50	50.00
133															65.00	59.38	57.69	70.83	
135															5.00		3.85	4.17	

Appendix *Continued*

SPS113	AI	CSP	CW	EIP	EIW	FN	GC	HM	MBS	MGR	NBR	TSBH	WBNP	YNP	AN	HE	HO	SH	TLH
128											13.16								
130	75.00	70.51	40.91	54.00	76.00	66.67	25.81	4.76	55.71	57.50	48.68	100.00	47.92	57.14					
132	25.00	29.49	59.09	46.00	24.00	33.33	74.19	95.24	44.29	42.50	38.16		52.08	42.86					
135															5.00				11.54
137															10.00	33.33		16.67	
139															20.00		15.38	12.50	19.23
141																3.33		8.33	
143																			3.85
145																	19.23		19.23
147															10.00				19.23
149															5.00	10.00	57.69	25.00	
151															50.00	53.33	7.69	29.17	26.92
154																		8.33	
BM4513	AI	CSP	CW	EIP	EIW	FN	GC	HM	MBS	MGR	NBR	TSBH	WBNP	YNP	AN	HE	HO	SH	TLH
132	89.06	67.95	81.82	100.00	100.00	98.15	95.31	85.71	100.00	93.59	100.00	100.00	100.00	83.93					
134	10.94	32.05	18.18			1.85	4.69	14.29		6.41				16.07					
139																3.13	7.69		3.85
141																			3.85
143															30.00	31.25	3.85	16.67	23.08
145															15.00	9.38	3.85		30.77
147															5.00	15.63	38.46	37.50	7.69
149															40.00	15.63	15.38	12.50	23.08
151															5.00		11.54	25.00	3.85
154																15.63		4.17	
160															5.00		15.38		
162																9.38			3.85
164																	3.85		
166																		4.17	
TGLA227	AI	CSP	CW	EIP	EIW	FN	GC	HM	MBS	MGR	NBR	TSBH	WBNP	YNP	AN	HE	HO	SH	TLH
73	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00					16.67
79																			22.22
83															15.00		3.85	16.67	
85																31.25		4.17	
90															5.00	3.13			5.56
92															40.00	25.00	19.23	66.67	11.11
94															15.00	28.13	19.23	4.17	5.56
96															10.00	9.38	3.85	8.33	11.11
98																	3.85		
101															15.00	3.13	42.31		27.78
106																	7.69		

Appendix Continued

PRL NULL 158 162 164	AI 100.00	CSP 96.15 3.85	CW 100.00	EIP 100.00	EIW 100.00	FN 100.00	GC 100.00	HM 100.00	MBS 100.00	MGR 100.00	NBR 100.00	TSBH 100.00	WBNP 100.00	YNP 100.00	AN 16.67 77.78 5.56	HE 3.13 96.88	HO 11.54 88.46	SH 12.50 87.50	TLH 30.77 69.23
PRL2 242 246 248	AI 100.00	CSP 4.05 95.95	CW 100.00	EIP 100.00	EIW 100.00	FN 100.00	GC 100.00	HM 100.00	MBS 100.00	MGR 100.00	NBR 100.00	TSBH 100.00	WBNP 100.00	YNP 100.00	AN 15.00 80.00 5.00	HE 3.13 96.88	HO 11.54 88.46	SH 12.50 87.50	TLH 30.77 69.23
RM185 90 92 94 96 98 100 102 104 106 108	AI 100.00	CSP 96.15 4.00 3.85	CW 100.00	EIP 96.00 4.00	EIW 100.00	FN 100.00	GC 100.00	HM 100.00	MBS 100.00	MGR 100.00	NBR 100.00	TSBH 100.00	WBNP 100.00	YNP 100.00	AN 5.00 20.00 10.00 15.00 30.00 10.00 10.00	HE 3.57 3.57 21.43 60.71 3.57 7.14	HO 19.23 11.54 34.62 20.83 12.50 16.67 4.17	SH 8.33 33.33 4.17 11.54 30.77 12.50 42.31	TLH 7.69 3.85 3.85 11.54 30.77 3.85 42.31
BM7233 100 103 104 105 106 108 113 114 115 116 117 118 119 121 122 124	AI 29.69 70.31	CSP 64.47 11.84 2.63 15.79 2.63	CW 100.00	EIP 78.00 12.00 8.00	EIW 84.00	FN 91.67 6.25 2.08	GC 100.00	HM 95.24 4.76	MBS 79.03	MGR 90.00	NBR 68.42 9.21	TSBH 95.71 4.29	WBNP 80.43 2.17 4.35 13.04	YNP 75.00 25.00	AN 25.00 5.00 40.00 5.00 20.00 5.00	HE 25.00 75.00	HO 30.77 11.54 34.62 7.69	SH 16.67 12.50 8.33 54.17	TLH 42.31 3.85 15.38 7.69 15.38 3.85 8.33

