Diversity of Kabardian horses and their genetic relationships with selected breeds in the Russian Federation based on 17 microsatellite loci

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Abstract. 17 standard microsatellite loci, used for horse identification and origin control, were deployed for studying the genetic structure of Kabardian breed. The observed (Ho) and expected (He) heterozygosity, level of polymorphism (Na), polymorphism information content (PIC), and fixation index (Fis) were calculated. The results of microsatellite analysis were used for description of the Kabardian breed at the population level. The distribution and frequency of microsatellite alleles was compared with Russian populations of Arab and Thoroughbred horses.

1. Introduction
The great interest in Kabardian breed is based by its exceptionally high adaptive qualities to the conditions in medium and high mountains as well as its perspective use in endurance (Fig. 1). The international studbook of Kabardian breed is conducted from the VNIIK in compliance with a centralized registration of this breed.

The genetic diversity of populations is primarily characterized by heterozygosity and existence of a great number of alleles. But it is difficult to maintain such diversity in the process of development and improvement of horse breeds and it can be a problem in small populations. Conservation of genetic diversity is one main goal of population work in farm animals because diversity is important for successful adaption to environmental changes and reactions on long-term selection (natural or artificial). Breed characterization on morphological and genetic level is the first step in development of selection strategy and priority setting for breed conservation.
Analysis of recent publications points out that microsatellites are a suitable marker system for assessing the genetic diversity of breeds [1-3] because they have a high frequency in the mammal genome, a high level of polymorphism, a codominant inheritance and an ease automation of analysis [4]. Microsatellites can be used for genetic characterization of breed structure, evaluation of the degree of "purity of blood", the study of genetic distances between populations and breeds, mating planning [5].

This study was conducted as part of an international project, which goal is complex research of the genome Kabardian breed. The project started in 2013 and was funded by the «VolkswagenStiftung» (Germany). It was planned through collaboration of Humboldt University Berlin, KBSU, Institute of Agriculture KBSC RAS and "VNIK" with active participation of the International Association of Kabardian breed (IKHA). As an important contribution and participation, scientists from the Institute of Radio Systems and Management SFU provide an opportunity to study the dynamics of physiological, biochemical and kinematic indicators in the online mode which allows already an analysis of the obtained data sets, promising to further identify potential genetic markers.

2. Materials and methods

2.1 Sampling and DNA extraction

Whole blood samples and hair bulbs were collected from 303 horses Kabardian breed from farms in Kabardino-Balkaria, of Krasnodar Region, Adygea, and Karachay-Cherkessia (Khabezsky District). Blood samples were collected in sterile 9 ml vacuum tubes containing anticoagulant (K3EDTA). The collection was carried out by qualified specialists. When taking materials, all paragraphs of article №10 of the federal law on protection animals from cruelty were strictly complied.

Genomic DNA was extracted from blood samples using standard method in biomedical KBSU center. DNA from hair bulbs was isolated using a set of «ExtraGene TM DNA Prep 200" (Laboratory Izogen, Russia).

2.2 Microsatellite analysis

Studies were carried out in the VNIK laboratory of genetics. 17 microsatellite loci included in the reference list of the International Society of Animal Genetics (ISAG) for individual identification and paternity verification (Table 1). DNA amplification was performed by PCR using a multiplex horse primer set StockMarks®. For electrophoretic separation, a 4-channel automatic analyzer ABI 3130 (Applied Biosystems, United states) was used after manufacturers instruction [6, 7].

Number of genotypes, allele frequencies, number of alleles per locus (NV), polymorphic information content (PIC) [17], fixation index (Fis) [18], level of polymorphism (Na) [19], observed (Ho) and
expected (He) heterozygosity [20] were determined using software Microsoft Excell, Statistica (StatSoft) and original electronic database «Kabardian horse base».

3. Results and discussion

3.1 Estimation of genetic diversity

The characterization of the microsatellite allele pool of Kabardian breed was the first result (n = 303). Basic data obtained from population genetic analysis are shown in Table 2. A total of 165 different alleles were obtained from the analyzed 17 polymorphic loci with an average allele number per locus of 9.7 ranging from 6 for locus HTG7 up to 15 for ASB17. Most loci had a PIC values higher than the desired value of 0.6 indicating a high degree of information of these markers in the assessment of genetic diversity.

