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Original article

Cytogenetic and genetic study of a Y-linked microsatellite polymorphism in Polish Black-and-White cattle breed

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ABSTRACT

The aim of the current study was to characterize Polish Black-and-White cattle by morphological study of the Y chromosome. A total of 14 Y-linked microsatellites from UMN and INRA group were genotyped and assessed for polymorphism in a total 22 bulls. Cytogenetic studies in Polish Black-and-White bulls showed the existence of two morphological forms of Y chromosome. Among the 22 karyotypic analyzed bulls, 12 had submetacentric and 10 metacentric Y chromosome. The centromeric index of Y chromosome measured as percentage length of the p arm to total length ratio in the first case was 28 ± 3.97% and in the second 47 ± 7.28%, whereas the relative size of these chromosomes remained within the same range. Morphology and G- and C-banding patterns of both forms of Y chromosome were typical for other cattle breeds originating from *Bos taurus*. Out of a total of 14 microsatellite loci examined, 13 showed specific alleles for two forms of Y chromosome. In a pool of 62 alleles, 43 (69.3%) were common in the two groups of cattle, 19 (30.7%) can be considered as specific for the group; among them 8 were typical for metacentric group of Y chromosome and 11 for submetacentric.

1. Introduction

Polish Black-and-White cattle can be found in the whole Poland, but dominated in areas of northern, central and western parts of Poland. This breed is characterized by bidirectional type of use and typical traits for autochthonous population, such as: high disease resistance and excellent adaptation to the difficult environmental conditions.

In *Bos taurus* and *Bos indicus* karyotypes the autosomes and X chromosomes are morphologically similar and the difference between these two species lies only in the morphology of Y chromosome. The Y chromosome dimorphism in bovines has been studied since 1964. The morphologic difference between the Y chromosomes of the two subspecies can be attributed to a pericentric inversion (Goldammer et al., 1997; Di Meo et al., 2005). Di Meo et al. (2005), concluded that a transposition of the centromere or a pericentric inversion occurred, which differentiated the Y chromosome of *B. taurus* from that of *B. indicus*. Iannuzzi et al. (2001) found an abnormal Y chromosome originated from a pericentric inversion of the Yq arm (Yq11 q12.2) in Podolian cattle. Previously it was generally accepted that Y chromosome of *B. taurus* breeds is metacentric, and the dimorphism of both types of Y chromosome does not occur simultaneously in the same breed (Ford et al., 1980). According to some authors, several native Brazilian breeds present Y chromosome dimorphism within the same breed (Britto and Mello, 1999). Such dimorphism has also been found in other breeds over different countries (Xin and Lin, 1993; Meghen et al., 1994; Jaszczak et al., 1998; Giovambattista et al., 2000). Comparative FISH-mapping was performed to extend the existing cytogenetic maps and improve the understanding of karyotype evolution of these small chromosomes in bovids. According to this study Y chromosomes in *B. taurus* cattle are small submetacentric (Iannuzzi and Di Meo, 1995; ISCNDB2000, 2001). However, Y chromosome in *B. indicus* has been described as small acrocentric and the conventional staining method does not allow to distinguish it from small autosomes (Halnan and Watson, 1982; Di Berardino et al., 2001). According to Mayer (1984), Goldammer et al. (1997) and Stranzinger et al. (2007) the difference in the Y chromosome can be identified as a pericentric inversion with an additional possible loss of genetic material. Comparative banding

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studies of acrocentric and metacentric Y chromosomes, as well as *in situ* hybridization and Southern blotting with male bovine specific DNA probes of *B. taurus* and *B. indicus* indicate that a pericentric inversion is responsible for the morphologic differences between both chromosomes (Goldammer et al., 1997).

The value of markers polymorphism in the Y chromosome studies has been widely recognized and used not only in study of human evolution (Hammer et al., 2001; Kayser et al., 2001) but also in forensic genetics (Gill et al., 2001). However, the information of Y-linked microsatellite polymorphism in farm animals is still limited. As molecular biology technology develops, DNA polymorphism has been widely used in the study of animal breed resources (Kawka et al., 2010; Kawka et al., 2012a,b; Parada et al., 2012). Only Giovambattista et al. (2000) analyzed the polymorphism in Argentinian and the Bolivian cattle, as well as Hanotte et al. (2000) in 69 African local species from 22 countries of African Sahara using INRA124 marker. Among all markers reported so far on the bovine Y chromosome (BTAY), only four have been found to be polymorphic in cattle and related to bovid species (Hanotte et al., 1997).

