ОШ МАМЛЕКЕТТИК УНИВЕРСИТЕТИНИН ЖАРЧЫСЫ. АЙЫЛ ЧАРБА: АГРОНОМИЯ, ВЕТЕРИНАРИЯ ЖАНА ЗООТЕХНИЯ

ВЕСТНИК ОШСКОГО ГОСУДАРСТВЕННОГО УНИВЕРСИТЕТА. СЕЛЬСКОЕ ХОЗЯЙСТВО: АГРОНОМИЯ, ВЕТЕРИНАРИЯ И ЗООТЕХНИЯ

JOURNAL OF OSH STATE UNIVERSITY. AGRICULTURE: AGRONOMY, VETERINARY AND ZOOTECHNICS

e-ISSN: 1694-8696
№2(11)/2025, 44-60

ЗООТЕХНИЯ

УДК: 636.1.082.251(575.2)(04)
DOI: 10.52754/16948696_2025_2(11)_4

THE MORPHOLOGICAL AND GENETIC CHARACTERISTICS OF KYRGYZ HORSES

МОРФОЛОГИЧЕСКИЕ И ГЕНЕТИЧЕСКИЕ ХАРАКТЕРИСТИКИ КЫРГЫЗСКИХ ЛОШАДЕЙ

КЫРГЫЗ АТТАРЫНЫН МОРФОЛОГИЯЛЫК ЖАНА ГЕНЕТИКАЛЫК МҮНӨЗДӨМӨЛӨРҮ

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THE MORPHOLOGICAL AND GENETIC CHARACTERISTICS OF KYRGYZ HORSES

Abstract

The relevance of this study lies in the necessity to preserve and selectively improve the indigenous Kyrgyz horse – a rare and unique breed group adapted to the extreme conditions of high-mountain environments. The genetic characteristics of this population have previously been studied to a very limited extent, which hinders effective management of its gene pool. The purpose of this study was to determine the intrapopulation genetic structure of Kyrgyz horses from the north-eastern region of the Kyrgyz Republic using single nucleotide polymorphisms (SNPs) and morphological traits. To achieve this, SNP genotyping was carried out using the Equine80k array (Illumina), alongside morphometric assessment of exterior traits, cluster and correlation analyses (UPGMA, PCoA, Dice, Mantel tests), and binary coding of phenotypic data. According to SNP analysis, the population was divided into two genetic clusters: the first comprised 22 horses with a genetically homogeneous structure, while the second included 3 individuals (KIR8, KIR18, KIR20) displaying higher genetic distance. Based on morphological traits, two clusters were also identified: the first group consisted predominantly of dark-coated horses (92%) with body measurements below average breed standards (up to 136 cm at the withers in 83% of cases); the second group comprised light-coloured horses (chestnut, grey, buckskin – 83%) with more developed conformation features (height at withers >136 cm, chest girth >158 cm – 92%, cannon bone girth >18 cm – 100%). However, results of the variation analysis and Mantel test (r = -0.07396; p = 0.0499) indicated no statistically significant correlation between morphological traits and SNP data, suggesting that traditional exterior features are independent of the molecular markers used. The practical significance of this study lies in establishing a foundation for future selective breeding and DNA-based certification of Kyrgyz horses, and in the development of scientifically grounded programmes for conserving native populations under mountain livestock systems

Keywords: conformation; SNP genotyping; markers; polymorphism; statistical analysis; cluster analysis

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Морфологические и генетические характеристики кыргызских лошадей

Аннотация

Актуальность исследования обусловлена необходимостью сохранения и селекционного совершенствования аборигенной кыргызской лошади – редкой и уникальной породной группы, приспособленной к экстремальным условиям высокогорья. При этом генетические характеристики данной популяции ранее были изучены крайне ограниченно, что затрудняет эффективное управление её генофондом. Целью настоящей работы было установление внутрипопуляционной генетической структуры кыргызских лошадей северо-восточного региона Кыргызской Республики с использованием однонуклеотидных полиморфизмов (SNP) и морфологических показателей. Для достижения цели использовались методы SNP-генотипирования с применением массива Equine80k (Illumina), морфометрические измерения экстерьера, кластерный и корреляционный анализы (UPGMA, PCoA, Dice, Mantel), а также бинарное кодирование фенотипических данных. По данным SNP-анализа вся выборка была разделена на два кластера: в первом – 22 лошади с генетически однородной структурой, во втором – 3 особи (KIR8, KIR18, KIR20) с повышенной генетической дистанцией. По морфологическим признакам также выделены два кластера: первый включал особей преимущественно

