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# Genetic diversity of the Kazakh Tobet dog and comparison with free-ranging dog populations

The Kazakh Tobet is a traditional livestock guardian dog (LGD) breed in Kazakhstan. A comparison of genetic diversity between the traditional breed and free-ranging (outbred) dogs makes it possible to better understand whether the genetic diversity of this breed is more similar to that of a structured breed population or an unstructured, free-ranging dog population. The aim of this study was therefore to assess the genetic diversity of the Kazakh Tobet and compare it with the genetic diversity of free-ranging dogs. A total of 107 Tobet samples from three regions of Kazakhstan and Mongolia and 55 free-ranging dogs were genotyped using 18 polymorphic microsatellite loci. The main parameters of genetic diversity — including mean number of alleles (Na), effective alleles (Ne), observed (Ho) and expected heterozygosity (He) and fixation index (F) — were evaluated. Tobet dogs showed a high level of genetic diversity (Na = 10.722, Ho = 0.781, He = 0.805 for the total populations), comparable to the values of outbred dogs (Na = 9.556, Ho = 0.776, He = 0.791). All four Tobet populations showed signs of internal diversity. Fixation index values were low or negative in most populations, suggesting that there is no strong inbreeding.

These results confirm the position of the Kazakh Tobet as a genetically rich and structurally complex LGD breed that is maintained without strict reproductive isolation. They also illustrate a paradox in the conservation of the Kazakh Tobet: while the high genetic diversity and admixture reflect the breed's adaptive success and functional selection history, formal recognition of the breed and long-term conservation require a strategic framework. In the case of the Kazakh Tobet, this does not mean imposing rigid reproductive isolation, but rather implementing a scientifically guided, open breeding system — that supports genetic monitoring, preserves functional traits, and protects against both genetic erosion and uncontrolled hybridization.

*Keywords:* Genetic profile, gene pool, genetic diversity, inbreeding, microsatellite marker, population genetics, Tobet breed.

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## *Introduction*

The Kazakh Tobet is one of the oldest and culturally most important guard dog breeds in Kazakhstan. Historically used by Kazakh nomad shepherds to guard livestock during seasonal migrations and was primarily selected for behavioral and functional traits such as alertness, endurance, weather resistance and independence. Despite its cultural importance, the breed remained largely uncharacterized at the genomic level until recently. Fragmented breeding practices, lack of centralized registration and increased crossbreeding with local or import breeds have raised concerns about the preservation of the genetic identity and functional capacity of the Kazakh Tobet.

To assess the current genetic status of the Kazakh Tobet, comparisons with free-ranging (or outbred) dog populations are an important benchmark. Free-ranging dogs, which reproduce without pedigree control or artificial selection, generally show a high degree of heterozygosity and allelic richness. These parameters make them a meaningful reference point for assessing whether a traditional breed such as the Tobet exhibits patterns of diversity consistent with purebred breeds, or whether it resembles the broader, genetically variable population of free-ranging dogs.

In parallel, several studies have begun in recent years to investigate the genetic characterization of working breeds. It has been shown that many of these breeds frequently interbreed with local free-ranging dogs [1, 2]. This has led to a paradigm shift: instead of interpreting genetic admixture as a threat to breed purity, it is increasingly seen as a mechanism that improves adaptability and preserves working traits, espe-

cially under extensive pastoral conditions. However, the Kazakh Tobet remains underrepresented in these discussions and its genetic diversity compared to outbred dog populations has not been systematically studied.

In this study, we present a comprehensive assessment of the genetic diversity and structure of the Kazakh Tobet in comparison to free-ranging dog populations. Using 18 polymorphic microsatellite markers, we analyzed samples of Kazakh Tobet from three regions of Kazakhstan and Mongolia. These were compared with a reference population of outbred dogs. The main genetic parameters, including observed and expected heterozygosity, average number of alleles, effective allele number and fixation index were evaluated. The main objective of this work is to quantify the genetic diversity of the Kazakh Tobet, determine its relationship to the outbred dog gene pool, and provide an empirical basis for strategies to preserve the breed.

## *Materials and methods of the research*

### *Objects of the research*

The research protocol was reviewed and approved by the Bioethics Committee of the RSE at REM Institute of Molecular Biology and Biochemistry named after M.A. Aitkhozhin, under the Committee of Science of the Ministry of Science and Higher Education of the Republic of Kazakhstan (Protocol No. 1, August 18, 2023). The study was conducted in accordance with the "Bioethical Rules for Conducting Research Involving Humans and Animals", the legislation of the Republic of Kazakhstan, and the principles of the European Convention on Bioethics. Importantly, no invasive experiments were performed on animals during the study. All research procedures involved the collection of biological materials from dogs through minimally invasive methods, posing no harm or distress to the animals.

