Evaluation of 11 microsatellite loci for their use in paternity testing in Yugoslav Pied cattle (YU Simmental cattle)

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ABSTRACT: Eleven microsatellite loci (*TGLA227, BM2113, TGLA53, ETH10, SPS115, TGLA126, TGLA122, INRA023, ETH3, ETH225, BM1824*) were evaluated for their use in paternity testing in the Yugoslav Pied cattle (YU Simmental cattle) population in Serbia. A total of 40 animals were tested. At the 11 tested loci, a total of 91 alleles were detected. The mean number of alleles per locus was 8.273. Polymorphism information content (PIC) values ranged from 0.58 to 0.88 with the mean value of 0.72. The most informative loci were: *TGLA53* (14 alleles, PIC = 0.88), *TGLA227* (11 alleles, PIC = 0.82), *INRA023* (11 alleles, PIC = 0.86), *BM2113* (9 alleles, PIC = 0.80). Combined power of discrimination (CPD) for the 11 microsatellite loci was 0.999. The results of the present study confirm that the analysed set of 11 microsatellite markers recommended by ISAG is suitable for paternity testing in Yugoslav Pied cattle in Serbia.

Keywords: molecular markers; informativeness; bovines; pedigree; Serbia

Accurate pedigree information is required for a successful breeding program and improvement of productivity in the animal industry. Misidentification of parentage can lead to breeding inaccuracy, causing great financial losses in herd management and in the beef industry (Cervini et al., 2006). A small misidentification percentage excessively endangers genetic patterns estimation. The paternity misidentification rate of 11% would result in a decrease of 11–15% in the genetic trend for milk traits (Banos et al., 2001). Pedigree errors may reflect in the structure of selection indexes (Přibyl et al., 2004; Řehout et al., 2006). Unfortunately, the extensive application of artificial insemination (AI) in cattle breeding causes an increase of pedigree errors. Thus, pedigree verification through paternity testing is necessary if we want to achieve optimal genetic progress in cattle breeding.

Traditionally, pedigree verification in cattle was based on blood groups and biochemical polymorphism analyses. However, a high frequency of incorrect cattle paternity was obtained using traditional markers. In addition, blood typing cannot be done retrospectively, e.g. after a sire is dead. DNA-based tests, specifically the analysis of microsatellites, offer several advantages over conventional parentage testing systems: any sample containing the animal's DNA, such as hair, saliva, milk, blood or semen can be used; sampling is noninvasive and samples can be used retrospectively from stored tissue or semen samples. Due

to their high polymorphisms, many microsatellites are useful in paternity testing. Accessibility of thousands of microsatellite mar-kers for cattle resulted in the creation of different only partly overlapping sets of markers used for parentage analysis.

In 2006, the International Society for Animal Genetics (ISAG) recommended 9 microsatellite loci (*TGLA227*, *BM2113*, *ETH10*, *SPS115*, *TGLA126*, *TGLA122*, *INRA023*, *ETH225*, *BM1824*) as the International Panel of Microsatellites for Cattle Parentage Testing (ISAG Panel) with suggestion that other markers should be added to this panel to increase efficacy in parentage testing (ISAG Conference, 2006). Recently, additional three markers (*BM1818*, *ETH3* and *TGLA53*) were suggested as candidate loci in cattle parentage analysis (ISAG Conference, 2008).

In some developed countries, paternity identification using microsatellite markers is established for their cattle populations (Curi and Lopes, 2002; Radko et al., 2005; Cervini et al., 2006; Řehout et al., 2006; Radko 2008; Tian et al., 2008; Carolino et al., 2009; Ozkan et al., 2009). In addition, microsatellites were used for the analyses of genetic diversity in cattle (Čítek and Řehout, 2001; Grzybowski and Prusak, 2004a,b; Zhou et al., 2005; Czerneková et al., 2006; Čítek et al., 2006; Zaton-Dobrowolska et al., 2007). Polymorphisms of genes associated with milk production parameters and quality of milk were investigated in Czech Fleckvieh (Kučerová et al., 2006; Matějíček et al., 2007) since such loci can be taken into account as a suitable supplement to conventional breeding procedures (Přibyl, 1995).

