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SHORT COMMUNICATION

Y-specific microsatellite polymorphisms in a range of bovid species

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Summary

At least five dinucleotide $(CA)_n$ microsatellite repeat arrays have been assigned to the bovine Y-chromosome, with one marker (*INRA124*) shown to be polymorphic. We describe here the assessment of a panel of four Y-specific microsatellite markers for polymorphism in a range of cattle and related species. It was possible to amplify all the markers in the animals sampled and all showed variation. Three of the microsatellite loci (*INRA124*, *INRA189* and *BM861*) displayed putative taurine- and zebu-specific alleles which can be useful indicators of male-mediated gene flow in hybrid populations. In the future these microsatellites, in combination with other Y-specific markers should provide a high-resolution Y haplotype system for evolutionary studies in both domesticated cattle and other related species.

Keywords: genetic diversity, microsatellite, taurine cattle, Y-chromosome, zebu cattle

Y-chromosome-specific microsatellites have been used in humans for discrimination of closely related populations and to determine relationships among these populations (Hammer *et al.* 1997; Pérez-Lezaun *et al.* 1997; Seielstad *et al.* 1999). With the exception of the pseudoautosomal region, the whole of the Y-chromosome acts as a single non-recombining unit, which is male-specific and effectively haploid. This ensures that original combinations of mutational events along male lineages are preserved as single linked unambiguous haplotypes. The genetic diversity at these haplotypic arrays may then be analysed both within and among populations. It is well established that herd animals have increased variability in male reproductive success (Nowak 1991). In addition, it has been demonstrated that domesticated cattle can display marked sex-related differences in population mixing and migration patterns (MacHugh *et al.* 1997; Bradley et al. 1998). Taking these factors into consideration, it is therefore highly likely that male lineages will reflect different evolutionary histories than those of female lineages. These Y-specific microsatellite polymorphisms should therefore provide a starting point for an informative paternal analogue to mitochondrial DNA for studies of genetic variation in cattle and related species.

In the present study, primer and sequence data pertaining to Y-chromosome-specific microsatellites from scientific literature and GenBank were consulted. Four markers were located (Bishop *et al.* 1994; Vaiman *et al.* 1994; Kappes *et al.* 1997), each comprising at least one dinucleotide repeat region of nine or more repeat units. Of these four repeat segments, all were of the $(dC\text{-}dA)_n\cdot(dG\text{-}dT)_n$-type (this array being the most common dinucleotide repeat motif found in vertebrate genomes; Hamada *et al.* 1982); two are perfect microsatellites and the other two imperfect. Of the four, only one marker (*INRA124*) has been previously shown to be variable (Hanotte *et al.* 1997). Table 1 shows information on these four microsatellites.

The four bovine Y-specific microsatellites were assessed for polymorphism in a panel of male animals from the following breeds and species: Hereford (European *Bos taurus*, n = 6); N'Dama (African *Bos taurus*, n = 6); Ongole (Indian *Bos indicus*, n = 3); Nellore (Indian *Bos indicus*, n = 12); Plains Bison (*Bison bison*, n = 6); Mithan (*Bos [Bibos] gaurus*, n = 11); Swamp Buffalo (*Bubalus arnee*, n = 6); and Yak (*Bos grunniens*, n = 6). Three samples of male sheep (African *Ovis aries*, n = 3) were also assayed to assess the specificity of the primers to the *Bovini* family. Six female Jersey animals were also included to check marker specificity for the cattle Y-chromosome. In addition, a small number of females (n = 1–6) from the other species and subspecies were screened to ensure Y-chromosome specificity for each marker.

Twenty to thirty nanograms of DNA was used as template in an 11 μl reaction volume. 0·5 U of PLATINUM *Taq* polymerase (GIBCOBRL) were used with the buffers supplied (1·5 mM $MgCl_2$;

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Table 1 Y-specific microsatellite loci used to assay haplotic variation in the cattle surveyed.

