Genetic polymorphism of 17 microsatellite DNA loci in main lines of Kostanay breed horses

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Abstract: With the rapid development of DNA technology nowadays, the number of genetic markers identified in horses reached several dozen. The use of gene markers in breeding at stud farms in many countries has become one of the essential criteria for the selection process[1]. Unfortunately, as to the horses bred in Kazakhstan, including Kostanay horse breed, no such work was conducted. Therefore, studying the possibilities of marker-assisted selection in horse breeding and the use of results in practice of Kazakhstani studs are the urgent necessity, and the study of genetic polymorphism of main lines of local horse breed, i.e. Kostanay breed, is a topical issue. According to the research, the main lines of Kostanay breed horses differ significantly by the presence and frequency of alleles of microsatellite loci, as each line of 17 microsatellite DNA loci has its own genetic structure.
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1 Introduction

In the breeding of animals, breeding by lines and families is very important. This allows you to concentrate valuable hereditary properties of the breed. Breed advantages accumulate in lines and families that make up its structure, and provide an opportunity to improve the breed.

The breed is continuously forming new lines with disappearance of the old ones. However, the life span of each line is related to the hereditary capacity of line founders and followers, and the efficiency of breeding work with the given line.

Due to the accumulation of hereditary properties (genotype) of the mother, each line's genetic similarity to the founder reduces with time.

Therefore, to extend the life of the lines, prevent their "leaving to dam" and preserve their valuable properties, new selection methods are necessary.

To be able to attribute a horse to the given line, knowledge of its origin is not enough. It requires comprehensive assessment of belonging to this line and investigation of the genotype and other features. Therefore, a comprehensive genetic examination of Kostanay breed lines is very important today.

An important task of modern agricultural genetics is the effective selection of parent pairs with gene markers (MAS - marker assisted selection)[2,3,4,5,6,7,8].

Highly informative DNA markers provide possibility of selective intervention in the process of breeding. In recent years, the studies of gene pools in livestock are mainly based on DNA microsatellite polymorphism [9,10,11,12,13].

Molecular markers have become fundamental tools for basic research programs on animal genomics as well as for applied marker-assisted breeding studies in livestock[14].

In horses, they promise to find utility not only for performing the indirect selection of animals based on markers linked to traits of interest but also for the assessment of conservation priorities and strategies (ie . parentage determination and mating plan optimization)

Molecular markers have revolutionized our ability to characterize genetic variation and rationalize genetic selection. They have proven to be effective and reliable tools for the analysis of genome architectures and gene polymorphisms in animals[15,16]. So far, the field of horse genomics that has seen the greatest advancement with the use of molecular marker technology has been population genetics. For instance, SSR and AFLP markers have been exploited for assessing genetic diversity within as well as between horse[17,18,19,20,21].

Knowledge of inbreeding levels and genetic relatedness among animals within studs of a given horse breed is expected to have a significant impact on the conservation of local resources and preservation of gene pools.

In this study, the analysis of molecular markers allowed us to assess the genetic diversity, of the Kostanay horse breed.

Practical implications: by preserving the highly polymorphic alleles identified in lines studied,

it is possible to keep valuable qualities transmitted to descendants from line originator; the effectiveness of monitoring the reliability of origin for stud horses using 17 microsatellite DNA loci was 99.999%, so the study of the reliability of origin of horses bred in Kazakhstan should use these 17 panels; the use of highly polymorphic microsatellite DNA loci as universal genetic markers proves to be efficient in genetic monitoring of the breed, lines and families, and assessing their genetic diversity and in preparation of breeding programs.

2 Materials and methods

The material for the study was based on samples of blood and hair of the main lines of Kostanay horse breed: Neon (n=29), Fort (n=18), Beaver (n=15), Windbreak (n=8) and Zeus (n=6). Blood samples were taken from the jugular vein by a standard technique of 10-15 ml[22]. Follicles were taken in the amount of 20-25 pieces per animal; paper bags were used for storage prior to DNA extraction. DNA was extracted from blood samples (suspension of white blood cells) and hair follicles using QIAGEN mini kit (Germany).

