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POLYMORPHISM OF ISSR AND IRAP MARKERS IN GENOMES OF MUSK-OXEN *(OVIBOSMOSCHATUS)* AND HORSE *(.EOUUS CABALLUS)* OF ALTAIC BREED

Glazko Valeriy Ivanovich - academician of Russian Academy of Agricultural Sciences (foreign member), academician of Russian Academy of Natural Sciences, professor of center of nanobiotechnologies of RSAU-MTAA, doctor of agricultural sciences; prof., dr; e-mail: vglazko@yahoo.com.
Bardukov Nickolay Vladimirovich - student of Educational department of RSAU-MTAA Russian State Agrarian University - MTAA named after K.A.Timiryazev
Pheophilov Anton Vladimirovich - scientific worker of center of nanobiotechnologies of RSAU-MTAA
Sipko Taras Petrovich - chief scientific worker of A.N. Severtsov Institute of Ecology and Evolution of RAS, candidate of biological sciences
Elkina Mariya Aleksandrovna - postgraduate student of center of nanobiotechnologies of RSAU-MTAA
Glazko Tatyana Theodorovna - academician of Russian Academy of Natural Sciences, professor of department of Animal Science of RSAU-MTAA, doctor of agricultural sciences, prof., dr.

Abstract: the polyloci genotyping of three populations of musk-oxen (Ovibos moschatus), living in East Greenland, the Taymyr Peninsula and Wrangel Island, and the Altaic breed of horses, using two types of molecular genetic markers - ISSR-PCR andIRAP-PCR was carried out. The level ofpolymorphism of most of the markers in the domestic horse was significantly higher than that in the musk-oxen. The differences in the characteristics ofpolymorphism of ISSR-PCR and IRAP-PCR markers between different versions of these markers and between species were identified. Sequencing ofDNA fragments, whose presence distinguished spectra ISSR-PCR markers Altaic horses from the musk-oxen, indicates that the species-specific DNA segments flanked by inverted repeats of microsatellites, can be formed as a result of a recombination between the evolutionarilv more "ancient" and a "young" mobile genetic elements.

Key words: musk-ox, genome scanning, ISSR-PCR, IRAP, inverted repeats, microsatellites, mobile genetic elements, endogenous retroviruses.

The emergence of new methods for the simultaneous genotyping of many DNA allowed passing from a comparison of the genetic structures of populations on a limited number of genes (usually no more than 10-15 loci) to genomic comparisons. Polyloci genotyping methods have been called "genome scanning" [9]. Today, such techniques have been developed enough, one of them suggests genotyping of genomic DNA fragments of different length, flanked by inverted repeat microsatellite loci (Inter-Simple Sequence Repeats - ISSR-PCR markers) [20]. It is believed that microsatellite loci are relatively evenly distributed over the genome of higher mammals, the frequency of their occurrence is largely dependent on the number of nucleotides in the elementary unit of tandem re-

peats (dinucleotide microsatellites are more frequent than trinucleotide, etc.) rather than the core motif, and about 5% of them form the inverted repeats at relatively short distances (100 ~ 2000 bp) [2, 17]. At the same time, the accumulated evidence that the genomic distribution and polymorphism of microsatellite loci may be substantially determined not by the length of the elementary unit of the repeat but by its core motif, and microsatellites may be associated with the distribution of individual species-specific retrotransposons [16].

In order to evaluate the dependence of the results of comparisons of populations' genetic structure using genome scanning methods, a comparative analysis of spectra of the ISSR-PCR and IRAP-PCR (Inter-Retrotransposon Amplified Polymorphism) [7] markers, using as primers in polymerase chain reaction (PCR) microsatellite regions and terminal sites of retrotransposons in the populations of musk-oxen and horses was carried out in this study. In order to explain the possible origin of one of the amplification products of ISSR-PCR markers' spectra sequencing and analysis of its nucleotide sequence were performed.