Marker	Primer sequences (5′–3′)	Repeat motif	No. of alleles	Range of alleles (bp)	Reference
INRA124 (DYS6)	For-GATCTTTGCAACTGGTTTG Rev-CAGGACACAGGTCTGACAATG	$(GT)_4(A)(TG)_9$ $(N)_{43}(TG)_9$	2	130–132	Vaiman *et al.* 1994
INRA126 (DYS7)	For-GTTGTTGCCTCTGCAGAGTAGG Rev-GACACTCTTTCTATTTTCAAGG	$(TG)_{11}$	3	182–186	Vaiman *et al.* 1994*
INRA189	For-TACACGCATGTCCTTGTTTCGG Rev-CTCTGCATCTGTCCTGGACTGG	$(TG)_{22}$	9	68–124	Kappes *et al.* 1997†
BM861	For-TTGAGCCACCTGGAAAGC Rev-CAAGCGGTTGGTTCAGATG	$(GT)_6(C)(TG)_{10}$	6	144–158	Bishop *et al.* 1994

*Published amplification primers differ from those presented here.
†No published primers available so those presented here were designed from sequences submitted to GenBank.

50 mM KCl; 20 mM Tris·HCl [pH 8·4]). The concentration of dNTPs was 200 μM, with the exception of dCTP, which was reduced to 20 μM. [α-^{32}P] dCTP radionucleotide was randomly incorporated during the PCR amplification by the addition of 0.5 μCi [α-^{32}P] dCTP at 3 000 Ci mmol^{-1} per reaction. 3·0 ρmoles of both the forward and the reverse primers were also added. The reactions were carried out using a HYBAID *TouchDown* thermal cycling machine with the following cycling parameters: 93 °C for 3 min, followed by 40 cycles of 93 °C for 40 s, 65–55 °C for 40 s and 72 °C for 40 s. Annealing temperatures were decreased from 65 to 55 °C successively for each of the first 21 cycles (i.e. decrements of 0·5 °C per cycle: *TouchDown* PCR). A final extension of 5 min at 72 °C was also carried out. The radiolabelled products were then assessed for length variation using standard 6% polyacrylamide sequencing gel electrophoresis and autoradiography. DNA sequenced from the phage vector M13mp18 was run with the amplified test samples to size the allelic variation. This technique was precise enough to reproducibly resolve single repeat unit differences. Table 2 shows the allele sizes and frequencies for the various populations screened with these markers. Fig. 1 shows sample autoradiograms for a panel of animals screened with each marker.

Table 2 Allele size ranges and frequencies for *INRA124*, *INRA126*, *INRA189* and *BM861* in a variety of cattle types and related species

Marker	Length (bp)	Hereford (6)	N/Dama (6)	Ongole (3)	Nellore (12)	Plains bison (6)	Mithan (11)	Swamp buffalo (6)	Yak (6)	Togo sheep (3)
INRA 124	130	0	0	100	100	67	100	0	0	0
(DYS6)	132	100	100	0	0	33	0	100	100	100
INRA 126	182	17	17	100	100	100	100	0	100	0
(DYS7)	184	67	17	0	0	0	0	100	0	33
	186	17	67	0	0	0	0	0	0	67
INRA 189	68	0	0	0	0	0	100	0	0	0
	84	0	0	0	0	0	0	83	0	0
	88	0	0	100	100	0	0	0	0	0
	90	0	83	0	0	0	0	0	0	0
	96	0	0	0	0	67	0	17	0	0
	98	100	0	0	0	33	0	0	0	0
	102	0	0	0	0	0	0	0	100	0
	104	0	17	0	0	0	0	0	0	0
	124	0	0	0	0	0	0	0	0	100
BMS861	144	0	0	0	0	33	0	0	0	0
	146	0	0	0	0	67	0	0	0	0
	148	0	0	0	0	0	0	0	100	0
	150	0	0	0	0	0	82	0	0	0
	156	0	0	100	100	0	18	0	0	0
	158	100	100	0	0	0	0	100*	0	100

*Of the six animals sampled, only one gave an amplification product with marker *BM861*.