Кыргыз аттарынын морфологиялык жана генетикалык мүнөздөмөлөрү

Аннотация

Изилдөөнүн актуалдуулугу – кыргыздын жергиликтүү аттарын сактоо жана селекциялык жакшыртуу зарылчылыгынан келип чыгат. Бул аттар – сейрек жана уникалдуу тукумдук топ болуп, тоо тектүү катаал шарттарга ылайыкташкан. Бирок бул популяциянын генетикалык өзгөчөлүктөрү мурунку изилдөөлөрдө абдан чектелип каралган, бул анын генофондун эффективдүү башкарылышын кыйындатат. Учурдагы изилдөөнүн максаты – Кыргыз Республикасынын түндүк-чыгыш аймагындагы кыргыз аттарынын популяция ичиндеги генетикалык структурасын бир нуклеотиддик полиморфизмдер (SNP) жана морфологиялык көрсөткүчтөр аркылуу аныктоо. Максатка жетүү үчүн Equine80k (Illumina) массивин колдонуу менен SNP-генотипирлөө ыкмалары, экстерьерди морфометриялык өлчөө, кластердик жана корреляциялык анализдер (UPGMA, PCoA, Dice, Mantel), ошондой эле фенотиптик маалыматтарды бинардык коддоо колдонулду. SNP-аналитика боюнча толук тандоо эки кластерге бөлүндү: биринчи кластерде – 22 аттар, генетикалык жактан бирдей структурага ээ, экинчи кластерде – 3 жаныбар (KIR8, KIR18, KIR20), генетикалык аралык жогору. Морфологиялык белгилер боюнча да эки кластер аныкталды: биринчи кластерде негизинен кара түстөгү аттар (92 %) жана орто стандарттардан төмөн дене түзүлүшү (83 % үчүн холка бийиктиги 136 тёмных мастей (92 %) с телосложением ниже средних стандартов (до 136 см в холке у 83 %), второй – особей светлых мастей (рыжая, серая, игреневая, 83 %) с развитыми экстерьерными показателями (высота в холке >136 см, грудь >158 см – у 92 %, пясть >18 см – у 100 %). Однако результаты вариационного анализа и теста Манте́ля (r = -0,07396; p = 0,0499) показали отсутствие статистически значимой связи между морфологическими признаками и SNP-данными. Это свидетельствует о независимости традиционных экстерьерных характеристик от используемых молекулярных маркеров. Практическое значение исследования заключается в формировании базы для будущей селекционной работы и DNA-паспортизации кыргызских лошадей, а также в разработке генетически обоснованных программ по сохранению аборигенных популяций в условиях горного животноводства

см чейин), экинчи кластерде – жеңил түстөгү аттар (чоң боро, күрөң, игрене, 83 %) жана өнүккөн экстерьердик көрсөткүчтөр (холка бийиктиги >136 см, көкүрөк >158 см – 92 %, пясть >18 см – 100 %). Бирок вариациялык анализдин жана Мантел тестинин (r = -0,07396; p = 0,0499) жыйынтыктары морфологиялык белгилер менен SNP-даноонун ортосунда статистикалык маанилүү байланыш жоктугун көрсөткөн. Бул традициялык экстерьердик өзгөчөлүктөрдүн колдонулган молекулярдык маркерлерден көз карандысыз экенин билдирет. Изилдөөнүн практикалык мааниси – кыргыз аттарынын келечектеги селекциялык иштери үчүн база түзүүдө, DNA-паспортторун жасоодо жана тоо шартындагы мал чарбачылыгында жергиликтүү популяцияларды сактоо боюнча генетикалык негизделген программаларды иштеп чыгууда.

Ключевые слова: экстерьер; SNP-генотипирование; маркеры; полиморфизм; статистический анализ; кластерный анализ

Ачкыч сөздөр: экстерьер; SNP-генотипирлөө; маркерлер; полиморфизм; статистикалык анализ; кластердик анализ

Introduction

The development of highland animal husbandry is impossible without sustainable, ecologically adapted breeds capable of ensuring productivity in extreme climatic conditions. In the Kyrgyz Republic, where more than 75% of the territory is occupied by mountains, the indigenous Kyrgyz horse is a strategically important genetic resource. This population was formed under the influence of natural selection, herd maintenance, and folk breeding. However, in recent decades, the increased introduction of improved breeds, spontaneous interbreeding, and the weakening of breeding control pose a threat of genetic erosion. Under these conditions, the importance of molecular genetic approaches is particularly increasing, which help to objectively assess the intrapopulation structure, identify unique lineages, and lay the foundation for the targeted conservation and reproduction of the indigenous gene pool.

In the Republic of Kyrgyzstan, horses of their own selection, well adapted to local natural and climatic conditions, are traditionally bred. The direction of horse breeding in the republic is mainly productive, with a herd-based method of maintenance. To a lesser extent – sports, working horses, and horse breeding. Horse breeding is aimed at meeting the needs of farmers in draft power and horseback riding, the production of horse meat and koumiss, and equestrian games. The main body of horse stock consists of the local Kyrgyz improved horse, its crossbreeds, the Novokyrgyz breed and a small group of indigenous Kyrgyz horses. According to S.D. Omurzakov (2011) there are 6 main breeds of horses bred in the republic: Novokyrgyz, which occupies 55% of the total population (195-200 thousand units); the second largest – local Kyrgyz improved – 40% (150-200 thousand units); then trotting horses – 3-4% (10-12 thousand units); representatives of the Don breed – 1-1.5% (3-3.5 thousand units); English thoroughbred horse breed – 0.5-1% (1 thousand units) and crossbreeds of different breeds – 1%.

Since the early 2020s, several significant studies have been conducted to assess the genetic diversity of traditional horse breeds using high-density SNP chips. The study by A. Pozharskiy et al. (2023) conducted the first GWAS (Genome-Wide Association Studies) analysis and SNP typing of indigenous Kazakh horses, identifying 60 significant polymorphisms associated with body size and weight, which highlights the high breeding value of such populations. The researchers noted a weak intra-breed structure, which was also observed in the populations of Kyrgyz horses, despite pronounced external differences.