In Yugoslav Pied cattle only cytogenetic investigations were done (Soldatovic et al., 1993, 1994a,b; Vucinic et al., 1996). There have been no investigations using molecular techniques and no estimations of microsatellite informativeness and their efficiency for paternity testing in the cattle population in Serbia. The objective of this study was to evaluate 11 microsatellite markers from the ISAG panel for their use in paternity testing and pedigree verification in Yugoslav Pied cattle (YU Simmental cattle) in Serbia.

Table 1. Informativeness of analyzed markers in the Yugoslav Pied cattle population in Serbia

Microsatellite marker	Chromosome	Observed size range (bp)	n_A	FNA	Ho	He	HWE	PIC
TGLA227	18	78–114	11	0.274	0.733	0.851	0.00324*	0.82
BM2113	2	122–142	9	0.333	0.607	0.817	0.01505	0.80
TGLA53	16	150–184	14	0.235	0.656	0.893	0.00000*	0.88
ETH10	5	206–220	7	0.600	0.522	0.557	0.13205	0.58
SPS115	15	242–254	6	0.515	0.719	0.656	0.78688	0.60
TGLA126	20	114–124	6	0.438	0.667	0.654	0.95571	0.59
TGLA122	21	138–162	9	0.424	0.452	0.728	0.00163*	0.70
INRA023	3	198–220	11	0.167	0.774	0.889	0.26167	0.86
ETH3	19	112–124	6	0.288	0.742	0.793	0.29764	0.75
ETH225	9	134–146	6	0.530	0.581	0.645	0.02655	0.62
BM1824	1	176–190	6	0.364	0.710	0.760	0.65137	0.70
Mean			8.273	0.379	0.651	0.750		0.72

n_A = number of alleles

FNA = frequency of the most frequent allele

Ho = observed heterozygosity

He = expected heterozygosity

PIC = polymorphism information content

*$P < 0.05$, significantly deviated from Hardy-Weinberg equilibrium (HWE)

MATERIAL AND METHODS

Eleven microsatellites used in this study are recommended by the International Society for Animal Genetics (ISAG) for cattle paternity testing (Table 1). Sperm or hair samples were taken from 40 heads of Yugoslav Pied cattle. Genomic DNA from sperm and hair roots was isolated with DNeasy® Blood and Tissue Kit (Qiagen, Valencia, CA) and kept frozen at –18°C until further processing. Microsatellites were amplified using the "StockMarks for Cattle® Bovine Genotyping Kit" (Applied Biosystems Inc., Foster City, CA) in multiplex reactions according to the manufacturer's recommendations. The reactions were performed in a programmable thermal cycler MultiGene Gradient (Labnet International Inc.). The fluorescent labelled PCR products were submitted to fragments analysis by capillary electrophoresis, with an automated sequencer ABI PRISM 310 (Applied Biosystems), using the GeneScan-350 ROX® Size Standard (Applied Biosystems), according to the manufacturer's specifications. Results were read and interpreted using GeneScan® and Genotyper® software, respectively. Standard statistical procedures were used to assess the informativeness of selected microsatellite markers. The number of alleles (n_A), frequency of the most frequent allele (FNA), observed and expected heterozygosity (Ho and He), polymorphism information content (PIC), power of discrimination (PD) and power of exclusion (PE) were calculated for each microsatellite marker. Combined power of discrimination (CPE) and combined power of exclusion (CPE) were calculated for the whole set of studied markers (Nei, 1987; Garza and Williamson, 2001). Allele frequencies, PIC, PD and PE were determined by the PowerStatsV12 freeware, Promega Corporation, USA (Brenner and Morris, 1990). Observed and expected heterozygosity as well as calculations for Hardy-Weinberg Equilibrium (HWE) were performed) in Arlequine ver. 3.1 (Excoffier et al., 2006) according to Guo and Thompson (1992).

