

Dinukleotidny polymorphism of fine-fleece breeds of sheep on microsatellites

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Abstract: Results of scientific research work on determination of polymorphism with using the dinucleotide microsatellite locuses (Msma11, Msma14, Msma20, Msma24, Msma26) among fine-fleece breeds of sheep. For research was engaged 250 livestock of sheep of CIS countries. Results of research showed that polymorphism of all 5 dinucleotide microsatellite locuses are high.

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1. Introduction

The majority of the fine-fleece breeds which are cultivated in the territory of CIS countries, are genetically close populations. They were created in the 20-40th years of the XX century by dint of import merino breeds (Rambouillet, Precos, Australian merino). The Grozny breed representing adapted for conditions of Russia the Australian merino, still is widely used for improvement of wool efficiency in zones of cultivation of fine-fleece sheep. Microsatellites as markers well proved when studying genetic linkages between populations of sheep [1, 2, 3].

The gene pool characteristic with use of microsatellite markers of agricultural animals is one of effective approaches for identification of their relationship between breeds, populations and lines, and the assessment of degree of an inbredness found research for polymorphism with use DNA-mikrosatellites[4, 5, 6].

The research DNA-mikrosatellites is represented to one of perspective receptions for individual control of an origin and an assessment of genetic structure of populations [7, 8, 9, 10].

The microsatellite analysis is one of the most widespread methods of detection of genetic polymorphism. With its help carry out a genetic typing, marking of genes and locuses of quantitative signs and genomic mapping [11, 12, 13].

Microsatellites are neutral markers, it does them possible for an assessment of a genetic variety of populations. They also are suitable for determination of reliability of an origin of posterity, effect identification "a bottle neck" in population, a genetic categorization of breeds [14, 15].

So far in Kazakhstan researches of a genetic variety of breeds of sheep on the basis of use of DNA technologies weren't conducted. In this regard studying of a gene pool of various breeds of sheep with use of marker technologies represents undoubted relevance.

2. Material and Methods

Materials

As materials of research samples of blood of fine-fleece breeds of sheep served, are taken from 250 sheep representing 5 fine-fleece breeds: Dagestan mountain merino of n = 50; transbaikal fine-fleece n = 50; Kazakh arharomerinos, n = 50; Kazakh fine-fleece, n = 50; Stavropol n = 50;

For an assessment of studied breeds of sheep the following locuses dinucleotide mikrosatellites were used: McMA11, Msma14, Msma20, Msma24, Msma26.

Methods

DNA emitted phenol - a chloroformic method with proteinase use To, and also with use of a set of BioNobile DNA Extraction Kit (BioNobile, Finland), according to the instruction of the producer.

PCR carried out in the amplifikators cyclers of Mastercycler (Eppendorf) of Eppendorf firm.

PCR reaction for 5 microsatellites carried out in 25 мкл reactionary mix of the following structure: Reactionary mix for carrying out PCR contained 1 мкл DNA, on 1 мкл each primer, 2,5 мкл 10X of the buffer for amplification, 17,5 мкл H₂O, 2 мкл total dNTP (final concentration of each triphosphate is equal in reactionary mix of 250 microns) and 1 мкл Taq-polymerases.

Elektroforetichesky division of fragments of DNA carried out during 5 h. For division of fragments used various outletting solutions.

3. Results

The microsatellite locuses investigated by us belong to anonymous as so far their genetic and phenotypical functions are completely unknown.

Conditions of the electrophoretic analysis of products of reaction, provide position division of the next alleles not less than on 2 mm that is sufficient for sure genotyping.

The carried-out analysis showed existence of high polymorphism at 5 fine-fleece breeds of sheep of CIS countries on all 5 locuses of microsatellites where total number of alleles for each breed of sheep averaged: 6,9 – the Dagestan mountain merino, 7,2 –

transbaikal fine-fleece, 7,1 – Kazakh arharomerinos, 7,9 – Kazakh fine-fleece, 6,5 – Stavropol.

As a result of the conducted researches on the above breeds of sheep we revealed all alleles which are present at allelic "ladders". The "mutant" fragments having intermediate length in comparison with fragments of allelic "ladders" in this group also it wasn't revealed. On the one hand, it testifies to high specificity and stability of the studied individualizing systems in the optimized conditions.

For definition of level of genetic variability on each locus counted observed and expected heterozygosity. Our researches showed high level of heterozygosity at 5 fine-fleece breeds of sheep of CIS countries on the tested 5 dinukleotide locuses of microsatellites (table 1).