A set of primers was used for analysis, including 17 microsatellite loci recommended by the International Society for Animal Genetics (ISAGThe laboratory is a member of this society, and every 2 years passes the comparative analysis. The extracted DNA was amplified in the "Mastercycler" thermocycler (Germany) with a set of "Stock Marks for Horses" primers according to the manufacturer's recommendations. Electrophoresis of the amplification products was carried out on "ABI Prism 310" Genetic Analyzer (by Applied Biosystems, USA). The obtained graphical results were interpreted and documented using GeneMapper^{TM} software for automatic interpretation of fragment analysis results.

Interpretation of graphical images of obtained individual genetic profiles and genotyping of animals were carried out with the control sample and results of participation in the international comparative tests (World Horse Comparison Test).

Genetic and statistical analyzes were performed by standard techniques (Y.E. Merkuryeva, 1977; Ch. Lee, 1978, L.A. Khrabrova, A.M. Zaitsev, 2005). The following indicators were calculated: allele frequencies, observed (Ho) and expected (He) heterozygosity, effective number of alleles (level of polymorphism, Ae); number of alleles per locus (Na); fixation index Fis; alleles were identified specific to a particular population - "private" alleles (Pa); genetic distances and the level of genetic similarity; effectiveness of traceability for individual loci and the overall sample. Statistical analysis of the data was carried out using MathCAD 2001 software and Excel.

3. Results

3.1 Genetic variation of main lines of Kostanay breed horses by 17 microsatellite DNA loci

Testing of the examined animals evidenced that the main lines of Kostanay breed horses differ significantly by the presence and frequency of alleles of microsatellite loci, as each line of 17 microsatellite DNA loci has its own genetic structure.

The frequency of alleles in loci on comparative analysis of allele pools of the main lines of Kostanay breed horses in each line is different, including in the line of Neon: VHL20, ANT4, NMS7, HTG6, ASB2, HMS26 ASB17, LEX3, CA425 loci, line of Fort: VHL20, HMS7, HTG6, ASB2, HMS2, CA425, line of Beaver: VHL20, AHT4, HTG6, AHT5, CA425, line of Zeus: VHL20, AHT5, ASB23 line of Windbreak: HTG6, HTG7 loci showed high polymorphism, i.e. differ in their genetic variation. In HTG10 locus we encountered a large number of alleles occurring in all the lines, so compared to other loci this one showed high polymorphism. We identified specific alleles of 5 lines of 17 DNA loci.

The widest range of allele distribution in Kostanay horse breed is in Neon line (number of alleles encountered - 121, and number of private alleles - 10), average of Beaver lines (number of alleles encountered - 115 and number of private alleles - 10) and Fort (number of alleles encountered - 114 and number of private alleles - 9). And in the lines of Zeus (number of alleles generally encountered - 94 and number of private alleles - 5) and Windbreak (number of alleles encountered - 83 and number of private alleles - 2) the indicators are lower compared to the other lines (Figure 1).

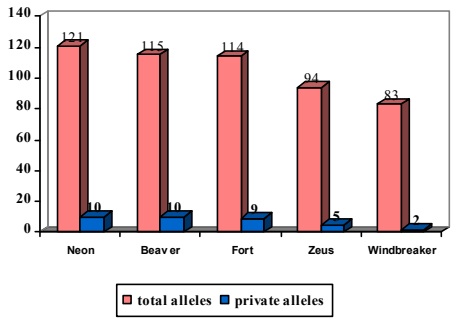

Figure 1. Total number and number of private alleles for 17 microsatellite DNA loci in the main lines of Kostanay horse breed

For 17 microsatellite DNA loci, we encountered ten "private" alleles in Neon line (VHL20^{R}, HTG4^{H}, HTG6^{H}, AHT5^{I}, HMS6^{Q},

ASB23[N], HMS2[P], ASB17[K], LEX3[E] and HMS1[G]), nine private alleles in Fort line (HTG4[N], AHT4 [L,Q], HMS7[Q], HMS6[R], ASB23 [T,R], LEX3[G], CA425[E]), ten - in Beaver line (HTG4[G,J], AHT4[M,N], AHT5[S], ASB23[U,O], ASB17[H,Q], LEX3[T]), five alleles in Zeus line (VHL20[J], HTG4[F], HTG6[Q], AHT5[L], HMS2[N]) and two alleles in Windbreak line (ASB23[F], CA425[H]).

