

Population Analysis and Genetic Structure of Two Kazakh Cattle Breeds Using 150K SNP

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ABSTRACT

Kazakh white-headed and Auliekol breeds are mostly spread through Kazakhstan and are animals with dual purpose productivity directions including meat and milk. Kazakh native cattle breeds are still largely unexplored based on the genetic markers and are of great interest in the country. In this study, we for the first time applied high-density SNP genotyping for two indigenous cattle breeds to study genetic diversity, population structure and relationships with the different foreign breeds. Using of GGP Bovine 150K SNP array the principal component analysis (PCA), populations estimates of the genetic structure (ADMIXTURE), genetic diversity and distances (Fst), and phylogenetic tree showed that the Kazakh white-headed and Auliekol breeds have certain separate genetic structure that differ from European and Russian cattle breeds. The two of these Kazakh cattle breeds have their genetic background according to observed results as distinct breeds. The obtained results will be aided in the development breeding system and conservation programs of the Kazakh white-headed and Auliekol breeds of beef cattle in the Kazakhstan.

1. Introduction

Over the course of a thousand years, adaptation and migration of humans has led to the domestication of cattle capable of living in different environments, which have unique animal genetic profiles (Ahmad *et al.* 2020). Over the past several decades, commercial cattle breeds have been developed with improved production when properly kept in many countries, including Kazakhstan (Boichard and Brochard 2012). Commercial breeds such as the Kazakh white-headed and Auliekol are among the most common breeds for meat and dairy production.

Kazakh white-headed breed (KWh). A large role in the formation and development of the specialized

beef cattle breeding industry in Kazakhstan belongs to the Kazakh white-headed breed, whose animals are successfully bred in many regions of the country. According to the Ministry of Agriculture of the Republic of Kazakhstan, as of January 1, 2021, there are more than 393 thousand heads of the Kazakh white-headed breed in 14 regions (Ministry of Agriculture of the Republic of Kazakhstan 2021). The Kazakh white-headed breed was bred by crossing the Kazakh and Kalmyk breeds, using the gene pool of the Hereford breed (Plakhtyukova 2020). Animals of this breed combine excellent meat qualities and early maturity inherited from the Herefords, as well as adaptive and maternal properties characteristic of local cattle, which makes it possible to successfully breed the breed in all regions of the republic with extensive natural pastures (Ombaev 2013). Breeding work was also carried out to improve the genealogical

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structure of the breed (Kineev 2014). Animals of the Kazakh white-headed breed are characterized by good reproductive, feeding and fattening qualities (Omibaev 2013). The main breed advantage is that individuals can do without water for a long time. The advantages of the Kazakh white-headed breed include unpretentiousness and endurance, good adaptation to both hot steppe conditions and cold north, resistance to various diseases of cattle, high-quality meat, good weight gain - at 18 months, young growth reaches 450 kg, which is considered a good indicator of early maturity.

Auliekol breed (AK). The course adopted for the intensification of beef production required the creation of new genotypes of beef cattle, which well combine a high growth rate with low feed costs for growth and a large live weight (Kineev 2014). Work on the creation of such a breed was carried out in the breeding farm "Moskalevsky" in Kostanay region. The breeding program included obtaining and breeding "in itself" three-breed crosses from the use of Charolais, Aberdeen-Angus bulls, and Kazakh white-headed cows (Kineev 2014). Breeding developments have led to the creation of a new meat breed that combines well the large live weight and growth rate of the ball-legged cattle, the high quality of meat, and the closeness of the Aberdeen-Angus, and the unique adaptive properties of the Kazakh white-headed breed to breeding conditions. An important property of the breed is also its adaptability to breeding in various natural and climatic conditions of the republic, its lability to the impact of changed environmental factors.

At this time, detailed population-genetic studies of the Kazakh breeds of cattle have not been carried out. Research of Kazakh cattle breeds, as a rule, were limited to local studies and used mainly microsatellites as one of the most common molecular markers. (Shamshidin *et al.* 2019; Plakhtukova *et al.* 2020; Abdelmanova *et al.* 2021) and SNP markers mostly in Kazakh white-headed breed (Yurchenko *et al.* 2018). The results of these studies indicate that it was necessary to perform detailed studies on modern and accurate molecular markers such as SNP. Single Nucleotide Polymorphism (SNP) genotyping is one of the most productive methods for genomic prediction and animal breeding and selection (Sermyagin *et al.* 2018).