Biological samples were obtained from Kazakh Tobet dogs during field expeditions, exhibitions, and specialized breed-related events. Evaluation of each dog's conformity to the Kazakh Tobet breed standard was conducted by certified cynologists from the national organization "KANSONAR". These experts met the qualification requirements and had extensive experience with both national Kazakh breeds and the broader Central Asian Shepherd Dog group, to which the Kazakh Tobet is classified. The assessments were based on the official breed standard for the Kazakh Tobet, approved by the Decree of the Ministry of Ecology and Natural Resources of the Republic of Kazakhstan No. 101 dated March 30, 2023. For comparative purposes, a control group of free-breeding (outbred) dogs was included. These dogs, known for their high genetic variability and adaptive potential, were sampled from the animal welfare organizations "Tailed Paradise" and "New Chance".

Two types of biological material were collected from Kazakh Tobet and outbred dogs:

- buccal swabs were obtained by gently brushing the inner cheek surface. Samples were placed in sterile tubes containing phosphate-buffered saline (PBS). This non-invasive and painless procedure was used for both Kazakh Tobet and outbred dogs.

- peripheral blood samples (up to 50 ml) were collected from the leg vein using EDTA-coated vacuum tubes by a licensed veterinarian experienced in research sampling. All procedures were carried out under sterile conditions and were minimally invasive.

All samples were promptly transported in a portable refrigerated container to the Institute of Genetics and Physiology. Upon arrival, samples were stored at −80 °C until further molecular genetic analyses were conducted.

Additionally, each sampled dog was photographed, and owners were asked to complete a detailed questionnaire. The questionnaire gathered data on the dog's age, sex, origin, current place of residence, physical description, and measurements. All data were digitized and entered into an electronic database. Informed consent was obtained from each dog's owner prior to the collection of samples and genetic testing.

## *Methods of the research*

### *DNA extraction*

DNA was extracted using "QIAamp Fast DNA Tissue Kit" (Qiagen, Germany) kits according to the manufacturer's protocol. The qualitative and quantitative characteristics of the isolated DNA were determined using Qubit4.0 (Invitrogen, USA) or 2100 Expert (Agilent Technologies, USA).

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*Microsatellite analysis*

Microsatellite analysis was performed with the SeqStudio™ Genetic Analyser (Thermo Fisher Scientific, USA) using the Thermo Scientific Canine Genotypes Panel 1 (Thermo Fisher Scientific, USA), which contains 19 loci recommended by ISAG for dog (AHTk211, CXX279, REN169O18, INU055, REN54P11, INRA21, AHT137, REN169D01, AHTh260, AHTk253, INU00 5, INU030.

*Methods for bioinformatics and statistical processing of microsatellite analysis data*

Genetic evaluation based on allele frequencies was performed using the programs GenAlEx 6.5 [3] and Cervus [4]. We evaluated indicators such as the average (Na) and effective (Ne) number of alleles, the observed (Ho) and expected (He) heterozygosity, and the agreement with the Hardy-Weinberg distribution.

*Results*

Genomic DNA was extracted from all collected biological samples of Kazakh Tobet dogs (n = 163), and its quality and quantity were assessed using standard protocols. Likewise, DNA was isolated from biomaterials collected from outbred dogs (n = 55), followed by evaluation of DNA integrity and concentration.

Microsatellite genotyping was conducted for 18 autosomal loci in 107 Kazakh Tobet dogs representing four geographic populations: South Kazakhstan (n = 73; hereafter Pop 1), East Kazakhstan (n = 16; Pop 2), North Kazakhstan (n = 8; Pop 3), and Bayan-Ölgii, Mongolia (n = 4; Pop 4). Additionally, 55 outbred dogs were genotyped as a comparative group. Allele frequencies were calculated for each locus, and key parameters of genetic variability were assessed for both Kazakh Tobet and outbred dogs (Tables 1, 2).

