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Genetics and Breeding

Comparative analysis of genome positioning of invert repeats of (AG)9C and (GA)9C in bovinae and caprinae species

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Abstract. *Comparative analysis of amplification products of DNA fragemnts, flanking by invert repeats $(AG)_9C$ and $(GA)_9C$, in Bovinae (Bos taurus, Bison bison, Bison bonasus) and Caprinae (Ovis aries, Ovis canadensis) species was carried out. The presence the high conservative DNA fragments in investigated species was revealed. The possible relation of such conservatism with connection of microsatellite loci belonging to dinucleotide purine-pyrimidine tracks was discussed.*

Keywords: polymorphism, invert DNA repeats, microsatellite loci, purine-pyrimidine tracks

Introduction

The main aim of using the molecular-genetic marker using was to reveal the common and particular traits of population-genetic structures for reconstruction of divergence ways and to search the genetic tags of artificial and natural selection. However, the results obtained depended on range and properties of using molecular-genetic markers' polymorphism of the different genome elements (nucleotide positions in structural genes' codons, structural genes themselves, noncoding sequences in different types of repeats), which, as it is known, vary essentially. A good example of it was the dramatic contrast in polymorphism of genes which code histones and immunoglobulins, another example corresponded to differentiation at the mutation frequency in some microsatellite loci of more than 100 times (Thuillet et al., 2002.). Hence, population-genetic estimation of genetic structures of investigated species highly depended on polymorphism of genomic elements used as molecular-genetic markers.

Particular interest represented the genome comparisons between domesticated and closely related wild species of animals. At present, there are approximately 3.4 billion domesticated cattle, buffalo, sheep and goats in the world which provide a major source of protein nutrition for 6.6 billion humans (FAO, 2007). The growth of domesticated ruminant populations, especially cattle, has mirrored the rapid expansion of the human population. Examples of co-evolution between cattle gene pools and human population were known (Tellam et al., 2009). The comparative analysis of single-nucleotide polymorphisms (SNPs) in different Bovinae species allowed to reveal DNA fragments in which the density of such SNPs distinguished cattle from closely related wild species (MacEachern et al., 2009; MacEachern et al., 2009; MacEachern et al., 2009). It was shown that this part of them could be considered as “domestication signatures”. At the same time, SNP marked polymorphism of the limited genome part, as a rule, to code the proteins. It was known, however, that more than 46 % of cattle genome were presented disseminated (LINE, SINE) and tandem repeats (Elsik et al., 2009). The peculiar feature of cattle genome, distinguishing it from other mammalian genomes was the rather high frequency, in particular, of microsatellite with core motif (AG) (Elsik et al., 2009).

Recently estimation of degree of polymorphism of so called anonymous DNA fragments - DNA fragments flanked by invert repeats of decanucleotides (RAPD - Random Amplified Polymorphic DNA) or microsatellite loci (ISSR - Inter-Simple Sequence Repeat) had become widely used (Zietkiewicz et al., 1994). These markers allowed to move from traditional studies of single loci polymorphism (structure genes, microsatellite loci) to an analysis of multiloci spectra representing polymorphism of many genomic fragments. The use of microsatellite loci as primers in PCR to reveal polymorphism of various sites of genomic DNA was based on the fact that microsatellite presented in genomes with very high frequency and ISSR method marking such polymorphism lead to reveal multiloci and polymorphic spectra of genomic fragments. A relatively higher degree of ISSR markers' polymorphism may be caused by high mutation frequency of microsatellite loci: it was supposed that in such loci it was 1000 times higher in average than in structure genes and microsatellite loci themselves were distributed equally across the whole genome (Thuillet et al., 2002). At the same time evidences were obtained that mutation rates in these loci varied essentially and were closely connected with its core motif and localization inside the chromosome (Sheth et al., 2006). Specificity of multilocus spectra of ISSR markers and their polymorphism may

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depend on the nucleotide sequences of flanks of such anonymous DNA sequences.

