Female segregation patterns of the putative Y-chromosome-specific microsatellite markers *INRA124* and *INRA126* do not support their use for cattle population studies

L. Pérez-Pardal*, L. J. Royo*, I. Álvarez*, F. A. Ponce de León[†], I. Fernández*, R. Casais[‡] and F. Goyache*

*SERIDA-Somió, C/ Camino de los Claveles 604, E-33203 Gijón (Asturias), Spain. [†]Department of Animal Science, University of Minnesota, 480 Haecker Hall, 1364 Eckles Avenue, St Paul, MN 55108, USA. [‡]SERIDA-Jove, Jove del Medio, E-33299, Gijón (Asturias), Spain

Summary

Here we tested the segregation and paternal compatibility of markers *INRA124* and *INRA126* on female DNA in 10 different cattle families, in order to clarify the usefulness of these microsatellites for the study of male-mediated population processes in cattle. Their performance was compared with that of four microsatellites located in the PAR-BTAY (*UMN0108*, *UMN0803*, *UMN0929* and *UMN0905*) and another one male-specific microsatellite (*INRA189*). *INRA124* and *INRA126* amplified the same sized fragment in both sexes. Same size alleles were sequenced and the high homology found allowed us to rule out non-specific female amplification. *INRA124* showed full parental compatibility, whilst the locus *INRA126* showed 55% parental incompatibility. Based on these observations, it is recommended that markers *INRA124* and *INRA126* should not be used in studies to characterize male-mediated genetic events in cattle.

Keywords cattle, *INRA124*, *INRA126*, phylogeny, Y-chromosome, Y-specific markers.

Assessment of neutral genetic variability on the Y-chromosome in livestock is of high interest to ascertain male genetic legacies throughout the history of a species (MacHugh & Bradley 2001). The number of studies on bovine Y-chromosome (BTAY) polymorphisms has quickly increased (Vaiman *et al.* 1994; Hanotte *et al.* 1997; Liu *et al.* 2003). Such studies have focused on the assessment of male-mediated migration patterns and admixture between *Bos taurus* and *Bos indicus* (Hanotte *et al.* 2000; Li *et al.* 2007). The number of polymorphic-specific markers identified on BTAY is limited (Vaiman *et al.* 1994; Kappes *et al.* 1997; Liu *et al.* 2003) and most population genetic studies in cattle have included *INRA124* and *INRA126* (Edwards *et al.* 2000, 2007; Giovambattista *et al.* 2000; Hanotte *et al.* 2000; Li *et al.* 2007), which were identified and reported to be male-specific by Vaiman *et al.* (1994). Indeed, the chromosomal location of the X- and Y-specific regions on both BTAX and BTAY has remained controversial (Liu *et al.* 2003).

This research aims to clarify the usefulness of the markers *INRA124* and *INRA126* for the study of male-mediated

population processes in cattle. This is justified by the uncertainty of their physical location on BTAY and the lack of systematic tests for the amplification of these markers on female DNA. Here, we test the amplification of these markers on female DNA and their segregation and paternal compatibility (markers clonally inherited from father to son) on 10 different cattle families (see Pedigrees in Fig. 1). Their performance is compared with that of five BTAY microsatellites, four located in the pseudo-autosomal region (PAR) of BTAY (*UMN0108*, *UMN0803*, *UMN0929* and *UMN0905*; Liu & Ponce de León 2007) and another male-specific microsatellite (*INRA189*; Kappes *et al.* 1997) (Table 1). This will contribute to the ascertainment of the ability of these markers to reflect only the evolutionary histories of male lineages.

As expected, *INRA189* amplified only from males (Table 2). The four microsatellites located in the PAR-BTAY (*UMN0108*, *UMN0803*, *UMN0905* and *UMN0929*) amplified alleles of the same size range both on male and female DNA, showing full parental compatibility except for *UMN0108* and *UMN0905* (see Table 2, Appendix S1). *INRA124* amplified in both sexes with alleles of the same sizes and showed full parental compatibility. *INRA126* showed a more erratic behaviour. It amplified in both sexes with alleles of the same sizes, but the frequency of parental incompatibility was 55% (see Table 2). Alleles of the same

Address for correspondence

F. Goyache, SERIDA-Somió, C/ Camino de los Claveles 604, E-33203 Gijón (Asturias), Spain.
E-mail: fgoyache@serida.org

Accepted for publication 18 January 2009

Figure 1 Description of analysed pedigrees of the Asturiana de los Valles beef cattle. Sires are identified using numbers from 1 to 10 whilst dams are identified with letters from A to S. Offspring are identified combining the corresponding number and letter with exception of two twin female calves that are identified by adding the symbols α and β . Paternity was confirmed in all cases using the 11 genomic StockMarks Kit for Cattle Bovine Genotyping (Applied Biosystems Inc.).