Table 1

**Genetic variability of Kazakh Tobet dogs**

| Population | Locus | Na | Ne | Ho | He | F |
|---|---|---|---|---|---|---|
| Pop1 | AHTk211 | 6,000 | 3,628 | 0,616 | 0,724 | 0,149 |
| | CXX0279 | 10,000 | 5,613 | 0,792 | 0,822 | 0,037 |
| | REN169O18 | 11,000 | 5,721 | 0,808 | 0,825 | 0,021 |
| | INU055 | 9,000 | 4,992 | 0,849 | 0,800 | -0,062 |
| | REN54P11 | 10,000 | 5,709 | 0,877 | 0,825 | -0,063 |
| | INRA21 | 8,000 | 5,557 | 0,918 | 0,820 | -0,119 |
| | AHT137 | 14,000 | 6,337 | 0,904 | 0,842 | -0,074 |
| | REN169D01 | 11,000 | 6,307 | 0,932 | 0,841 | -0,107 |
| | AHTh260 | 12,000 | 4,329 | 0,712 | 0,769 | 0,074 |
| | AHTk253 | 9,000 | 2,791 | 0,466 | 0,642 | 0,274 |
| | INU005 | 10,000 | 3,335 | 0,603 | 0,700 | 0,139 |
| | INU030 | 7,000 | 4,315 | 0,781 | 0,768 | -0,016 |
| | FH2848 | 9,000 | 5,789 | 0,877 | 0,827 | -0,060 |
| | AHT121 | 12,000 | 7,860 | 0,795 | 0,873 | 0,090 |
| | FH2054 | 12,000 | 6,563 | 0,767 | 0,848 | 0,095 |
| | REN162C04 | 13,000 | 6,149 | 0,833 | 0,837 | 0,005 |
| | AHTh171 | 12,000 | 8,167 | 0,877 | 0,878 | 0,001 |
| | REN247M23 | 9,000 | 4,372 | 0,685 | 0,771 | 0,112 |
| | Mean | 10,222 | 5,418 | 0,783 | 0,801 | 0,027 |
| | SE | 0,495 | 0,341 | 0,029 | 0,015 | 0,024 |
| Pop2 | AHTk211 | 5,000 | 4,303 | 0,813 | 0,768 | -0,059 |
| | CXX0279 | 6,000 | 3,657 | 0,813 | 0,727 | -0,118 |
| | REN169O18 | 6,000 | 4,697 | 0,875 | 0,787 | -0,112 |
| | INU055 | 7,000 | 5,389 | 0,875 | 0,814 | -0,074 |
| | REN54P11 | 7,000 | 4,031 | 0,938 | 0,752 | -0,247 |
| | INRA21 | 6,000 | 3,969 | 0,625 | 0,748 | 0,164 |
| | AHT137 | 9,000 | 7,314 | 0,938 | 0,863 | -0,086 |
| | REN169D01 | 8,000 | 5,069 | 0,875 | 0,803 | -0,090 |
| | AHTh260 | 7,000 | 4,303 | 0,688 | 0,768 | 0,104 |
| | AHTk253 | 7,000 | 3,737 | 0,750 | 0,732 | -0,024 |
| | INU005 | 7,000 | 4,096 | 0,625 | 0,756 | 0,173 |