In recent years, SNP genotyping, mostly 150K and 50K SNP markers, typing of cattle has been widely used to reveal the genetic structure of animals. At present, genotyping based on the SNP method has not been used to study the genetic diversity of Kazakh cattle breeds, so this study is of great interest

for the characterization of the bovine genome. For the first time in Kazakhstan, two local Kazakh breeds of cattle were selected and genotyped using 150K SNP genotyping. This study was aimed at genotyping two local Kazakh cattle breeds by 150K SNP, determining the genetic diversity and structure of cattle, and comparing with global domestic cattle breeds using DRYAD database of SNP.

2. Materials and Methods

To examine the two Kazakh cattle breeds, ear tissue were collected from 200 specimens, including 100 cows of Auliekol (AK) and 100 animals of Kazakh-white-headed (KWh) cattle breeds. Hair follicles were sampled from bulls and sires, and the animals were weighed at birth, at 6 months, at 8 and 12 months and also which had the best meat and milk productive qualities. Genomic DNA was extracted and typed in Neogen Agrigenomics, Lincoln, Nebraska, USA, according to the manufacturer's protocol by GeneSeek GGP Bovine 150K, which contains 150 000 SNPs ("Illumina Inc.", USA). To compare the genetic structure of the Kazakh white-headed and Auliekol breeds with their progenitors Hereford and Kalmyk cattle, as well as other breeds of the Russian and European populations were included. A comparative analysis was carried out with Russian and Eurasian cattle breeds, including Ala-Tau (AT)-Bestuzhev (BEST)-Black Pied (BIPi)-Buryat (Bur)-Hereford (Her)-KalmykR (KalR)-KalmykS (KalS)-Kazakh Whitehead Russian population (KWh_Rus)-Kholmogory (Khol)-Kostroma (Kost)-Tagil (Tag)-Ukrainian Grey (Ukr_Grey)-Ukrainian White-headed (Ukr_Wh)-Yakut (Yak)-Yaroslavl (Yar) using available data from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.68hv7>.

Quality control and filtration of genotyping data for each SNP and each sample was performed using the PLINK software package 1.9 (Purcell *et al.* 2007) following filters (the corresponding commands in the PLINK program are given in brackets): Call-rate for all SNPs under study for an individual sample is not lower than 90% (--mind), Call rate for each of the studied SNPs for all genotyped samples is not less than 90% (--geno), the frequency of occurrence of minor alleles (MAF) is more than 0.01 or 0.05 (--maf 0.01), deviation of SNP genotypes from the Hardy-Weinberg distribution in the set of tested samples with a reliability of p-9 value <10⁻⁶ (--hwe). In addition, the linkage disequilibrium of the studied SNPs was assessed (LD estimate) with $r^2 < 0.2$ with a step of 50 kb (--indep-pairwise). To analyze the genotyping data of SNP markers (population genetic

and phylogenetic analysis), a database of whole-genome SNP genotypes was formed using high-density genotypes of 15 other beef and combined cattle breeds include Kazakh Whitehead in the Russian population studied by A. Yurchenko *et al.* (2018).

To assess the population structure and phylogenetic relationships of the studied cattle populations were used SNP data. Based on the obtained SNP genotypes in the R package *diveRsity* (Keenan *et al.* 2013) calculated the following population genetic parameters: observed (H_o) heterozygosity, unbiased expected heterozygosity (uHe), allelic diversity (Ar), coefficient F_{is} inbreeding (95% confidence interval). Principal component analysis (PCA) was performed in the PLINK 1.9 program, followed by plotting in R the *ggplot2* package (Wickham 2009). Population differentiation was estimated using Arlequin program (Excoffier and Lischer 2010) and based on the F_{st} values the phylogenetic tree was constructed by Neighbor-joining methods implemented in MEGAX software (Kumar *et al.* 2018). The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei 1987). The optimal tree is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. This analysis involved 17 nucleotide sequences. Evolutionary analyses were conducted in MEGA11 (Tamura *et al.* 2021). Population structure of the studied cattle breeds was performed by Admixture 1.3 (Alexander *et al.* 2009) with graphical representation using the Genesis (Buchmann and Hazelhurst 2014).

