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FOUR BOVINE MICROSATELLITES SHOWING POLYMORPHISM IN RIVER BUFFALO (*BUBALUS BUBALIS*)

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SUMMARY

Seven polymorphic bovine microsatellites namely BMS2361, RM327, BMS1352, BMS1282, BM143, BL1043 and BMC4203 were tested in five unrelated herds of Egyptian river buffalo (Bubalus bubalis). Four were found to be polymorphic while two were monomorphic and one did not amplify any specific fragments. The fragment size and the allele frequency were determined.

The results verify the genetic similarity between cattle and buffalo, and emphasize the possibility of using bovine microsatellite markers for constructing a buffalo linkage map.

Keywords: buffalo, polymorphism, microsatellite, molecular markers

INTRODUCTION

Recently, much attention is focused towards the use of molecular methods like microsatellite analysis, as a genetic marker in improvement of animal breeds. In addition to biodiversity studies, microsatellites are used for construction of genetic linkage maps. These maps can be used for mapping quantitative trait loci (QTL), mapping of disease resistance genes and finally as a tool in marker-assisted selection (MAS).

Microsatellites are a sub class of non-coding DNA, which is tandemly repeated. They mainly constitute double or triple short nucleotides, tandemly repeated, and were first discovered by Hamada and Kakunaga in 1982.

Microsatellites are found through the genomes of probably all higher organisms, and are now extensively used in many different species. Many microsatellite loci possess extreme genetic variability in terms of varying number of repeating units, a situation that might be explained by high mutation rates (Weber and Wong, 1993).

Buffalo is the most important farm animal present in Egypt. It is mainly used as a dual-purpose animal for producing milk and meat. According to the Egyptian Government report in 1995, its population counts around 2.245 million animals (NIC, 1997).

The Karyotype of river buffalo consists of 50 chromosomes, including five pairs of meta or submetacentric chromosomes. Biarmed pairs correspond to the fused 1 / 25; 2 / 23; 8 / 19; 5 / 28 and 16 / 29 chromosomes of cattle. The remaining 20 pairs are acrocentric and include the sex chromosomes (Report of the committee for the

standardization of banded karyotypes of the river buffalo, 1994). Syntenic conservation between cattle and river buffalo has been reported (for review see: Othman and El Nahas, 1998).

The aim of the present study was to test the polymorphism of seven bovine microsatellites in buffalo.

MATERIALS AND METHODS

Blood sampling and DNA isolation

Blood samples were collected under aseptic conditions using EDTA as an anticoagulant. The samples were collected from twenty five buffaloes raised in five different farms. There was no relationship between these farms, but within farms the relationship is not known. DNA was isolated from blood using standard salting out method described by Miller *et al.* (1988). Briefly, cells were washed twice or more in a solution consisting of 0.32 M sucrose, 1 mM tris HCl pH 7.5, 5 mM $MgCl_2$, 1 % Triton X 100. Leukocytes were lysed and digested overnight at 37°C with 0.2 ml of 10% SDS and 100µl of a proteinase K (5mg/ml) and 2 mM Na_2 EDTA. Finally DNA was recovered by ethanol precipitation, picked up with a heat-sealed Pasteur pipette, washed briefly in 70% ethanol and resuspended in 200µl TE buffer. DNA concentration was adjusted to 50 ng/µl, and all samples were kept frozen until use.

PCR conditions and optimization

Each PCR reaction was carried out in a total volume of 10 µl, containing 50 ng genomic DNA, 10 pmol of each primer, 200 µM of each dNTP, standard buffer conditions and 1.0 U Taq polymerase. PCR primer sets are presented in Table 1. One primer from each primer pair was labelled with one of the fluorescent dyes (FAM, HEX, TET). Genomic DNA was denatured for 3 min at 95°C, and PCR was run for 35 cycles at 95°C for 15 sec, the actual annealing temperature for 15 sec, and 72°C for 30 sec, and a final extension step of 5 min at 72°C. After PCR amplification 2µl from each sample were analyzed on 2% agarose gel. Unsuccessful amplification was subjected to PCR optimization by changing in the annealing temperature.

Fragment analysis

The fluorescence labelled fragments were analyzed using an ABI 373 DNA sequencer, and the fragment sizes were determined by using the GeneScan® -350 [ROX] size standard (PE Applied Biosystems, Warrington, UK).

RESULTS

Polymorphism

Four out of seven bovine microsatellites (RM327, BMS1352, BMS1282, BMS2361) tested in river buffaloes were found to be polymorphic. Two others were monomorphic (BM143, BL1043) while one did not amplify specific fragments (BMC4203). The number of animals tested for each microsatellite varied between 18 and 23. Information about number, size and frequency of alleles is given in Table 2. Fig.1 shows a computer-printed image of the laser-scanned polyacrylamide gel showing a monomorphic (a) and a polymorphic microsatellite (b).