The aim of this study was to characterize Polish Black-and-White cattle by investigating the morphology of the Y chromosome and to genotype 14 Y-linked microsatellite polymorphism in a total 22 Polish Black-and-White bulls.

2. Material and methods

2.1. Cytogenetic analysis

Cytogenetic examinations were performed on 22 bulls of Polish Black-and-White cattle from private farms located in north-eastern Poland. Chromosome preparations were made from cultured lymphocytes. Whole blood was set up in culture with mitogen phaseoline according to a standard method. Chromosome slides were stained by the routine Giemsa´s staining method, GTG-banding (Seabright, 1971) and CBG-banding (Sumner, 1972). Measurements of chromosome X and Y were made directly on routinely stained preparations by means of a light microscope with a CCD camera connected to a computer supplied with the Multiscan software. A total of 30 metaphases (randomly chosen) from each animals were examined. The centromeric index of chromosome Y for five bulls with metacentric and submetacentric type was calculated as a percentage of the p arm length to the sum of the p and q arms lengths (Halnan and Watson, 1982). The estimation of the relative size of Y chromosome has been simplified and according to the recommendations of Halnan and Watson (1982), was expressed only as a percentage of X chromosome. The significance of differences in two groups of bulls was evaluated by a one way analysis of variance (ANOVA).

2.2. Genetic analysis

For analysis of genetic polymorphism, genomic DNA was isolated from blood using Wizard Genomic DNA Isolation KIT (Promega). Each sample of 22 individuals was examined both spectrophotometrically and electrophoretically. The primer sequences of investigated 14 Y-linked microsatellite *loci* designed by the UMN (University of Minnesota) and INRA (French National Institute for Agricultural Research) groups (Table 1) were performed. One primer from the given pair has been labeled with one of the four dyes – 6-FAM, VIC, NED and PET.

The amplification of selected microsatellite *loci* was performed using a thermal cycler PTC-200 Engine (MJ Research). The PCR mixture consisted of 10 ng of template DNA, 100 pmol of each primer, 10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 0.5 mM of each nucleotide, 0.01% Triton X-100 and 0.5 units of *Taq* polymerase (Polgen) in a final volume of 10 μl. The PCR conditions were optimized for all primer pairs. The PCR was performed using one cycle at 94 °C during 5 min, followed by 30–35 cycles, each consisting of denaturation during 45 s at 94 °C, annealing during 45 s at 54–65 °C and extension during 90 s at 72 °C. One last cycle (elongation step) was performed during 10 min at 72 °C. The fluorescent PCR products were separated by electrophoresis using the four-capillary Genetic Analyzer 3130 (Applied Biosystems). The results were visualized and the genotyping was completed with GenScan 2.1. software. In addition, Gene Mapper software (Applied Biosystems) was used to automatically determine allele sizes for the individual markers. The statistical analysis of obtained results was performed using Cervus software (Kalinowski et al., 2007). It included following population parameters: frequency of alleles, observed and expected heterozygosity (H_o and H_e) and the polymorphic information content (PIC).

3. Results and discussion

3.1. Cytogenetic study

All animals analyzed in this study presented $2n = 60$, showing that the chromosome number does not vary within the species *B. taurus* (Iannuzi, 1996). All autosomes were acrocentric, and the X chromosome, one of the largest in the karyotype, was submetacentric.