Table 1 Description of the microsatellite markers tested.

Marker	Primer sequences [5'–3']	No. alleles ¹	Size range ¹	Location ²	Reference
UMN0108	GATCCATCCACATTGCTCCA-up CCAAGCGTCCATCAATTTC-dn	5	190–208	PAR	Liu <i>et al.</i> (2003)
UMN0803	GATCCACATCCCCCTCAC-up CTGCTTCTCTTGTCGCTAA-dn	2	260–279	PAR	
UMN0929	ACCAGCTGATACACAAGTGC-up GGTCAGAGAATGAAACAGAG-dn	8	174–196	PAR	
UMN0905	ATCAACCGTGGTAGCTCTAA-up CTAGAATGTAAACCAGCTGC-dn	4	158–164	PAR	
INRA124	GATCTTTGCAACTGGTTTG-up AGGACACAGGTCTGAGAATG-dn	2	130–132	Y	Vaiman <i>et al.</i> (1994)
INRA126	GTTGTTGCCTTCGAGAGTAGG-up GACACTCTTCTATTTCAAGG-dn	5	179–187	Y	Vaiman <i>et al.</i> (1994)
INRA189	TTTTGTTTCCCGTGCTGAG-up GAACCTCGTCTCTTGAGCC-dn	2	152–160	MSY	Kappes <i>et al.</i> (1997)

¹No. alleles and size range found in the 10 cattle families tested in this study.

²Following Liu & Ponce de León (2007): PAR and MSY mean the pseudoautosomal and male-specific regions of BTAY respectively.

size, on loci *INRA124* and *INRA126*, were sequenced from two individuals of each sex (including a female zebu in *INRA124*). For *INRA124*, we found one polymorphism with respect to the published sequence (Genbank accession no. X71546) (Appendix S1). For *INRA126*, three polymorphisms were found with respect to the published sequence (Genbank accession no. X71553) (Appendix S1). Altogether these results allowed us to rule out non-specific female amplification.

Liu *et al.* (2003) suggested three explanations as to why a Y-specific marker amplifies the same product from both male and female DNA: (i) the common origin of sexual chromosomes, with X- and Y-specific regions still sharing homologous sequences across the genome; (ii) the existence of multiple copies of a given BTAY-specific marker along the chromosome; and (iii) non-specific PCR amplification of female DNA samples. If amplification products of BTAY microsatellites on female DNA differ in size to those obtained

Table 2 Identification of genotyped individuals and their genotypes for the seven Y-chromosomes tested. The male calf offspring is that amplifying for the microsatellite *INRA189*. Genotypes showing locus incompatibility are in bold. Each sample was genotyped three times for all markers using different PCR amplifications to verify consistency of obtained results (Appendix S1). Identical results were found regardless of PCR condition.