Materials and methods

We studied genomic DNA of 80 musk-oxen from Greenland, the Taymyr Peninsula andWrangel Island, as well as the group of the Altaic breed horses (116 horses). ISSR-PCR using two dinucleotide microsatellites $(AG)_9C$ and $(GA)_9C$, and two trinucleotide $(GAG)_6C$, $(CTC)_6C$ as primers was performed for a comparative analysis of the genetic structure of animals (estimate share of polymorphic loci, the polymorphic information content of each locus - Polymorphic Information Content, PIC). IRAP-PCR spectra were obtained by adding to the reaction mixture of the terminal site of 5GCAGTTATGCAAGTGGGATCAGCA3' retrotransposon LTR SIRE-1 [7] and retrotransposon-like elements R173 family of monocots' repeats PawS 5 [12]. Each amplificate product (amplicon) was considered as a single locus.

It should be noted that only stable reproducible, at least in triplicate, a part of the spectrum of amplification products (amplicons) containing relatively light DNA fragments (from 1500 to 100 bp) were taken into account in order to prevent errors.

DNA was extracted from peripheral blood a set of reagents of DNA-Extran-1 (JSC "Syntol". Moscow) .PCR was performed on the thermocycler Tertsik ("DNA-Tcchnology". Russia) using a set of reactions RT-PCR (JSC "Syntol". Moscow) with the following parameters: initial denaturation (t = 94°C, 2 min.) denaturation (t = 94°C, 30 sec.), annealing (t = 55°C, 30 sec.), elongation (t = 72°C, 2 min.) - 35 cycles, final elongation (t = 72°C, 10 min.). Electrophoretic analysis of amplification products was performed using 1.5% agarose gel in 1 x TBE buffer with the addition of ethidium bromide to a final concentration of 0.5 fig / ml. The results of PCR were visualized by the transilluminator UVT-1 ("Biokom". Russia). The amplified DNA fragments were determined by a marker molecular weight 100 bp + 1.5 Kb + 3 Kb (12 fragments from 100 to 3000 bp) M27 (SibEnzyme, Russia).

In order to study the mechanisms of origin of genome fragments flanked by inverted sequences, we sequenced and analyzed a fragment of 420-450 bp specific for the spectrum of domestic Altaic horse, which has been obtained using $(AG)_9C$ as a primer in PCR. This fragment was extracted from gel using a set for the elution of DNA from agarose gels Diatom DNA Elution ("Isogene". Russia) according to protocol recommendations. We used the following primer: CAG-CTG-CAG-AGA-GAG-AGA-GAG-AGA-GC (on 5' end of the added restriction endonuclease site for PstI) for PCR with the eluted DNA fragment. The amplification program was adjusted a little: initial denaturation (t = 94°C, 2 min.), denaturation (t = 94°C, 30 sec.), annealing (t = 55°C, 30 sec.), elongation (t = 72°C, 50 sec.) - 20 cycles, final elongation (t = 72°C, 5 min.). The resulting PCR products were directly inserted into a pUC19 vector by DNA ligase. The competent cells of *E. Coli* JM109 were

transformed by a ligation product. Then the cells were cloned. And in the end we chose colonies using a white-blue selection. Plasmid DNA was isolated and purified according to a standard protocol. After that it was treated with restriction enzymes to further check the length of the inserts by agarose gel. We added primers to the phage M13 in selected samples. Sequencing of a DNA fragment of clone was performed using a set of reagents ABI PRISM ® BigDye ™ Terminator v. 3.1 with subsequent analysis of reaction products on an automated DNA sequencer ABI PRISM 3730 Applied Biosystems.

DNA sequencing was carried out in the Inter-institute Center for Collective Use "GENOME" IMB Academy of Sciences (http://www.genome-centre.narod.ru/), organized with the support of the RFBR.

The genetic distances were counted by the method of Nei M. (1972) [10] using TFPGA. Dendrograms of genetic relationships have been built using the same program.

Search for homologous sequences in GenBank was carried out using BLASTn algorithms (http://blast.ncbi.nlm.nih.gov). Identification of inverted repeats within the sequenced fragment was performed using the program http://mobyle.genouest.org/cgi-bin/Mobyle/, homology search in the archives of repetitions performed with the use of programs http://www.repeatmasker.org/ and http://www.girinst.org/censor/.

Results and Discussion

The characteristics of the spectra of amplification products obtained from musk-oxen DNA fragments, flanked by inverted repeats of two dinucleotide microsatellites and one trinucleotide and two terminal sites of retrotransposons, are presented in tables 1-2.