The cytogenetic examination of bulls has shown that in Polish Black-and-White cattle two morphologic forms of Y chromosome – submetacentric and metacentric were observed (Fig. 1). Among the 22 bulls analyzed, submetacentric Y chromosome was observed in 12 animals. A metacentric type of Y chromosome occurred in the remaining 10 bulls. The centromeric index of Y chromosome in metacentric type was 47 ± 7.28% and submetacentric type – 28 ± 3.97%. The difference between them was statistically significant at $P \leqslant 0.01$ (Table 2). The relative size of the submetacentric and metacentric type of Y chromosome, as expressed by the Y:X ratio, was 31 ± 3.7% and 32 ± 4.1%, respectively. An analysis of G–banding patterns of submetacentric and metacentric Y chromosomes have not revealed differences between them. Following C-banding usually the Y chromosome was dark throughout. In some preparations the long arm stained faintly and the short arm and centromeric region stained darkly. Similarly, the centromeric index and the relative size of the Y chromosome in the Polish Black-and-White bulls studied here were characteristic of the European *B. taurus* breeds (Halnan and Watson, 1982). The presence of two types of Y chromosome – submetacentric and metacentric within one breed, observed in the case of Polish Black-and-White cattle, has been also noticed in other breeds of European origin. It was reported by Jaszczak et al. (1998), who proved the differences in the Y chromosomes (meta- to submetacentric) in Piemontese bulls. In crosses between *B. taurus* and *B. indicus*, a very significant difference in Y chromosomes is visible – *B. taurus* with a meta- to submetacentric and *B. indicus* with acro- to telocentric Y chromosome. These visible morphological differences, however are not always cause the significant fertility problems and minor variations mostly disturb the reproduction processes (Stranzinger et al., 2007).

3.2. Genetic study

Characteristics of cattle groups with meta- and submetacentric Y chromosome based on observed heterozygosity (H_o), expected heterozygosity (H_e) and PIC index are presented in Table 3. Mean heterozygosity for 14 analyzed markers were similar in both groups. The H_o ranged from 0.00 to 1.00 in both groups of cattle.

Table 1
Characteristics of 14 cattle microsatellite markers used in the study.

Microsatellite	Sequence of microsatellite	Number of alleles	Length of alleles (bp)	GenBank no.
UMN0304	TGATATTCACAAGGCCGCTG GGCTGTGGTATACTATGGAG	10	210 to 232	AF483758
UMN0307	GATACAGCTGAGTGACTAAC GTGCAGACATCTGAGCTGTG	12	101 to 162	AF483750
UMN0406	GTTGAGGACTCTTGCATCTG TGCTTCATCCTTCATTCCAC	14	140 to 172	AF483760
UMN0905	ATCAACCGTGGTAGCTCTAA CCAGAATGTAAACCAGCTGC	12	160 to174	AF483748
UMN0920	GTTGAGGACTCTTGCATCTG CACAGGCCTAGAAGATTGAG	16	254 to 290	AF483763
UMN0929	ACCAGCTGATACACAAGTGC GGTCAGAGAATGAAACAGAG	10	176 to 193	AF483749
UMN2303	TACTTGCTTGAGACTTACTG TGTGAACACATCTGATTCTG	20	98 to 132	AF483753
UMN2404	GGTACAATTGAAAATATG TGTACCTACACTGATATGTT	15	85 to 112	AF483769
UMN2405	CCTGCCATCCATTGTGAAGA CTGCTTACCTGGTCAGGATT	12	140 to 176	AF483770
UMN2706	TTGTTGAGGACTCTTGCATC CCACATATCAGGCAAAGTCAT	20	109 to 150	AF483772
UMN2713	GTACCTACACTAATATGTTCA CCAAAGAAAGTTCAGGTACA	20	94 to 124	AF483773
UMN3008	TTGTGGAGGACTATTCATGG TCTGGACTCGACAGGACACC	16	172 to 214	AF483755
INRA124	GATCTTTGCAACTGGTTTG AGGACACAGGTCTGAGAATG	12	58 to 67	X71546
INRA189	TTTTGTTTCCCGTGCTGAG GAACCTCGTCTCCTTGTAGCC	7	43 to 44	X73941

