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Research paper

Analysis of Genetic Diversity in three Kazakh Sheep using 12 Microsatellites

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Abstract

The genetic structure of three Kazakh sheep breeds was examined by using 12 microsatellite loci. A total of 144 alleles were detected from the 12 STR loci, with a mean value of 12.0. The highest allele diversity was found at the locus *CSRD247* (16 alleles). PIC value showed that all studied STR markers are more informative and appropriate for genetic analysis of three Kazakh sheep populations. Beside of *INRA006*, all markers had high level of genetic variability. As Fixation index shows, the excess of the heterozygosity was observed only in loci *MAF065*. Obtained number of private alleles in Edilbai, Kazakh Arkhar Merino and Kazakh Fine-wool sheep were 25, 17 and 15 respectively. Genetic diversity was higher in Edilbai population than in other two populations. The genetic variability was lower in Kazakh Arkhar Merino sheep than in the Edilbai and Kazakh Fine-wool sheep breeds. The genetic distance was the largest between Edilbai and Kazakh Arkhar Merinos. Also, the moderate differentiation was observed between Edilbai and Kazakh Arkhar Merinos.

Keywords: *Edilbai, Kazakh Fine-Wool, Kazakh Arkhar Merino, Microsatellites, Gene Diversity..*

1. Introduction

Sheep breeding is one of the leading animal agricultural sectors in Kazakhstan due to its vast expanses and favorable climatic conditions for breeding of these animals, and also, due to the high productivity of this industry. Sheep are grown not only to obtain meat, but also as a source of wool, sheep skin, lamb, fat, milk, and a valuable organic fertilizer-manure. Edilbai, Kazakh fine-wool and Kazakh Arkhar Merinos are special sheep breeds bred in Kazakhstan [1].

Edilbai sheep breed is a representative of the national selection, which was carried out about 200 years ago. In the breeding process, sheep, selected for breeding, were the most adapted to the natural and climatic conditions of nomadic sheep breeding. Edilbai sheep breed is tolerable to the summer drought and winter cold and easily produce numerous offspring. In addition, according to the morphphysiological composition, sheep have the ability to easily gain fat on a sparse, thinned pasture fodder [2].

Kazakh Arkhar Merinos is a breed of fine-wooled sheep of meat-woolly direction. The breed was created on the basis of the interspecific hybridization of wild sheep argali with uterine fins: Novo-Caucasian merinos, prekos and rambulie. This sheep breed has a sturdy constitution, with a strong, well developed skeleton which allows them to graze on the mountainous pastures, usually inaccessible for other sheep breeds [3-4].

Kazakh fine-wool sheep breed is a breed of meat-woolly direction. It is derived by crossing Kazakh sheep with prekos sheep breed. Due to the careful selection and wide application of linear breeding, the Kazakh fine-wool breed combined the valuable features of local sheep breed -large size, quality meat, endurance and ability to year-round pasture maintenance [2].

Unfortunately, the genetic diversity of the breeds mentioned above was not described in details.These days, the research onthe genetic diversity of animals is widely performed with the help of microsatellite markers and many research works were carried out to describe genetic diversity of sheep [5-9].

Microsatellite DNA markers can serve as a tool for genetic identification of breeds, types, lines of agricultural animals, for determination of the genetic structure and assessing genetic distances between groups of animals, assessing the magnitude and direction of the gene flow between populations and as a determination of the effective population size of endangered and small populations [10-12].

Despite of the emergence of the new methods of genotyping, microsatellite markers play an important role in the genetic testing of different breedsof agricultural animal populations due to such important advantages as uniform distribution in the genome, great allelic diversity, high information content, Mendelian inheritance and ease of automation of determination [13-14].

2. Materials and methods

The blood samples were taken from 45 unrelated individuals of the 3 sheep breeds, where 15 animals of every breed were chosen randomly. Genomic DNA was extracted from white blood cells using Gene JET Genomic DNA Purification Kit. In this paper, 12 microsatellites recommended by ISAG (Table 1) were used to study genetic diversity of three Kazakh sheep breeds. The polymerase chain reaction was performed in a thermal cycler

(Bio-Rad). The 12 microsatellites were previously tested by PCR analysis and screened for the successfully amplified STR markers. The PCR reaction volume, conditions and grouping to multiplexes have been adjusted according to Baumung R. 2006 [15]. PCR products were attached at *ABI PRISM 310 Genetic Analyzer (Applied Biosystems)*. For the interpretation of the allele variants and fragment sizes, there was used *GeneMapper* software. Statistical processing of data was carried out by software *GenAlex 6.5 and Excel microsatellite Toolkit* (version 3.1) [16-17].

