J. Anim. Breed. Genet. ISSN 0931-2668

ORIGINAL ARTICLE

Application of bovine microsatellite markers on Saola (*Pseudoryx nghetinhensis*)

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Received: 13 July 2004;
accepted: 22 November 2004

Summary

The aim of the present study was to assess the applicability of bovine microsatellite markers on Saola (*Pseudoryx nghetinhensis*). A total of 127 microsatellite markers were tested on a male and a young female Saola. An efficient amplification was observed for 123 markers (96.8%), 73 markers (59.3%) were polymorphic. Four loci (*BM2304*, *BMS1928*, *BMS779* and *ILSTS006*) on cattle chromosomes 1, 4, 7 and 8, respectively, failed to amplify in Saola. Two cattle Y-chromosome-specific microsatellite markers (*INRA126* and *BM861*) were successfully amplified from both sexes in Saola. However, two additional markers (*INRA124* and *INRA189*) on Y-chromosome failed to amplify in the female animal. These results show that most of the bovine microsatellite markers are applicable in Saola and therefore they can be used to study the phylogenetic relationships and the genetic diversity of the Saola population.

Introduction

Saola (*Pseudoryx nghetinhensis*), a new bovid species, was discovered in the montane evergreen forests of Vu Quang, Vietnam, and described by Dung *et al.* (1993). It is an endangered species with only a few hundred representatives surviving in the wild. Although very few studies have been carried out on Saola because of the poor material availability and the unknown number of chromosomes, studies of the molecular phylogeny by Hassanin & Douzery (1999) and Gatesy & Arctander (2000) suggested that *Pseudoryx* belongs to Bovinae.

Due to their extremely high level of polymorphism, microsatellite markers, which consist of tandemly repeated sequences, have been used for comparative studies of genetic diversity (MacHugh *et al.* 1997), genetic differentiation among closely related populations (Martin-Burriel *et al.* 1999), genetic linkage maps (Kappes *et al.* 1997), phylogenetic analysis (Ritz *et al.* 2000) and studies of population genetic (Bruford & Wayne 1993). Furthermore, microsatellite markers can be used for studies of population variation in geographically isolated or endangered species (Moore *et al.* 1995). In addition, the applicability of bovine microsatellite markers for genetic analysis in many species, for example sheep (*Ovis aries*, De Gortari *et al.* 1997), goat (*Capra hircus*, Pepin *et al.* 1995), African buffalo (*Syncerus caffer*, Van Hooft *et al.* 1999) and water buffalo (*Bubalus bubalis*, Moore *et al.* 1995) was reported. These studies let assume that a characterization of Saola, as a supposed Bovinae, is pertinent with bovine microsatellite markers.

Materials and methods

DNA from a male and a female Saola (Figure 1) was extracted from muscle tissue following the standard protocol of Laird *et al.* (1991). DNA from Brown Swiss cattle (*Bos taurus*) was extracted from whole blood (Higuchi 1989) and used as positive control.

A total of 127 bovine microsatellite markers distributed on the entire cattle genome and showing a

Figure 1 Saola (*Pseudoryx nghetinhensis*) Vietnam, 1996. EBRV-CNST.

high frequency of polymorphism in cattle were chosen for PCR amplification from the CaDBase (Roslin Institute Edinburgh, Roslin BioCentre, UK), the Cattle Genome Mapping Project database (MARC, Clay Center, NE, USA) and the BOVMAP database (INRA, Jouy en Josas, France, Table 1). Amplification reactions were performed in 25 μl reaction volumes following the standard protocol. The forward primer of each marker was 5′-labelled, with either a *FAM*, a *JOE* or a *TET* fluorescent tag. After the first denaturation step at 95°C for 5 min, samples were subjected to 35 cycles of denaturation at 95°C for 30 s, 52–60°C annealing for 30 s and extension at 72°C for 30 s. The final cycle was followed by an extension at 72°C for 7 min. Gel electrophoresis was performed with a 377 ABI sequencer (Applied Biosystems, Perkin-Elmer Corp., Foster City, CA, USA). Subsequent gel analysis and fragment sizing were performed using ABI 672 Genescan software and Genotyper (version 2.1; Applied Biosystems) software.

