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REINDEER POPULATION AS AN INDICATOR OF SUSTAINABLE DEVELOPMENT OF THE ARCTIC TERRITORY

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ABSTRACT

The objectives of this study are to carry out genetic monitoring of domestic reindeer of Nenets breed raised on the territory of Taimyr; establishment of the genetic bank of domestic reindeer of Nenets breed; as well as a comparative analysis with populations of the Nenets breed from other breeding regions (Nenets and Yamalo-Nenets Autonomous Okrug, Murmansk Oblast and the Komi Republic) using DNA markers. The microsatellites were used in the studies as DNA markers of genetic diversity. Genotyping was carried out by the methods of the Laboratory of molecular selection basis of L.K. Ernst Federal Research Center for Animal Husbandry. Statistical data processing was performed using the GenAlEx 6.5, SplitsTree 4.14.5 software, as well as the software packages “diveRsity”, “pophelper”, and “adegenet” for the R computing environment. As a result of the study, it was found that the population of reindeer of Taimyr breed in the village of Tukhard is characterised by the minimum level of polymorphism and genetic diversity, as indicated by the minimum values of all population genetic indicators. The authors assume that the patterns that have been identified are primarily the result of breeding programmes on the samples presented in this study (such studies have not been conducted before). The results of the PCA analysis and the phylogenetic dendrogram of genetic relationships, built on the basis of the matrix of Jost's D pairwise genetic distances using the NeighborNet, revealed a similar genetic structure of Taimyr populations, which, most likely, could be due to the close geographical localisation of the studied individuals. The authors come to the conclusion that a low level of both allelic and genetic diversity in the future may negatively affect the adaptation of animals to environmental conditions.

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INTRODUCTION

Domestic reindeer husbandry and hunting for wild reindeer in Taimyr are the main activities of daily living of the five small indigenous northern peoples—Dolgans, Nenets, Nganasans, Evenks, and Enets. Under the influence of anthropogenic, technogenic, pyrogenic and other factors of external influence on the ecology of the Taimyr Peninsula, there is a decrease in livestock

production, in particular, reindeer herding. Industrial concerns PJSC MMC Norilsk Nickel, PJSC Norilskgazprom, JSC Taimyrgaz and others operating in the region, whose activities have a negative impact on the habitat of Taimyr's reindeer. The economic transformations in Taimyr, which took place in the late 19th century – early 20th century, led to a crisis in the branches of traditional nature management of the

indigenous inhabitants of the Taimyr Peninsula: hunting, fishing, including reindeer husbandry. One of the ways to identify the negative impact of the anthropogenic factor on the fauna of the peninsula is to carry out genetic studies of Taimyr animals, the results of which, together with the results of research in the field of ecology, economics and other sciences, will help improve the natural and economic conditions of reindeer farms to preserve reindeer husbandry and increase the production of reindeer meat.

At present, due to unfavourable natural and anthropogenic factors, there is a sharp decline in the number of both domestic and wild reindeer, which leads to the loss of genetic diversity necessary for survival in new habitats (Stolpovsky, 2010). The most abundant native breed of domesticated reindeer (*Rangifer tarandus*) is the Nenets breed. It is one of the four officially recognised domesticated reindeer breeds in the Russian Federation. It is bred in the Taimyr (Dolgano-Nenets) Autonomous Okrug, the Murmansk Oblast (Kola Peninsula), the Nenets Autonomous Okrug (AO), the Komi Republic, the Yamalo-Nenets and Khanty-Mansiysk Autonomous Okrugs. Breeding of domestic reindeer in the regions has its own characteristics, which are conditioned by the difference in the geography, the specificity of the fodder base and the ethnocultural component (Kharzinova *et al.*, 2017).

The Taimyrsky district of the Krasnoyarsk Krai is the world's largest section of tundra – from the Putorana plateau in the south to the Kara Sea and the Laptev Sea in the north (Belikov and Kupriyanov, 1985). Until the first half of the 20th century, the indigenous ethnic inhabitants of the Taimyr Peninsula were actively engaged in reindeer husbandry (Yuzhakov, 2017a, 2017b). Due to the crisis in domestic reindeer husbandry, the Dolgans and Nganasans almost completely switched to the wild reindeer hunting. The further development of reindeer husbandry and an increase in the productivity of animals must be associated with the improvement of breeding programmes through modern methods of selection and breeding based on the use of molecular genetic methods.