Table 1. Primers (5→3) and annealing temperatures for the seven microsatellites used in this study

Microsatellite	Primers (5→3)	References	Optimised annealing temperature in buffalo
BMS1282	ACTCTTCCACAGTTGGCCTG CCTCCTTCCTCCAGAGCC	Kappes *et al.* (1997a) Stone *et al.* (1995)	58
BMS1352	GACTCCAGGTGCAGGAAGAG TCTGCAAGGAATGACAGTGC	Kappes *et al.* (1997a) Stone *et al.* (1995)	58
BMS2361	ACACAACCCAAATGTTACCAA ATTGTGCAGAGACCAAGTGC	Kappes *et al.* (1997a) Stone *et al.* (1997)	58
BMC4203	GCAAATGTAAGCTGAAGGCC CCTGGGAAATCCCATGGAC	Kappes *et al.* (1997a,b)	58
BL1043	AGTGCCAAAAGGAAGCGC GACTTGACCGTTCCACCTG	Kappes *et al.* (1997a) Smith *et al.* (1997)	58
RM327	ATACGCCGCAAGAAATGATA GCAGTCCTGAGAGTAGTAAACTCTG	Kappes *et al.* (1997a) McGraw *et al.* (1997)	56
BM143	ACCTGGGAAGCCTCCATATC CTGCAGGCAGATTCTTTATCG	Bishop *et al.* (1994) Kappes *et al.* (1997a)	60

Table 2. Names, annealing temperatures, and allele sizes for bovine microsatellites tested in river buffalo *(Bubalus bubalis)*.

Name	No. of alleles		Allele sizes and frequencies					Allele size in cattle
	cattle	buffalo						
RM327 (n=21)	12	4	69 0.071	71 0.119	73 0.167	77 0.643		81-111
BMS1352 (n=23)	8	4	94 0.130	98 0.370	102 0.457	108 0.043		85-105
BMS1282 (n=18)	9	2	144 0.806	148 0.194				141-159
BMS2361 (n=23)	7	5	109 0.109	113 0.109	117 0.565	123 0.022	125 0.196	121-137

DISCUSSION

Based on gene mapping data, Hediger *et al.* (1991) studied the relationship between cattle and sheep genomes. They reported that sheep and cattle karyotypes are similar, and that large parts of the respective linkage maps are also similar. Compared to cattle (2n=60), buffalo (2n=50) has 5 meta and submetacentric chromosomes arose from 5 Robertsonian translocations. The chromosome arms, which can be matched to bovine homologues on the basis of chromosome bands, will basically have the same genes or microsatellites.

Cytogenetic analysis (Report of the committee for the standardization of banded karyotypes of the river buffalo, 1994), as well as *in situ* hybridization studies of coding genes (Hassanane *et al.*, 1993 & 1994; Iannuzzi, 1997) have also demonstrated a close similarity between the cattle and buffalo genomes.

In the present study, four out of seven bovine microsatellites showed polymorphism in buffalo. This result is in agreement with the findings of Moore *et al.* (1995), testing bovine microsatellites in swamp and river buffaloes. Some bovine microsatellites are informative in sheep as well (More *et al.*, 1991 & 1994). The number of alleles has been found to vary between the two types of buffalo: swamp and river (Barker *et al.*, 1997).

Genetic polymorphism described in buffalo is not limited to microsatellites (type II markers). Polymorphism in coding genes (type I markers) has been reported for a coat colour gene (Klungland *et al.*, 2000) and the kappa case in gene (Sulimova *et al.*, 1996 and Mitra *et al.*, 1998). There have been also some reports on the polymorphism in ribosomal and mitochondrial DNA (Amano *et al.*, 1994; Tanaka *et al.*, 1995; Hu *et al.*, 1997; Lau *et al.*, 1998; and Simonsen *et al.*, 1998).

As stated by Hediger *et al.* (1991), conservation of microsatellites and flanking sequences between cattle and sheep, together with the similarities in their genomes, have important implications for the construction of linkage maps in the two species. This means that data obtained from cattle are of potential interest in buffalo, and that bovine microsatellites were useful source for molecular markers that could be used for genetic improvement in buffalo.