Table 1

Shares of polymorphic loci of the ISSR PCR amplification profiles with respect to populations of musk-oxen, %

Primer	Taymyr	Wrangel	Greenland	Over three populations
$(AG)_9C$ (12 loci)	8,33	0,00	16,67	25,00
$(GA)_9C$ (14 loci)	42,86	42,86	35,71	50,00
$(GAG)_6C$(12 loci)	16,67	8,33	16,67	25,00
LTR SIRE - 1 (12 loci)	41,67	41,67	8,33	50
PawS 5 (7 loci)	14,29	14,29	0	14,29

The unique loci for ISSR-markers were found for each population. There were no polymorphism for locus length of 730 bp in a population of Wrangel Island by $(GA)_9C$ primer, and fragments of 210 bp were not observed in all animals (PIC = 0,392), whereas the presence of this fragment have been always noted in other populations.

Taymyr population is characterized by polymorphism at locus 530 bp by primer $(AG)_9C$ (PIC = 0,438) and at locus 270 bp by primer $(GAG)_6C$ (PIC = 0,404).

A locus 700 bp (PIC = 0,5) was marked in Greenland animals by $(AG)_9C$ primer, and was not found in animals from other populations. Polymorphism was discovered at locus 890 bp (PIC = 0,307) by $(AG)_9C$ primer and 330 bp (PIC = 0,307) by $(GAG)_6C$ primer in this population of musk-oxen. Polymorphism was not found at locus 1180 bp by primer $(GA)_9C$. It was revealed a significant difference in frequency of occurrence of locus 780 bp by primer $(GA)_9C$, marked in 54% of animals of Greenland population (PIC = 0,434), while this figure is 5% at the Taymyr and Wrangel musk-oxen (PIC = 0,052 and 7% PIC = 0,070, respectively).

Table 2

Polymorphic information content (PIC) **of the amplification profiles with respect to populations of musk-oxen**

Primer	Taymyr	Wrangel	Greenland	Over three populations
$(AG)_9c$	0,037	0,000	0,067	0,066
$(GA)_9C$	0,133	0,140	0,162	0,177
$(GAG)_6C$	0,063	0,035	0,067	0,084
LTR-S IRE-1	0,176	0,179	0,039	0,177
PawS 5	0,055	0,067	0,000	0,056
Averaged over all ISSR-PCR markers	0,080	0,063	0,102	0,113
Averaged over all IRAP-PCR markers	0,131	0,138	0,025	0,132

The values of index PIC, which determines the share of heterozygous variants in the population, are presented in table 2. Based on this data, we can conclude according to the results obtained using ISSR-markers, a native population of Greenland has the largest number of heterozygous variants (PIC = 0,102), then the Taymyr population, formed by animals from Nunivak Island and Banks Island [14] (PIC = 0,080), follows and the latter - a population of Wrangel Island. It is important to emphasize that the relatively reduced heterozygosity for ISSR-PCR markers coincides with the results of a comparative analysis of these three populations by polymorphism of microsatellite loci [13]. Taymyr population differed from a population of Greenland with a reduced level of heterozygosity, and a population of Wrangel Island has a pronounced deficiency of heterozygotes at 5 microsatellite loci, polymorphism, which were estimated in this paper [13]. It's known that coefficient of inbreeding of a population of Wrangel Island calculated by the method of Kislovsky is significantly higher than of the other two populations [14]. Thus, the data obtained using microsatellite loci and ISSR-PCR markers are qualitatively coincided. They indicate that the characteristics of genetic heterogeneity are relatively reduced with an increase of the estimated coefficient of inbreeding in the reintroduced populations of musk-oxen in comparison with the native population of Greenland.

The following data were obtained using a comparative analysis of polymorphism of DNA fragments, flanked by terminal inverted repeats regions of retrotransposons of these three populations. There were no unique fragments in the spectra of amplification products from any population, however, we found significant differences in frequency of occurrence of some of them. No polymorphic loci were found in a population from eastern Greenland using PawS 5 primer, and only one polymorphic locus 210 bp obtained by LTR SIRE-1 primer was present. A polymorphic locus of250 bp was found in populations from Wrangel Island and Taymyr Peninsula by PawS 5 primer, polymorphism of six loci obtained using LTR SIRE-1 primer was marked: 210, 230, 320, 370, 480 and 510 bp length. Besides a locus 480 bp is polymorphic in Taymyr population and a locus 510 bp polymorphic in Wrangel population.