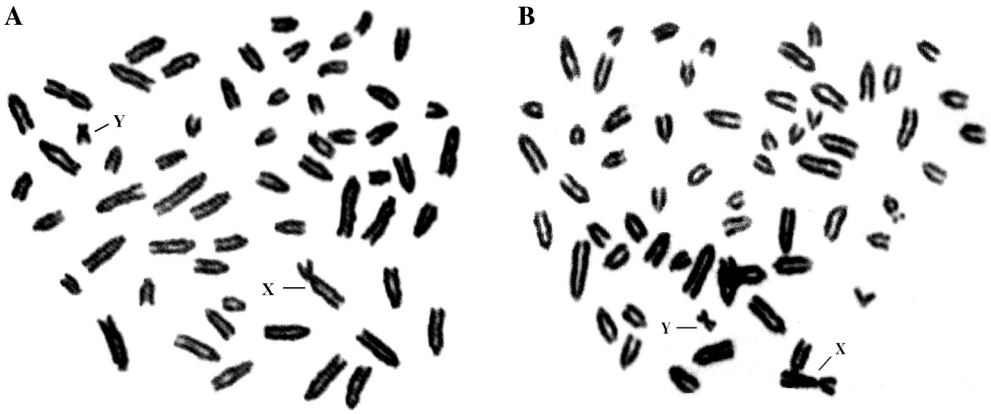

Fig. 1. Metaphase chromosomes of Polish Black-and-White bulls. A – with submetacentric Y chromosome; B – with metacentric Y chromosome.

Table 2
Centromeric index and relative size of Y chromosome in selected individuals of Polish Black–and–White cattle.

Type of chromosome Y	Number of bulls	Number of metaphase	Centromeric index (%) $(p/p+q) \times 100$	Relative size (%) Y/X × 100
Metacentric	5	150	47 ± 7.28**	32 ± 4.1
Submetacentric	5	150	28 ± 3.97**	31 ± 3.7

** Values within the column differs significantly at $P \leqslant 0.01$.

In turn, the values of the H_e estimated for population analyzed, ranged from 0.00 to 0.83 (metacentric group) and from 0.00 to 0.82 (submetacentric group). Both mean values (H_o and H_e) occurred relatively high ~0.80 what indicates the high genetic variability in the population. As regards the PIC, the highest values were observed for 6 *loci* in metacentric and for 3 *loci* in submetacentric group. The lowest values of the PIC in metacentric group (0.00, 0.36, 0.37 and 0.38) were recorded for *locus* UMN3008, UMN0920, UMN2405 AND INRA124, respectively. In submetacentric group, the lowest PIC (0.00, 0.42, 0.46 and 0.49) were observed for *locus* UMN3008, UMN2303, UMN0929, UMN2405 and INRA189, respectively.

Out of a total of 14 microsatellite *loci* examined, 13 showed different alleles for both groups (Table 4). One microsatellite *locus* (UMN3008) had no specific alleles in any bull group. In a total pool of 62 microsatellite alleles, 43 (69.3%) were common for the two bull groups. The most common alleles were observed at *locus* UMN0406 (5 of the 7 identified alleles) and *loci* UMN0304, UMN0307, UMN0905, UMN0929, UMN2303, UMN2404, UMN2706 and UMN2713 – 4 common alleles.

Table 3
Population parameters in studied group of bulls.

Locus	Observed heterozygosity			Expected heterozygosity			PIC		
	Metacentric	Submetacentric	Overall	Metacentric	Submetacentric	Overall	Metacentric	Submetacentric	Overall
UMN0304	1.00	1.00	1.00	0.77	0.80	0.78	0.69	0.72	0.72
UMN0307	1.00	1.00	1.00	0.63	0.64	0.66	0.52	0.54	0.58
UMN0406	0.60	0.60	0.60	0.76	0.71	0.74	0.68	0.64	0.69
UMN0905	1.00	1.00	1.00	0.63	0.77	0.70	0.52	0.69	0.64
UMN0920	0.80	0.80	0.80	0.50	0.67	0.59	0.36	0.56	0.49
UMN0929	0.80	0.60	0.70	0.80	0.56	0.73	0.71	0.46	0.66
UMN2303	0.40	0.40	0.40	0.43	0.48	0.64	0.38	0.42	0.55
UMN2404	1.00	1.00	1.00	0.76	0.82	0.79	0.68	0.75	0.74
UMN2405	1.00	1.00	1.00	0.52	0.61	0.63	0.37	0.49	0.54
UMN2706	1.00	1.00	1.00	0.70	0.70	0.69	0.61	0.61	0.63
UMN2713	1.00	0.90	0.95	0.83	0.71	0.79	0.76	0.61	0.73
UMN3008	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
INRA124	1.00	0.90	0.95	0.52	0.65	0.60	0.37	0.57	0.52
INRA189	1.00	1.00	1.00	0.78	0.61	0.71	0.69	0.49	0.63
MEAN	0.82	0.80	0.81	0.62	0.62	0.65	0.52	0.54	0.58

Table 4
Common and specific alleles for two analyzed groups of Polish Black-and-White bulls.

