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MICROSATELLITE SEQUENCES OF Y HETEROSOME IN *BOVIDAE* FAMILY

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Genetic conservatism at the level of microsatellite sequences permits within-family comparisons of a species. Using this assumption, we examined the polymorphism of microsatellite sequences specific to the Y chromosome (INRA124, BM861, INRA126, INRA 189) in cattle, sheep and goats belonging to the Bovidae family.

The three polymorphic microsatellite sequences: BM861, INRA126, INRA 189 were found useful for analysing genetic conservatism in the investigated Bovidae species, while the fourth INRA 124 marker was shown to be monomorphic.

Key words: *BOVIDAE*, CHROMOSOME Y, MICROSATELLITE SEQUENCES

Genetic conservation studies were most often concentrated on sex chromosomes (Kozubska-Sobocińska et al., 2005).

A large number of papers reported DNA conservation between different mammalian species, with the use of anonymous DNA markers such as microsatellite loci (Moore et al., 1995; De Gortari et al., 1997; Slate et al., 1998). Microsatellite markers have been efficiently used in genome mapping projects, pedigree determination and population genetics in humans and animals. Cross-species utilization of microsatellite loci enables the construction of comparative maps between related species (O'Brien et al., 1993).

The aim of the study was to evaluate genetic conservation of microsatellite markers *INRA124, INRA126, INRA189* and *BM861,* specific to the non-conjugating fragment of the Y chromosome, in different *Bovidae* species.

Materials and methods

The material for experiment comprise blood samples of about 1 ml per animal, taken into tubes with EDTA as anticoagulant. The samples were taken from: 30 bulls and 3 cows of Polish Red breed, 20 ram-lambs and 3 ewes of the Romanov breed and 15 kids and 3 goats.

DNA isolation from peripheral blood leukocytes was carried out according to Kawasaki (1990) as modified by Coppieters et al. (1992).

Amplifications of the DNA isolated from individual animals were performed through PCR using 4 pairs of starter sequences (Edwards et al., 2000) enabling simultaneous amplification of all the markers tested in a single reaction sample (multiplex-type PCR).

PCR reaction was performed according to the PCR-Protocol under the following conditions: starting denaturation at 95 °C for 15 minutes, 31 cycles of denaturation at 94 °C for 45 seconds, annealing at 59 °C for 45 seconds, extension at 72 °C for 1 minute, final extension at 72 °C for 60 minutes. One starter from each pair of starter sequences was labeled with a fluorescent dye to enable for simultaneous analysis the PCR products in a single gel lane during electrophoretic

separation. In the PCR reaction we used the following components: 20 ng double-stranded DNA, buffer for PCR reaction, mixture of dNTP deoxynucleotides (1.25 mM for each nucleotide), DNA Ampli Taq Gold polymerase (5 units/µl), deionized water and 2.5 pmol of each starter sequence. The PCR reaction products were subjected to electrophoresis in 4 % denaturing polyacrylamide gel in an ABI PRISM 377 DNA sequencer. The size of the analysed DNA fragments was determined in base pairs using Genotyper software (Applied Biosystems).

Results and discussion

The results of cross-species amplification in 4 microsatellite loci specific for the non-conjugating fragment of the Y chromosome in cattle, sheep and goat are presented in Table 1.

Table 1.

Y-specific microsatellite loci used to assay haplotypic variation in cattle, sheep and goats

Locus	Range of alleles (bp)					
	Cattle (No. of animals)		Sheep (No. of animals)		Goat (No. of animals)	
	♂	♀	♂	♀	♂	♀
INRA124	128 (30)	– (3)	128 (20)	– (3)	128 (15)	– (3)
INRA126	180 (9)	– (3)	182 (11)	– (3)	182 (6)	– (3)
	182 (7)	– (3)	184 (9)	– (3)	184 (9)	– (3)
	184 (3)	– (3)				
	188 (9)	– (3)				
	190 (2)	– (3)				
INRA189	80 (11)	– (3)	108 (20)	– (3)	80 (8)	– (3)
	96 (10)	– (3)			96 (7)	– (3)
	100 (9)	– (3)				
BM861	158 (30)	– (3)	156 (12)	– (3)	158 (15)	– (3)
			162 (8)	– (3)		

The analysis of allele lengths, determined using Genotyper 2.0 software, showed the presence in the three species of a monomorphic allele of 128 bp at the *INRA124* locus. For the *BM861* sequence, one allele of 158 bp was found in cattle and goats and two alleles of 156 and 162 bp in sheep. More polymorphic was the *INRA 126* locus, for which five alleles (180, 182, 184, 188 and 190 bp) were found in cattle and two alleles (182 and 184 bp) in sheep and goats. For the third locus (*INRA 189*), three alleles were identified in cattle (80, 96 and 100 bp), two in goats (80 and 96 bp) and one in sheep (108 bp).

In the experimental groups of females (cows, ewes and goats) no amplification products were found at the *INRA124, INRA126, INRA189* and *BM861* loci.

The analysis of haplotype variation performed in the present study for microsatellite sequences specific for the non-conjugating fragment of the Y heterosome in cattle, sheep and goats, showed that three polymorphic sequences (*INRA126, INRA189* and *BM861*) are suitable for assessing genetic conservation in the *Bovidae* species investigated and revealed the monomorphic nature of the *INRA124* locus.

The monomorphic allele of the *BM861* locus was also found in cattle, as confirmed by Liu et al. (2003), but this is not consistent with a study by Edwards et al. (2000), who considered the *INRA124* and *BM861* loci to be polymorphic. The discrepancy between these results can be attributed to a small number of species that we compared (only three species — 30 bulls, 20 rams and 15 kids), while Edwards et al. (2000) compared nine species and each species was represented by a small number of animals (from 3 to 12).