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Continuation of Table 1

| Population | Locus | Na | Ne | Ho | He | F |
|---|---|---|---|---|---|---|
| Pop2 | INU030 | 5,000 | 4,197 | 0,625 | 0,762 | 0,179 |
| | FH2848 | 6,000 | 4,452 | 0,813 | 0,775 | -0,048 |
| | AHT121 | 10,000 | 7,014 | 0,938 | 0,857 | -0,093 |
| | FH2054 | 9,000 | 5,333 | 0,813 | 0,813 | 0,000 |
| | REN162C04 | 8,000 | 4,531 | 0,750 | 0,779 | 0,038 |
| | AHTh171 | 8,000 | 3,580 | 0,750 | 0,721 | -0,041 |
| | REN247M23 | 5,000 | 2,926 | 0,625 | 0,658 | 0,050 |
| | Mean | 7,000 | 4,589 | 0,785 | 0,771 | -0,016 |
| | SE | 0,343 | 0,264 | 0,026 | 0,012 | 0,027 |
| Pop3 | AHTk211 | 4,000 | 3,048 | 0,375 | 0,672 | 0,442 |
| | CXX0279 | 6,000 | 5,120 | 0,875 | 0,805 | -0,087 |
| | REN169O18 | 5,000 | 3,556 | 0,875 | 0,719 | -0,217 |
| | INU055 | 5,000 | 3,879 | 0,750 | 0,742 | -0,011 |
| | REN54P11 | 6,000 | 4,414 | 0,750 | 0,773 | 0,030 |
| | INRA21 | 6,000 | 4,741 | 1,000 | 0,789 | -0,267 |
| | AHT137 | 6,000 | 4,923 | 1,000 | 0,797 | -0,255 |
| | REN169D01 | 8,000 | 5,818 | 0,875 | 0,828 | -0,057 |
| | AHTh260 | 8,000 | 6,400 | 0,750 | 0,844 | 0,111 |
| | AHTk253 | 8,000 | 6,400 | 0,875 | 0,844 | -0,037 |
| | INU005 | 6,000 | 3,200 | 0,625 | 0,688 | 0,091 |
| | INU030 | 6,000 | 4,414 | 0,875 | 0,773 | -0,131 |
| | FH2848 | 5,000 | 4,741 | 0,750 | 0,789 | 0,050 |
| | AHT121 | 7,000 | 5,333 | 0,750 | 0,813 | 0,077 |
| | FH2054 | 6,000 | 5,120 | 0,875 | 0,805 | -0,087 |
| | REN162C04 | 6,000 | 4,267 | 0,625 | 0,766 | 0,184 |
| | AHTh171 | 6,000 | 4,129 | 0,625 | 0,758 | 0,175 |
| | REN247M23 | 6,000 | 3,765 | 0,500 | 0,734 | 0,319 |
| | Mean | 6,111 | 4,626 | 0,764 | 0,774 | 0,018 |
| | SE | 0,254 | 0,230 | 0,039 | 0,012 | 0,045 |
| Pop4 | AHTk211 | 5,000 | 3,704 | 0,900 | 0,730 | -0,233 |
| | CXX0279 | 7,000 | 2,941 | 0,900 | 0,660 | -0,364 |
| | REN169O18 | 5,000 | 4,082 | 0,900 | 0,755 | -0,192 |
| | INU055 | 6,000 | 4,000 | 0,600 | 0,750 | 0,200 |
| | REN54P11 | 5,000 | 3,448 | 0,500 | 0,710 | 0,296 |
| | INRA21 | 6,000 | 5,000 | 0,800 | 0,800 | 0,000 |
| | AHT137 | 9,000 | 6,452 | 0,900 | 0,845 | -0,065 |
| | REN169D01 | 8,000 | 5,714 | 0,900 | 0,825 | -0,091 |
| | AHTh260 | 6,000 | 3,704 | 0,900 | 0,730 | -0,233 |
| | AHTk253 | 3,000 | 1,504 | 0,200 | 0,335 | 0,403 |
| | INU005 | 4,000 | 2,667 | 0,700 | 0,625 | -0,120 |
| | INU030 | 5,000 | 4,167 | 0,900 | 0,760 | -0,184 |
| | FH2848 | 7,000 | 4,762 | 0,900 | 0,790 | -0,139 |
| | AHT121 | 7,000 | 5,405 | 0,800 | 0,815 | 0,018 |
| | FH2054 | 7,000 | 4,000 | 0,300 | 0,750 | 0,600 |
| | REN162C04 | 6,000 | 4,348 | 0,900 | 0,770 | -0,169 |
| | AHTh171 | 6,000 | 3,571 | 1,000 | 0,720 | -0,389 |
| | REN247M23 | 6,000 | 5,128 | 0,900 | 0,805 | -0,118 |
| | Mean | 6,000 | 4,144 | 0,772 | 0,732 | -0,043 |
| | SE | 0,333 | 0,276 | 0,054 | 0,027 | 0,062 |
| All (n=107) | AHTk211 | 6,000 | 3,813 | 0,654 | 0,738 | 0,113 |
| | CXX0279 | 11,000 | 5,335 | 0,811 | 0,813 | 0,002 |
| | REN169O18 | 11,000 | 5,632 | 0,832 | 0,822 | -0,011 |

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Continuation of Table 1

| Population | Locus | Na | Ne | Ho | He | F |
|---|---|---|---|---|---|---|
| All (n=107) | INU055 | 10,000 | 5,110 | 0,822 | 0,804 | -0,023 |
| | REN54P11 | 10,000 | 5,516 | 0,841 | 0,819 | -0,027 |
| | INRA21 | 8,000 | 5,408 | 0,869 | 0,815 | -0,066 |
| | AHT137 | 14,000 | 7,198 | 0,916 | 0,861 | -0,064 |
| | REN169D01 | 11,000 | 6,439 | 0,916 | 0,845 | -0,084 |
| | AHTh260 | 13,000 | 4,733 | 0,729 | 0,789 | 0,076 |
| | AHTk253 | 11,000 | 3,085 | 0,514 | 0,676 | 0,239 |
| | INU005 | 10,000 | 3,433 | 0,617 | 0,709 | 0,130 |
| | INU030 | 7,000 | 4,504 | 0,776 | 0,778 | 0,003 |
| | FH2848 | 9,000 | 5,943 | 0,860 | 0,832 | -0,034 |
| | AHT121 | 12,000 | 8,255 | 0,813 | 0,879 | 0,075 |
| | FH2054 | 13,000 | 6,584 | 0,738 | 0,848 | 0,129 |
| | REN162C04 | 15,000 | 6,293 | 0,811 | 0,841 | 0,035 |
| | AHTh171 | 12,000 | 6,895 | 0,850 | 0,855 | 0,005 |
| | REN247M23 | 10,000 | 4,233 | 0,682 | 0,764 | 0,107 |
| | Mean | 10,722 | 5,467 | 0,781 | 0,805 | 0,034 |
| | SE | 0,547 | 0,321 | 0,025 | 0,013 | 0,020 |