3. Results

In this study, we analyzed 200 individuals from two Kazakh cattle breeds. After filtering data, 1755 variants were removed due to missing genotype rate >10%, and 1 individual was excluded with more than 10% missing genotypes as well as 5363 variants removed above the set minor allele frequency (MAF)

threshold (--maf 0.05). As a result of the quality control retained 112723 SNPs and 198 individuals.

The results of the assessment of the genetic diversity of the Kazakh white-headed and Auliekol cattle breeds are summarized in Table 1.

As shown in Table 1, the Kazakh white-headed breed and Auliekol breed are characterized by a relatively high level of genetic diversity among the studied breeds. A high level of genetic diversity may be due to the use of natural mating when breeding the Kazakh white-headed breed in contrast to artificial insemination. The established in significant excess of heterozygotes can be considered as an indication of a low level of genomic inbreeding in the studied population of Kazakh white-headed cattle ($F_{is} = -0.007 [-0.007; -0.007]$), in comparison with the Aulekol breeds ($F_{is} = -0.0121 [-0.0165; -0.0085]$).

As shown in the first two components (Figure 1), the breeds of cattle studied by us that are divided into five groups. The first cluster is formed by the Hereford and native Kazakh white-headed and exported Russian Kazakh white-headed cattle breeds. The second group is formed by only Auliekol breed of Kazakh cattle. The formation of a consolidated isolated cluster by the Kazakh white-headed breed, despite the active use of crossing with Herefords, can be considered as an indication of the preservation of ingenious genomic components that distinguish this breed from Hereford cattle. The third, fourth and fifth groups are the most numerous, represented by the cattle breeds of Russia and Eurasia. According to the first and third components (Figure 2), two Kazak cattle breeds with Hereford clearly separated from all other breeds.

The genetic affinity of the Kazakh white-headed breed is confirmed minimum values of genetic distances between these breeds ($F_{st} = 0.027$) versus 0.035-0.164 compared to other breeds of cattle (Table 2) and Aulekol breed ($F_{st} = 0.048$) versus 0.041-0.146, and the formation of a single branch on the phylogenetic tree (Figure 3), built on the basis of the values of the F_{st} .

Table 1. Parameters of the genetic diversity of the Kazakh white-headed and Auliekol cattle breeds*

Breed	n	H_o	Ar	uF_{is} [95 CI]
Kazakh WH	99	0.36±0	1.91±0.001	-0.0076 [-0.0124; -0.0031]
Auliekol	99	0.36±0	1.92±0.001	-0.0121 [-0.0165; -0.0085]

*cattle breeds are presented in descending order the degree of genetic diversity assessed by the uHe indicator, H_o - observed heterozygosity, Ar - allelic diversity, uHe - unbiased (undistorted) expected heterozygosity, uF_{is} - unbiased inbreeding coefficient, calculated based on uHe values, CI is the confidence interval