To estimate such possibility we made a comparative analysis of amplification products from anonymous DNA sequences, flanked by reverse repeats of purine/pyrimidine tracks (GA)9C and (AG)9C in genomes of domesticated and wild *Bovinae* and *Caprinae* species. Purine/pyrimidine nucleotide motifs attracted the special attention because their localization in promoters of many genes was shown and their possibility to take part in regulation of gene expression was experimentally approved (Walter et al., 2007). Moreover, dinucleotides AG participated in canonical border between introns and exons (Jin et al., 2005).

Material and methods

We investigated blood samples from the following cattle breeds: Grey Ukrainian (20 heads), Yakutian (18 heads, Cherga, Altai region, Russia), white headed Ukrainian (12 heads, Ukraine), *Bison bison* (8 heads, reproducing in biosphere reserve "Askania-Nova", Ukraine, blood samples from the collection of Dr. N.Yasinetskaya), *Bison bonasus* (10 heads, Belovezhskaya Pushcha, Belarus - given by Dr. T.Sipko); 10 heads of Kulunda sheeps (Cherga), Askanian multi prolificacy karakul (18 heads, Markeevo, Ukraine) and 5 heads of *Ovis canadensis* (collection of Dr. T.Sipko). To estimate polymorphism of DNA fragments flanked by invert repeats of microsatellite loci we used as primers sequences (GA)9C and (AG)9C. Genomic DNA was extracted from lymphocytes of peripheral blood according to classical method (Nasuda et al., 2005). PCR conditions were: initial denaturation - 2 minutes at 95°C, denaturation - 30 secs at 95°C, annealing 30 secs at 55°C, elongation - 2 minutes at 72°C, 37 cycles, final elongation - 7 minutes at 72°C.

Amplification products (amplicons) were separated in 2% agarose gel (marker of molecular weights - DNA fragments GeneRuler™ 100 bp DNA Ladder Plus, «MBI Fermentas», USA) and colored by ethidium bromide with further visualization in UV-light.

Results and discussion

An analysis of amplification spectra, according to fragments between invert repeats of microsatellite loci, revealed the following interspecific differences. 22 DNA fragments in summary were identified for primers $(GA)_9C$ and $(AG)_9C$ (Table 1, Figure 1 A) with length ranging from 0,4 to 2,5 kb¸ (assumed that each amplicon corresponded to a certain locus). Only these amplicons which were reliably reproduced and easily typed in spectra (called «major amplicons») were included in the analysis. All three investigated animal species produced common amplicons in 1.0; 1.4; 1.7; 2.1 kb length while using $(AG)_9C$ as primer (Table 1).

Table 1. Length of DNA fragments (amplicons) from cattle, *Bison bonasus* and *Bison bison*, received in PCR by using of $(AG)_9C$ and $(GA)_9C$ as primers.

Amplicon length, kb	Primer $(AG)_9C$			Primer $(GA)_9C$		
	Cattle	*Bison bonasus*	*Bison bison*	Cattle	*Bison bonasus*	*Bison bison*
0,4	-	-	-	-	-	-
0,5	+	-	+	+	+	+
0,6	-	-	+	+	+	-
0,7	-	-	-	+	-	-
0,8	-	-	-	-	-	-
0,9	-	-	+	-	-	-
1,0	+	+	+	-	-	-
1,1	+	-	+	+	+	+
1,2	-	-	-	+	-	-
1,3	-	-	-	-	-	-
1,4	+	+	+	-	-	-
1,5	-	-	-	+	-	+
1,6	-	-	-	+	+	-
1,7	+	+	+	-	-	-
1,8	-	-	-	-	-	-
1,9	-	-	-	-	-	-
2,0	-	-	-	-	-	-
2,1	+	+	+	-	-	-
2,2	-	-	+	-	-	-
2,3	-	-	-	-	-	-
2,4	-	-	-	-	-	-
2,5	-	-	-	+	-	-

«+» and «- » — presence and absence of DNA fragment of certain length in amplification spectrum respectively.

