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Microsatellite analysis of Kalmyk cattle

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Abstract. Development of specialized beef cattle breeding contributes to increase in beef production, which directly affects the country's food security. Currently, increasing productivity of animals is the major trend of cattle breeding development, which in turn requires improvement of breeding. The effectiveness of breeding work depends on the assessment of genetic value of breeding animals. To control authenticity of animal origin is a prerequisite for conducting breeding work. One of the main directions of cattle breeding in Kalmykia is breeding of Kalmyk cattle. The aim of the research was to study genetic diversity of Kalmyk cattle populations using microsatellite analysis. The study was conducted in the Regional Research and Production Center for Reproduction of Kalmyk State University. 60 Kalmyk cattle from 'Plodovitoe' agricultural production company in Maloderbetovsky district were studied. PCR analysis was performed by 9 microsatellite loci: BM1824, BM 2113, INRA023, SPS 115, TGLA 122, TGLA 126, TGLA 227, ETH 10, ETH 225. It was found that the average number of alleles was 10.1, while the number of alleles per locus varied from 7 (BM 1824, SPS 115, ETH 10) to 18 (TGLA 122). The loci with the largest range of alleles were BM 2113 (12), INRA 023 (12), TGLA 122 (18) and TGLA 227 (12). The most informative loci were INRA 023, TGLA 122 and TGLA 227. The level of observed heterozygosity varied from 0.67 (ETH 10) to 0.83 (SPS 115, TGLA 227, ETH 225), and expected heterozygosity — from 0.86 (BM 1824, SPS 115, ETH 10) to 0.92 (BM 2113, INRA 023, TGLA 227). Analysis of fixation index data showed that 8 loci had negative index (BM 1824 (–0.22), BM 2113 (–0.26), INRA 023 (–0.26), SPS 115 (–0.18), TGLA 122 (–0.12), TGLA 126 (–0.10), ETH 10 (–0.28), ETH 225 (–0.04) and 1 locus (TGLA 227) had positive index (1.0). The results of the analysis of microsatellite loci showed that level of genetic diversity in the studied herd of Kalmyk cattle is high.

Key words: Kalmyk breed, microsatellites, genetic diversity, heterozygosity, polymorphism

Conflict of interests. The authors declare that they have no conflict of interests.

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Introduction

Beef cattle breeding is one of the priority areas of agriculture in Russia and plays an important role in the development of agro-industrial complex [1, 2]. The development of specialized beef cattle breeding helps to increase beef production, which directly affects the country's food security [3]. Increasing animal productivity is the main direction [4], which in turn requires improving breeding.

The effectiveness of breeding work depends on assessment of genetic value of breeding animals. A mandatory condition for conducting breeding work is controlling authenticity of animal origin. Genetic certification of animals has become a necessary procedure for breeding registration and a reliable identification method in many countries [5]. To establish origin of animals, molecular genetic analysis of satellite DNA is the most effective and accurate [6, 7]. It represents sequences that are repeated many times in the genome [8, 9]. Each repetitive sequence is called motif — a short nucleotide repeat [10]. These motifs are restricted to unique sequences called single-copy sequences [11]. One of the most informative types of satellite DNA are microsatellite sequences, also known as STR loci (STR — short tandem repeat). These sequences consist of repeats of short motifs that vary in the number of repeats [12]. Molecular genetics methods based on satellite DNA analysis have become valuable tools for animal breeding. They make it possible to identify more accurately genetically valuable individuals and use them for development of more productive and sustainable livestock populations [5, 13].

The study of genetic differences between lines and breeding herds is important for purebred breeding. Genetic diversity research allows to understand how genes vary within and between populations. This is of great importance for determining genetic basis of breed qualities and effective selection [14].

One of the main areas of cattle breeding in Kalmykia is pedigree breeding of Kalmyk cattle which is characterized by high productive qualities, strong constitution, relative longevity, and endurance. In addition, Kalmyk cattle are resistant to unfavorable climatic conditions and are unpretentious in maintenance and feeding. Thus, Kalmyk breed is unique [15, 16].