3. Discussion

A total of 144 alleles were indentified from the 12 STR loci of the three sheep breeds. The number of identified alleles per locus varied from 7 in *D5S2* to 16 in *CSRD247*, with a mean of 12.0. In the analysis of survey findings, the highest and the lowest effective number of alleles have been found in marker *INRA005* (6.9) and *McM042* (3.1) respectively, and the alleles effective number mean was 4.592 for the 12 markers. The maximum and minimum observed and expected heterozygosity ranged from (*Ho*) 0.888 and (*He*) 0.891 to (*Ho*) 0.466 and (*He*) 0.718 accordingly. The mean value of these two parameters were 0.720 and 0.811. Calculating polymorphic information content value *(PIC)* showed that the greatest *PIC* value was 0.870, while the smallest was 0.667, with a mean of 0.777. Fixation index was between 0.375 and –0.096. Total number of alleles, private alleles, mean number of alleles and effective number of alleles were larger in Edilbai sheep than the Kazakh Arkhar Merino and Kazakh Finewool. Observed heterozygosity ranged from 0.678±0.051 to 0.744±0.048 among the breeds. The highest expected heterozygosity was observed in Edilbai, whereas the lowest value was obtained from Kazakh Arkhar Merino. Moreover, Edilbai breed had higher fixation index (Table 1).

Table 1: Genetic diversity of Kazakh sheep breeds based on the 12 microsatellites (*Na* – number of alleles, *Ne* – effective number of alleles, *Ho* – observed heterozygosity, *He* – expected heterozygosity, *PIC* – polymorphic information content, *F* – fixation index)

Locus	*Na*	*Ne*	*Ho*	*He*	*PIC*	*F*
CSRD247	16	5.685	0.666	0.869	0.844	0.233
D5S2	7	3.536	0.688	0.753	0.705	0.086
INRA005	13	6.938	0.866	0.885	0.863	0.021
INRA006	10	3.378	0.466	0.746	0.698	0.375
INRA023	14	5.767	0.777	0.891	0.870	0.127
INRA63	13	5.481	0.844	0.863	0.840	0.022
INRA172	12	3.495	0.600	0.718	0.689	0.164
MAF065	12	4.176	0.866	0.790	0.752	-0.096
MAF214	12	3.194	0.600	0.765	0.720	0.215
McM042	11	3.131	0.666	0.719	0.667	0.073
McM527	12	4.574	0.711	0.840	0.810	0.153
OarFCB20	12	5.751	0.888	0.889	0.867	0.001
Mean	12.0	4.592	0.720	0.811	0.777	0.112

4. Discussion

In order to measure the genetic diversity and variability of the three sheep breeds were used 12 microsatellites, located on the different chromosomes of the sheep and differed in their base pairsize. From literature sources it is known that the information about genetic diversity is useful for farmers to improve their breeds and adapt livestock populations to the changing environment. The highest genetic diversity was detected at the marker *CSRD247* with 16 alleles, this value being higher than the 13 alleles found in four populations of Nguni sheep (N.W. Kunene et al.) [18]. However, *INRA005* marker showed high level of effectiveness. All 12 loci studied were highly informative, with an overall mean of *PIC* value 0.77 and also all markers had the *PIC* value higher than 0.5 [19]. In this study, the *PIC* value was higher than the value reported by J.Y. Bai [20]. Depending on the high value of *PIC*, these 12 polymorphic microsatellite markers are suitable for studies of the genetic diversity, structure and mating system of Kazakh three sheep breeds. In addition, there were identified several STR markers with high levels of genetic diversity: *INRA005, INRA63* and *OarFCB20*. Fixation index results showed that all of the markers were observed under random mating, with the exception of *MAF065*. After estimating the number of alleles in the populations separately, we found that the Edilbai sheep had the highest genetic diversity (100 alleles). The Kazakh Finewool's number of alleles (95 alleles) was higher than that of the Kazakh Arkhar Merino (85 alleles). The examined sheep populations differed from each other by the private alleles. A total of 57 private alleles were detected for the three sheep breeds. 25 out of 57 private alleles were observed in Edilbai sheep, whereas Kazakh Arkhar Merino and Kazakh Fine-wool sheep had 17 and 15 private alleles accordingly. The most common private alleles were found in the following loci: allele 196 at the locus *INRA023* of the Kazakh Arkhar Merino breed (frequency of occurrence – 0.167), allele 130 at the locus *INRA006* of the Kazakh fine-wool sheep breed (frequency of occurrence – 0.267), allele 162 in the *INRA172* locus of the Edlbai breed (frequency of occurrence – 0.167). The frequencyof the remaining private alleles was less than 0.1. These private alleles were proven to be essential for the characterization of the investigated sheep breeds [21-24]. Comparing genetic diversity parameter among the populations, the greatest mean number of alleles 8.333±0644 was at the Edilbai breed, which is higher than the results of E. Sh. Seidani et al. (2009) and A. Ferrando (2014) [25-26]. Therefore, it can be concluded that the selection of superior animals is taking place in the population of Edilbai sheep breed. Furthermore, the effective number of alleles was calculated among the breeds, where *Ne* value ranged from 3.902±0.477 to 4.975±0.461.The obtained indicators were similar to the result of S.B.S. Gaouaret al. (2016) and greater than the values reported by X. C. Zeng et al. (2010) [27-28]. In order to compare the genetic variability of the three sheep breeds, the observed and expected heterozygosity was estimated for each pair of populations (Table 2).