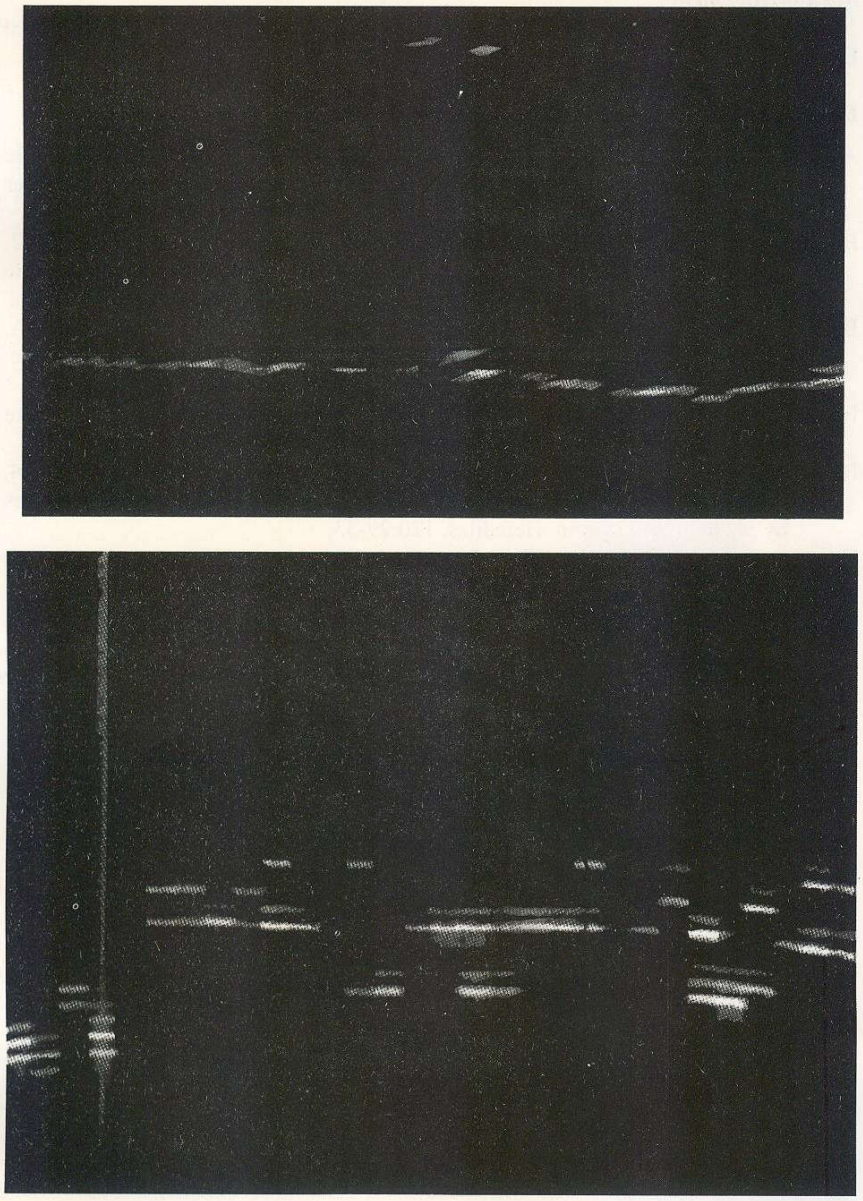

Figure 1. A computer-printed image of the laser-scanned polyacrylamide gel showing a monomorphic (upper) and a polymorphic microsatellite (lower).

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استخدام التوابع الدقيقة لـــ DNA الأبقار لدراسة تعدد المظاهر فى الجاموس

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تم فى هذا البحث اختبار سبعة من الواسمات الجينية للأبقار لمقارنتها بجينوم الجاموس المصرى. هذه الواسمات هى:

BMS2361, RM327, BMS1352, BMS1282, BM143, BL1043 and BMC4203

و لاختبار هذه الواسمات تم أولا جمع عينات دم جاموس من خمسة مزارع مختلفة من أنحاء مصر وليس بين حيوانات هذه المزارع اية درجة قرابة وراثية. تم عزل المادة الوراثية DNA من الدم . أجرى بعد ذلك تفاعل انزيم البلمرة PCR مع بادئات خاصة بكل واسم على حدة .تم بعد الكشف عن نجاح ذلك تفاعل إنزيم البلمرة بواسطة اخذ بعض من ناتج التفاعل وتفريده كهربائيا وصبغه بمادة بروميد الاثيديوم. ثم إعادة الواسمات التي لم يحدث بها نجاح لتفاعل إنزيم البلمرة تم إعادتها مرة ثانية مع إجراء بعض التعديلات فى تفاعل انزيم البلمرة PCR optimization . تم بعد ذلك تحديد أطوال الاليلات وتكرارها باستخدام جهاز Automated DNA sequencer . بينت نتائج الدراسة أن الأربعة واسمات الأولى أظهرت تعدد المظاهر الوراثية فى الجاموس بينما لم تعطى الثلاثة واسمات الأخرى تعدد المظاهر الوراثية فى الجاموس. تبين الدراسة إمكانية استخدام الواسمات الجينية للأبقار فى الجاموس .والتى يمكن استخدامها بعد ذلك فى عمل الخريطة الجينية الارتباطية للجاموس وتحديد أماكن الجينات المسئولة عن الصفات الكمية مثل اللبن واللحم Q TL علاوة على جينات مقاومة الأمراض. يمكن فى النهاية باستخدام الخريطة الارتباطية عمل برامج تربية تسمى MAS وهذا اختصار لـــ (marker assisted selection) وذلك بغرض تحسين إنتاجية الحيوان ومقاومته للأمراض.