The purpose of the research was to study genetic diversity of Kalmyk cattle populations using microsatellite analysis.

Materials and methods

The research was conducted at the Regional Research and Production Center for Reproduction of Kalmyk State University. 60 Kalmyk cattle from 'Plodovitoe' agricultural production company in Maloderbetovsky district were studied. Genetic testing was carried out using molecular genetic analysis to control reliability of origin and identification of animals based on PCR analysis for 9 microsatellite loci: BM1824, BM 2113, INRA023, SPS 115, TGLA 122, TGLA 126, TGLA 227ETH 10, ETH 225.

For molecular genetic analysis, whole blood was taken from jugular vein. To isolate DNA, kit of M-Sorb Synthol reagents (on magnetic particles) was used. DNA extraction from whole blood was performed according to standard kit protocol. To amplify the isolated DNA, kit of Synthol reagents for polymerase chain reaction and mixture of primers were used. The polymerase chain reaction was carried out on a Bio-Rad C1000 Touch thermal cycler; amplification modes were selected depending on specificity of each pair of primers.

The PCR products were detected by electrophoretic separation on agarose gel using AmpliSens kit. Detection was performed in Wide Mini-Sub Cell GT horizontal electrophoresis chamber. Visualization was carried out using a Clinx Science Instruments ChemiScope 6200Touch gel documentation system.

Microsoft Office software package Excel (Microsoft, USA) was used for processing the experimental data.

All microsatellite loci used in the analysis belong to the list recommended by the International Society of Animal Genetics (ISAG).

Results and discussion

Genetic analysis of Kalmyk cattle population from 'Plodovitoe' agricultural company was carried out using microsatellite loci. The results of the study showed the presence of genetic diversity in this population.

STR analysis of cattle was characterized according to the following indicators: range of alleles, number of alleles per locus, number of informative alleles per locus, frequency of occurrence, expected heterozygosity.

It was found that average number of alleles was 10.1, while number of alleles per locus varied from 7 (BM 1824, SPS 115, ETH 10) to 18 (TGLA 122) (Fig. 1). Loci BM 2113, INRA 023, TGLA 122 and TGLA 227 have the largest range of alleles, and number of alleles per locus is 12, 12, 18 and 12, respectively. The most informative loci for Kalmyk breed were INRA 023, TGLA 122 and TGLA 227. Average frequency of one allele per locus varied from 0.06 to 0.14 (Fig. 2).

