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Polymorphism of bovine microsatellite DNA sequences in the lowland European bison

Barbara GRALAK, Małgorzata KRASIŃSKA, Cezary NIEMCZEWSKI, Zbigniew A. KRASIŃSKI and Maciej ŻURKOWSKI

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Investigations of genetic polymorphism of microsatellite DNA sequences were conducted in 22 individuals of the European bison *Bison bonasus* (Linneaus, 1758) from Białowieża Primeval Forest. For this purpose 27 cattle microsatellite primer pairs were used. Among the 27 microsatellite markers examined, an amplification product was obtained for 21 loci. This rendered it possible to identify total of 40 alleles in the bison population tested. In addition, eight loci were proved to be monomorphic. A majority of the 40 alleles identified was identical with the alleles identified at the corresponding loci in cattle. Only two alleles seem to be specific for the European bison. The value of heterozygosity for the examined loci in bison population from Białowieża was low and ranged from 0.13 to 0.53. Hence, the polymorphism information content was low as well. Based on our results the microsatellite DNA markers identified in cattle may be used to analyse the genetic structure of the population of European bison.

Institute of Genetics and Animal Breeding, Polish Academy of Sciences, Jastrzębiec, 05-552 Wólka Kosowska, e-mail: b.gralak@ighz.pl (BG, CN), Mammal Research Institute, Polish Academy of Sciences, 17-230 Białowieża (MK), Białowieża National Park, 17-230 Białowieża (ZAK), Research Station for Ecological Agriculture and Preserve Animal, Polish Academy of Sciences Popielno, 12-222 Wejsuny (MŻ)

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Introduction

From the middle of the 18th century until the beginning of 20th century, Białowieża Forest has been the only forest in the world where a natural population of lowland European bison *Bison bonasus* Linnaeus, 1758 has lived. At the beginning of the 20th century, *B. bonasus* was threatened with total extinction. The restoration of the European bison in captive breeding centers started in 1929. Only 12 animals (4 males and 8 females bred in reserves and zoological gardens) took part in the restoration of the species (Slatis 1960). Experiments of returning this species to the natural environment began in the Białowieża Forest in 1952, ie 50 years ago (Pucek 1991). During the years 1991–2001 there were 600–700 European bisons in Poland, of which 25% were in captive breeding centers and 75% in free-ranging populations. At the end of year 2000 a total of 2864 European bison

lived throughout the world. This included 1154 (40%) animals in captive breeding centers and 1710 (60%) in free-ranging populations. Although the species have been saved from extinction, they remain threatened. The largest world population of the European bison reside in both parts of the Białowieża Forest (Polish and Bielarussian). The population numbers about 600 animals (Krasiński and Krasińska 2004).

The history of the European bison was characterised by a high inbreeding (Pucek *et al.* 2004). The mean coefficient of inbreeding of the world population amounted to F = 0.201, in the lowland line to F = 0.324 and in the lowland--Caucasian line to F = 0.193 (Olech 1987). Later studies showed that coefficients of inbreeding in the two lines were much higher: F = 0.439 and F = 0.263, respectively (Olech 1998). The genetic variability of the European bison was examined on the basis of the variability and differentiation of: blood proteins (Gębczyński and Tomaszewska-Guszkiewicz 1987, Hartl and Pucek 1994), blood group systems (Sipko *et al.* 1995, 1996), mitochondrial DNA (Tiedemann *et al.* 1998, Burzyńska *et al.* 1999), kappa-casein genes (Sipko 1994, Kamiński and Zabolewicz 1997) and the DQB and DRB genes of the major histocompatibility complex (Udina *et al.* 1994, Udina and Shaikhaev 1998).