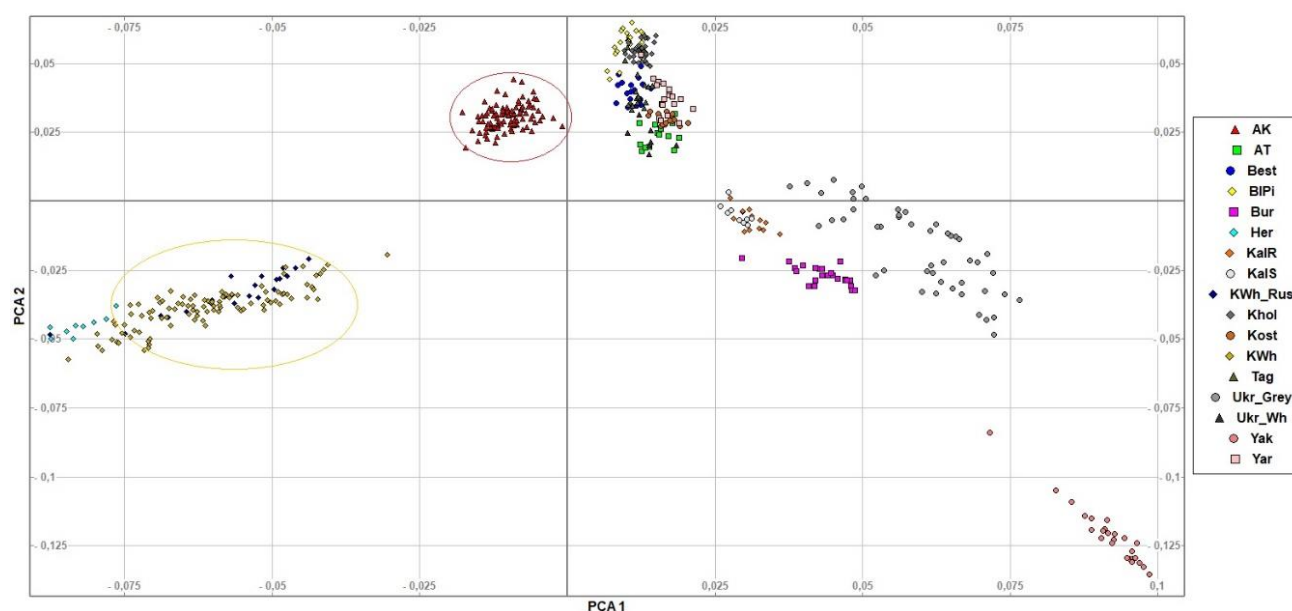


Figure 1. The principal component analysis (PCA1 and PCA2) of the studied cattle breeds

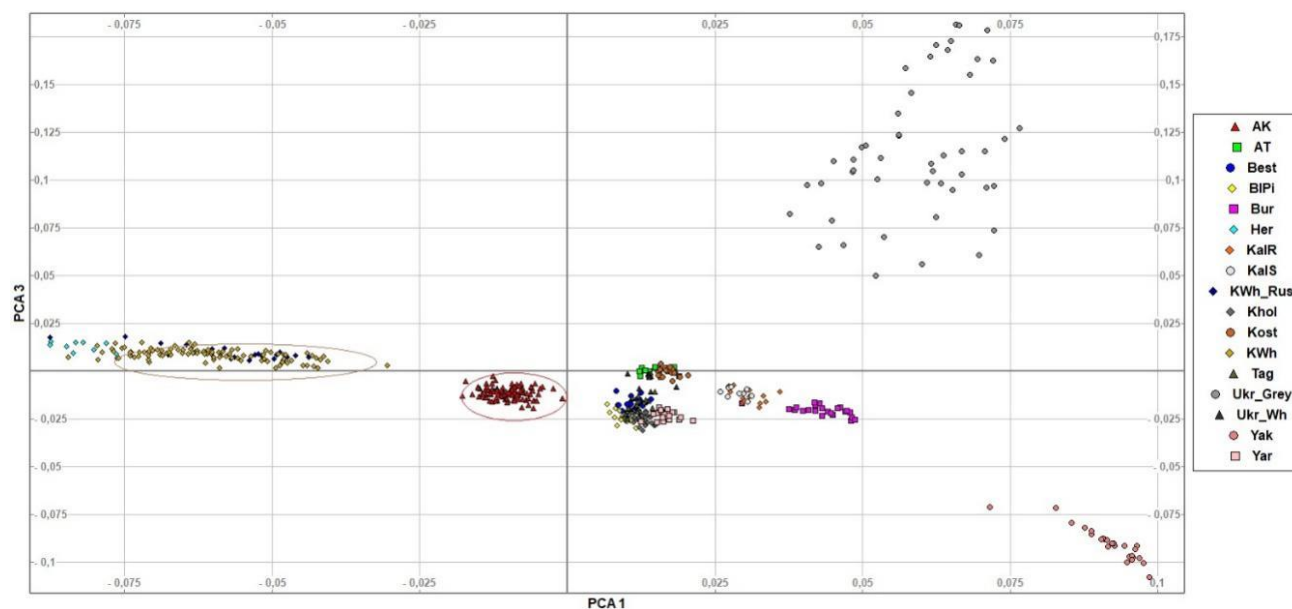


Figure 2. The principal component analysis (PCA1 and PCA3) of the studied cattle breeds

Analysis of the genetic structure of the studied cattle breeds (Figure 4) shows that at $K = 2$, Hereford and Yakut breeds form their own clusters, while other breeds exhibit varying degrees of admixing. The Kazakh white-headed breed exhibits a greater proportion of the ancestral genomic components of the Hereford breed compared to the Yakut. The share of ancestral genomic components in the structure of the Kazakh white-headed breed is also preserved at higher K from 3 to 9. It can also be seen that

from $K = 3$ the Auliekol breed has its own genetic structure, which differs from all studied Eurasian cattle breeds (Figure 4). At $K = 10$, the Kazakh white-headed breed exhibits its own genetic structure, which is different from the Hereford cattle, while traces of the Hereford cattle are preserved (Figure 5 Supplementary). The revealed genetic structure of the Kazakh white-headed breed is preserved even with an increase in the number of clusters K from 14 to 20 (Figure 5 Supplementary).