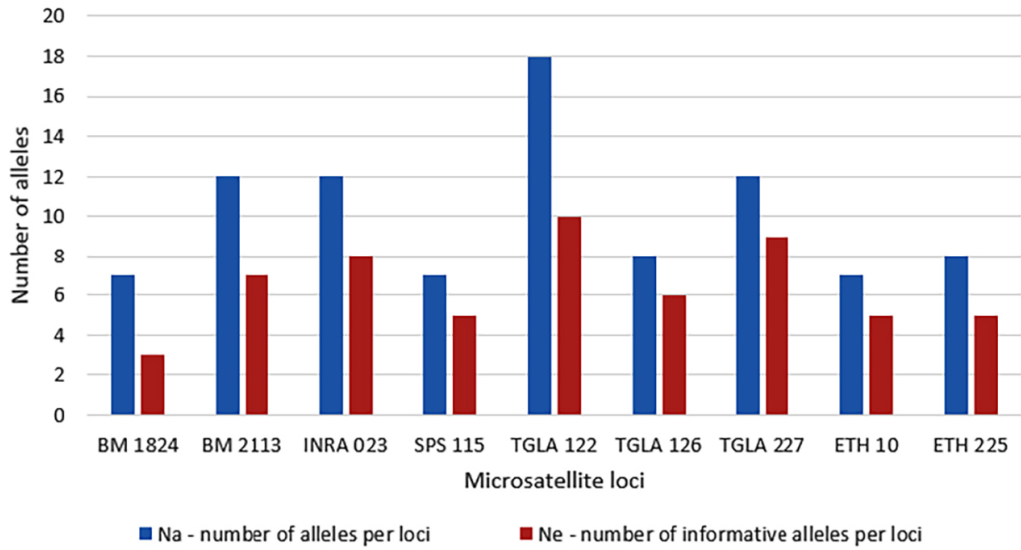

Fig. 1. The number of alleles in the studied loci
Source: created by the authors

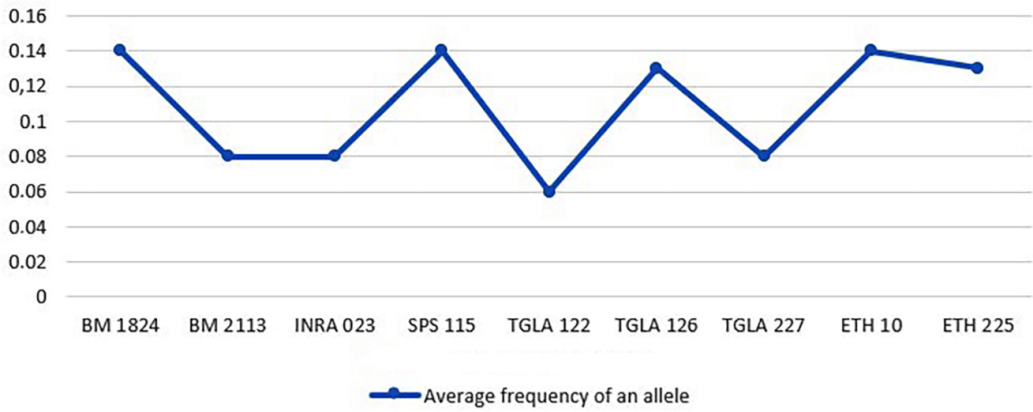

Fig. 2. Frequency of alleles
Source: created by the authors

Heterozygosity analysis allows to assess genetic differentiation. The level of observed heterozygosity varied from 0.67 (ETH 10) to 0.83 (SPS 115, TGLA 227, ETH 225), and the expected heterozygosity ranged from 0.86 (BM 1824, SPS 115, ETH 10) … 0.92 (BM 2113, INRA 023, TGLA 227) (Fig. 3).

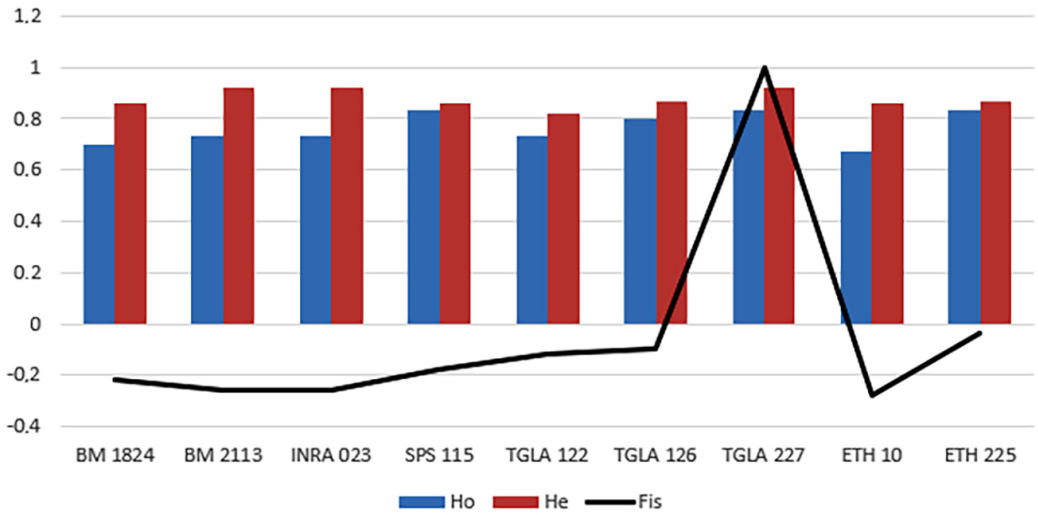

Fig. 3. The level of heterozygosity
Source: created by the authors

A comparison of expected and observed heterozygosity in Kalmyk cattle showed that for all 9 studied loci, the expected heterozygosity exceeds the observed one.