Similar work was also carried out in the neighbouring regions of Central Asia. Thus, D. Kabylbekova et al. (2023) performed a molecular genetic evaluation of Kazakh horses of the Zhab and Adai types using SNP analyses of the LCORL and PRKAG3 loci associated with meat productivity. The researchers revealed a high level of homozygosity in the studied groups and limited polymorphism, especially in horses from the Zhetysu and Mangystau regions. Despite this, it has been found that certain genotypes correlate with muscle development, chest volume, and growth rate, which confirms the possibility of using these SNP markers as tools for the genetic assessment of productive qualities in traditional horse breeding. However, the internal structure and degree of divergence between regional groups have not been sufficiently disclosed, which underscores the need for broader genomic research involving high-density SNP chips.

Reproductive traits play an important role in the development of effective breeding programmes. The study by M.D. Gomez et al. (2020), based on an analysis of more than 696,000 pedigree records of eight Spanish horse populations, has shown that the age of the first foaling, the intervals between foaling, and the duration of reproductive life have a moderate to high heritability (up to 0.42 in mares and 0.28 in stallions). Particularly high values were recorded for sports breeds. The data obtained confirmed the importance of including these traits in genetic selection models, which is also relevant for traditional breeds in need of sustainable breeding strategies.

The role of the mutation in the DMRT3 gene, also known as the Gait Keeper mutation, was examined in detail in the study by T. Kristjansson et al. (2014), where it was found to be directly related to the quality and types of gaits in Icelandic horses. The AA genotype enhanced lateral gaits, while carriers of the CA genotype showed better performance in diagonal gaits (trot, gallop). These data highlight the influence of individual genes on movement coordination and exterior characteristics, but it should be emphasised that systematic studies of similar mutations in Central Asian indigenous breeds are still insufficient.

E.T. Todd et al. (2020) identified potential lethal haplotypes in the breed of purebred riding horses, focusing on the consequences of inbreeding and breeding without considering the genetic stability of populations. It was found that mutations in the LY49B gene are associated with foetal mortality, despite the high frequency of heterozygotes. This highlights the importance of including genetic monitoring in breeding programmes, especially when working with confined or native populations where latent lethal alleles can negatively affect abundance and reproduction.

Thus, the current scientific situation requires an integrated approach to the study of the gene pool of the Kyrgyz aboriginal horse using modern molecular genetic methods and parallel analysis of exterior features. The purpose of this study was to identify the intrapopulation genetic structure of Kyrgyz horses in the north-eastern region based on high-density SNP typing using an Equine80k microchip, and to analyse the morphometric characteristics of individuals.

Materials and Methods

Location and dates of research. The field stage of sampling and morphometric measurements was conducted in June 2024 at the Laboratory of Animal Genetics of the National Academy of Sciences of the Kyrgyz Republic (Bishkek) with a trip to the mountainous upper valley of the Tonsky district of the Issyk Kul region. Laboratory processing and data analysis were performed in the period from July to October 2024.

Objects of research and ethics. The study involved 25 animals of indigenous Kyrgyz horse breeds from private farms in the villages of Temir-Kanat (6 units), Kok-Sai (5), Zher-Uy (4), Tuura-Suu (5), and Toguz-Bulak (5). The animals were identified using a unique marking code with the prefix KIR (Kyrgyz breed). All procedures complied with the European Convention for the Protection of Vertebral Animals Used for Scientific Purposes (ETS No. 123, 1986) and the recommendations of the World Organisation for Animal Health (OIE) (2021) on bioethics. Biomaterial sampling was carried out painlessly, without sedation, and did not cause stress in the animals.

Sampling and morphometry. For genetic analysis, hair follicles were selected by DNA genotyping: at least 20 hairs with roots from the withers, manually. The samples were stored in paper envelopes at a temperature of +4°C until DNA isolation. The selection was carried out according to the requirements of ISAG (International Society for Animal Genetics, 2017). External measurements in horses were carried out before sampling according to generally accepted zootechnical standards using measuring tape and compasses. The main external indicators included height at the withers (1), oblique body length (2), chest circumference (3), and metacarpal circumference (4) (Fig. 1).

Figure 1. Main indicators of the exterior

Source: created by the authors

SNP genotyping. It was performed using a set of Equine 80k HTS biochips and reagents manufactured by Illumina Inc. (USA) using the iScan genetic system for 145 SNP markers recommended by the International Society of Animal Genetics (ISAG) and approved by the Eurasian Economic Commission, Collegium (2020) (Isakova et al., 2018; 2021).

Data processing and analysis. The allelic data obtained during SNP genotyping, and the external morphological features of Kyrgyz horses, were encoded in binary form: the value “1” corresponded to the presence or severity of the trait, and “0” – to its absence. This encoding method allowed unifying heterogeneous data for statistical and cluster analyses. The following methods were used to identify the population structure and assess the degree of correspondence between molecular and phenotypic traits:

- The UPGMA (Unweighted Pair Group Method with Arithmetic Mean) method, implemented through the SAHN algorithm, was used to build dendrograms and identify clusters based on the level of genetic similarity between individuals. The method is widely used in population genetics due to its simplicity of interpretation and visual visualisation of groupings.

- The Dice coefficient (Dice, 1945) was used to calculate the degree of similarity of binary matrices, which is especially important when comparing data of different origins (morphological and genetic). It allows objectively assessing the overlap of features between two objects.