Table 2. Pairwise genetic differentiation (Fst) between the from different cattle breeds

	AK	Ala Tau	Bestuzhev	Black pied	Buryat	Hereford	KalmykR	KalmykS	Kazakh whiteheaded (Russian)	Kholmogory	Kostroma	KWh	Tagil	Ukrainian grey	Ukrainian whiteheaded	Yakut	Yaroslavl
AK	0.0000																
Ala_Tau	0.04871	0.0000															
Bestuzhev	0.05200	0.05952	0.0000														
Black_Pied	0.05978	0.06785	0.04651	0.0000													
Buryat	0.06613	0.06571	0.07477	0.08657	0.0000												
Hereford	0.08490	0.11436	0.11327	0.11797	0.13487	0.0000											
KalmykR	0.05451	0.05990	0.06356	0.07293	0.04240	0.12359	0.0000										
KalmykS	0.04164	0.04577	0.04952	0.06004	0.02915	0.10999	0.01322	0.0000									
Kazakh_ white headed (Russian)	0.05958	0.08040	0.08241	0.08911	0.09755	0.03942	0.08735	0.07324	0.0000								
Kholmogory	0.06169	0.07047	0.05983	0.05858	0.08482	0.12123	0.07368	0.05950	0.09341	0.0000							
Kostroma	0.07877	0.03617	0.09331	0.10190	0.10088	0.14804	0.09352	0.08123	0.11279	0.10249	0.0000						
KWh	0.04838	0.06944	0.07137	0.07877	0.08425	0.03565	0.07410	0.06093	0.02768	0.07964	0.09910	0.0000					
Tagil	0.04952	0.05447	0.04543	0.03736	0.06990	0.10946	0.05777	0.04465	0.07893	0.05066	0.08972	0.06736	0.0000				
Ukrainian_ grey	0.08436	0.08705	0.09556	0.10427	0.08494	0.15553	0.08253	0.07050	0.11895	0.10381	0.11913	0.10484	0.09100	0.0000			
Ukrainian_ whiteheaded	0.04881	0.05101	0.05281	0.05660	0.06652	0.11361	0.05616	0.04096	0.08010	0.06142	0.08742	0.06702	0.04804	0.08863	0.0000		
Yakut	0.14651	0.16508	0.16684	0.17475	0.11589	0.23544	0.13575	0.12911	0.19018	0.17118	0.19599	0.16405	0.16176	0.15701	0.17105	0.0000	
Yaroslavl																	0.0000