For many microsatellite loci, indicator of excess or deficiency of heterozygotes is fixation index. Positive index value indicates lack of heterozygotes, negative index value indicates excess of heterozygotes. Analysis of fixation index data showed that for 8 loci this indicator was negative (BM 1824 (–0.22), BM 2113 (–0.26), INRA 023 (–0.26), SPS 115 (–0.18), TGLA 122 (–0.12), TGLA 126 (–0.10), ETH 10 (–0.28), ETH 225 (–0.04) and for one locus (TGLA 227) it was positive (1.0).

Thus, results of molecular genetic analysis of microsatellite loci showed that level of genetic diversity in the studied herd of Kalmyk cattle was high.

Conclusion

1. We found that the average number of alleles was 10.1 in 9 studied STR loci of Kalmyk cattle, with a frequency of one allele of 0.06…0.14. The level of observed heterozygosity varied from 0.67 to 0.83, and expected level was 0.86…0.92. The fixation index for 8 loci was negative (from –0.28 to 0.04) and for 1 locus (TGLA 227) it was positive (1.0).

2. The results of the study of microsatellite loci of Kalmyk breed indicate that the allele pool of the breed is diverse. To further improve the breed in the future, it is necessary to study genetic structure of Kalmyk breed.

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Микросателлитный анализ крупного рогатого скота калмыцкой породы

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Аннотация. Развитие специализированного мясного скотоводства способствует увеличению производства продукции говядины, что напрямую влияет на продовольственную безопасность страны. Увеличение продуктивности животных — основное направление развития современного скотоводства, что в свою очередь требует совершенствования племенного дела. Эффективность племенной работы зависит от оценки генетической ценности племенных животных. Племенная работа ведется при обязательном контроле достоверности происхождения животных. Одним из основных направлений скотоводства в Калмыкии является племенное разведение крупного рогатого скота (КРС) калмыцкой породы. Цель исследования — изучение генетического разнообразия популяций КРС калмыцкой породы с использованием микросателлитного анализа. Исследование было проведено на базе Регионального научно-производственного центра по воспроизводству Калмыцкого государственного университета. Для исследования был взят КРС калмыцкой породы, принадлежащий СПК «Плодовитое» Малодербетовского района, в количестве 60 голов, проведен ПЦР-анализ по 9 микросателлитным локусам: BM1824, BM 2113, INRA023, SPS 115, TGLA 122, TGLA 126, TGLA 227, ETH 10, ETH 225. Установлено, что среднее число аллелей составляет 10,1, при этом число аллелей на локус варьировалось от 7 (BM 1824, SPS 115, ETH 10) до 18 (TGLA 122). Локусы с наибольшим диапазоном аллелей — BM 2113 (12), INRA 023 (12), TGLA 122 (18) и TGLA 227 (12). Наиболее информативными оказались локусы INRA 023, TGLA 122 и TGLA 227. Уровень наблюдаемой гетерозиготности варьировал от 0,67 (ETH 10) до 0,83 (SPS 115, TGLA 227, ETH 225), а показатели ожидаемой — 0,86 (BM 1824, SPS 115, ETH 10) … 0,92 (BM 2113, INRA 023, TGLA 227). Анализ данных показателя индекса фиксации показал, что у 8 локусов данный показатель отрицательный (BM 1824 (–0,22), BM 2113 (–0,26), INRA 023 (–0,26), SPS 115 (–0,18), TGLA 122 (–0,12), TGLA 126 (–0,10), ETH 10 (–0,28), ETH 225 (–0,04) и у 1 локуса (TGLA 227) положительный (1,0). Результаты проведенного анализа по микросателлитным локусам показали, что у исследуемого стада КРС калмыцкой породы уровень генетического разнообразия высок.

Ключевые слова: калмыцкий скот, микросателлиты, генетическое разнообразие, гетерозиготность, полиморфизм

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