- Principal Coordinates Analysis (PCoA) was applied to visualise the intrapopulation structure based on the distance matrix. This method helped to reflect the genetic differences between individuals in the form of a two- or three-dimensional scheme, which is convenient for subsequent interpretative analysis.

- The Mantel test (1967) was used to determine the degree of statistical dependence between two distance matrices based on morphological and SNP data. This helped to determine whether there is a correlation between phenotypic and genetic diversity in the study population.

All data processing and mathematical calculations were performed in the NTSYSpc software, version 2.2 (Rohlf, 1998). To assess the reliability of the obtained clusters, bootstrap analysis with

1,000 repeated samples was used, implemented in the FreeTree software suite (Pavlíček et al., 1999). Visualisation of dendrograms was performed using the TreeView programme (Page, 1996).

Results

The study analysed the population of indigenous Kyrgyz horses, distributed mainly in the mountainous regions of Naryn, Issyk-Kul, and Osh regions. The population is estimated at about 50 thousand animal units. Comprehensive studies of phenotypic and genotypic characteristics conducted by scientists of the National Academy of Sciences of the Kyrgyz Republic have allowed identifying the Kyrgyz horse as a separate breed group. This paper presents the results of the phenotypic and molecular analysis of this population.

DNA polymorphism is so great that any two organisms, no matter what kind, reproducing sexually, will always carry differences in their DNA, which can be identified in one way or another. Analysing a large number of SNPs at once allows determining a population feature and provides more valuable information, including the genealogy of individuals and their traits that may be useful in breeding.

To identify potential SNPs related to colour and exterior characteristics, horses were sampled and grouped according to the main morphological characteristics – colour, height at the withers, oblique body length, chest circumference, and pastern circumference. According to these indicators, the horses were divided into several groups, as shown in Table 1. This distribution allowed identifying variability within the population in key external characteristics.

Table 1. Grouping by morphological characteristics of the horse phenotype

No.	Morphological indicators	Groups	Number of animals
1	Coat (5 groups)	chestnut	10
		black	4
		grey	7
		red	3
		liver-chestnut	1
2	Height at the withers (4 groups)	up to 135cm	4
		from 135 to 136cm	4
		from 136 to 139cm	4
		over 139cm	5
3	Oblique body length (3 groups)	up to 142cm	11
		from 142 to 145cm	7
		over 145cm	7
4	Chest circumference (3 groups)	up to 158cm	9
		from 158 to 165cm	8
		over 165cm	8
5	Metacarpal girth (3 groups)	up to 17.5cm	6
		18cm	13
		18.5cm	6

Source: compiled by the authors

An analysis of the data in Table 1 shows that within the studied population of Kyrgyz horses there is significant variability in key morphological features. By colour, the most numerous group is the chestnut (10 units), followed by the grey (7) and black (4) coats, which reflects the diversity of coat colour in the population. The distribution of height at the withers, oblique body length, chest circumference, and metacarpal circumference also demonstrates heterogeneity: the number of individuals is almost evenly distributed among the selected groups, indicating the presence of diversity in exterior parameters. This indicates the preservation of phenotypic diversity within the population, which is an important factor for maintaining its adaptive and breeding qualities. Thus, grouping by morphological features helped to identify several morphological subgroups within the population, which was used to compare with genetic data and identify potential SNPs associated with external characteristics.

Genotyping and analysis of the genetic structure. A two-level cluster analysis, biplot analysis, was used to visually display information about both samples and variables of the Kyrgyz horse data matrix (Fig. 2).