Differences in the characteristics of the gene pool obtained using ISSR and IRAP markers provide some interest. Thus, the lowest heterozygosity was detected in Wrangel population according to PIC calculated using ISSR-PCR markers, as well as the microsatellite loci (table 2). This population is characterized by the smallest value of the coefficient of inbreeding [14]. Comparative analysis of the distribution of allelic variants of ISSR-markers are in good agreement with known histories of the formation of reintroduced populations of Taymyr Peninsula, Wrangel Island and the Greenland population, as demon-

stated by dendrogram (Fig. 1) constructed on the basis of genetic distances of ISSR-PCR markers between these groups of musk-oxen (table 3). The results show the efficiency of using ISSR-PCR markers for the objective assessment of population-genetic parameters, the history of the origin of animal groups, the degree of their similarity and the comparative level of inbreeding.

Table 3

Values of genetic distances between three populations of musk-oxen by ISSR-PCR and IRAP-PCR markers

Compared populations	ISSR markers		IRAP markers	
	Values of Nei genetic distances	Values of Nei identity index	Values of Nei genetic distances	Values of Nei identity index
Taymyr/Wrangel	0,0185	0,9817	0,0108	0,9893
Taymyr / Greenland	0,0357	0,9649	0,0217	0,9785
Wrangel / Greenland	0,0284	0,9720	0,0276	0,9728

Population characteristics obtained by IRAP-markers are qualitatively different from those detected by ISSR-markers, except the clustering of populations in the construction of dendrograms of genetic relationships (fig. 1,2). Genetic distances calculated from amplicons obtained by PawS 5 and LTR SIRE-1 primers coincide to a lesser extent with the history of the origin of populations (distance between the Taymyr and Greenland populations were smaller than the distance between the Wrangel and Greenland populations) (table 3). But the main differences are in polymorphic loci and the values of index PIC (tables 1 and 2).

The values of PIC and the proportion of polymorphic loci in the native population of the East Greenland, calculated using the IRAP-markers were lowest for both terminal parts of retrotransposons. By contrast, polymorphic loci and the index of PIC were the greatest in the most inbred population of Wrangel Island. This pattern was observed in spectra of amplification products obtained using two primers, PawS 5 and LTR SIRE-1, that increases the reliability of the identified patterns.

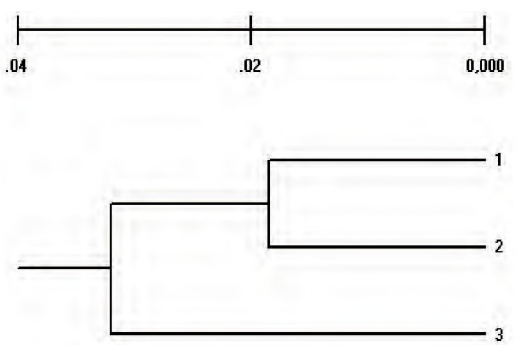

Fig. 1. Dendrogram of genetic relationships based on the genetic distance values, inferred from the distribution of ISSR-PCR markers in different populations of musk-oxen: 1 - population of Taymyr; 2 - population of Wrangel Island; 3 - population of East Greenland

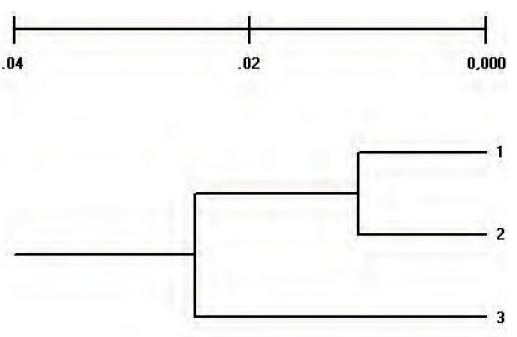

Fig. 2. Dendrogram of genetic relationships based on the genetic distance values, inferred from the distribution of IRAP-PCR markers in different populations of musk-oxen: 1 - population of Taymyr; 2 - population of Wrangel Island; 3 - population of East Greenland

It should be noted that a lot of relatively large securely replicating amplification products (in repeated assays) were obtained using LTR SIRE-1 and PawS 5 primers (12 and 7 loci, respectively). This fact may indicate that DNA fragments complementary to the terminal sites of these retrotransposons are widespread all over the genome.