Table 1. Observed and expected heterozygosity at 5 fine-fleece breeds of sheep by results of 5 dinukleotide of microsatellites

Breeds	Observed heterozygosity	Expected heterozygosity
Dagestan mountain merino	0,72	0,74
Transbaikal fine-fleece	0,77	0,78
Kazakh arharomerinos	0,72	0,75
Kazakh fine-fleece	0,73	0,77
Stavropol	0,75	0,79

The carried-out analysis of 5 fine-fleece breeds of sheep on 5 dinukleotide locuses showed high level of heterozygosity on each locus. Data of observed heterozygosity on locuses (HO) were at average value 0,73 on all studied breeds.

The received results showed that level of observed heterozygosity at the tested breeds of sheep little differs from level of expected heterozygosity that testifies to statistical reliability of these indicators.

For level definition "genetic purity" fine-fleece sheep used Bayes's method by means of the BAPS 3.2 program, thus initially each breed was considered as separate taxonomical unit. Genetic distances between breeds are presented in table 2.

Table 2. Matrix of genetic distances (DA) between 5 fine-fleece breeds of sheep

	The Stavropol fine-fleece	Dagestan mountain merino	the Transbaikal fine-fleece	Kazakh fine-fleece
Dagestan mountain merino	0,130			
Transbaikal fine-fleece	0,050	0,098		
Kazakh fine-fleece	0,090	0,105	0,040	
Kazakh arharomerinos	0,111	0,124	0,097	0,122

The smallest genetic distance was between transbaikal fine-fleece and Kazakh fine-fleece breeds (0,040). And the greatest result was noted between the Dagestan mountain merino and the Stavropol fine-fleece breed (0,130). On dendrogramma fine-fleece breeds of sheep form three clusters (figure 1).

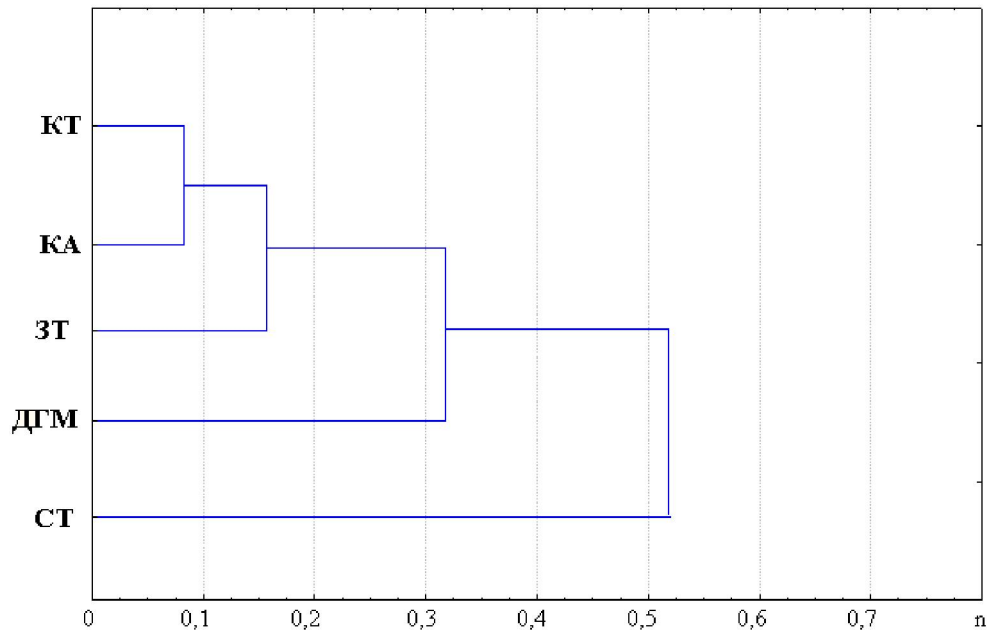


Figure 1. Dendrogramma of genetic relationship between 5 fine-fleece breeds of sheep.

The first cluster created Kazakh fine-fleece and Kazakh arharomerinos, then they join transbaikal fine-fleece, after the Dagestan mountain merino and Stavropol breeds. Thus, dendrogramma representing each animal as taxonomical unit, among fine-fleece of communications gives deeper characteristic.

Thus, the conducted researches showed high informational content of the used genetic markers. Microsatellites, possessing a number of specific characteristics, promoted in recent years to their broad application at an assessment of rare species and the breeds, the closed populations where are widely used various forms of an inbreeding and an outbreeding, an assessment of genealogy of certain outstanding producers, in preservation of disappearing populations of animals.

Conclusion.

In summary we will note that, despite high temporary stability of frequencies of alleles of studied microsatellite loci, a contribution of a stochastic component of variability it is rather powerful and in order that to minimize it, it is necessary to increase the volume of selections and quantity of analyzed locuses.

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