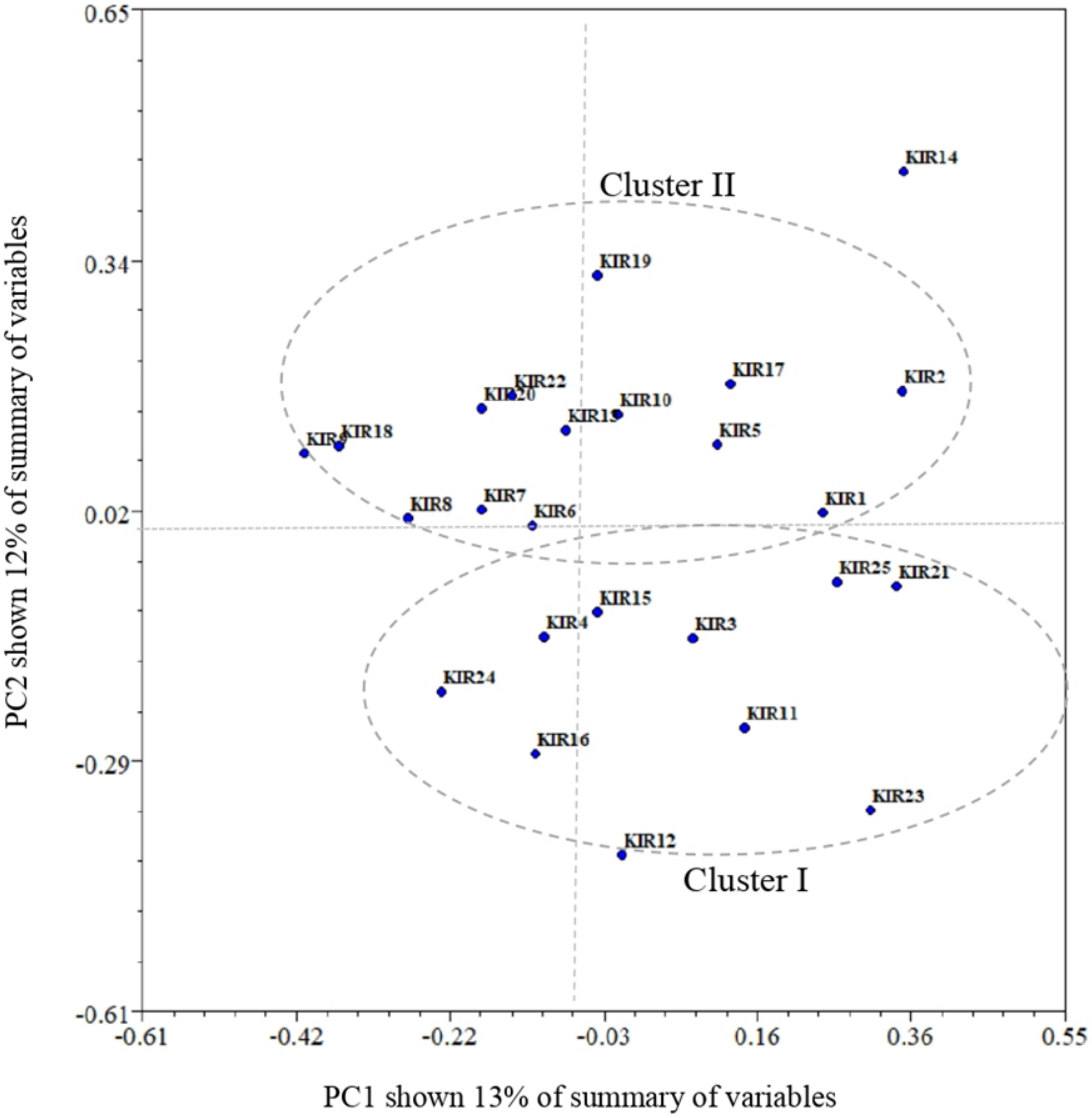

Figure 2. Biplot (principle of main coordinates)

Source: compiled by the authors

The figure shows a biplot based on SNP data, which displays the distribution of 25 Kyrgyz horses in a two-dimensional space defined by two main coordinates (PC1 and PC2). The first main coordinate (PC1) explains 13% of the variation, and the second (PC2) explains 12% of the variation in the overall variability of the features. The analysis showed the division of the studied population into two main clusters: Cluster 1 and Cluster 2. The Cluster 1 contains 22 individuals united by close genetic similarity, and the Cluster 2 includes 3 animals that differ from the main group.

This separation may be conditioned by differences in the allelic frequencies of SNP markers, reflecting the genetic structure and variability within the population. The division into two clusters indicates the presence of genetic differentiation, which may occur due to geographical isolation, breeding processes, or historical genetic differences between subgroups of horses. The reasons for the differences between clusters can be both phenotypic features (for example, colour or exterior parameters) and genetic variations due to hereditary factors. Given that PC1 and PC2 together account for about 25% of the total variation, most of the genetic diversity remains outside the two-dimensional representation, which is typical for complex biological systems with many features.

This division is important for understanding the intrapopulation structure and can be used for further analysis of associations between genotype and phenotype, and for optimising breeding work and preserving the genetic diversity of Kyrgyz horses. To visualise the genetic differentiation of the studied horses based on SNP marker data, a cluster tree was constructed using the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) method based on the standard Nei distance. The reliability of clustering was assessed using bootstrap analysis. The results are shown in Figure 3.