Table 2

**Genetic variability of outbred dogs**

| Pop | Locus | Na | Ne | Ho | He | F |
|---|---|---|---|---|---|---|
| Outbred (n=55) | AHTk211 | 6,000 | 4,569 | 0,764 | 0,781 | 0,022 |
| | CXX0279 | 8,000 | 5,004 | 0,727 | 0,800 | 0,091 |
| | REN169O18 | 10,000 | 5,490 | 0,855 | 0,818 | -0,045 |
| | INU055 | 9,000 | 4,549 | 0,636 | 0,780 | 0,184 |
| | REN54P11 | 10,000 | 5,879 | 0,818 | 0,830 | 0,014 |
| | INRA21 | 7,000 | 4,632 | 0,818 | 0,784 | -0,043 |
| | AHT137 | 12,000 | 5,996 | 0,891 | 0,833 | -0,069 |
| | REN169D01 | 11,000 | 5,955 | 0,891 | 0,832 | -0,071 |
| | AHTh260 | 12,000 | 5,891 | 0,873 | 0,830 | -0,051 |
| | AHTk253 | 7,000 | 3,085 | 0,709 | 0,676 | -0,049 |
| | INU005 | 12,000 | 3,658 | 0,636 | 0,727 | 0,124 |
| | INU030 | 6,000 | 3,168 | 0,709 | 0,684 | -0,036 |
| | FH2848 | 8,000 | 6,044 | 0,727 | 0,835 | 0,129 |
| | AHT121 | 13,000 | 8,509 | 0,891 | 0,882 | -0,010 |
| | FH2054 | 11,000 | 5,762 | 0,764 | 0,826 | 0,076 |
| | REN162C04 | 12,000 | 4,844 | 0,873 | 0,794 | -0,100 |
| | AHTh171 | 11,000 | 5,123 | 0,764 | 0,805 | 0,051 |
| | REN247M23 | 7,000 | 3,555 | 0,618 | 0,719 | 0,140 |
| | Mean | 9,556 | 5,095 | 0,776 | 0,791 | 0,020 |
| | SE | 0,550 | 0,308 | 0,022 | 0,013 | 0,020 |

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The percentage of polymorphic loci in the sample of Kazakh Tobet dogs was 100 % and a total of 193 alleles were identified. Pop 1 had the highest average number of alleles per locus (Na=10.222±0.495). In comparison, Pop 2 and Pop 3 showed moderate genetic diversity, with mean Na values of 7.000±0.343 and 6.111±0.254, respectively. The lowest genetic diversity among the four populations was observed in Pop 4, where the mean Na value was 6.000±0.333. The highest number of alleles was found at loci REN162C04, AHT137, AHTh260, FH2054, AHT121 and AHTh171, each with 12 to 15 alleles. The lowest number of alleles was found for the AHTk211 locus with 6 alleles. The average number of effective alleles for the all samples analyzed was 5.467±0.321 and ranged from 4.144±0.276 in Pop 4 to 5.418±0.341 in Pop 1. The highest observed heterozygosity was found in Pop 2 (Ho=0.785±0.026), followed by Pop 1 (Ho=0.783±0.029) and Pop 3 (Ho=0.764±0.039). The highest expected heterozygosity was found in Pop 1 (He=0.801±0.015), while Pop 3 (He=0.774±0.012) and Pop 2 (He=0.771±0.012) also showed significant but

slightly lower heterozygosity. In contrast, Pop 4 had the lowest heterozygosity values, with Ho at 0.772±0.054 and He at 0.732±0.027. The fixation index F was negative in Pop 2 (-0.016±0.027) and Pop 4 (-0.043±0.062), indicating an excess of heterozygotes in these populations. In contrast, Pop 1 (0.027±0.024) and Pop 3 (0.018±0.045) had positive F-values, indicating a slight lack of heterozygotes.