Studies of each line of Kostanay horse breed identified the total number and average number of alleles per locus (%) of DNA microsatellite in the main lines pf Kostanay horse breed (Table 1).

Loci	Main lines of Kostanay horse breed									
	Neon		Fort		Beaver		Zeus		Windbreak	
	n=28		n=18		n=15		n=6		n=8	
	Na	NV	Na	NV	Na	NV	Na	NV	Na	NV
VHL20	8	4.908	8	4.908	7	4.294	7	4.294	5	3.067
HTG4	5	3.067	5	3.067	6	3.681	4	2.454	4	2.454
AHT4	6	3.681	7	4.294	8	4.908	6	3.681	4	2.454
HMS7	7	4.294	8	4.908	7	4.294	5	3.067	3	1.840
HTG6	8	4.908	8	4.908	8	4.908	8	4.908	6	3.681
AHT5	7	4.294	6	3.681	8	4.908	5	3.067	5	3.067
HMS6	6	3.681	6	3.681	5	3.067	4	2.454	4	2.454
ASB23	6	3.681	7	4.294	8	4.908	6	3.681	6	3.681
ASB2	8	4.908	8	4.908	7	4.294	4	2.454	6	3.681
HTG10	9	5.521	9	5.521	9	5.521	8	4.908	7	4.294
HTG7	6	3.681	5	3.067	5	3.067	6	3.681	4	2.454
HMS3	7	4.294	7	4.294	5	3.067	5	3.067	4	2.454
HMS2	7	4.294	7	4.294	6	3.681	6	3.681	4	2.454
ASB17	8	4.908	5	3.067	6	3.681	6	3.681	4	2.454
LEX3	10	6.135	7	4.294	7	4.294	5	3.067	6	3.681
HMS1	6	3.681	5	3.067	6	3.681	4	2.454	4	2.454
CA425	7	4.294	6	3.681	7	4.294	5	3.067	7	4.294
average	7.118	4.366	6.706	4.114	6.765	4.150	5.529	3.392	4.882	2.995

Table 1. Number of alleles (Na) encountered in 17 microsatellite DNA loci and average number of alleles per locus (NV, %) in the main lines of Kostanay horse breeds.

The studies have shown that the number of private alleles in the lines of Neon and Beaver are the same, in the lines in Fort - average, and in the lines of Zeus and Windbreak the number of private alleles compared with the other lines are fewer.

To maintain genetic inbreeding diversity, the average number of alleles (NV) for all studied markers in a particular breed is of great interest. Neon line was the most polymorphic with NV = 4.366 (Figure 2).

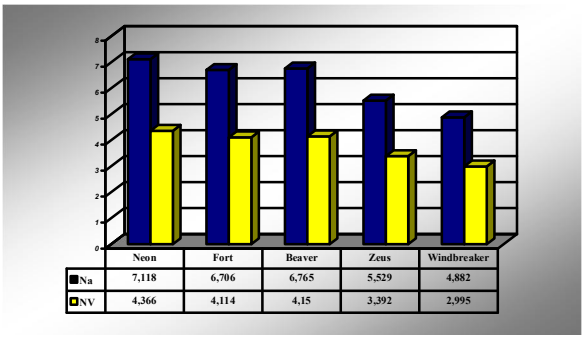

Figure 2. Number of alleles (Na) encountered in 17 microsatellite DNA loci and average number of alleles per locus (NV)

For each investigated locus, genetic analysis of Kostanay horse breeds line showed that each line has its inherent genetic structure. This proves that the genetic variation of main lines of Kostanay breed horses for 17 microsatellite DNA loci is high and has sufficient gene pool; The observed features enable more efficient use of individual loci for various purposes of genetic and population studies.