When detailing the biodiversity of many animal species microsatellite DNA sequences are the most commonly used group of genetic markers. They are blocks of short (2–6 base pairs), repeating in tandem nucleotide sequences, comparatively evenly distributed over the *Eucaryota* genome. They are characterized by a high degree of polymorphism and heterozygosity and demonstrate considerable individual differentiation. Because of the comparatively easy identification of the polymorphism of these markers by molecular methods, they are being used to characterize the genetic structure and variation of farm animal populations (Martin-Buriel *et al.* 1999, Gralak *et al* 2001, Fan *et al.* 2002), wild animals such as a bison (Mommens *et al.* 1998, Wilson and Strobeck 1999), deer (Nagata *et al.* 1998), caribou and reindeer (Cronin *et al.* 2003).

The present investigation is aimed at estimating the value of DNA microsatellite sequences of cattle for the analysis of the genetic structure of the population of European bison in Białowieża Forest.

Material and methods

The material for examination originated from 22 individuals of the lowland European bison *Bison bonasus bonasus*, culled by workers of the Białowieża National Park during the winter of 2001 and 2002. Five animals (2 males and 3 females), aged 4 to 22 months, came from captive breeding centers in Białowieża, while 17 (1 male and 16 females), aged 6 months to 18 years, came from the free-ranging population of the Białowieża Forest.

Blood samples were collected in test tubes containing EDTA as an anticoagulant and stored frozen at –20°C. The genomic DNA was extracted using the Wizard® Genomic Purification Kit (Promega).

The amplification of DNA fragments of 27 microsatellite loci, identified in cattle, was conducted in five multiplex PCR reactions (Table 1). Multiplex I consisted of a commercial kit for cattle parentage control (StockMarks® for Cattle Paternity Bovine II v.2 PCR Typing Kit, Applied Biosystems) and

Table 1. Composition of multiplexes for the bovine microsatellite loci examined.

Multiplex	Locus	Allelic range (bp)	Reference
I	TGLA227	64–115	Georges and Massey 1992
	BM2113	116–146	Bishop *et al*. 1994
	TGLA53	147–197	Georges and Massey 1992
	ETH10	198–234	Solinas Toldo *et al*. 1993
	SPS115	235–265	Moore and Byrne 1993
	TGLA126	104–131	Georges and Massey 1992
	TGLA122	134–193	Georges and Massey 1992
	INRA023	193–235	Vaiman *et al*. 1994
	ETH3	90–135	Solinas Toldo *et al*. 1993
	ETH225	135–165	Steffen *et al*.1993
	BM1824	170–218	Bishop *et al*. 1994
II	CSRM60	93–111	Moore *et al*. 1994
	INRA005	137–143	Vaiman *et al*. 1992
	ILSTS005	181–193	Brezinsky *et al*. 1993
	HEL1	98–118	Kaukinen and Varvio 1993
	HEL5	151–181	Kaukinen and Varvio 1993
	BM1818	252–272	Bishop *et al*. 1994
III	INRA037	120–146	Vaiman *et al*. 1994
	CSSM66	179–199	Barendse *et al*. 1994
	ILSTS006	281–304	Brezinsky *et al*. 1993
	MM12	107–133	Mommens and Coppieters 1994
	INRA032	161–190	Vaiman *et al*. 1994
IV	HEL9	143–171	Kaukinen and Varvio 1993
	INRA063	175–188	Vaiman *et al*. 1994
	ETH185	220–238	Steffen *et al*. 1993
V	ETH152	191–207	Steffen *et al*. 1993
	HEL13	177–197	Kaukinen and Varvio 1993

comprised 11 loci. These markers were amplified according to the manufacturer's recommendations. The remaining four multiplexes contained respectively: II – 6 loci, III – 5 loci, IV – 3 loci and V – 2 loci were elaborated by Lubieniecka *et al*. (2001) and amplified using 10–50 ng DNA template, 1 unit AmpliTagGold™ (Applied Biosystem) with reaction buffer consisting of 50 mM KCl, 10 mM Tris-HCl pH 8.3, 1.5 mM MgCl2, 200 μM each dNTP and 0.05–0.18 μM each primer. PCR reaction was performed in 10 μl reaction volumes using a GeneAmp 9600 thermal cycler (Applied Biosystem). The PCR products were separated in 5% Long Ranger gel (FMC Bioproducts) on an ABI Prism 377 DNA sequencer using the internal size standard GeneScan-500 ROX (Applied Biosystem). Fragment sizes were determined using the GeneScan v. 3.1 software (Applied Biosystem, Foster City).