Identification		INRA189		UMN0108		UMN0803			UMN0905			UMN0929			INRA124			INRA126		
Bull	Cow	Offs	B	C	O	B	C	O	B	C	O	B	C	O	B	C	O	B	C	O
1	A	1A	152	--	190-194	190-194	279	279	279	164	164	164-168	164	164-168	174-176	174-192	130-132	130	181	187
	B	1B	--	--	200-210	194-210	279	279	279	164	164	164	164	164	176-192	174-192	130-132	130	181	181
2	C	2C α	152	--	190-198	190-194	279	279	279	164	166	164	164	164-166	174-176	174-184	130-132	130	187	187
		2C β	--	--	194-198	194-198	279	279	279	164	166	164	164	164-166	174-184	174-184	130	130	181	187
3	D	3D	152	--	194-202	194-208	279	279	279	164	166	164	164	164-166	174-192	176-192	130-132	130	183	181
	E	3E	--	--	190-194	194-202	279	279	279	166	166	166	164	164-166	176-184	176-194	130-132	130	181-185	183
4	F	4F	152	--	190-194	192-202	279	279	279	164	164	164	164	164	174-192	180-186	130-132	132	181	183
	G	4G	--	--	194-208	194	279	279	279	166	166	166	164	164-166	188-194	192-194	130-132	132	183	183
5	H	4H	--	--	194-202	190-202	279	279	279	166	166	166	164	164	176-192	192	132	130-132	183	181
	I	5I	152	--	194-200	200-208	260	260-279	260-279	164	166	166	164	166	174-194	176-192	130-132	130	185	179
6	J	6J	152	--	192-200	194	260-279	279	279	164	164	164-166	164	164-166	174-176	174-194	130	130	187	181
	K	6K	--	--	192-194	192	279	279	279	164	166	164	164	164-166	174-180	180-194	130	130	183	183
7	L	7L	152	--	190-194	192-194	260	260-279	279	260-279	166	164-166	166	164-166	174-192	174	130	130	183	183
8	M	8M	152	--	192-194	194	192-194	279	279	166	164	164	164	164-166	174-184	174	130-132	130-132	181	181
9	N	9N	160	--	190-194	194-200	279	279	279	158-166	164-166	158-166	164	164-166	176-192	188-192	130	130	183-187	185
	O	9O	--	--	190-194	194	279	279	279	164-168	164-166	164	164	164-166	174-176	174-176	130-132	130-132	181	183
10	P	9P	--	--	194-200	190-200	279	279	279	166	158	166	164	164	192-194	192-194	130-132	130-132	183	187
	Q	9Q	--	160	192-200	190-200	279	279	279	164	158-164	164	164	158-164	176-184	184-192	130-132	130	179-187	179-187
10	R	9R	--	160	200-202	194-200	260	260-279	260-279	164	164	164	164	164-166	176-192	176-192	130-132	130	185	183
	S	10S	152	--	200-208	200-208	279	279	279	162-164	164-168	162	162	164	192-196	174-184	130-132	130-132	181	185

from male DNA, the Y-specificity of a microsatellite should be accepted. Here, we report that both markers do not meet with this 'rule of thumb' and phylogenetic studies in cattle should therefore be questioned.

This is the first time in which amplification of the microsatellite *INRA124* on female DNA is reported (Fig. S1). Moreover, even though this marker has previously only been found to be polymorphic between *B. taurus* and *B. indicus* (Hanotte *et al.* 1997, 2000; Edwards *et al.* 2007; Li *et al.* 2007), we have also found polymorphism within both *B. taurus* and *B. indicus*. This was checked by genotyping 200 taurine male samples belonging to 37 European cattle breeds, four Brahman, two Brangus and four Indian Zebu sire samples (Table S1). Three different alleles (130, 132 and 134 bp) were found in the taurine samples and other three (126, 128 and 132) on the zebu males.

INRA124 has been frequently used in studies assessing admixture between *B. taurus* and *B. indicus* (Hanotte *et al.* 2000; Edwards *et al.* 2007; Li *et al.* 2007). As this marker undoubtedly amplifies the same PCR product on female taurine and zebu DNA, the occurrence of recombination events cannot be rejected. Therefore, the studies assessing male-mediated introgression events carried out on the basis of this marker alone should be interpreted with caution.

Some doubts on the Y-specificity of the marker *INRA126* already existed. PCR products of the same size from both sexes have been reported on cattle (Liu *et al.* 2003), yaks (Edwards *et al.* 2000) and saola (Nguyen *et al.* 2005). This could indicate that, across bovids, the X-chromosome has retained a homologous sequence to the Y-chromosomal segment containing the *INRA126* marker (Edwards *et al.* 2000) or that the location of the locus *INRA126* is on the PAR-BTAY (Li *et al.* 2007). Here, we show that *INRA126* amplifies from female DNA but also that this locus has a large variability within family. The large number of paternal incompatibilities that have accumulated on locus *INRA126* (see Table 2) cannot be easily explained, even though unexpectedly high mutation rates together with chromosomal recombination could be the basis of the large variability assessed on this locus (Li *et al.* 2007). Whatever the explanation might be, the use of the locus *INRA126* to characterize male evolutionary histories is questionable and not recommended.

Recently, Anderung *et al.* (2007) indicated the occurrence of hybridization between indicine and taurine cattle in Africa, and the presence of indicine X chromosomal recombinant forms. From our results, recombination events involving loci *INRA124* and *INRA126* cannot be rejected, and therefore use of these markers may distort actual indicine male-mediated ancestries.