Appendix *Continued*

BMS2270	AI	CSP	CW	EIP	EIW	FN	GC	HM	MBS	MGR	NBR	TSBH	WBNP	YNP	AN	HE	HO	SH	TLH
66	25.81	47.44	63.64	70.83	66.00	29.63	28.13	78.57	52.86	60.00	69.74	81.43	67.39	51.79					
68	74.19	34.62	36.36	29.17	22.00	70.37	57.81	21.43	28.57	28.75	28.95		19.57	48.21					
70		15.38			12.00		14.06		18.57	11.25	1.32	18.57	13.04						
80																3.33	3.85		
82																23.33		33.33	11.54
84																10.00	3.85	12.50	34.62
86																			7.69
88															10.00		30.77		
90		2.56													10.00	26.67	11.54	12.50	26.92
92															20.00		19.23	20.83	3.85
94															5.00			4.17	7.69
96																30.00			
98															55.00	6.67	30.77	16.67	7.69
ILSTS065	AI	CSP	CW	EIP	EIW	FN	GC	HM	MBS	MGR	NBR	TSBH	WBNP	YNP	AN	HE	HO	SH	TLH
NULL	100.00	97.40	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00					
131		2.60													50.00	43.33	37.50	36.36	16.67
133																		4.55	16.67
135															22.22	20.00	8.33	9.09	12.50
137															16.67	30.00	4.17	40.91	4.17
139																			8.33
141																6.67	50.00	9.09	8.33
143															11.11				33.33
HEL11	AI	CSP	CW	EIP	EIW	FN	GC	HM	MBS	MGR	NBR	TSBH	WBNP	YNP	AN	HE	HO	SH	TLH
142		12.16	9.09	4.17		12.96				11.11	1.32		2.17	10.71					
148		4.05		8.33	48.00			7.14	50.00		3.95		30.43	23.21					
153		4.05		20.83			3.13		2.86	5.56				5.36					
155	90.63	32.43		12.50	26.00	12.96	73.44		7.14	41.67	17.11	4.29	8.70	7.14					
156		2.70		6.25	8.00	33.33	15.63		21.43		10.53		23.91	12.50					
157					6.00					1.39									
159		6.76	36.36	2.08		7.41		9.52					17.39	10.71					
160	1.56																		
161	1.56	27.03		31.25		25.93	3.13	83.33	8.57	9.72	2.63	64.29	4.35	23.21					
163		5.41							1.43	6.94	6.58	31.43	4.35	5.36					
165	1.56												2.17						
167			4.55						5.71				4.35						
171				4.17	4.00				1.43	2.78	27.63		2.17						
173	4.69		50.00	10.42	8.00	1.85	4.69			18.06	30.26			1.79					
175						5.56			1.43	2.78									
179															11.11	37.50	46.15	25.00	7.69
183															16.67			25.00	7.69

Appendix Continued

HEL11	AI	CSP	CW	EIP	EIW	FN	GC	HM	MBS	MGR	NBR	TSBH	WBNP	YNP	AN	HE	HO	SH	TLH
185															11.11	12.50	3.85	8.33	
187		5.41													22.22	18.75	23.08	4.17	38.46
189															22.22	28.13	19.23	4.17	15.38
191															16.67	3.13	3.85	33.33	19.23
195																			3.85
197																			3.85
203																	3.85		3.85
BM1314	AI	CSP	CW	EIP	EIW	FN	GC	HM	MBS	MGR	NBR	TSBH	WBNP	YNP	AN	HE	HO	SH	TLH
137	100.00	95.95	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00					
143																			3.85
145																			3.85
147															5.56	25.00			
153															5.56	31.25	3.85		
155															66.67	34.38	61.54	33.33	23.08
157		4.05													16.67		34.62	58.33	42.31
159															5.56				19.23
163																6.25			3.85
165																		8.33	
167																3.13			3.85
CSSM36	AI	CSP	CW	EIP	EIW	FN	GC	HM	MBS	MGR	NBR	TSBH	WBNP	YNP	AN	HE	HO	SH	TLH
158	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	10.00	9.38	16.67	29.17	34.62
162																	8.33		3.85
167																			
169																3.13	4.17		
171															5.00	12.50	4.17	12.50	
173															5.00	9.38	29.17		42.31
175															20.00			8.33	7.69
177																			7.69
179															55.00	43.75	33.33	16.67	3.85
181															5.00	21.88		33.33	
185																	4.17		