Expected heterozygosity (H_e) (Ott 1992) and polymorphic information content (PIC) (Botstein *et al*. 1980) estimated for each locus were based on allele frequencies obtained by direct counting.

Results

Our results are presented in Table 2. Among the 27 microsatellite sequences examined an amplification product was obtained for 21 loci. Six microsatellites

Table 2. Polymorphism of bovine microsatellite sequences in European bison (n = 22).

Locus	Alleles (bp)	Allele frequencies	Heterozygosity (H_e)	PIC
TGLA227	74	1.000	0	0
BM2113	127	1.000	0	0
TGLA53	150	0.682	0.44	0.34
	152	0.318		
ETH10	211	0.273	0.51	0.44
	213	0.091		
	215	0.636		
SPS115	252	0.341	0.45	0.35
	256	0.659		
TGLA126	111	0.182	0.27	0.23
	115	0.682		
	121	0.136		
TGLA122	141	0.841	0.27	0.23
	165	0.159		
INRA023	194	1.000	0	0
ETH3	119	0.409	0.53	0.43
	121	0.045		
	123	0.546		
ETH225	156	0.477	0.50	0.37
	158	0.523		
BM1824	180	0.250	0.37	0.30
	182	0.750		
CSRM60	89	1.000	0	0
INRA005	0	0	0	0
ILSTS005	0	0	0	0
HEL1	0	0	0	0
HEL5	0	0	0	0
BM1818	262	0.619	0.47	0.36
	264	0.381		
INRA037	120	1.000	0	0
CSMM66	172	0.025	0.26	0.24
	180	0.150		
	196	0.850		
ILST006	282	1.000	0	0
MM12	113	0.452	0.50	0.37
	115	0.548		
INRA032	173	1.000	0	0
HEL9	143	0.932	0.13	0.12
	161	0.045		
	163	0.023		
INRA063	0	0	0	0
ETH185	228	1.000	0	0
ETH152	197	0.029	0.46	0.37
	199	0.677		
	203	0.294		
HEL13	0	0	0	0

failed to amplify or produced trace signal (INRA005, ILSTS005, HEL1, HEL5, INRA063, HEL13). It was possible to identify a total of 40 alleles. Eight loci were proved to be monomorphic. The remaining 13 microsatellites were polymorphic, with number of alleles two or three. For all polymorphic loci heterozygosities (H_e) ranged from 0.13 (HEL9) to 0.53 (ETH3). The polymorphism information content (PIC) varied from 0.12 (HEL9) to 0.44 (ETH10).

Discussion

Primers for cattle microsatellites were used for assaying microsatellite variation in American bison (Mommens *et al*. 1998, Schnabel *et al*. 2000). In our study the DNA microsatellite sequences identified in cattle were used for analysis of the genetic structure of European bison. The identification of individual alleles was possible for 21 out of the 27 loci examined. For remaining 6 loci, the quality of the amplification product made it impossible to obtain a clear result. A majority of the total number of 40 alleles identified was identical in length with those found in cattle at corresponding loci. Only two of them, allele 74 bp long at the monomorphic locus TGLA227 and allele 89 bp at locus CSRM60, seem to be specific for the European bison. In the case of microsatellite TGLA227, the allele 73 bp was identified by Mommens *et al*. (1998) in the American bison as the only one at a given locus and was accepted as specific for this species. Considering that the difference estimated by Mommens *et al*. (1998) and our study was only one base pair between alleles, it is highly probable that this can be the same allele in both cases. However results should be confirmed by DNA sequencing.