3.2. Level of heterozygosity for 17 microsatellite DNA loci in the main lines of Kostanay horse breed

The results of research conducted to determine the level of observed (Ho) and expected (He) heterozygosity for 17 microsatellite DNA loci showed that in the line of Zeus the observed level of heterozygosity was 0.824, and in line of Windbreak the expected level of heterozygosity was 0.829 which is higher compared to the levels of heterozygosity in other lines.

According to the studies, intermediate correlation of levels of observed and expected heterozygosity of microsatellite loci was not identified, but the assessment of excess or deficiency in heterozygosity is the most effective indicator of fixation index.

Fixation index accurately reflects excess or deficiency of heterozygosity for most of the studied loci - in case of excess of heterozygotes its value was negative, otherwise - positive (Figure 3).

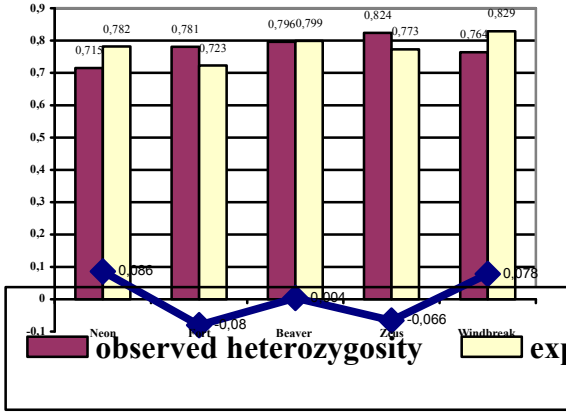

Figure 3. Level of observed (Ho) and expected (He) heterozygosity and fixation index (Fis) in the lines studied

According to the information in Figure 3, the ratio of observed (0.796) and expected (0.799) heterozygosity in line of Beaver is in equilibrium, because fixation index is 0.004. The analysis demonstrated that the coefficient of fixation (Fis) was negative in the lines of Zeus (-0.066) and Fort (-0.080), which indicates a shift of genetic equilibrium in these groups towards the excess of heterozygotes.

Positive value indicates a lack of heterozygotes observed in the lines of Neon (0.086) and Windbreak (0.078). This proves the lack of heterozygosity due to frequent occurrence of inbreeding in these lines.

In determining the level of heterozygosity for the maintenance of genetic balance in the lines, the index of fixation is important. Since this index quantitatively reflects the deviation of frequencies of heterozygous genotypes from theoretically predicted by Hardy-Weinberg proportion of heterozygotes for random mating within a population, it can be considered as one of the criteria of inbred population.

Also, the level of heterozygosity in horses can be considered as an additional feature in the selection process.

3.3 Determination of polymorphism level of the main lines of Kostanay horse breed

The level of polymorphism is an indicator of effective application of alleles in this population; this figure is associated with alleles encountered in all investigated loci and shows equality of distribution of alleles.

Average figure of single locus of polymorphic frequency of alleles in DNA microsatellites is: in the line of Neon - 3.7, Fort - 3.0, Beaver - 3.6, Zeus - 3.7 and 3.5 in the line of Windbreak. If we take the level of polymorphism in percentage terms, then in the lines of Windbreak, Neon and Beaver it is 21%, in the line of Zeus - 20%, with the lowest percentage of 17% is in the line of Fort.

Of 17 microsatellite DNA loci in the main lines of Kostanay horse breed, the level of polymorphism of loci HTG7, HMS1 and HMS6 compared to other loci is lower, and the highest level of polymorphism is observed for locus ASB23.

The average level of polymorphism calculated per one locus for the entire sample under study ranged from 3 (Fort line) to 3.73 (Zeus line).

For 17 microsatellite DNA loci in the analysis of allele pool of the studied lines of Kostanay horse breed, reliable information on polymorphism rate of each marker (Table 2) was used.