Table 2: Population-genetic parameters between the studied sheep breeds. (*TNA* – total number of alleles, *Pa* – private alleles, *MNA* – mean number of alleles, *Ne* – effective number of alleles, *Ho* – observed heterozygosity, *He* – expected heterozygosity, *F* – fixation index)

Population	*TNA*	*Pa*	*MNA*	*Ne*	*Ho*	*He*	*F*
Kazakh Arkhar Merino	85	17	7,083±0.633	3.902±0.477	0,678±0,051	0,702±0,033	0.035
Kazakh Finewool	95	15	7.917±0.557	4.900±0.538	0,744±0,048	0,770±0,022	0.036
Edilbai	100	25	8.333±0644	4.975±0.461	0,739±0,052	0,777±0,023	0.058
Total	280	57	7,778±0,354	4,592±0,289	0,720±0,029	0,750±0,016	0.043

The highest average level of observed heterozygosity was found in Kazakh Fine-wool population (0.744±0.048), which did not differentiate a lot from that of Edilbai (0.739±0.052), although, these values were much higher than those for the Kazakh Arkhar Merinos breed (0.678±0.051). The expected heterozygosity ranged from 0.777±0.023 to 0.702±0.033 among the breeds. Population-genetic studies showed that the total observed and expected heterozygosity were 0.720±0.029 and 0.750±0.016 respectively. Comparable genetic variability has been reported by E. Guang-Xin et al. (2016) and R. Ocampoet al. (2015) in 14 native Chinese sheep and Colombian sheep breeds [29-30]. Among the populations, Fixation index (*F*) varied from 0.035 for Kazakh Arkhar Merinos to 0.058 for Edilbai ($0 < F < 1$) [31]. As a result, Fixation index revealed the random-mating events in all populations. Evaluation of the genetic distance in populations showed that the greatest values observed were between Kazakh Arkhar Merinos and Edilbai (0.469), however, the smallest genetic distance was between Kazakh Arkhar Merinos and Kazakh Fine-wool (0.259). This could be explained bythe direction of productivity, Kazakh Arkhar Merinos and Kazakh Fine-wool have been fine wool direction sheep breeds and Edilbai is coarse wool sheep. F_{st} values varied from 0.040 for Kazakh Fine-wool to 0.063 for Edilbai (Table 3). Obtained genetic divergence indicated that the moderate differentiation was observed between Edilbai and Kazakh Arkhar Merinos ($F_{st} > 0.05$) [32]. A little genetic differentiation was found between Fine-wool and Kazakh Arkhar Merinos, as well as between Edilbai and Kazakh Finewool. The F_{st} values were lower between Kazakh Fine-wool and Kazakh Arkhar Merinos than those between Edilbai and Kazakh Finewool (Table 3).

Table 3: Genetic distance (above diagonal) and differentiation (below diagonal) of three sheep populations

	AK	KT	ED
AK	**0.000**	0.259	0.469
KT	0.040	**0.000**	0.401
ED	0.063	0.046	**0.000**

5. Conclusion

To study the genetic structure of three sheep breeds, there were selected 12 microsatellite loci recommended by ISAG. Due to the fact that the number of alleles, the PIC index and the level of heterozygotes were high, all studied 12 microsatellite markers were highly effective for analyzing the genetic structure and variability of three Kazakh sheep breeds. According to the results of the study, these sheep breeds differ from each other not only in terms of morphological features and productivity, but also in the number of alleles, frequency of their occurrence and in the detected private alleles. In addition, the level of genetic differentiation between the Edilbay and the Kazakh Arhar Merinos was higher compared to the same index between the Kazakh Arkhar Merinos and Kazakh fine wool breeds.

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