Comparison of number of alleles of bovine microsatellite markers in three species of bovid: European bison (present study), American bison (Mommens *et al*. 1998) and three Polish cattle breeds (Lubieniecka *et al*. 2001) indicates that the genetic variability of European bison is very low (Table 3). This may result from the fact that European bison have passed through a genetic bottleneck. However an earlier study (Gębczyński and Tomaszewska-Guszkiewicz 1987) demonstrated that heterozygosity based on 20 loci in *Bison bonasus* and *B. bison* was very similar, although American bison have not experienced such a severe bottleneck. When a larger number of loci (69) and somewhat different methods were used (Hartl and Pucek 1994), it was concluded that a genetic variability in European bison had been reduced by a bottleneck. Hence it seems that the average heterozygosity used by earlier authors is not sufficient to estimate of genetic variability.

Three is the maximum number of alleles at a given locus, and 0.53 is the highest heterozygosity. Comparing these data with polymorphisms of individual loci and the values obtained for the heterozygosity between Polish Red, Polish Black--and-White, and Polish Red-and-White breeds (Lubieniecka *et al*. 2001) and the European bison examined, it may be stated that those parameters are much lower in European bison. Referring to the same microsatellite sequences in cattle at individual loci 3 to 13 alleles (depending on the breed) were identified and

Table 3. Number of alleles of bovine microsatellite markers in three species of *Bovidae* family: European bison (present study), American bison (Mommens *et al.* 1998) and three Polish cattle breeds (Lubieniecka *et al.* 2001): "–" – not tested.

Locus	European bison	American bison	Cattle
TGLA227	1	1	–
BM2113	1	9	6–8
TGLA53	2	6	9–12
ETH10	3	3	7–8
SPS115	2	6	4–6
TGLA126	3	6	4–5
TGLA122	2	5	8–14
INRA023	1	1	7–9
ETH3	3	3	7–9
ETH225	2	3	6–8
BM1824	2	8	3–5
CSRM60	1	–	6–7
INRA005	0	4	2–3
ILSTS005	0	–	2–4
HEL1	0	0	4–7
HEL5	0	4	5–9
BM1818	2	5	4–8
INRA037	1	–	7–13
CSMM66	3	–	8–10
ILST006	1	–	6–7
MM12	2	–	5–9
INRA032	1	–	6
HEL9	3	–	9–12
INRA063	0	0	4–6
ETH185	1	–	8–9
ETH152	3	–	6
HEL13	0	3	4–5

heterozygosity reached 0.88 (Peelman *et al.* 1998, Lubieniecka *et al.* 2001). Moreover, a similar comparison with the results of Mommens *et al.* (1998) for American bison, using identical cattle microsatellites, points to a clearly lower polymorphism in European Bison examined in our study. For example, at locus BM2113 in American bison were identified nine alleles (heterozygosity 0.83) and only one in European bison (Table 3). It is also interesting that 38% of the microsatellite sequences examined in European bison (8 loci) were monomorphic, while in American bison only two revealed no polymorphism – TGLA227 and INRA023 (Mommens *et al.* 1998).

Reasons for such a small genetic differentiation of the population of European bison examined from the Białowieża Forest lie in the history of the *Bison bonasus* species, which became almost extinct at the beginning of the 20th century and was reestablished from just a dozen individuals. Lowland European bison demonstrates a lower genetic differentiation than the Caucasian European bison, what remains in agreement with the lower number of founders of the lowland line (Olech 1987, 1989). The share of genes from ancestors renders it possible to determine the genetic variability in the entire population of European bison. In the lowland line of European bison the share of one pair of genes M 45 PLEBEIER and F 24 PLANTA is currently dominant and reaches almost 90% (Olech 1989).

Our investigations confirm that microsatellite sequences identified in cattle may be successfully used for a genetic characterisation of the population of European bison; our study confirms the strong conservatism of the regions flanking microsatellite sequences of three species: cattle, American bison and European bison.

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