Results

Although some application failures were observed, 96.8% of the microsatellites from cattle could be successfully amplified on Saola by PCR (Table 1). Seventy-three markers (59.3%) were polymorphic, 50 markers (40.7%) were homozygous and only four markers (*BM2304*, *BMS1928*, *BMS779* and *ILSTS006*) failed to amplify. Four loci (*INRA124*, *INRA126*, *INRA189* and *BM861*) on cattle Y-chromosome gave PCR products when amplified with DNA from male Saola. However, *INRA124* and *INRA189* failed to amplify in the female animal. In the positive control (*Bos taurus*), all the microsatellite markers could be amplified with 88.4% of them being polymorphic.

Discussion

The positive amplification rate in Saola (96.8%) is higher compared with other species of the subfamily Bovinae previously reported, for example African buffalo (*Syncerus caffer* 83%, Van Hooft *et al.* 1999) and water buffalo (*Bubalus bubalis* 70%, Moore *et al.* 1995). In accordance with the previous phylogenetic analyses of Hassanin & Douzery (1999) and Gatesy & Arctander (2000), these results confirm that *Bos* is more closely related to *Pseudoryx* than to either *Sycerus* or *Bubalus*. However, 40.7% homozygous microsatellites in Saola were observed. The reason of this high percentage of homozygous microsatellites in Saola may be the limited number of animals tested. In addition, for some markers, some significant differences in the allele size range in Saola were observed in comparison with the positive control (data not shown). The amplification of two cattle Y-chromosome-specific microsatellite markers (*INRA126* and *BM861*) in both sexes in Saola suggests that the Saola X-chromosome retained a homologous sequence to the Y-chromosomal segment, which contains the *INRA126* and *BM861* microsatellites. Furthermore the non-amplification of the four loci (*BM2304*, *BMS1928*, *BMS779* and *ILSTS006*) on chromosomes 1, 4, 7, 8 in cattle could be attributed to the absence of the homologous sequence in this animal.

In conclusion, this study shows that a large fraction of bovine microsatellite markers can be amplified in Saola and that these markers are applicable for genetic studies on this species. This report provides further support that it is possible to use bovine microsatellite markers to establish genetic distances, to construct phylogenetic trees or to confirm parentage in Saola. In the future, cytogenetic and molecular genetic research will help to clarify the genome construction of Saola and will contribute to preserve this endangered species from extinction.

Acknowledgements

The samples from Saola were provided by the Laboratory of Embryo Technology, Vietnam Academy of Sciences and Technology, Hanoi, Vietnam. The authors wish to thank N.T. Uoc and L.V. Ty for sample collection and preparation and Š. Š. Kramer for technical assistance in the laboratory.

Table 1 List of bovine microsatellite markers tested on Saola (*Pseudoryx nghetinhensis*) with the cattle chromosome location, the allele size and the number of alleles in Saola