Based on our findings, it is recommended that the *INRA124* and *INRA126* markers should not be used in studies to characterize male-mediated genetic events. Assessment of paternal genetic legacies would require thorough examination of BTAY-specific marker amplifica-

tion patterns and segregation on female DNA. Previous cattle population studies using these markers alone should be interpreted with caution. New analyses using Y-specific markers are necessary to gain a better understanding of the different levels of admixture in breeds from different geographic areas and the ages of the admixtures (Anderung *et al.* 2007).

Acknowledgements

This study was partially funded by MEC-FEDER: CGL2005-03761/BOS and USDA-Hatch project MIN-16-019. L. Pérez-Pardal is funded by BES-2006-13545. We acknowledge Ligia Mercedes Jimenez Robayo, Maria Raquel Carvalho and Catarina Ginja for their help in sampling.

References

- Anderung C., Hellborg L., Seddon J., Hanotte O. & Götherström A. (2007) Investigation of X- and Y-specific single nucleotide polymorphisms in taurine (*Bos taurus*) and indicine (*Bos indicus*) cattle. *Animal Genetics* **38**, 595–600.
- Edwards C.J., Gaillard C., Bradley D.G. & MacHugh D.E. (2000) Y-specific microsatellite polymorphism in a range of bovid species. *Animal Genetics* **31**, 127–30.
- Edwards C.J., Baird J.F. & MacHugh D.E. (2007) Taurine and zebu admixture in Near Eastern cattle: a comparison of mitochondrial, autosomal and Y-chromosomal data. *Animal Genetics* **38**, 520–4.
- Giovambattista G., Ripoli M.V., De Luca J.C., Mirol P.M., Lirón J.P. & Dulout F.N. (2000) Male-mediated introgression of *Bos indicus* genes into Argentine and Bolivian Creole cattle breeds. *Animal Genetics* **31**, 302–5.
- Hanotte O., Okomo M., Verjee Y., Rege E. & Teale A. (1997) A polymorphic Y chromosomal microsatellite locus in cattle. *Animal Genetics* **28**, 308–22.
- Hanotte O., Tawah C.L., Bradley D.G., Okomo M., Verjee Y., Ochieng J. & Rege J.E. (2000) Geographic distribution and frequency of a taurine *Bos taurine* and an indicine *Bos indicus* Y specific allele amongst sub-Saharan African cattle breeds. *Molecular Ecology* **9**, 387–96.
- Kappes S.M., Keele J.W., Stone R.T., McGraw R.A., Sonstegard T.S., Smith T.P., Lopez-Corrales N.L. & Beattie C.W. (1997) A second-generation linkage map of the bovine genome. *Genome Research* **7**, 235–49.
- Li M.H., Zerabruk M., Vangen O., Olsaker I. & Kantanen J. (2007) Reduced genetic structure of north Ethiopian cattle revealed by Y-chromosome analysis. *Heredity* **98**, 214–8.
- Liu W.-S. & Ponce de León F.A. (2007) Mapping of the bovine Y chromosome. *Electronic Journal of Biology* **3**, 5–12.
- Liu W.-S., Beattie C.W. & Ponce de León F.A. (2003) Bovine Y chromosome microsatellite polymorphism. *Cytogenetic and Genome Research* **10**, 53–8.
- MacHugh D.E. & Bradley D.G. (2001) Livestock genetic origins: goats buck the trend. *Proceedings of the National Academy of Sciences of the United States of America* **98**, 5382–4.
- Nguyen T.T., Ménétrey F., Genini S., Nguyen V.L., Vögeli P., Nguyen B.X. & Stranzinger G. (2005) Application of bovine

microsatellite markers on Saola (*Pseudoryx nghetinhensis*). *Journal of Animal Breeding and Genetics* **122**, 195–8.

Vaiman D., Imam-Ghali M., Moazami-Goudarzi K., Guerin G., Grohs C., Leveziel H. & Saidi-Mehtar N. (1994) Conservation of a syntenic group of microsatellite loci between cattle and sheep. *Mammalian Genome* **5**, 310–4.

Supporting information

Additional supporting information may be found in the online version of this article.

Figure S1 Genmapper image of *INRA124* amplification (green lines due to VIC labelling of PCR products). Bands of different size form the GenScan 500-LIZ standard (orange).

Table S1 Number of genotyped individuals (N) and allele frequency found on locus *INRA124* in 37 bovine cattle breeds and some Zebu cattle samples.

Appendix S1 PCR conditions and DNA sequences of the markers used.

Please note: Wiley-Blackwell is not responsible for the content or functionality of any supporting information supplied by the authors.