Species-specific for cattle and *Bison bonasus* fragments were absent in this spectrum but were observed in *Bison bison* (0.6; 0.9; 2.2 kb). By using $(AG)_9C$ as primer the widest amplicon spectrum from investigated representatives of *Bovinae* subfamily took place in *Bison bison* (9 fragments of lenght from 0.5 to 2.2 kb length) - in cattle and *Bison bonasus* 6 and 4 fragments, respectively (Table 1).

The distribution of amplicon spectra while using $(GA)_9C$ was different. Thus, the widest spectrum belonged to cattle (8 fragments of lenght from 0.5 to 2.5 kb length), and it's interesting that amplicons with size of 0.7; 1.2 and 2.5 kb were found only in this animal group, and fragments of 0.5 and 1.1 kb length were common for all individuals of investigated animals. Species-specific amplicons were not observed for *Bison bison* and *Bison bonasus*. Cattle and *Bison bonasus* had two common amplicons (0.6 and 1.6 kb), cattle and *Bison bison* had one - 1.5 kb (Table 1). So, the estimation of interspecific differentiation varied essentially depending on type of primer and amplicon length. Species-specific amplicons with primer $(AG)_9C$ were found in *Bison bison* and with primer $(GA)_9C$ were found in cattle. It's clear that the multilocus character of ISSR-PCR was convenient and likely to be irreplaceable for intergenomic comparisons.

When we compared genetic structures which had been gained by using $(GA)_9C$ and $(AG)_9C$ primers taking in account the distribution of amplification products from Grey Ukrainian animals, *Bison bonasus* and *Bison bison* (each zone by molecular mass was considered as one locus, an absence of a fragment was considered as homozygote in recessive allele) an auroch turned out to be the closest to cattle.