The inclusion of 3 experimental groups with 3 females of each species was aimed to prove that the analysed microsatellites are located in the male-specific part of the Y chromosome.

It is worth noting the results of Hanotte et al. (1997), who for the INRA126 locus found an allele of 182 bp in both male and female yaks. The occurrence of this allele in both sexes was attributed to the presence in the X chromosome of the yak of a sequence homologous to the fragment of the Y chromosome containing the *INRA126* microsatellite.

Our analysis of haplotype variation in microsatellite sequences specific for the Y heterosome in cattle, sheep and goats showed the suitability of three polymorphic sequences (*INRA126, INRA189* and *BM861*) for assessing genetic conservation in the analysed *Bovidae* species and revealed the monomorphic nature of the *INRA124* locus.

POSLEDOWNOSCI MIKROSATELITARNE Y HETEROSOMY W RODZINIE *BOVIDAE*

S t r e s z c z e n i e

Konserwatyzm genetyczny na poziomie sekwencji mikrosatelitarnych umożliwia porównania gatunków w obrębie rodzin. Wykorzystując to założenie podjęto próbę analizy sekwencji mikrosatelitarnych specyficznych dla chromosmu Y (*INRA124, BM861, INRA126, INRA189*) u bydła, owiec i kóz należących do rodziny *Bovidae*.

Wykazano przydatność trzech polimorficznych sekwencji mikrosatelitarnych specyficznych dla chromosomu Y do oceny konserwatyzmu genetycznego u badanych gatunków z rodziny *Bovidae*, natomiast czwarty marker *INRA124* określono jako monomorficzny.

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ПОСЛІДОВНОСТІ МІКРОСАТЕЛІТІВ Y ГЕТЕРОСОМИ У СІМЕЙСТВІ *BOVIDAE*

Р е з ю м е

Консервація генів на рівні послідовностей мікросателітів дозволяє порівняти види, які входять в родини. Використовуючи це припущення, ми розглянули поліморфізм специфіки послідовностей хромосоми Y (*INRA124, BM861, INRA126, INRA189*) великої рогатої худоби, овець і кіз, що належать до сім'ї Bovidae.

Три поліморфні послідовності мікросателітів: BM861, INRA126, INRA 189 були визнані корисним для аналізу консервації генів у досліджуваному виді Bovidae, в той час як четвертий маркер INRA 124 виявився мономорфним.

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ПОСЛЕДОВАТЕЛЬНОСТИ МИКРОСАТЕЛЛИТОВ Y ГЕТЕРОСОМЫ В СЕМЕЙСТВЕ BOVIDAE

А н н о т а ц и я

Консервирование генов на уровне последовательностей микросателлитов позволяет сравнить виды, которые входят в семейство Bovidae. Используя это предположение, мы рассмотрели полиморфизм специфики последовательностей хромосомы У (INRA124, BM861, INRA126, INRA189) большого рогатого скота, овец и коз, что принадлежат семье

Bovidae. Три полиморфные последовательности микросателлитов: BM861, INRA126, INRA 189 были признаны полезным для анализа консервирования генов в исследуемом виде Bovidae, в то время как четвертый маркер INRA 124 оказался мономорфним.

1. *Coppieters W*. Rechtstreekse genotypering von stress (on) gevoelligheit bij verkens met behulp met van DNA onderzoek / Coppieters W., Van Zeveren A., Van De Weghe A. et al. // Vlaams Diergeneeskunding Tijdschrift. — 1992. — 61 ; 68–72.

2. *De Gortari M. J.* Extensive genomic conservation of cattle microsatellite heterozygosity in sheep / De Gortari M. J., Freking B. A., Kappes S. M. et al. // Anim Genet. — 1997. — 28 (4), 274–90.

3. *Edwards C. J.* Y-specific microsatellite polymorphisms in a range of bovid species / Edwards C. J., Gaillard C., Bradley D. G. et al. // Anim.Genet. — 2000. — 31 : 127–130.

4. *Hanotte Q*. A polymorphic Y chromosome microsatellite locus in cattle / Hanotte Q., Okomo M., Verjee Y. et al. // Anim.Genet. — 1997. — 28 : 308–322.

5. *Kawasaki E. S.* Sample preparation from blood, cell and other fluids / In : «PCR Protocols ; A Guide to methods and applications». — NY : Acadamic Press, 1990. — 146–152.

6. *Kozubska-Sobocińska A*. Comparative hybridization of Y chromosome in selected species of *Bovidae* / Kozubska-Sobocińska A., Słota E., Pieńkowska-Schelling A. et al. // Ann. Anim. Sci. — 2005. — 5 (1), 5–9.

7. *Liu W.-S.* Bovine Y chromosome microsatellite polymorphisms. Cytogenet / Liu W.-S., Beattie C. W., Ponce de Leon F. A. // Genome Res. — 2003. — 102 : 53–58.

8. *Moore S. S.* A set of polymorphic DNA microsatellites useful in swamp and river buffalo (Bubalus bubalis) / Moore S. S., Evans D., Byrne K. et al. // Anim Genet. — 1995. — 26 (5), 355–9.

9. *O'Brien S. J.* Anchored reference loci for comparative mapping in mammals / O'Brien S. J., Womack J.E., Lyons L.A. et al. // Nat. Genet. — 3, 103–112.

10. *Slate J.* Bovine microsatellite loci are highly conserved in red deer (Cervus elaphus), sika deer (Cervus nippon) and Soay sheep (Ovis aries) / Slate J., Coltman D. W., Goodman S. J. et al. // Anim Genet. — 1998. — 29 (4), 307–315.

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