Locus	Alleles common for two groups of bulls	Allele specific for the group		Number of alleles
		Metacentric	Submetacentric	
UMN0304	214,220,222,226	–	218	5
UMN0307	100,148,154	–	146	4
UMN0406	150,158,162,164,168	144,156	–	7
UMN0905	164,166,168	–	158,160	5
UMN0920	256,258	–	242	3
UMN0929	176,178,186	192,194	182	6
UMN2303	101,103,105	115	–	4
UMN2404	82,84,94,96	–	92	5
UMN2405	139,155	–	153	3
UMN2706	121,127,129,133	–	139	5
UMN2713	99,101,103,105	95,115	–	6
UMN3008	180	–	–	1
INRA124	60,66	–	42,46	4
INRA189	35,37,39	41	–	4
TOTAL	43	8	11	62

Nineteen (over 30%) microsatellite alleles from a total pool of alleles occurring in the genome of the two analyzed bull groups can be considered as specific for the group. Of these alleles, 8 (42.1%) were typical for metacentric bulls and 11 (57.8%) for submetacentric. The most specific alleles occurred at the *locus* UMN0929 (3 of the 6 identified) (Table 4). Alleles specific for metacentric bull groups were identified at 5, while for submetacentric group – at 9 microsatellite *loci*. The most specific alleles for metacentric bulls were identified at *loci* UMN0406, UMN0929 and UMN2713 – 2 alleles. Two microsatellite *loci* were characterized by only one specific allele for this group of bulls (Table 4). However, in the case of submetacentric bulls, the most specific alleles were observed at *loci* UMN0905 and INRA124 – 2 alleles. The one characteristic allele for these bulls occurred in 7 analyzed microsatellite markers.

Thirty-eight bovine Y chromosome (BTAY) microsatellites were assessed for polymorphism in DNA samples obtained from 17 unrelated bulls by Liu et al. (2003). These microsatellites were also used for the construction of a first generation radiation hybrid map for BTAY. The polymorphic markers identified in this study and their related haplotypes should provide a powerful tool for study the origin and evolution of domestic cattle as well as bovid species. In turn, Cai et al. (2006) genotyped and also assessed two Y chromosome specific microsatellites UMN2404 and UMN0103 for polymorphism in a total of 423 unrelated males from 25 indigenous Chinese cattle breeds. Both microsatellites displayed specific indicine and taurine alleles in each bull examined. Similarly Perez-Pardal et al. (2010) indicated the usefulness of UMN0103 microsatellite for phylogeographic history of the different cattle strains. The cytogenetic and molecular studies of the Pantaneiro cattle breed were performed by Issa et al. (2006). The objective of these studies was to genetically characterize Pantaneiro cattle through its paternal ancestry by the morphology of the Y chromosome. The karyotype and mitochondrial DNA of 12 bulls were analyzed. Among studied animals three had a taurine (submetacentric) Y and nine had a zebuine (acrocentric) Y chromosome, suggesting breed contamination by Zebu cattle, once Pantaneiro is considered to be of European origin. The mitochondrial DNA was exclusively of taurine origin, indicating that the participation of zebuines in the formation of the breed occurred entirely through the paternal line. On the other hand, Xin et al. (2011) studied the correlations between Y chromosome polymorphisms and the carcass traits in five Chinese beef cattle populations by SSCP (single strand conformation polymorphism) and Y-STR (short tandem repeats) sequence analysis. Results showed that Y-STR UMN0929 alleles were correlated with carcass traits in beef cattle populations and could be implemented into the cattle breeding program for choosing individuals with better traits.

4. Conclusions

The karyotype of Polish Black-and-White cattle, regarding to the Y chromosome presents a dimorphism (metacentric and

submetacentric). Banding patterns of these two forms of Y chromosome were typical for the *B. taurus*. The group of bulls with submetacentric chromosome showed more specific alleles (11) in relation to metacentric group (8 specific alleles). Identification of such specific markers maybe useful in the investigation of cattle breeds origin.

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