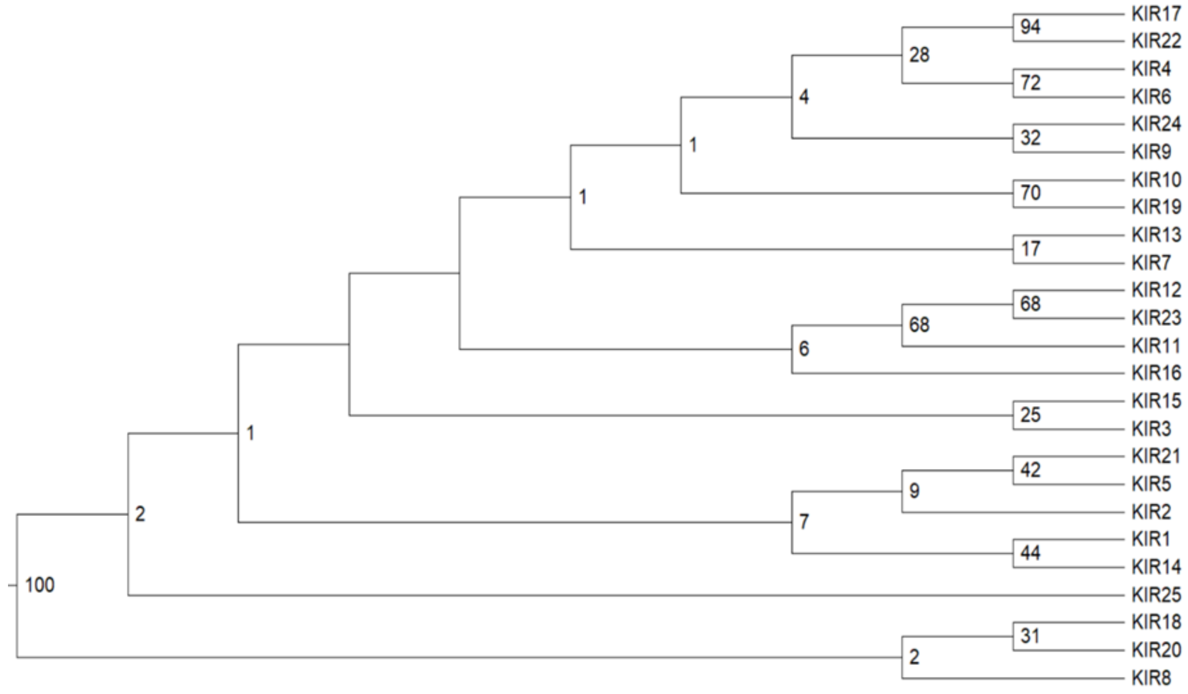

Figure 3. UPGMA of the standard Nei distance according to SNP markers

Source: created by the authors

The dendrogram (Fig. 3) shows that the studied population of Kyrgyz horses was divided into two main clusters. The numbers indicated between the branches are bootstrap values reflecting the reliability of the established clusters. The higher this value, the more stable the cluster is when generating samples multiple times using the Monte Carlo method. The analysis shows that most individuals form stable and genetically homogeneous subgroups, which indicates the presence of a certain intrapopulation structure. This is especially evident in the cluster combining samples KIR17, KIR22, KIR4, KIR6, and KIR24, which has high bootstrap values (94 and 72), which indicates a high reliability of their genetic proximity.

The first main group is of interest, in which the KIR8, KIR18, and KIR20 samples form a separate branch. Despite the relatively low bootstrap scores (maximum 31), their association into one cluster may indicate the presence of a common genetic feature that distinguishes them from other groups. To compare the results of the genetic analysis with the external features, clustering based on morphometric data was performed, based on the Nei and Li/Dice similarity coefficient. The reliability of clustering was also assessed by bootstrap analysis. The results are shown in Figure 4.

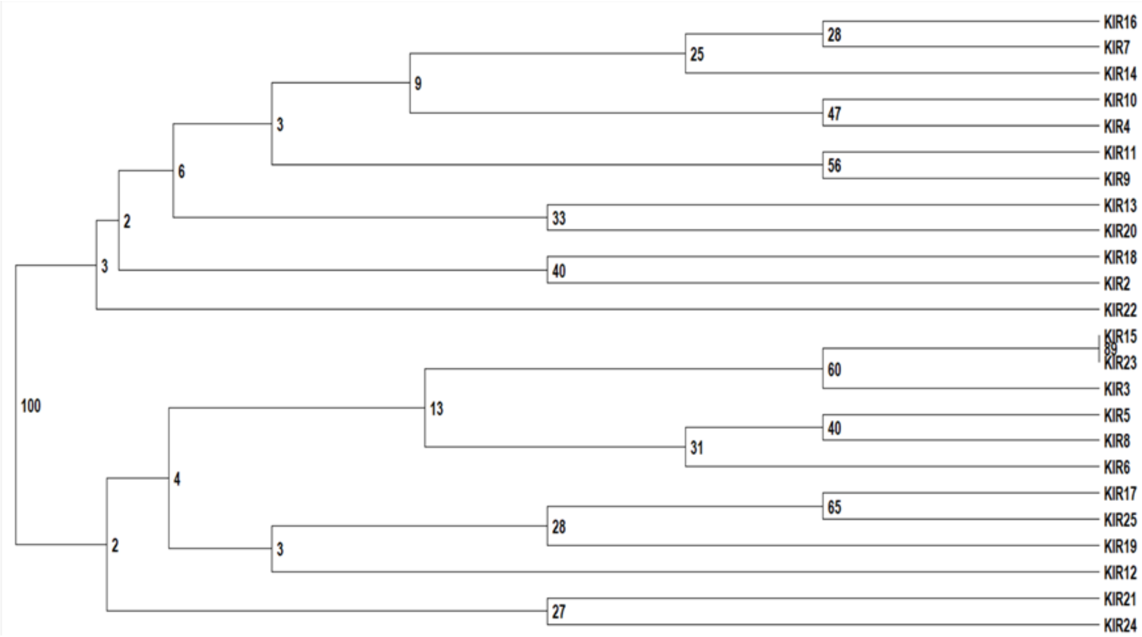

Figure 4. The dendrogram is constructed according to morphological features, the coefficient of Nei and Li/Dice

Source: created by the authors

According to the results of cluster analysis (Fig. 4), a group of Kyrgyz horses in the amount of 25 units was divided into two main clusters. The numbers located between the branches reflect bootstrap values indicating the degree of reliability of combining individuals into the corresponding subgroups. The Cluster 1 includes 12 animals with similarities in a number of morphological features. The majority of animals in this group have dark coats (chestnut and black colours) – 92% (11 animals). According to the main measurements of the exterior: height at the withers up to 136 cm was observed in 83% (10 animals), oblique body length up to 145 cm – in 83% (10 animals), chest circumference up to 165 cm – in 92% (11 animals), and metacarpal circumference up to 18 cm – in 75% (9 animals). Thus, the first cluster unites individuals with a relatively compact physique while maintaining breed standards, as well as predominantly dark colour.