We can expect the activation of transpositions of mobile genetic elements during the reintroduction of animals in new environmental conditions passing the effects of «bottleneck» [11]. These data suggest that genomic sequences flanked by inverted repeats of terminal sites of retrotransposons are involved in this variability, but not sites of microsattelites. Apparently, this might explain the differences between the groups of reintroduced musk-oxen of the Taymyr, Wrangel and East Greenland populations in the alternative poliloci polymorphism of ISSR-PCR and IRAP-PCR markers' spectra. It should be emphasized that the highest values of genetic distances were obtained as a result of comparing the Greenland population with the Wrangel and Taimyr's in spite of the different directions in estimates of polymorphism of both ISSR-PCR and IRAP-PCR markers, (table 3).

The genetic structure of the domestic Altaic horse was studied by using dinucleotide and trinucleotide microsatellites, and the flanks of the retrotransposons (LTR SIRE-1 and PawS 5) as primers. The obtained data are presented in table 4.

Table 4

Mean values of PIC and polymorphic loci share (%) in spectra of amplification products of ISSR-PCR and two IRAP-PCR markers derived from the flanks as primers LTR SIRE Raw and five horses in the group of the Altaic breed

Primer	Mean values of the polymorphic information content (PIC)	Polymorphic loci share, %
$(AG)_9C$ (13 loci)	0,325	77,0
$(GA)_9C$ (9 loci)	0,318	88,9
$(GAG)_6C$ (13 loci)	0,366	69,2
$(CTC)_6C$ (13 loci)	0,349	76,9
Averaged over all ISSR-PCR markers	0,340	77,1
LTR SIRE (17 loci)	0,260	82,4
Paw 5 (13 loci)	0,050	23,1

It's interesting that the share of polymorphic loci and PIC values of the spectrum of DNA amplification products of the Altaic horses which have been obtained in PCR using all primers, except PawS 5, are significantly higher than that of the musk-oxen (table 1, 2, 4). Moreover, the share of polymorphic loci of the Altaic horses in the spectrum of PawS 5 primer is higher with almost twice more amplicons in the spectrum, but the value of PIC is almost the same as that of the musk-oxen. Besides, the characteristics of the polymorphism of ISSR-PCR markers of the Altaic horses are almost identical to spectra obtained by all primers (table 4), while the spectra of $(GA)_9C$ primer of the musk-oxen are significantly more polymorphic than of $(AG)_9C$ and $(GAG)_6C$ primers (table 2), although the proportion of polymorphic loci of $(GA)_9C$ primer of the Altaic horses is somewhat higher than of other ISSR-PCR markers.

Thus, these data suggest that polymorphism of ISSR-PCR markers is not so much dependent on the length of the elementary unit of microsatellite locus used as a primer (di- or trinucleotide repeats) of studied species, the musk-oxen, and the domestic horse, but on its core motif, even if the motifs are quite close, as AG and GA. At the same time, the spectra of DNA amplification products obtained using the terminal parts of retrotransposons

as primers, originally described in plants, differ from each other: spectra flanked by LTR SIRE-1 are much more polymorphic than by PawS 5 in both species (table 1, 2, 4).

We isolated and sequenced the DNA fragment length of approximately 400-450 bp (the length was determined according to the DNA fragments of a marker of molecular weight) in order to find out which genetic elements may be involved in the formation of fragments of ISSR-PCR markers, presented in the spectrum of DNA amplification products of the Altaic horses obtained in PCR using $(AG)_9C$ as a primer. An amplicon of the same length was absent in the spectrum of amplification products of musk-oxen using the same primer.

In result the DNA fragment of 416 bp length was obtained. On its ends there are two inverted repeat sequences $(AG)_9$, which correspond to the primer that was used in the polymerase chain reaction.