Allelic distribution of studied microsatellite loci revealed that the breed is characterized by high number of effective alleles (Na: 5.2) representing a extensive genetic diversity. Genetic diversity plays an important role in the field of adaptive capacity of a population [23]. The Fixation index Fis being main criterion for inbreeding calculation only demonstrate in two loci (HTG4 with 0.039 and HTG6 with 0.059) a deficit of heterozygous genotypes compared with the theoretical possibility. This is explained by the small number of effective alleles.

Table 1. Names of used microsatellites and their references

| Locus  | Chromosome number | Repeat motif | References | Fragment length (bp) | N* | GenBank  |
|--------|-------------------|--------------|------------|----------------------|----|----------|
| AH74   | 24                | (AC)nAT(AC)n| Binns et al. (1995) | 146-170 | 10 | Y07733   |
| AH5    | 8                 | (GT)n       | Binns et al. (1995) | 118-130 | 6  | Y07732   |
| ASB2   | 15                | (GT)n       | Breen et al. (1997) | 240-270 | 14 | X93516   |
| ASB17  | 2                 | (AC)n       | Breen et al. (1997) | 87-129 | 11 | X93531   |
| ASB23  | 3                 | (TG)n и (TG)nТТ(TG)n | Irvin et al. (1998) | 175-211 | 12 | Y93537   |
| CA425  | 28                | (GT)n       | Eggleston-Scott et al. (1997) | 226-246 | 6  | U67406   |
| HMS1   | 15                | (TG)n       | Guerin et al. (1994) | 170-186 | 5  | X74630   |
| HMS2   | 10                | (CA)n(TC)n | Guerin et al. (1994) | 218-238 | 12 | X74631   |
| HMS3   | 9                 | (TG)n(СА)nTC(СА)n и (TG)n(TC)n(TG)n | Guerin et al. (1994) | 149-172 | 10 | X74632   |
| HMS6   | 4                 | (GT)n       | Guerin et al. (1994) | 157-171 | 10 | X74635   |
| HMS7   | 1                 | (AC)n(СА)n | Guerin et al. (1994) | 165-185 | 8  | X74636   |
| HTG4   | 9                 | (TG)nAT(AG)nAAG(GA) и ACAG(AAGG)n | Ellegren et al. (1992) | 120-140 | 6  | A169165  |
| HTG6   | 15                | (TG)n       | Ellegren et al. (1992) | 80-107 | 6  | A169167  |
| HTG7   | 4                 | (GT)n       | Marklund et al. (1994) | 118-130 | 4  | A169291  |
| HTG10  | 21                | (TG)n и ТАТС(TG)n | Marklund et al. (1994) | 92-118 | 7  | A169294  |
| LEX3   | X                 | (TG)n       | Coogle et al. (1996) | 142-164 | 5  | A075607  |
| VHL20  | 30                | (TG)n       | Van Haeringen et al. (1994) | 85-107 | 10 | X75970   |

* N – number of alleles [8-16].

PIC value for all loci was evaluated according to the allele frequency (average value of PIC = 0.754) ranging from 0.553 to 0.874 for HTG7 and ASB17 respectively. Most loci had a PIC values higher than the desired value of 0.6 [22] indicating a high degree of information of these markers in the assessment of genetic diversity.

The Fixation index Fis being main criterion for inbreeding calculation only demonstrate in two loci (HTG4 with 0.039 and HTG6 with 0.059) a deficit of heterozygous genotypes compared with the theoretical possibility. This is explained by the small number of effective alleles.
Analyzing the allele pool, seven private alleles were found which are not described for any foreign population (AHT4 T, ASB23 N, ASB23 Q, AHT5 Q, HMS1 R, HMS2 Q, HTG6 K from 19 breeds). Among Russian populations, Kabardian breed has the widest range of microsatellite alleles (n = 165). High level of microsatellite polymorphism indicates wide genetic background of the breed.