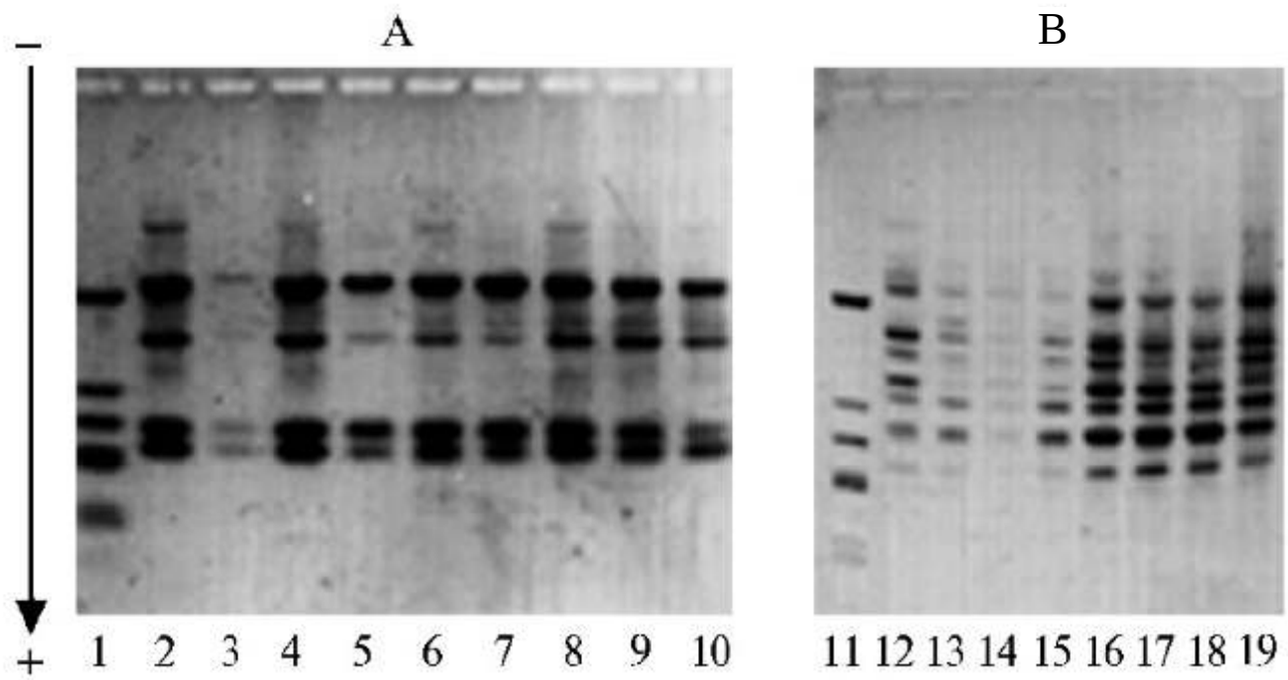

Figure 1. Examples of amplification product spectra, obtaining in PCR
A) With the use as primer of (GA)9C on the cattle DNA of different cattle breeds: 1 - marker of molecular weight; 2-4 - Grey Ukrainian breed; 5-7 - White Headed Ukrainian breed; 8-10 - Yakutian breed.
B) With the use as primer (AG)9C on the DNA of the breeds of Ovis aries: 11 - marker of molecular weight; 12-15 - Askanian multi prolificacy karakul; 16-19 - Kulunda sheep.

Major amplicons with sizes from 0.5 to 1.9 kb length, depending on the primer used, were revealed in breeds of *Ovis aries* (Kulunda sheep, Askanian multi prolificacy karakul) and *Ovis Canadensis.* The spectra of amplification products, obtained with the use of $(AG)_9C$ as primer in two sheep breeds had the low levels of polymorphism (Table 2, Figure 1 B). The common DNA fragments in the amplicon spectra of sheep breeds and *Ovis canadensis* had the length of 0.5; 0.6; 1.0 and 1.7 kb. Only animals of wild species had in amplicon's spectra the DNA fragment of 1.3 kb length; at the same time the DNA fragment in 1.1 kb which was observed in domestic sheep, was absent in amplicon's spectra of wild species. (Table 2).

By using $(GA)_9C$ as primer, amplicon's spectra included the DNA fragments of 0.5 to 1.9 kb in length. Both species had the common amplification products of 0.5; 1.5 and 1.9 kb length; 1.6 and 1.7 kb fragments were found only in domestic sheep, 1.4 kb fragment – only in *Ovis canadensis*. Intraspecific differences between sheep breeds by using the described primer $(GA)_9C$ had not been revealed. In other words, interspecific genetic differentiation, observed by ISSR-PCR markers in representatives of *Caprinae* family also varied essentially depending on the primer used.

We must pay attention tothe high precision of primer annealing in the experiments: the (AG)9C sequence could be considered as $(GA)_8GC$ and $(GA)_9C$ — as $(AG)_8AC$. But in spite of evident similarity between primers (both core motifs belong to purines), the amplification spectra gained differed essentially from each other (Table 1,2). At the same time, the presence of some major amplicons of the same length flanked by invert repeats $(AG)_9C$ and $(GA)_9C$ in

Table 2. Length of DNA fragments (amplicons) from Askanian multi prolificacy karakul (AMP), Kulunda sheep (KS) and *Ovis canadensis* (OC) received in PCR by using of $(AG)_9C$ and $(GA)_9C$ primers.

Amplicon length, kbp	Primer $(AG)_9C$			Primer $(GA)_9C$		
	AMP	KS	OC	AMP	KS	OC
0,4	-	-	-	-	-	-
0,5	+	+	+	+	+	+
0,6	+	+	+	-	-	-
0,7	-	-	-	-	-	-
0,8	-	-	-	-	-	-
0,9	-	-	-	-	-	-
1,0	+	+	+	-	-	-
1,1	+	+	-	-	-	-
1,2	-	-	-	-	-	-
1,3	-	-	+	-	-	-
1,4	-	-	-	-	-	+
1,5	-	-	-	+	+	+
1,6	-	-	-	+	+	-
1,7	+	+	+	+	+	-
1,8	-	-	-	-	-	-
1,9	-	-	-	+	+	+
2,0	-	-	-	-	-	-
2,1	-	-	-	-	-	-