The percentage of polymorphic loci in the sample of outbred dogs was 100 %, a total of 172 alleles were identified. The average number of alleles per locus (Na) was 9.556±0.550. The highest genetic diversity among the loci was found for the AHT121 locus, which had 13 alleles. The INU030 locus had the lowest number of alleles — only 6. The average Ne value was 5.095±0.308 and varied from 3.085 for the AHTk253 locus to 8.509 for the AHT121 locus. The average observed heterozygosity (Ho) for all analyzed loci was 0.776±0.022, which is close to the average expected heterozygosity (He), which was 0.791±0.013. The highest observed heterozygosity was recorded for the AHT137, REN169D01 and AHT121 loci, where Ho reached 0.891. The lowest observed heterozygosity was at the REN247M23 locus (Ho=0.618). The fixation index was generally close to zero (F=0.020±0.020), indicating that there is neither a significant lack nor excess of heterozygotes in the population. Negative F-values, indicating an excess of heterozygotes, were found for REN169O18 (-0.045), AHT137 (-0.069), REN169D01 (-0.071) and other loci. At the same time, positive F-values, indicating a slight heterozygote deficiency, were found at the loci INU005 (0.124), FH2848 (0.129) and REN247M23 (0.140).

HWE assessment in the analyzed sample of Kazakh Tobet dogs showed a deviation from HWE for seven loci (INRA21, AHT137, AHTh260, AHTk253, FH2054, REN162C04 and AHTh171 at P<0.0011) and in the analyzed sample of outbred dogs — for four loci (CXX0279 at P<0.05, INU055 at P<0.05, FH2054 and INU005 at P<0.001).

A comprehensive analysis of the genetic parameters of outbred and Kazakh Tobet dogs (Fig.) revealed relatively similar values for all parameters assessed. At the same time, the Kazakh Tobet population even showed a slightly higher genetic diversity compared to the outbred dogs, despite a relatively small deficit of heterozygotes: the F-fixation index was higher in the Kazakh Tobets (0.034) than in the outbred dogs (0.02).

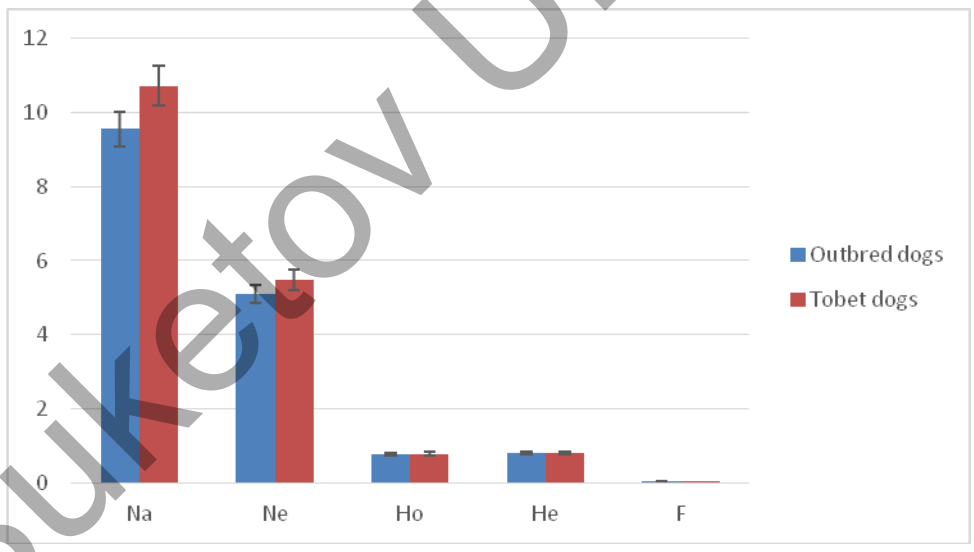


Figure. Comparative analysis of genetic diversity parameters of Kazakh Tobet and outbred dogs

*Discussion*

This study provides the population genetic analysis of the Kazakh Tobet, a traditional LGD breed, based on 18 highly polymorphic microsatellite loci. By analyzing 107 Kazakh Tobet dogs from three regions of Kazakhstan and Mongolia, and comparing them with 55 free-breeding dogs, we assessed the genetic diversity that define the contemporary gene pool of this indigenous breed.