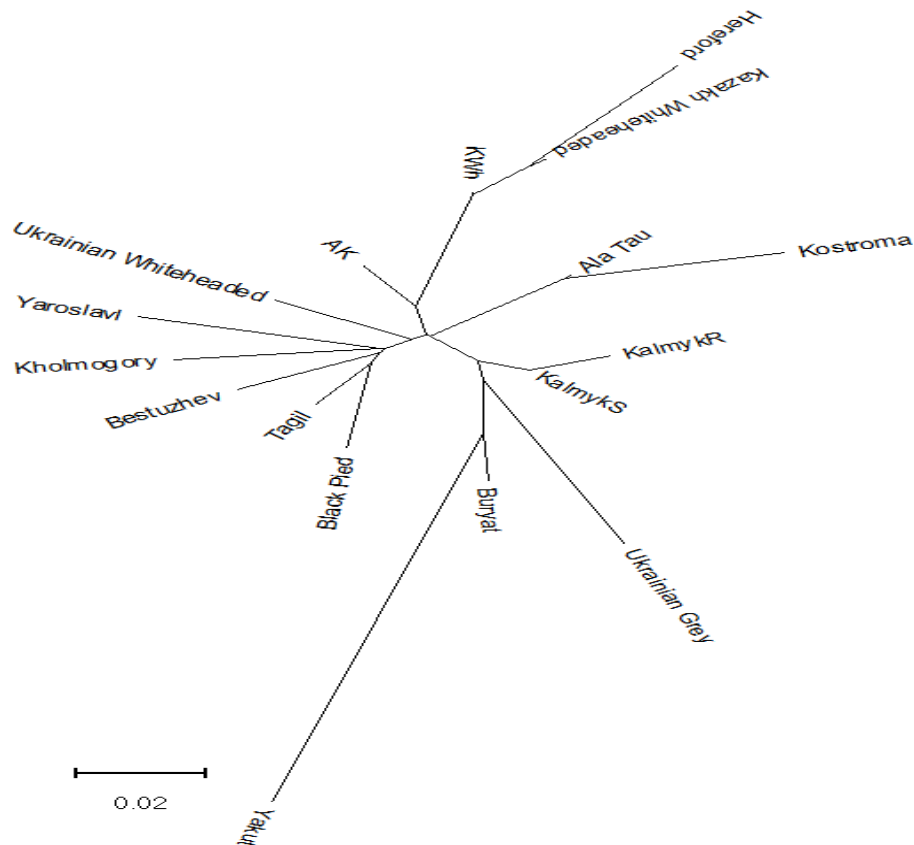


Figure 3. Phylogenetic tree of different cattle breeds constructed by Neighbour joining methods