spectra from species of one subfamily and from species of different subfamilies (*Bovinae*, *Caprinae*) remained highly conserved. So, by using $(AG)_9C$ as primer two amplicons in 1.0 and 1.7 kb length were common for representatives of *Bovinae* and *Caprinae*, and for $(GA)_9C$ spectra - only one DNA fragment in 0.5 kb length was common for all investigated animals.

The comparison of obtained amplification spectra evidenced that in some cases the length of DNA fragments flanked by invert repeats of $(AG)_9C$ and $(GA)_9C$ was highly conserved and remained the same in species of different subfamilies. Hence, despite high variability, the certain constancy of nucleotide sequences and distribution of invert repeats $(AG)_9C$ and $(GA)_9C$ were conserved in genomes of species of different subfamilies. Such conservatism was well agreed with considerations of Lima de Faria (1987) about non-random distribution of tandem repeat groups across chromosomes and about participation of some of them the structural and functional organization of linear eukaryotic chromosomes even in such small DNA fragments (2 kb) which are used in population research as ISSR markers of genomic polymorphism.

Heterogeneity of structure of eukaryotic chromosomes has been known for a long time. Two main morphological structures - centromere and telomere regions were the basis of linear chromosome formation. Telomere regions of most animal species as it was known to be built with the help of telomerase enzyme which activity of RNA dependent DNA polymerase amplified the telomere repeats (TTAGGG). Some insects in particular drosophila had telomere repeats built by retrotransposone replication (Walter MF et al., 2007).

The structure of centromere region included transposone and retrotransposone families (Jin et al., 2005; Nasuda et al., 2005; Casola et al., 2008; Zhang et al., 2008). Furthermore proteins taking part in folding of centromere region and kinetochore formation in that place had significant homology with transposase of DNA transposones group (Casola et al., 2008; Zhang et al., 2008). Data achieved build the basis to suppose that these two main morphological structures of eukaryotic chromosome appeared with the help of transposing elements, giving certain linear coordinates of a chromosome. Lima de Faria (1987) was the first who made such conclusion in his papers on the basis of observed non-random distribution of heterochromatin blocks across the length of chromosomes in some plant species. He advanced a hypothesis about "chromosome fields" - nucleotide sequences and concentration of different repeat families including centromere and telomere which are closely connected with chromosome morphology (Lima de Faria, 1987).

Recently a lot of evidences of close connection between molecular structure of genetic material and morphology of chromosomes had been received - that was called "chromosome phenotype" by Lima de Faria (1987). In particular the non-random distribution of retrotransposone families across chromosome length was shown in *Arabidopsis* species (Kendal and Suomela, 2005) and some fungi (Thon et al., 2006) species and retrotransposone families in centromere and telomere regions of chromosomes was shown for some plant species for example for maize (Jin et al., 2005). Such difference let us expect that polymorphism of some genomic elements which are widely used for researches of genetic population structure in plants and animals may depend on a distribution of such elements according to linear chromosome coordinates.

Conclusion

So, in spite of particular similarity between $(AG)_9C$ and $(GA)_9C$ nucleotide motifs, by using them as primers in PCR with genomic DNA of same animals, different amplification spectra were obtained.

It was the evidence of high precision in primer annealing and the reliable identification of genomic fragments flanked by above-listed sequences. The dependence of fragments' structure in spectra of amplification products in PCR in different conditions was revealed, but major fragments were observed and they reproduced in different conditions. The length of some amplicons was conserved not only in different species in borders of subfamily but also in representatives of different subfamilies. The conservatism revealed indicated on non-random genomic distribution of invert repeats $(AG)_9C$ and $(GA)_9C$ which was likely to be connected with their belonging to purine/pyrimidine dinucleotide motifs.

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