A search of homology in Genbank using the BLASTn algorithms has not allowed to discover the full homology of the fragment of sequenced regions. There are three main groups of fragments with almost complete homology - at position from 1 to 46 of selected DNA fragment in several regions in the genome of mouse *(Mils musculus),* approximately from 47 to 172 nucleotides - in the human genome *(Homo sapiens),* and the longest section homology in the DNA sequencing fragment, from positions 182 to 416, was detected in the sequenced genome of the domestic horse *(Equus cabctUus)*.

A searching of homology in the archives of highly repeated mammalian sequences using the program RepeatMasker and yielded the following data. Two areas of homology were identified using the program RepeatMasker. The first site - from positions 46 to 182 of the analyzed DNA fragment of the Altaic horses 416 bp length to the site from positions 5 84 to 445 of the endogenous virus of mammals with long terminal repeats 3 (LTR / ERVL-MaLR). We revealed two deletions (2 and 1 nucleotide), 21 transitions and 14 transversions, the percentage of divergence - 25.6% while comparing the analyzed area with the appropriate sequence of ERV 3 (fig. 3).

The second site of the analyzed sequence had a homology from 182 to 416 bp to nucleotide sequence of a fragment of the endogenous retrovirus containing long terminal repeats (ERV-1) from 1 to 232 bp (fig. 4).

There are four deletions (3 - in one nucleotide, and 1 - to 4 nucleotides), 16 transitions and 6 transversions, the percentage of divergence - 9.4% in the analyzed area in comparison with the sequence of an ERV-1.

It is assumed that endogenous retroviruses are directly derived from exogenous retroviruses and their recombination play a great role in genome evolution [5]. Endogenous retroviruses of mammals are divided into three classes according to their origin from exo-

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  UnnamedSequen      46 TCTATCGCTGTGTAGCAAATCACCCT--AATTTAATCTTATAAAACAATT 93
                           i    i   i    i     i--  i   i vvi         ii
C MLT1G3#LTR/ER     584 TCTATTGCTGCGTAACAAACCACCCCAAAACTTAGTGGCATAAAACAACC 535

  UnnamedSequen      94 ATTTTATTATTCTAATGGACTCTGTGCATCAGGAATTCTGAGAGGATTTA 143
                          v  v i   i      vi          v  v   iivi
C MLT1G3#LTR/ER     534 ATTTTATTATGCTCACGGATTCTGTGGGTCAGGAATTCGGACAGGGCACA 485

  UnnamedSequen     144 TTGGGCATGGCTTGACTCTGTTCT-TGATGGCATGGACCT 182
                          vi   v        v     i  i-i    v vv  i
C MLT1G3#LTR/ER     484 GCGGGGATGGCTTGTCTCTGCTCCACGATGTCTGGGGCCT 445
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Fig. 3. Sequence homology of the analyzed DNA fragment which is presumably specific to horses showing similarities from 46 to 182 positions of query to positions 584 to 445 of the endogenous retroviruses of mammals with long terminal repeats 3 (LTR/ERVL-MaLR)

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UnnamedSequen    182 TAATACTGACCCTGATAACAGTTTCCCTACATTAAACCCAGCATATATAC 231
                      i    i          v         i                  iv
  ERV1-1N-EC_LT    1 TGATACCGACCCTGATATCAGTTTCCCCACATTAAACCCAGCATATATGG 50

  UnnamedSequen  232 GGTTAAAACCAAAACACTCATTCCCTGCTTACTCTCTGGCTTCCTGGCTC 281

  ERV1-1N-EC_LT   51 GGTTAAAACCAAAACACTCATTCCCTGCTTACTCTCTGGCTTCCTGGCTC 100

  UnnamedSequen  282 CAGAATCCACTATCCTGCATG-AGACAGACACCACCAAGGACAAGCACCT 330
                                       v    -           i
  ERV1-1N-EC_LT  101 CAGAATCCACTATCCTCCATGGAGACAGACACCGCCAAGGACAAGCACCT 150

  UnnamedSequen  331 GAATTCACTATCTCCTCCATACAAAGCAATACAGGC-AGTGGGAA-GCTG 378
                      i     i          vii     vi       -i       -
  ERV1-1N-EC_LT  151 GGATTCATTATCTCCTCCCCGCAAAGAGATACAGGCTGGTGGGAAAGCTG 200