Thus, a comparative evaluation of 17 microsatellite DNA loci polymorphism in the main lines of Kostanay horse breed showed that almost every line has its own distinctive genetic structure.

During the systematic, purposeful selection associated with the inheritance of valuable qualities by the descendants, this genetic structure can be used as a universal genetic marker keeping them in each line.

Table 2. Characteristics of polymorphism of microsatellite DNA loci in the studied livestock (n=75)

Loci	Level of polymorphism (Ae)	Level of heterozygosity		Fixation index (Fis)	Average number of alleles per locus (NV)
		expected (He)	observed (Ho)		
VHL20	3.51*	0.790*	0.868*	-0.112	4.294*
HTG4	2.88**	0.700**	0.765**	-0.135	2.945**
AHT4	3.27**	0.801*	0.878*	-0.097	3.804*
HMS7	3.52*	0.809*	0.820*	-0.017	3.681**
HTG6	4.26*	0.875*	0.842*	0.035	4.663*
AHT5	3.54*	0.821*	0.764**	0.059	3.803*
HMS6	2.28**	0.606**	0.645	-0.083	3.067**
ASB23	4.24*	0.881*	0.886*	-0.008	4.049*
ASB2	3.67*	0.842*	0.655**	0.227	4.049*
HTG10	5.79*	0.935*	0.968*	0.017	5.153*
HTG7	2.94**	0.742**	0.775**	-0.259	3.190**
HMS3	3.43**	0.822*	0.856*	-0.030	3.435**
HMS2	3.85*	0.841*	0.759**	0.088	3.681**
ASB17	3.52*	0.821*	0.827*	-0.015	3.558**
LEX3	3.71*	0.819*	0.779*	0.041	4.294*
HMS1	2.05**	0.416**	0.470**	-0.088	3.067**
CA425	2.91**	0.754**	0.635**	0.142	3.926*
Average	3.5	0.781	0.776	-0.014	3.803

* loci with above average result

* loci with below average result

3.4 Genetic polymorphism of the main lines of Kostanay horse breed

Use of polymorphism of microsatellite loci of genome enables precise calculation between heterozygosity and genetic distance of breeds and animal populations.

Due to a very high level of polymorphism, this method is a good tool for the analysis of inner and intermediate population variability and determination of genetic distance between groups of organism.

In Figure 4, the comparative analysis of alleles taken from the literature and from the results of research is given. In nearly all loci the number of alleles known from the literature and the number of alleles identified in the result of our research are the same.

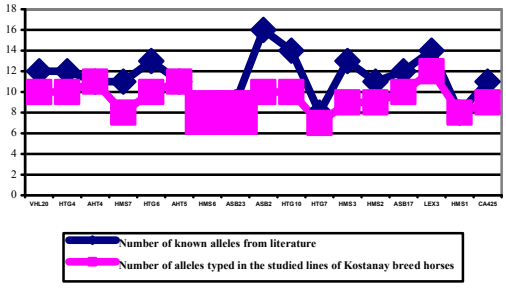

Figure 4 - Maximum number of identified alleles for 17 loci studied

In each of the 17 microsatellite loci studied, from 7 to 12 alleles were identified. The average level of polymorphism in all lines was 3.5 units. Accordingly, the loci were divided into two groups. The first group are the loci with the average level of polymorphic loci - VHL20, HMS7, HTG6, AHT5, ASB23, ASB2, HTG10, HMS2, ASB17 and LEX3.

In this group, the largest value of alleles per locus HTG10 was 5.79 units.

From the second level of polymorphism of the average figures, the group of lower loci was made up by loci HTG4, HMS6, HTG7, HMS1 and CA425, and the lowest value of effectively applied alleles in this group in locus HMS1 was 2.05 share units, and the other loci AHT4 and HMS3 refer to loci close to average figures of effectively applied alleles.

For 17 microsatellite DNA loci in the analysis of allele pool of the studied lines of Kostanay horse breed, reliable information on polymorphism rate of each marker (Table 3) was used.