The Kazakh Tobet demonstrated high levels of genetic variability across all metrics analyzed. A total of 193 alleles were identified, and 100 % of loci were polymorphic. The average number of alleles per locus (Na) reached 10.722—substantially higher than values reported for many molossoid and non-molossoid breeds. For example, the Kazakh Tobet parameters were higher than those of the Tibetan Mastiff (Na=7.7,

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panel of 10 STR loci [5]), the in English Bulldog (Na=6.455 and Ne=2.722; panel of 33 STR loci [6], the French Bulldog (Na=5.1; Ne=2.9; panel of 18 STR loci [7]). In addition, the genetic analysis performed on the basis of a panel of 10 STR markers showed lower values of observed heterozygosity (Ho) compared to Kazakh Tobets for such breeds from the molossoid group as Boxers, Staffordshire Bull Terriers and Rottweilers (Ho = 0.51, 0.63 and 0.47, respectively) when analyzing a panel of 15 markers [8], for the Tibetan Mastiff and French Bulldog (Ho = 0.694-0.76, and 0.6077, respectively) when analyzing a panel of 10 markers [9]. However, similar values of over 70 % observed heterozygosity were also found for several non-molossoid dog breeds: for the Korean Dongyonggi (Ho = 0.7266) when analyzing 10 microsatellite loci [10], for the Italian Pointer and the Podenco (Ho = 0.723 and 0.710-0.718) when analyzing a similar panel of 19 microsatellite loci [11], for the Yorkshire Terrier (Ho= 0.73) when analyzing 15 STR markers [8].

In population genetic studies of dog breeds, high observed heterozygosity is often interpreted as an indication of recent admixture, large effective population size or lack of strict reproductive isolation. Indeed, our own control group of outbred dogs exhibited high heterozygosity (Ho = 0.776), very similar to that of the Kazakh Tobet. This supports the conclusion that the Kazakh Tobet dogs are still kept in an open mating system with varying degrees of reproductive isolation.

However, recent genomic studies have shown that LGD breeds worldwide often do not exhibit strict reproductive isolation and show extensive genetic overlap with outbred dogs due to their traditional role in rural and nomadic livestock systems. Dutrow et al. were the first to point out the genetic link between purebred and free-ranging dogs [1]. Coutinho-Lima et al. showed the widespread nature of this relationship within LGD breeds and suggested that reproductive isolation may not be necessary to maintain highly specialized dogs [2]. This has led to the growing consensus that reproductive isolation is not a prerequisite for the preservation of important working traits. Rather, it is cultural and functional selection—based on performance and behavior in the field—that maintains the integrity of LGD populations. In this context, the high levels of heterozygosity observed in Kazakh Tobets are not indicative of breed degradation, but reflect the adaptive diversity maintained by an open breeding system. Similar results have been reported for other traditional LGD breeds such as the Turkish Kangal [12] and the Portuguese Castro Laboreiro dog [13], where genomic analyses revealed an extensive exchange of alleles with local outbred populations. From this perspective, the high genetic diversity observed in Kazakh Tobets should not be seen as a threat to the conservation of the breed, but rather as a sign of resilience and adaptability—traits that are essential for their survival in the harsh steppe and mountainous landscapes of Central Asia.

Although the genetic diversity of the Kazakh Tobet breed was high overall, it was not evenly distributed across all populations. There are clear differences between the four populations studied. The southern population showed the highest genetic diversity, but a slight deficit of heterozygotes indicated low inbreeding (positive F-value). The eastern and northern populations showed moderate diversity, with the former showing an excess of heterozygotes (negative F-values), indicating inbreeding, and the latter showing a slight deficit of heterozygotes. The Mongolian population was characterized by the lowest genetic diversity and the strongest evidence of crossbreeding (the lowest negative F-value).

Taken together, these results position the Kazakh Tobet as a genetically rich and structurally complex LGD breed maintained under conditions of semi-natural selection and open gene flow. They also illustrate a paradox in the conservation of the Kazakh Tobet: while the high genetic diversity and admixture reflect the breed's adaptive success and functional selection history, formal recognition of the breed and long-term conservation require a strategic framework. In the case of the Kazakh Tobet, this does not mean imposing rigid reproductive isolation, but rather implementing a scientifically guided, open breeding system — that supports genetic monitoring, preserves functional traits, and protects against both genetic erosion and uncontrolled hybridization.

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## Қазақ төбет иттерінің генетикалық әртүрлілігін бағалау және оны жабайы иттердің популяциясымен салыстыру

Қазақтың төбеті — Қазақстан аумағында малды қорғау қызметін орындаған дәстүрлі ит тұқымы. Текті тұқымды топтар мен жабайы иттердің популяциялары арасындағы генетикалық әртүрлілігін салыстырмалы талдау тұқымның генофондының құрылымдық популяция белгілеріне сәйкес келетіндігін немесе ұйымдастырылмаған, генетикалық жағынан әр түрлі топтардың сипаттамаларын сақтайтындығын анықтауға мүмкіндік береді. Бұл зерттеу жұмысының негізгі мақсаты — Қазақ төбетінің генетикалық әртүрлілік деңгейін бағалауға және оны жабайы иттердің көрсеткіштерімен салыстыруға бағытталған. Жұмыс аясында Қазақстанның үш өңірінен және Монғолиядан іріктелген төбет тұқымды иттердің 107 түрі, сондай-ақ жабайы иттердің 55 түрі талданды. Генотиптеу 18 полиморфты микросателлиттік локустар бойынша жүргізілді. Генетикалық вариацияның негізгі