  UnnamedSequen  379 CTGACAAATAAGGCCATGGGCTCTCTCTCTCTCTCTC 416
                          v ii    i  i      i   -----
  ERV1-1N-EC_LT  201 CTGACCAGCAAGGTCACGGGCTCCCTC-----CTCTC 232
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Fig. 4. Sequence homology of the analyzed DNA fragment which presumably is specific to horses showing similarities from 182 to 416 positions of query to positions 1 to 232 of the endogenous retroviruses of mammals with long terminal repeats 1 (LTR/ERV1)

genous retroviruses, which is estimated on the basis of sequence homology. Thus, a close relationship between the ERV class I and *Gammctretrovirus* and *Epsilonretrovims* is expected, the ERV class II - *Alpharetrovirus, Betaretrovirus, Deltaretrovirus,* and *Leniivinis:* the ERV Class III - Spumavirus [4].

Several studies have indicated that the efficiency of searching of homology of highly repeated sequences may depend on the selected databases and analysis software (e.g. [3]). So, we performed a homology searching of the sequenced DNA fragment of the Altaic horse, flanked by inverted repeat $(AG)_9C$ using a database program Giri. We obtained the following data. There are also three areas of homology, as in the case of algorithms of BLASTn, the first - from 1 to 39 bp, the second - from 46 to 166 bp, the third - from 182 to 416 bp. The first site coincides with the 39 nucleotides of the DNA transposon that was observed even in the genome DNA isolated from *Dam o rerio* [1]. The second site coincides with the consensus of a site of a long terminal repeat retrovirus-like element of mammls, ERV3 (Mammalian long terminal repeat, MLT1G2 subfamily - a consensus) in the opposite direction, from nucleotides 579 to 454 (25% divergence). First it was described in the human genome [15]. And the third fragment coincides with (the degree of divergence of 4%) the leading site presumably of non-autonomous horse endogenous retrovirus ERV1 [6].

Thus, all searches of homology of the sequenced DNA fragment, flanked by inverted repeat $(AG)_9C$ length of 416 nucleotides, suggest that this fragment is a result of ancient recombination between mobile elements (DNA transposon of fish and LTR ERV3, that typical for many mammalian species) and sequence of an endogenous retrovirus ERV1, that specific to a horse genome. The sequenced DNA fragment of the Altaic horse of 235 bp length shows a homology only to ERV1 domestic horse in the databases of repeats. It suggests its relatively later formation, in comparison, especially with a site of homology to the LTR ERV3. The sequence of homologous sites is shown in fig. 5.

A search of areas of its integration into the chromosomes of the domestic horse was carried out using a family of algorithms and programs BLASTn Map Viewer (*Eqims cabal-*

his (horse) genome view) in order to evaluate the characteristics of the isolated site of LTR ERV1 of the domestic horse's genome.

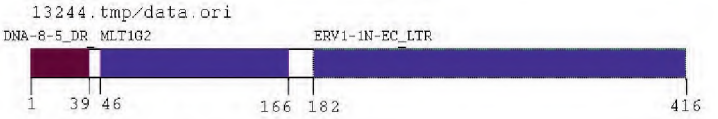

Fig. 5. Homology fragments in a sequenced fragment of the domestic Altaic horse which is flanked by two inverted repeat sequences $(AG)_9$: 1-39 - fragment from non-autonomous fish DNA transposon; 46-166 - fragment from LTR ERV3, which has been firstly described in the human genome; 182-416 - fragment from LTR ERV1 that specific to a horse genome

We obtained the following data. Actually the sequence ERV1 -1 -EC LTR is relatively regularly distributed on the chromosomes of a horse (about one sequence to one chromosome) [19]. However, some of sequenced fragments homologous to a leading site of an endogenous retrovirus are distributed in another way.