**Table 2. Number of alleles and genotypes, Na, He, Ho, PIC and Fis values for all analyzed 17 microsatellites**

| Loci  | Number of genotypes | NV | Na   | He   | Ho   | PIC   | Fis    |
|-------|---------------------|----|------|------|------|-------|--------|
| AHT4  | 48                  | 12 | 6.043| 0.835| 0.827| 0.815 | -0.009 |
| AHT5  | 24                  | 8  | 4.566| 0.781| 0.749| 0.750 | -0.043 |
| ASB17 | 75                  | 15 | 8.647| 0.884| 0.830| 0.874 | -0.065 |
| ASB2  | 46                  | 11 | 6.663| 0.850| 0.790| 0.833 | -0.076 |
| ASB23 | 41                  | 12 | 5.437| 0.816| 0.774| 0.791 | -0.055 |
| CA425 | 41                  | 12 | 4.723| 0.788| 0.502| 0.763 | -0.571 |
| HMS1  | 20                  | 8  | 3.189| 0.686| 0.640| 0.632 | -0.072 |
| HMS2  | 34                  | 10 | 5.075| 0.803| 0.729| 0.777 | -0.101 |
| HMS3  | 26                  | 7  | 5.067| 0.803| 0.726| 0.776 | -0.106 |
| HMS6  | 23                  | 7  | 4.621| 0.784| 0.754| 0.753 | -0.039 |
| HMS7  | 22                  | 7  | 4.898| 0.796| 0.737| 0.768 | -0.080 |
| HTG10 | 51                  | 12 | 6.136| 0.837| 0.810| 0.819 | -0.034 |
| HTG4  | 18                  | 7  | 2.622| 0.619| 0.644| 0.583 | 0.039  |
| HTG6  | 21                  | 9  | 3.337| 0.700| 0.744| 0.647 | 0.059  |
| HTG7  | 14                  | 6  | 2.583| 0.613| 0.612| 0.553 | -0.001 |
| LEX3  | 46                  | 13 | 7.939| 0.874| 0.542| 0.861 | -0.614 |
| VHL20 | 37                  | 9  | 6.442| 0.845| 0.796| 0.826 | -0.061 |
| mean value | 34.53 | 9.706 | 5.176 | 0.783 | 0.718 | 0.7542 | -0.108 |

**3.2 Genetic distance**

A comparison of the specific distribution of microsatellite alleles can help to find genetic differences to other breed [24, 6]. In this case, the breed improvers (Arabian and Thoroughbred) were chosen. A graphic distribution image of the alleles from 15 microsatellite loci is shown in Figure 2 (not included CA425 and LEX3).
Figure 2. Allele distribution from 15 microsatellite loci in Kabardian (n=303), Arabian (n=1662) and Thoroughbred (n=2795) horses.

Radar charts show that the distribution of microsatellite alleles differ for these three breeds. Some common alleles with high frequency can be found in all three breeds (e.g. HTG7 allele O) while a variety of markers demonstrates significant differences in the genetic structure. Great differences in allele frequencies between the Kabardian, Arabian and Thoroughbred horses were found in the distribution of alleles for loci HMS6 (Figure 3a). Allele P with a frequency of 0.718 is the most common in Arabian breeds while it has a frequency of only 0.329 in Kabardian. The allele O with a frequency of 0.568 in thoroughbred exists in Kabardian with the frequency of 0.139. Also in locus HTG6, the most typical allele in Kabardian is O with a frequency of 0.402 whereas its frequency in Arabian and Thoroughbred is 0.204 and 0.127 respectively (Figure 3b). On other hand, the allele I of this locus only exists in Kabardian horses with a frequency of 0.038.
4. Conclusions

The number of alleles is a direct indicator of genetic diversity [25] and obtained results can be stated a high degree of genetic diversity in the investigated Kabardian population compared to previously studied samples of other Russian and foreign breeds. The mean PIC is closely to that from Zaniskari pony (PIC = 0.732) [26] and slightly less than for Hucul and Kiso pony with values of 0.6661 [27] and 0.619 [28], respectively.

Summing up, we can conclude a pronounced genetic differentiation between the breeds of Kabardian, Arabian and Thoroughbred. The last both are breed improvers and were used in different periods to increase growth, improving exterior and performance of the Kabardian horses. Nevertheless, we can say that the Kabardian breed was slightly undergone cast-blood breed improvers, preserving their genetic identity. Beside many respects, that originated in the unique climatic and environmental conditions of the breeding region of the Kabardian horse selecting a special horse type. But we can’t ignore that selection work at all stages was not carried out the mass of unsystematic mating, and was by individual, rational and limited "foreign type" Thoroughbred and Arabian stallions.

The genotyping of 17 microsatellites is an effective method for the study of genetic and population characteristics of breeds and their certification. However, an effective research of phylogenetic relationships and microevolution is limited by small number of described specific alleles, insufficient population studies and lack of allele information from other breeds as well as knowledge about the allele frequencies in the time of formation of the diverse breeds.

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