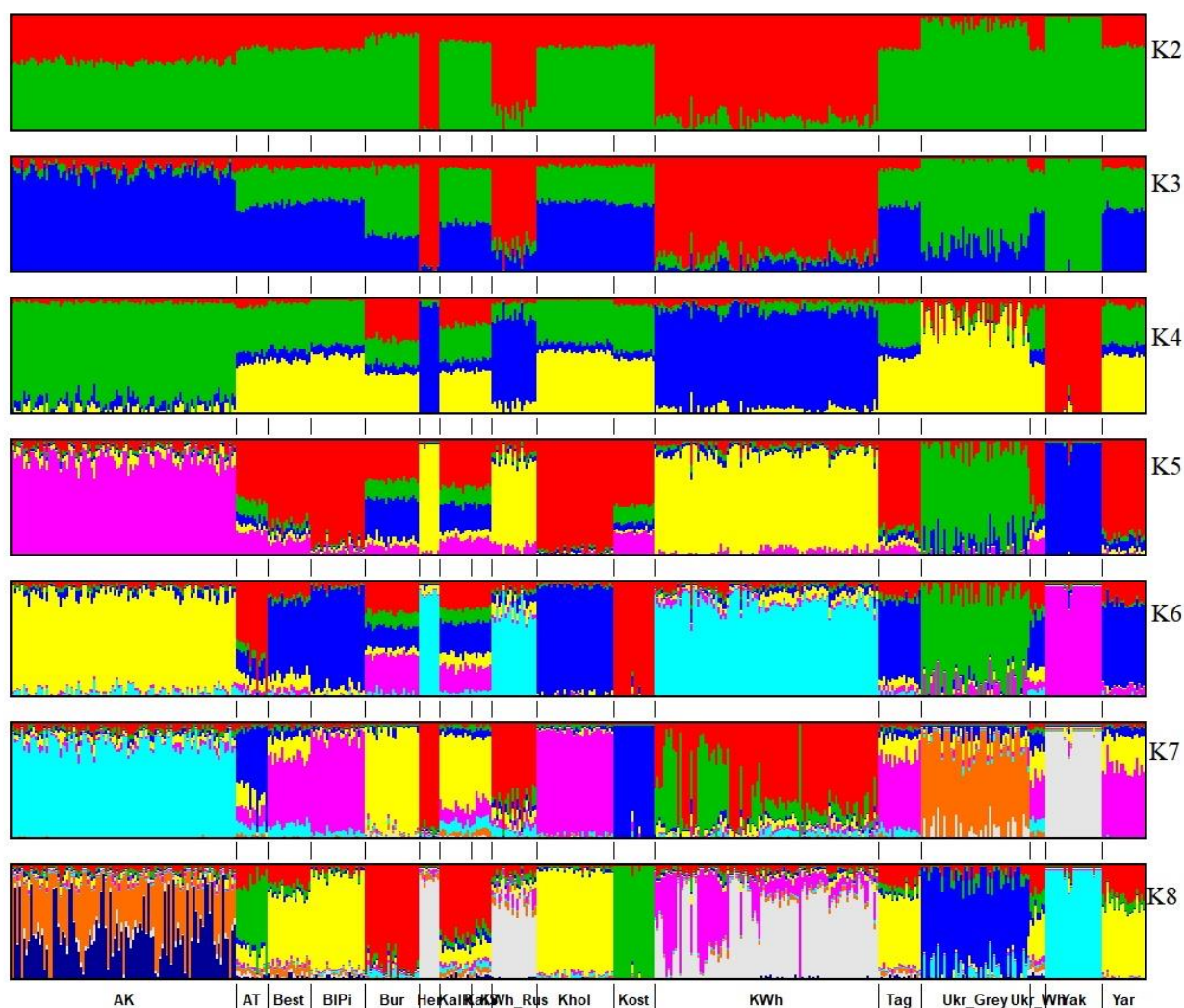


Figure 4. Genetic structure of seventeen Eurasian cattle breeds, determined from the results of the Admixture analysis for the number of clusters $K = 2-8$

4. Discussion

Assessment of the level of genetic diversity of the studied population of the Kazakh white-headed and Auliekol breeds is a very important issue for the country since Kazakhstan is located in the center of Eurasia, which is a plus for the export of meat and meat products to neighboring and distant countries and is also a country that consumes a large amount of meat products within the country. It was found that the Kazakh white-headed breed was characterized by a relatively high level of genetic diversity comparable to breeds such as Russia and Eurasia, which may be a consequence of the use of natural mating when breeding the Kazakh white-headed breed. An insignificant excess of heterozygotes (0.7%) was established, which can be considered as an indication

of a low level of inbreeding in the studied population of Kazakh white-headed cattle and Auliekol breed. The results of the principal component analysis, the calculation of genetic distances, and estimates of the genetic structure of the population showed that the preservation of ingenious genomic components in the Kazakh white-headed breed cattle, distinguishing it from the related Hereford breeds and other breeds of meat and combined directions productivity of Europe and Asia. Our results are consistent with the previous studies reported by A.S. Shamshidin *et al.* (2019), who showed Kazakh white-headed, Auliekol, and Alatau breeds clustered separately from the other cattle populations in PCA based on 11 STR loci. The same analysis with Kazakh white-headed and Auliekol breeds using 11 microsatellite loci was held as part of A.S. Abdelmanova *et al.* (2021) research group that