A comparison of the frequency localization of the sequenced fragment length of 235 nucleotides with a length of chromosomes enabled us to discover the close relationship between them ($r = 0,93$, $P < 0,001$; table 5). These data suggest that further transposition, recombination and evolution of endogenous retroviral sequences take place in the genome of a domestic horse even today. It's interesting that similar data on the correlation between the frequency of integration sites of endogenous retroviral sequences and lengths of chromosomes were observed in the bovine genome [3]. The authors of this paper noted that

Table 5

Length of chromosomes and quantity of homologous sites in each chromosome to a fragment from LTR ERV1 of 416 bp length

Chromosome	Length of a chromosome (Mb)	Number of homologous sites to a fragment from LTR ERV1	The ratio of the size of chromosomes to number of homologous sites to fragment from LTR ERV1
1	185,8	64	2,90
2	120,9	43	2,81
3	119,5	43	2,78
4	108,6	41	2,65
5	99,7	54	1,85
6	84,7	31	2,73
7	98,5	41	2,40
8	94,1	39	2,41
9	83,6	25	3,34
10	84,0	47	1,79
11	61,3	32	1,92
12	33,1	14	2,36
13	42,6	30	1,42
14	93,9	39	2,41
15	91,6	40	2,29
16	87,4	33	2,65
17	80,8	29	2,78
18	82,5	22	3,75
19	60,0	22	2,73
20	64,2	29	2,21

Continued

Chromosome	Length of a chromosome (Mb)	Number of homologous sites to a fragment from LTR ERV1	The ratio of the size of chromosomes to number of homologous sites to fragment from LTR ERV1
21	57,7	24	2,41
22	49,9	22	2,27
23	55,7	14	3,98
24	46,7	21	2,23
25	39,5	16	2,47
26	41,9	15	2,79
27	40,0	17	2,35
28	46,2	27	1,71
29	33,7	11	3,06
30	30,1	13	2,31
31	25,0	8	3,12
X	124,1	47	2,64

the integration sites of these sequences were often depleted of GC and enriched of AT. The relatively regular distribution through the length of horse chromosomes is described also for the sites homologous to a fragment of a long terminal repeat of endogenous retrovirus ERV3 betal [18].

Conclusion

1. It is shown on the populations of the musk-oxen and the Altaic horse that poliloci genome scanning using ISSR-PCR and IRAP-PCR markers may reflect different processes in the gene pools of animals, depending on the use of DNA markers, as well as the specific features of the investigated groups of animals (degree of inbreeding, belonging to the wild or domesticated form).

2. ft is shown on the example of the sequenced DNA fragment which distinguished the amplicon spectrum of the Altaic horse of $(AG)_9C$ primer from musk-oxen that these genome regions may be formed by recombination of mobile genetic elements. The studied fragment was formed by recombination between the sites of ancient transposable elements and a later version of ERVf, spread throughout the genome of a domestic horse. The lack of complete homology of the sequenced fragments of the genome sequences of thoroughbred horse suggests a breed-specific recombination, which can be used later to create a database of molecular genetic markers of "gene pool standard" of breeds of horses.

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ПОЛИМОРФИЗМ ПО ISSR- IIRAP-МАРКЕРАМ В ГЕНОМАХ ОВЦЕБЫКОВ (*Ovibos moschatus)* И ЛОШАДЕЙ *(Equus caballus)* АЛТАЙСКОЙ ПОРОДЫ

Аннотация: выполнено полилокусное генотипирование представителей трёх популяций овцебыков (Ovibos moschatus), обитающих в восточной Гренландии, полуострове Таймыр и острове Врангеля, а также лошадей алтайской породы, по двум типам молекулярно-генетических маркёров - ISSR-PCR и IRAP-PCR. Уровень полиморфизма по большинству из них у домашней лошади оказался существенно выше, чем у овцебыков. Обнаружены выраженные отличия по характеристикам полиморфизма ISSR-PCR- и IRAP-PCR-маркеров между отдельными вариантами маркеров и между видами. Секвенирование фрагмента ДНК, присутствие которого отличало спектры ISSR-PCR-маркеров алтайских лошадей от овцебыков, свидетельствует о том, что видоспецифичные участки ДНК, фланкированные инвертированными повторами микросателлитов, могут формироваться в результате рекомбинаций между эволюционно более «древними» и более «молодыми» мобильными генетическими элементами.

Ключевые слова: овцебык, геномное сканирование, ISSR-PCR, IRAP, инвертированные повторы, микросателлиты, мобильные генетические элементы, эндогенные ретровирусы.

Автор для корреспонденции: Глазко Валерий Иванович - д. с.-х. н., проф.. акад. РАСХН (иностр. член); e-mail: vglazko@yahoo.com