The Cluster 2 includes the remaining 13 animals, which are characterised by opposite characteristics. 83% of the animals (10 animals) have a predominant light colour (red, grey, liver-chestnut). Morphometric data also show differences: height at the withers exceeds 136 cm, oblique body length exceeds 145 cm, chest circumference exceeds 165 cm – these signs are characteristic of 92% (11 animals); metacarpal circumference of more than 18 cm is observed in all 12 horses in the second cluster (100%). These data indicate that the second cluster contains animals with a more massive and elongated physique, exceeding the standard breed indicators, and a light colour.

The marked differentiation of morphological features (Fig. 3 and 4) may indicate the presence of inbreed variability due to both genetic and breeding-climatic factors. The performed grouping of animals by phenotypic characteristics (Table 1) and subsequent cluster analysis based on morphological characteristics using the Nei and Li/Dice coefficients (Fig. 4) revealed two distinct clusters within the population of Kyrgyz horses under study.

The first cluster unites animals with predominantly dark hair colour and smaller exterior measurements, while the second cluster is characterised by a predominance of light colours and larger body measurements. Thus, visualisation based on morphological features demonstrated the degree of proximity of individual individuals grouped by appearance and colour within the population, and also allowed tracing stable differences within the breed. Additionally, to confirm and detail the results obtained, a grouping by morphological features based on SNP data was performed (Table 2). The obtained genotypic data correspond to the identified morphological clusters and confirm the existence of two stable phenotypic subgroups in the population of Kyrgyz horses.

Table 2. Grouping by morphological features based on SNP data

Groups	Source of the variation	Degrees of freedom	Components of variation	Variation (%)
(A) Samples without grouping	Between populations	24	Va = 42.27006	100.00 ***
	Within the population	27	Vb = 0.00000	0.00 **
	Total	51	42.27006	
(B) Data grouped by the place where it was collected	Between collection points	4	Va = 4.67276	11.03 NS
	Within the population	45	Vb = 37.70185	88.97 NS
	Total	49	42.37461	
(C) Samples were grouped by coat	Between groups	4	Va= -0.68679	-1.66***
	Between groups within a population	20	Vb=42.08095	101.66***
	Within the population	91	Vc= 0	0 NS
	Total	115	41.39416	
(D) Samples grouped according to the oblique length of the trunk	Between groups	2	Va= 0.16994	0.40 NS
	Between populations within a group	22	Vb= 42.14168	99.60 NS
	Within the population	25	Vc= 0	0 *
	Total	49	42.31161	
(E) Samples grouped by chest circumference	Between groups	2	Va= 0.40752	0.96 NS
	Between populations within a group	22	Vb= 41.97412	99.04 NS
	Within the population	25	Vc= 0	0 *
	Total	49	42.38164	
(F) Samples grouped according to the pastern circumference	Between groups	2	Va= 0.19344	0.46 NS
	Between populations within a group	22	Vb= 42.13287	99.54 NS
	Within the population	25	Vc= 0	0 *
	Total	49		

Note: (A) samples without grouping, (B) grouping by collection location, (C) grouping by coat, (D) grouping by oblique body length, (E) grouping by chest circumference, (F) grouping by metacarpal circumference. NS – no significant difference; *p-value >0.5; ** p-value >0.1; *** p-value >0.01

Source: created by the authors

Table 2 is a comparative analysis of the contribution of morphological groupings to the overall genetic variance by SNP markers. Option (A), where the analysis was performed without preliminary grouping, shows the maximum possible level of genetic variation between populations

(100%), which indicates the presence of pronounced differences in the studied material in the absence of a priori classifications. However, already at the first level of grouping – at the sampling site (Option B) – the proportion of intergroup variation sharply decreases to 11.03% and turns out to be statistically non-significant (NS), which indicates the absence of a significant spatial structure of the population at the level of SNP profiles.

The most interesting results are observed when grouped by phenotypic traits. In the case of the colour of the suit (Variant B), a negative value of variation between the groups is found (Va = -0.68679), which may indicate the artificiality or conventionality of this division and its inconsistency with the genetic structure. The intragroup variation is more than 100%, which also confirms the lack of genetic validity of the age differences. Similar results were obtained in the analysis of external indicators: oblique body length (D), chest circumference (E), and metacarpal circumference (F), where the contribution of intergroup variation ranges from 0.40-0.96% and is not statistically significant.

Thus, none of the analysed morphological features explains a significant part of the molecular variation. This indicates that the studied SNP markers are not directly associated with the selected morphological characteristics or exterior parameters. This result is typical for markers located outside the coding regions or not involved in the regulation of traits that are subject to polygenic heredity and strong environmental influences. Additionally, the Mantel test, comparing the matrices of genetic and morphological distances, showed a low and negative degree of correlation (r = -0.07396, p = 0.0499), which confirms the lack of congruence between the morphological and molecular genetic structure. This may be due to both the complex nature of phenotypic traits and the limited number of markers used in the analysis. In general, the data obtained indicate the need for an integrated approach to assessing the inbreed structure, including expanding the set of SNP markers, in-depth morphometric analysis, and consideration of environmental and breeding factors when interpreting the results.