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параметрлері есептелді: аллельдердің орташа саны (Na), аллельдердің тиімді саны (Ne), бақыланатын (Ho) және күтілетін гетерозиготалық (He) және фиксация индексі (F). Нәтижесінде жабайы иттермен салыстырғанда (Na = 9.556, Ho = 0.776, He = 0.791) төбет генетикалық әртүрліліктің жоғары деңгейіне ие екенін көрсетті (Na = 10.722, Ho = 0.781, He = 0.805). Зерттеуге қатысқан төбеттің барлық төрт популяциясында жоғары гетерогенділік тіркелді. Бекіту индексінің теріс немесе нөлге жақын мәндері көп жағдайда айқын инбридингтің жоқтығын көрсетеді. Ұсынылған нәтижелер Қазақ төбетінің қатаң репродуктивті оқшаулау болмаған жағдайда қалыптасқан генетикалық әр түрлі және ішкі сараланған тұқым екенін көрсетеді. Мұндай ерекшеліктер тау жыныстарын сақтау мәселелерінде белгілі бір парадокс тудырады: жоғары өзгергіштік және қоспа белгілерінің болуы оның бейімделгіш икемділігі мен функционалдық тұрақтылығын көрсетеді, бірақ сонымен бірге тану мен ұзақ мерзімді сақтаудың стратегиялық тәсілін қажет етеді. Төбет иттерінің жағдайында қатаң репродуктивті оқшаулауды енгізбей, тұрақты генетикалық бақылауды және негізгі тұқымдық белгілерді сақтауды, сонымен қатар генетикалық деградациядан да, бақылаусыз будандастырудан да қорғауды көздейтін ашық тұқымды өсірудің ғылыми негізделген үлгісін жасау дұрыс шешім деп санаймыз.

*Кілт сөздер:* генетикалық профиль, генофонд, генетикалық әртүрлілік, инбридинг, микросателлиттік маркер, популяциялық генетика, төбет тұқымы.

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## Оценка генетического разнообразия собак породы Казахский Тобет и её сравнение с популяциями беспородных собак

Казахский Тобет представляет собой традиционную породу собак, исторически выполнявшую функцию охраны скота на территории Казахстана. Сравнительный анализ генетического разнообразия породных и беспородных собак позволяет установить, соответствует ли генофонд породы признакам структурированной популяции или сохраняет характеристики неорганизованных, генетически разнородных групп. Настоящее исследование было направлено на оценку уровня генетического разнообразия Казахского Тобета и его сопоставление с показателями беспородных собак. В рамках работы были проанализированы 107 образцов собак породы Тобет, отобранных в трёх регионах Казахстана и Монголии, а также 55 образцов беспородных собак. Генотипирование осуществлялось по 18 полиморфным микросателлитным локусам. Были рассчитаны ключевые параметры генетической изменчивости: среднее количество аллелей (Na), эффективное число аллелей (Ne), наблюдаемая (Ho) и ожидаемая гетерозиготность (He), а также индекс фиксации (F). Результаты показали, что Тобет обладает высоким уровнем генетического разнообразия (Na = 10.722, Ho = 0.781, He = 0.805), сравнимым с показателями беспородных собак (Na = 9.556, Ho = 0.776, He = 0.791). Высокая гетерогенность была зафиксирована во всех четырёх популяциях Тобета, участвовавших в исследовании. Отрицательные или близкие к нулю значения индекса фиксации в большинстве случаев свидетельствуют об отсутствии выраженного инбридинга. Представленные результаты свидетельствуют о том, что Казахский Тобет является генетически разнообразной и внутренне дифференцированной породой, формировавшейся в условиях отсутствия строгой репродуктивной изоляции. Такие особенности создают определённый парадокс в вопросах сохранения породы: высокая изменчивость и наличие признаков примеси отражают её адаптивную пластичность и функциональную устойчивость, но одновременно требуют более стратегического подхода к признанию и долгосрочному сохранению. В случае Тобета целесообразным представляется не введение строгой репродуктивной изоляции, а разработка научно обоснованной модели открытого разведения, предусматривающей регулярный генетический мониторинг, сохранение ключевых породных признаков и защиту как от генетической деградации, так и от неконтролируемой гибридизации.

*Ключевые слова:* генетический профиль, генофонд, генетическое разнообразие, инбридинг, микросателлитный маркер, популяционная генетика, порода Тобет.

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