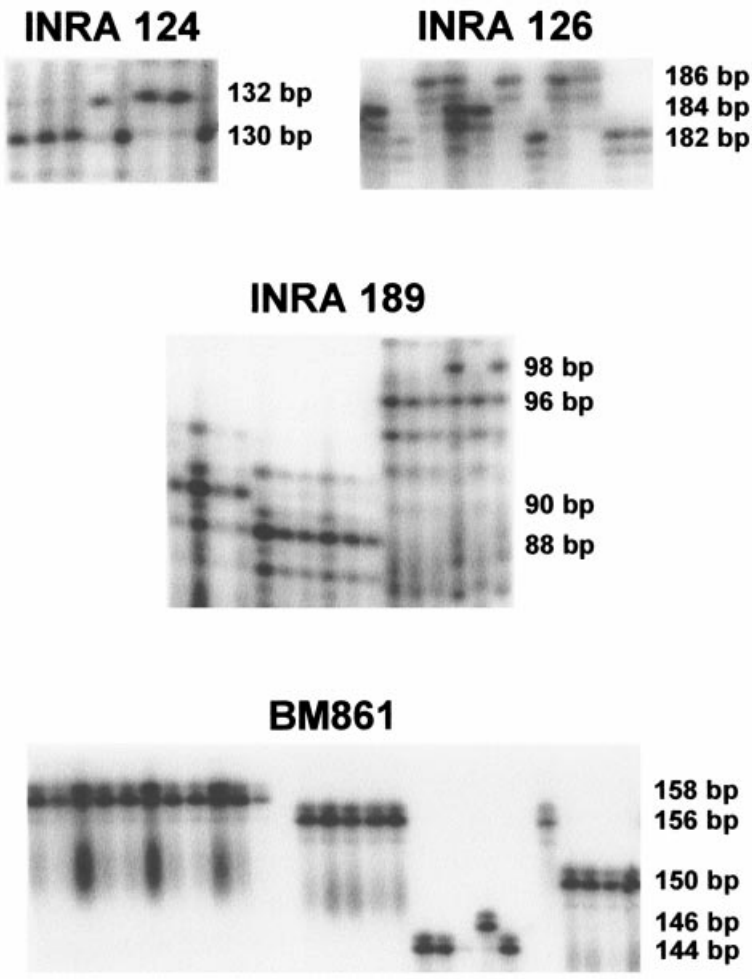

Fig. 1 Electronic scans of autoradiograms showing each of the four microsatellite markers amplified in a range of animals. Allele sizes are also indicated in base pairs.

Using the four microsatellite loci, a total of eleven alleles were detected in various populations of cattle. This number rose to twenty alleles when individuals from closely-related species were also surveyed with the same genetic markers. One Y-specific microsatellite used in this study (*INRA124*) had already been shown to be polymorphic by Hanotte *et al.* (1997). They found two length variants in several cattle populations, but failed to obtain amplification in samples from swamp buffalo, yak or mithan (domesticated gaur). This was not found to be the case here, as all four loci gave PCR products when amplified with DNA from American plains bison (*Bison bison*), mithan (*Bos [Bibos] gaurus*), swamp buffalo (*Bubalus arnee*), yak (*Bos grunniens*) and sheep (*Ovis*). In all but one case, the amplification of these Y-chromosome-derived markers was restricted to the male samples. However, *INRA126* displayed an amplification product in six female yaks of exactly the same size as that detected in the panel of male yaks (182 bp). This would indicate that the yak X-chromosome has retained an homologous sequence to the Y-chromosomal segment containing the *INRA126* microsatellite.

As Hanotte *et al.* (1997) showed, *INRA124* displays taurine- and zebu-diagnostic alleles. The genetic dichotomy often observed between zebu and taurine cattle is useful for detecting gene flow and introgression between the two taxa (MacHugh *et al.* 1997; Bradley et al. 1998). Both *BM861* (a similar marker to *INRA124*) and *INRA189* seem to show allele size variants specific to taurine and zebu in the cattle breeds studied. Although these loci have zebu- and taurine-diagnostic alleles, the presence of these alleles in related bovid species cannot be taken to be meaningful and care must be taken before making assumptions about inter-specific relationships based on these microsatellite data. There are at least three reasons why two samples can possess the same length variant at any one locus. These are: common descent from the same ancestor; introgressive hybridisation;

and convergent evolution (or independent mutation, i.e. identical in state but not identical by descent).

In the future this marker panel should prove a useful tool for evolutionary studies in bovid species as it may provide a high-resolution perspective on paternal relationships in a range of populations. Also, apart from the zebu/taurine diagnostic alleles mentioned previously, these microsatellites could provide additional evidence concerning the origins of African cattle.

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