Marker	Chromosome number in cattle	Alleles of Saola (bp)	Number of alleles in Saola	Marker	Chromosome number in cattle	Alleles of Saola (bp)	Number of alleles in Saola
AGLA17	1	217	1	BR4206	18	98	1
BM6438	1	271, 283, 285	3	BR4406	18	102	1
BM8139	1	111, 121	2	HAUT14	18	116, 124	2
BMS1928	1	–	–	ILSTS002	18	99, 103	2
BMS4015	1	138, 140, 146	3	ILSTS021	18	120	1
BMS574	1	130, 132	2	INRA063	18	185, 187	2
BMS711	1	111, 113	2	INRA121	18	111	1
INRA117	1	103, 109	2	TEXAN10	18	128	1
TGLA49	1	131, 135	2	TGLA227	18	68	1
BM2113	2	117, 119	2	BMS650	19	148, 151, 166	3
ETH121	2	184, 210	2	BMS745	19	65, 85	2
BM3020	3	154, 158, 160, 166	4	ETH3	19	157	1
BM6465	3	112, 118	2	BMS1120	20	125	1
INRA023	3	213, 217, 219	3	BMS1128	20	90, 92	2
BMC1410	4	264	1	BMS1282	20	152, 154	2
BMS1074	4	139, 153	2	TGLA126	20	113	1
BMS779	4	–	–	HEL5	21	164	1
AGLA293	5	188	1	TGLA122	21	168, 174	2
ETH10	5	209, 213, 217	3	BMS672	22	156, 160	2
ETH152	5	207, 209, 213	3	BMS875	22	107, 109, 111	3
BL1038	6	99, 101	2	HAUT24	22	122, 148	2
BM4621	6	143, 145	2	BM1818	23	264, 268	2
INRA133	6	226	1	MB026	23	234, 236, 240	3
BL1043	7	87	1	BMS1926	24	126	1
BMS1247	7	107	1	BMS2270	24	60	1
BMS1979	7	100	1	BMS2526	24	149, 155, 163	3
BMS522	7	144	1	BMS3024	24	140, 150	2
IDVGA90	7	166	1	CSSM023	24	222, 226	2
ILSTS006	7	–	–	BM4005	25	117, 125, 131	3
BM2304	8	–	–	BMS1353	25	110, 112	2
HEL9	8	149, 175	2	ILSTS102	25	124, 132, 150	3
RM372	8	116	1	BL1040	26	88	1
ETH225	9	156	1	BM1314	26	134, 138	2
MM12E6	9	104, 106	2	BM188	26	108, 110	2
TGLA73	9	129, 131, 139	3	BMS332	26	127	1
CSRM60	10	103, 105, 107	3	HAUT27	26	156, 162	2
ILSTS005	10	149, 151, 185	3	IDVGA59	26	260	1
INRA037	10	104	1	INRA081	26	151	1
BMS424B	11	257, 259	2	RM026	26	86	1
HEL13	11	154, 188	2	BM203	27	214	1
INRA032	11	146	1	INRA183	27	122	1
BMS2252	12	160, 174, 178	3	TGLA179	27	92	1
INRA005	12	148, 150	2	BL25	28	199, 203	2
BL1071	13	191, 195, 197	3	BMC6020	28	179, 181, 207	3
TGLA23	13	92, 94	2	BMS1714	28	120, 130	2
UWCA25	13	104, 108, 116	3	BM4602	29	116, 134	2
BL1029	14	143, 151, 153	3	BMC2228	29	175	1
BM6425	14	175, 177, 180	3	BMS1244	29	99, 127, 157, 183	4
CSSM66	14	174	1	BMS1857	29	135, 139, 161	3
BL1095	15	177, 183	2	BMS1948	29	95, 99, 119	3
HEL1	15	103	1	ILSTS015	29	261	1
MB085	15	217	1	BM6017	X	128	1
SPS115	15	269	1	BMC6021	X	193	1
ETH11	16	200, 202	2	BMS1616	X	85, 87	2

Table 1 Continued

Marker	Chromosome number in cattle	Alleles of Saola (bp)	Number of alleles in Saola	Marker	Chromosome number in cattle	Alleles of Saola (bp)	Number of alleles in Saola
INRA035	16	118, 124	2	BMS631	X	126	1
TGLA53	16	134	1	BMS911	X	103, 105	2
BM1862	17	208, 210	2	ILSTS017	X	116	1
BMS1825	17	145, 161	2	XBM11	X	180, 194, 246	3
ETH185	17	184	1	XBM7	X	193, 195	2
BM8151	18	124, 138, 148	3	BM861	Y	136	1
BMS1322	18	136, 140, 144	3	INRA124*	Y	130	1
BMS1355	18	164	1	INRA126	Y	191, 193	2
BMS2213	18	118, 120	2	INRA189*	Y	138	1
BMS2639	18	171	1				

Information concerning the bovine microsatellite markers used can be acquired from: http://locus.jouy.inra.fr/cgi-bin/bovmap/intro2.pl; http://www.marc.usda.gov/genome/genome.html; http://www.projects.roslin.ac.uk/cdiv/accessdb.html.

–, Not amplified.

*Not amplified in the female Saola.

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