RESULTS AND DISCUSSION

In this study, 11 microsatellite markers (*TGLA227, BM2113, TGLA53, ETH10, SPS115, TGLA126, TGLA122, INRA023, ETH3, ETH225, BM1824*), recommended by ISAG for cattle paternity testing, were evaluated for the first time for their use in paternity testing and pedigree verification in the Yugoslav Pied

Table 2. Power of discrimination (PD), combined power of discrimination (CPD), power of exclusion (PE) and combined power of exclusion (CPE)

No.	Microsatellite marker	PD	PE
1	*TGLA227*	0.922	0.552
2	*BM2113*	0.918	0.333
3	*TGLA53*	0.939	0.393
4	*ETH10*	0.755	0.246
5	*SPS115*	0.806	0.485
6	*TGLA126*	0.818	0.409
7	*TGLA122*	0.865	0.175
8	*INRA023*	0.953	0.472
9	*ETH3*	0.900	0.424
10	*ETH225*	0.814	0.230
11	*BM1824*	0.880	0.472
		CPD = 0.999	CPE = 0.996

cattle (YU Simmental cattle) population in Serbia. The results are presented in Tables 1 and 2.

Microsatellite markers are effective in parentage testing and pedigree verification if they are highly informative. The informativeness of a polymorphic marker depends upon the number of alleles and their relative population frequencies (Taylor, 1997). In the studied group of cattle, the n_A per locus ranged from 6 to 14. The mean n_A per locus was 8.273 and the total number of alleles was 91. PIC values ranged from 0.58 to 0.88 with the mean value of 0.72 (Table 1). High number of alleles and PIC values were observed for *TGLA53, TGLA227, INRA023, BM2113* and *TGLA122*. In addition, FNA of these loci was well below 0.5, with the lowest values in *INRA023, TGLA53, TGLA227* and *BM2113*, thus are the most informative markers among tested loci in this study. These four loci showed also the highest values of He and PD (Tables 1 and 2). Although all loci showed PIC values over 0.5, the informativeness of *ETH10, SPS115* and *ETH225* is low since their FNA values exceeded 0.5. CPD was 0.999, which is the required level of discrimination in a parentage analysis (Vankan and Faddy, 1999; Perez-Miranda et al., 2005).

The markers tested in this study were tested in other breeds of Simmental cattle (from Poland, Slovak Republic, Czech Republic) and appeared

highly polymorphic (Janík et al., 2001; Choroszy et al., 2006; Czerneková et al., 2006). However, the mean n_A per locus in the Yugoslav Pied cattle population analysed in our study (8.273) is higher than 7.90 found in Czech Pied and 7.45 found in Slovakian Pied (Czerneková et al., 2006) or 7.27 found in Simmental cattle from Poland (Choroszy et al., 2006). Moreover, total number of alleles we observed in Yugoslav Pied cattle (91) is higher than Janík et al. (2001) and Choroszy et al. (2006) found in Simmental cattle from Poland for the same set of loci (79 and 80, respectively). PIC values for the 11 evaluated loci in Yugoslav Pied cattle ranged from 0.58 to 0.88, with the mean PIC value of 0.72, which is comparable with the results obtained for the same set of loci in other Simmental breeds: mean PIC value was 0.757 in Czech Pied cattle, 0.642 in Slovakian Pied cattle (Czerneková et al., 2006) and 0.641 in Simmental cattle from Poland (Choroszy et al., 2006).

As shown in Table 1, Ho ranged from 0.452 (*TGLA122*) to 0.774 (*INRA023*), with the mean value of 0.651, whilst He varied from 0.557 (*ETH10*) to 0.893 (*TGLA53*), with an average of 0.750. Three loci (*TGLA227*, *TGLA53* and *TGLA122*) were significantly deviated from HWE ($P < 0.05$), while other loci conformed to HWE. The observed deviations from HWE at five loci could be a result of the specific selection programs. The means for n_A and He (Table 1) indicate high levels of genetic diversity in the population studied as found in other Simmental breeds of cattle (Janík et al., 2001; Choroszy et al., 2006; Czerneková et al., 2006).

The loci that appeared the most informative in Yugoslav Pied cattle in our study (*TGLA53*, *TGLA227*, *INRA023* and *BM2113*) were also highly polymorphic in Simmental cattle from Poland (Janík et al., 2001; Choroszy et al., 2006). High polymorphism at the *TGLA53* locus was also reported by Heyen et al. (1997) in American Simmentals.

In summary, the results of the present study confirm that the analysed set of 11 microsatellite markers recommended by ISAG is suitable for paternity testing and pedigree verification in Yugoslav Pied cattle (YU Simmental cattle) in Serbia.

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