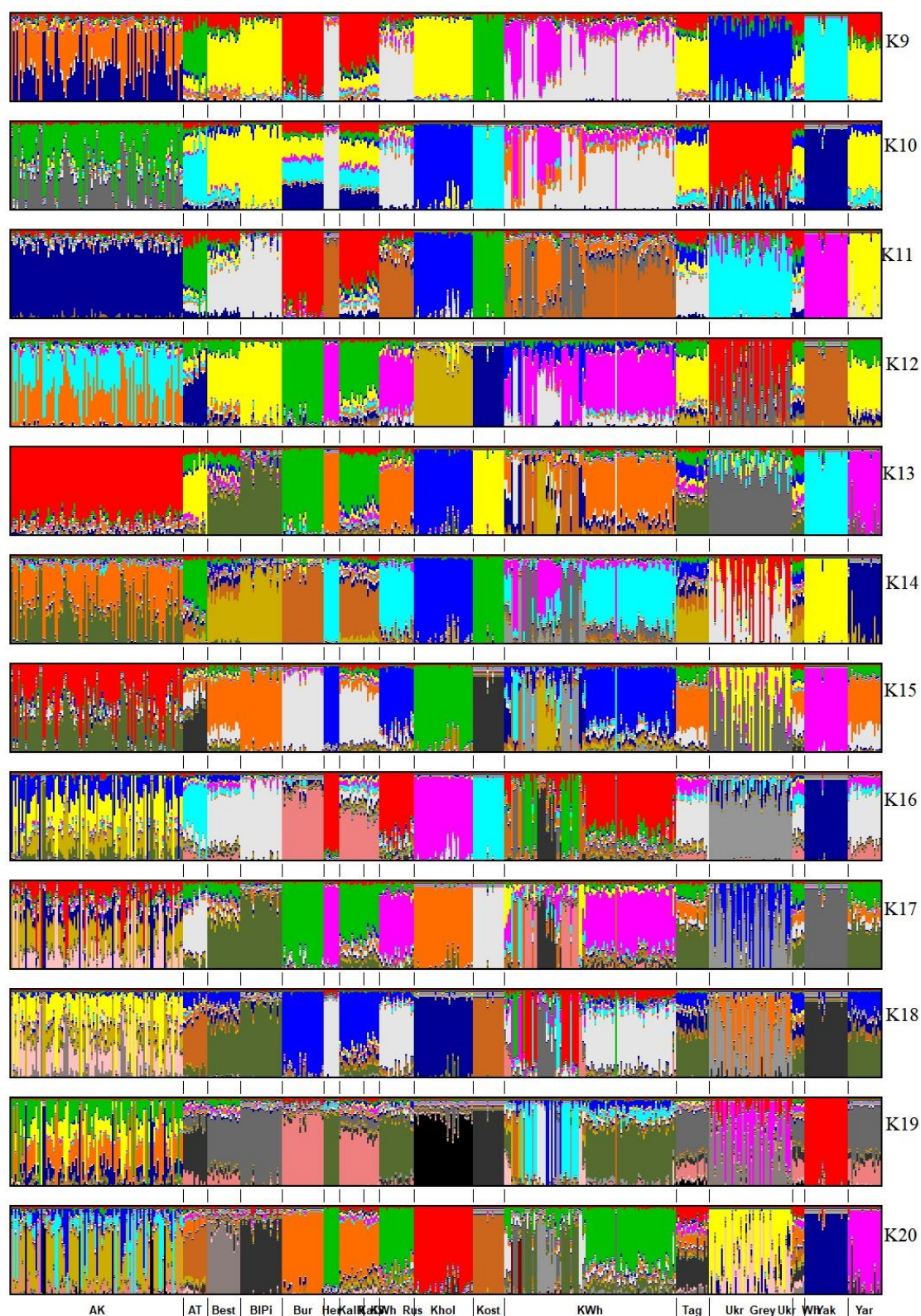


Figure 5. Genetic structure of seventeen Eurasian cattle breeds, determined from the results of the Admixture analysis for the number of clusters K = 9-20

demonstrated the ancient Kazakh native cattle genetic components was found significantly in the Kazakh white-headed cattle than the Hereford breed. These findings confirm that Kazakh white-headed cattle naturally preserved historical genetic structure to date. The Auliekol breed has shown its heterogeneity in the ADMIXTURE, but this breed has formed its own cluster of genetic diversity. As M. Kineev (2014) described, the Auliekol breed was created to improve the meat production qualities and this shows the genetic diversity of the population. The results of ADMIXTURE analysis of two populations of the Kazakh white-headed breed in Kazakhstan and Russia show that both populations have the same patterns and, in comparison with other breeds, are close in genetic structure to the Hereford breed. These findings are similar to the investigations of A. Yurchenko *et al.* (2018), and since the Hereford breed participated in the formation of the Kazakh white-headed breed. The structure of the tree was derived in comparison with other breeds, which show that the Kazakh white-headed is a separate breed. Even in comparison with the Kazakh white-headed breed, the Russian population has a slightly different genetic structure. According to Yamanaka *et al.* (2018) Kazakhstan cattle breeds, divergent mtDNA subhaplogroups were observed, and in terms of mtDNA, Kazakhstan cattle populations also significantly differed from the worldwide breeds, however closely related to Hereford. Also, for a complete work on Kazakh breeds of cattle, genome-wide studies, including mitochondrial and Y-chromosomal markers, are interested.

In conclusion, the genetic sequence is critical for the successful breeding and selection of cattle. Kazakh breeds of cattle are currently poorly studied and require further study and comparison with world breeds since Kazakhstan is one of the main others of meat and meat products to neighboring countries. The novelty of the research is that for the first time data on the genetic diversity of the Kazakh white-headed and Auliekol cattle breeds based on high-density SNP analysis genotypes. The results obtained will find application in the development of programs breeding and conservation of the Kazakh white-headed and Aulekol cattle breed livestock.

The genetic sequence is critical to the successful breeding and selection of cattle. Kazakh breeds of cattle are currently little studied and require further study and comparison with world breeds, since Kazakhstan is one of the main suppliers of meat and meat products

to neighboring countries. The novelty of the study lies in the fact that it was revealed that the Kazakh white-headed and Auliekol breeds differ from their progenitors in terms of genetic structure and each has its own genetic profile that distinguishes them from the rest of the populations of Russia and Europe. Also given that genetic consistency is critical to successful breeding and selection of cattle, future efforts are needed to compile a detailed genetic description of the Kazakh white-headed and Auliekol breed. These studies expand the interest of farmers and scientists in the breeds of cattle in Kazakhstan.

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