A comparable study was conducted by J. Yun et al. (2022), where the genetic structure of Mongolian horses from four geographically isolated regions was analysed using 14 microsatellite markers. The researchers revealed a high level of polymorphism and significant interpopulation differentiation, especially between the southern and northern groups. The use of PCoA and FCA helped to visualise a clear genetic separation, and cluster analysis confirmed the existence of two main genetic clusters. These results correlate with the data of the present study, which also shows structural heterogeneity within traditional populations, despite morphological proximity. The study demonstrates that in the absence of phenotypic differences, molecular markers can reveal a hidden population structure caused by spatial isolation and genetic drift.

A similar approach was implemented in the study of traditional Kazakh horses (Genetic structure and genome-wide..., n.d.), where, based on PCA, ADMIXTURE data and distance analysis, it was shown that intrapopulation differences between regional groups do not reach statistical significance. The genetic homogeneity helped to consider the studied animals as a single breed, which contrasts with the results obtained in the analysis of Kyrgyz horses, where pronounced structuring and the presence of several genetic clusters were established. Additionally, a GWAS analysis was performed in the Kazakh study, which helped to identify markers associated with the development of bone and nervous systems, whereas in the Kyrgyz sample, the main focus was on population structure and morphometry. Thus, in both cases, the high breeding value of Central

Asian horses was confirmed, but the differences revealed emphasise the influence of local breeding practices on the development of a genetic profile.

An additional perspective was provided by J. Metzger et al. (2014), based on genome-wide sequencing of both purebred and mixed-breed horses. The researchers identified more than 10 million SNPs and over a million indels, of which a significant part had not been previously described. It was found that mixed-breed animals were dominated by unique mutations associated with metabolic and morphogenetic processes, whereas purebred animals were dominated by muscle activity and neurodevelopment. Although the current study also used high-density SNP analysis, the focus was on inbreed structure rather than differences between types of ancestry. Nevertheless, both approaches demonstrate the value of large-scale genotyping for identifying signs of selective breeding and studying adaptations.

V. Rossokha et al. (2022) evaluated the informative value of individual SNPs and polymorphisms of the LY49B, EDNRB, and CSN3 genes in three horse breeds using PCR-RFLP. The researchers found the presence of a lethal LWFS mutation in the Ukrainian horse breed and showed that the analysis of individual genes can serve as a diagnostic tool in breeding. In contrast to the focused methodology used in research, the current study was based on the analysis of thousands of markers within the Equine80k platform, which revealed a more complete picture of intra-breed differentiation. Thus, mass SNP genotyping demonstrated a higher resolution than targeted study of individual loci.

Another example of an applied molecular approach is presented in the paper by M.A. Ayala-Valdovinos et al. (2016), where mutagen-specific PCR (MS-PCR) was developed for genotyping a mutation in the EDNRB gene associated with lethal white foal syndrome. The proposed method helped to quickly and accurately identify carriers, replacing more time-consuming diagnostic methods. Despite the fact that the research had a narrow applied focus, it emphasised the importance of integrating genetic tests into the breeding system. In contrast, the Kyrgyz study focused on population structure and a comprehensive analysis of morphometric and genetic traits, which demonstrates a broader assessment of the genetic potential of the population.

A study of eight native Japanese horse breeds and Japanese thoroughbred mounts using SNP genotyping by T. Tozaki et al. (2019) revealed relatively low genetic diversity in most local breeds, except Hokkaido. Cluster and phylogenetic analyses have shown that the genetic relationships between the breeds generally correspond to their geographical distribution. Japanese thoroughbred horses differed from both local Japanese breeds and foreign thoroughbreds, demonstrating unique genetic characteristics. The study confirmed the origin of Japanese horses from Mongolian ancestors who migrated through the Korean Peninsula.

Thus, a comparison with the results of other researchers demonstrated a steady trend: morphological parameters often do not reflect real genetic diversity, especially in populations with open reproduction and without targeted breeding based on external characteristics. The inclusion of high-density SNP analysis and in-depth morphometry in a single methodology helps to more accurately assess the inter-breed structure, identify hidden clusters, and develop sound strategies for preserving the genetic resource. The totality of the data presented highlights the need for an integrated approach to the study of traditional horse breeds, considering both molecular and phenotypic aspects, and the socio-cultural conditions of their breeding.

Conclusions

As a result of a comprehensive analysis of the genetic and morphological structure of indigenous Kyrgyz horses, the presence of a certain genetic diversity within the population was established, which was confirmed by the results of cluster and statistical analysis of SNP marker data. The study revealed two main genetic clusters reflecting the inter-breed structure, while the phenotypic variation in the main morphological features was significant and heterogeneous.

The SNP markers used did not correlate with morphological parameters, which indicated the complex nature of the inheritance of phenotypic traits and the significant influence of non-genetic factors such as housing conditions and habitat. This pointed to the limitations of traditional morphological criteria in assessing the genetic structure of the breed and emphasised the importance of the integrated application of both classical morphometric methods and advanced biotechnologies.

To increase the efficiency of breeding work, it was recommended to develop and implement DNA markers linked to key morphological and productive traits, which would speed up the selection process and improve the quality of breed characteristics. The obtained data on the genetic structure and intra-breed variability could become the basis for the creation of adapted breeding programmes and strategies for preserving the genetic resource of Kyrgyz horses.

In the future, it was recommended to expand the sample of the studied animals and increase the density of SNP genotyping, which would allow for a deeper study of the relationship between genotype and phenotype, and consider the influence of environmental and breeding factors. Additional study of morphological features using modern methods of morphometry and statistical analysis would contribute to a more accurate assessment of breed characteristics and adaptive potential.

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