Table 3 - Characteristics of polymorphism of microsatellite DNA loci in the studied livestock (n=75)

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* loci with above average result
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The average number of effective alleles per locus of the studied line and level of polymorphism are closely linked. Because the ratio of the level of polymorphism to the average number of alleles describes the equilibrium of alleles distribution. According to Figure 6, 92% of all alleles encountered in 17 microsatellite loci are effective alleles. The loci below this figure also include eight loci - VHL20, AHT4, HTG6, HMS6, ASB2, LEX3, HMS1 and CA425, and the lowest ratio figure in locus NMS1 is 66.8 percent. Low-percentage ratios describe inequality of alleles distribution. The remaining nine loci were not lower than the ratio of the average number of alleles to a single locus. These loci of alleles are special in uniform distribution of alleles.

For 17 loci of microsatellite DNA studied, the general observed (0.776) and expected (0.781) heterozygosity rates can be called similar. Also, when comparing individual loci, we can see the difference between them. It was identified that the lowest level of heterozygosity in locus HMS1, if the level of its expected heterozygosity was 0.416 share units, the level of controlled heterozygosity was 0.470. And the highest level of heterozygosity was observed in locus NTG10, as the level of expected heterozygosity was 0.935 share units, and the level of observed heterozygosity was 0.968 share units (Figure 5).

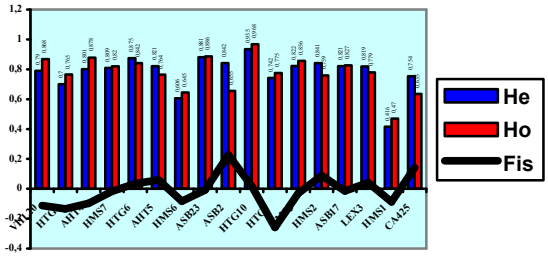

Figure 5 - Expected (He), observed (Ho) heterozygosity and fixation index (Fis) in 17 loci studied

The studied 17 microsatellite DNA loci of main lines of Kostanay horse breed in determining the prevalence level of expected and observed heterozygosity in loci HMS7 (-0.017), ASB23 (-0.008), ASB17 (-0.015) was close to zero, this means that the level of heterozygosity in the normal state.

During the research, the level of fixation in loci VHL20 (-0,112), HTG4 (-0,135), AHT4 (-0,097), HMS6 (-0,083), HTG7 (-0,259), HMS3 (-0,030) and HMS1 (-0,088) showed a negative value, which means that in these loci the level of heterozygosity was in excess. The reason for this is that in the selection of animals of these lines a genetic balance was not preserved, heterozygous genotypes were increased, and this may reduce the genetic quality of lines. During breeding, especially during selection, you need to pay attention to the genotype along with phenotype, exterior and other zootechnical properties.

Application of the fixation index in controlling the level of heterozygosity, preservation of genetic balance for 17 microsatellite DNA loci is critical in the control of breed heterozygosity. Calculated fixation index provides relationship between individuals of certain population and general population.

The use of microsatellite markers is effective with certain genetic structures of breeds, with some differentiation between the line and family of animals, with refinements in heterozygosity, control of inheritance of economically useful properties.

3.5 Effectiveness of DNA microsatellites in the control of origin of the main lines of Kostanay horse breed

One of the main applied values of DNA microsatellite in horse breeding is control of the origin of breeding animals. To date, the only

effective way to control reliability of identification of origin and genetic evaluation of horses is based on the method of genetic polymorphism.

The comparative analysis of loci conducted on the effectiveness of origin of horses in the line of Neon HMS2 (0,690 share units), in the line of Fort HMS6 (0,719), in line of Beaver AHT5 (0,628) and in line of Windbreak AHT4 (0,656) demonstrated a high loci effectiveness in controlling the origin of lines (Table 4)

Table 4. Efficiency of origin control of horses of Kostanay breed

Loci	General lines				
	Neon	Fort	Beaver	Zeus	Windbreak
	(n=28)	(n=18)	(n=15)	(n=6)	(n=8)
VHL20	0.402	0.473	0.590	0.570	0.600
HTG4	0.417	0.557	0.606	0.262	0.274
AHT4	0.437	0.335	0.383	0.464	**0.656**
HMS7	0.559	0.402	0.439	**0.573**	0.548
HTG6	0.516	0.369	0.491	0.484	0.520
AHT5	0.635	0.553	**0.628**	0.385	0.441
HMS6	0.532	**0.719**	0.311	0.431	0.612
ASB23	0.322	0.579	0.554	0.481	0.571
ASB2	0.493	0.590	0.421	0.527	0.559
HTG10	0.618	0.499	0.465	0.505	0.499
HTG7	0.593	0.544	0.348	0.381	0.624
HMS3	0.408	0.663	0.547	0.347	0.575
HMS2	**0.690**	0.355	0.455	0.565	0.488
ASB17	0.628	0.475	0.559	0.230	0.598
LEX3	0.599	0.621	0.613	0.368	0.295
HMS1	0.568	0.380	0.271	0.385	0.484
CA425	0.558	0.391	0.413	0.412	0.422
Average	0.99999	0.99999	0.99998	0.99987	0.99999

In general, when using 17 microsatellite DNA loci, effective origin control of the investigated Neon lines, Fort and Windbreak was 0.99999, or 99.999%, and the lines of Beaver - 99.998%. The most demanded control of origin is for the lines of Zeus. Its efficiency was 0.99987 or 99.987%.

4. Discussion

Use of polymorphism of microsatellite loci of genome enables precise calculation between heterozygosity and genetic distance of breeds and animal populations.

Due to a very high level of polymorphism, this method is a good tool for the analysis of inner and intermediate population variability and determination of genetic distance between groups of organism.

The use of microsatellite markers is effective with certain genetic structures of breeds, with some differentiation between the line and family of animals, with refinements in heterozygosity, control of inheritance of economically useful properties.

5. Summary & Conclusions

It was established that the main lines of Kostanay breed horses differ significantly by the presence and frequency of alleles of microsatellite loci, as each line of 17 microsatellite DNA loci has its own genetic structure.

Analysis of 17 microsatellite DNA loci of the main lines of Kostanay horse breed revealed a pronounced genetic differentiation between them.

In each of the 17 microsatellite loci studied, from 7 to 12 alleles were identified on average for all lines.

The average number of alleles (NV) averaged 3.8 - from 5.2 in locus NTG10 to 2.9 in locus NTG4, the number of effective alleles (Ae) - 3,5 - from 5.8 in NTG10 to 2.05 in locus NMS1.

A widest range of alleles (121 alleles for 17 loci) and the maximum number of "private" alleles (Pa = 10) were detected in the line of Neon Kostanay breed. For the remaining lines, allele pool included about 100 alleles, including several "private" alleles.

The observed (0.796) and expected (0.799) level of heterozygosity in line of Beaver is in equilibrium, because fixation index is 0.004. Also, the coefficient of fixation (Fis) was negative in the lines of Zeus (-0.066) and Fort (-0.080), which indicates a shift of genetic equilibrium in these groups towards the excess of heterozygotes. Positive value indicates a lack of heterozygotes observed in the lines of Neon (0.086) and Windbreak (0.078). This proves the lack of heterozygosity due to frequent occurrence of inbreeding in these lines.

Among the main lines of Kostanay horse breed, based on the genetic polymorphism, the highest level of polymorphism (Ae) was found in Beaver and Neon lines, the level of observed heterozygosity (Ho) - in the line of Zeus, and the level of expected heterozygosity (He) in the line of Windbreak, the number of "private" alleles (Pa) was specific to the line of Neon.

When controlling the origin of horses for 17 microsatellite DNA loci, a high degree of effectiveness of this control was identified in Neon lines, Fort and Windbreak - 0.99999 or 99.999%, in the line of Beaver - 99.998%. The most demanded origin control is for the line of Zeus. Its efficiency was 0.99987 or 99.987%.

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