METHODOLOGY

Despite some similarities in the tasks of preserving the genetic diversity of domesticated reindeer, as well as preserving the genetic diversity of wild species, there are differences associated, first of all, with the different

nature of variability of wild and domestic animals. Populations of wild animals, as a rule, are outwardly similar to each other, morphologically homogeneous; but they are highly polymorphic in biochemical and physiological characteristics. This polymorphism not only provides the possibility of further evolution of the species, but also serves as a condition for its ability to adapt to changing environmental conditions (Moiseeva *et al.*, 2006), which under the influence of humans has changed very much

Currently, one of the tasks of studying the population of the Taimyr breed of reindeer is to investigate their genetic characteristics. Studies of the genetic variability of reindeer in Taimyr were carried out using methods corresponding to the stages of genetics development. Until the middle of the 20th century genetic changes was taken into account by the number of gene mutations with a visible morphological manifestation. Since the 1950s, the detection of antigenic properties of blood in various types of farm animals has been widely used. In the 1960s the method of gel electrophoresis began to be used in population genetics. With the improvement of methods for studying the variability of traits, more subtle molecular genetic methods began to be used (Bazelyanskaya, 2010).

Genetic studies of blood groups in the reindeer husbandry were associated with the development of scientifically grounded methods of stock breeding to increase the fodder base. The first studies in Taimyr were carried out by Savaderova (1983): introduction of immunogenetic methods into stock breeding of reindeer, study of the antigenic properties of blood in domesticated reindeer. The work was carried out to establish the genetic status of breeds and offsprings of domestic reindeer. Among the goals and objectives set were the following: differentiation of domesticated reindeer in line breeding, identification of related groups, control of the genealogy of young stock improves the productivity of animals. As a result of the study, a high frequency of occurrence of antigenic factors to antibodies of A1, Z, V sera specific to cattle blood was determined. At the current stage of research, it was possible to give the low-down on the antigenic composition of the reindeer blood of the Potapovsky domestic breed.

During further research, it was found that domestic and wild reindeer have their own gene pool and evolve as relatively independent units (Shubin and Efimtseva,

1988). With the introduction of new methods of genetic research, work was carried out to determine the genetic lines of the Taimyr wild reindeer (Kolpashchikov *et al.*, 2007), as well as to study the genetic variability of individual groups of the Taimyr wild reindeer population (Kuznetsova and Kolpashchikov., 2007). The results of this work show that intensive genetic exchange takes place between groups of reindeer in different regions.

RESULTS AND DISCUSSION

The aim of the study was to characterise the genetic diversity of the Taimyr population of the domestic Nenets reindeer breed in the village of Tukhard and the village of Khatanga, as well as a comparative analysis with the populations of the Nenets breed in other breeding regions (Nenets and Yamalo-Nenets Autonomous Okrug, Murmansk Oblast and the Komi Republic) and the possibility of defining further research tasks.

When the method of ISSR analysis was developed, domestic deer of the Tyva Republic (Kol, 2007) and wild deer of Taimyr herds were studied. When studying samples of wild reindeer from Western Taimyr using ISSR markers, it was found that the migratory flow of reindeer in this area is made up of animals of two genotypes (Bazelyanskaya, 2016). When analysing the polymorphism of the control region of mitochondrial DNA in samples of wild deer from Taimyr, a high level of haplotype (H) and nucleotide (R) diversity (0.987 and 0.018, respectively) was revealed, indicating a long-term existence of this population with a fairly stable growth (Kholodova *et al.*, 2011). Further use of new methods for genetic studies of samples of domesticated reindeer from Taimyr made it possible to reveal a high level of inbreeding in herds, the number of which is less than 500 head. A number of polymorphic protein systems of the blood have been discovered and their genetic determination has been studied (Goncharov *et al.*, 2009). At the present stage, genetic studies of reindeer to preserve the population and increase productivity are carried out in different regions of Russia: (Yuzhakov, 2017a, 2017b), Kharzinova *et al.* (2017) and others. Genetic monitoring of animals is carried out in order to preserve biodiversity – (Stolpovsky, 2010b). At the L. K. Ernst Federal Research Centre for Animal Husbandry, the work was carried out to introduce the method of artificial insemination of reindeer, to develop a

technology for cryopreservation of reindeer semen and to create a cryobank of sperm (Study of physiological and biochemical changes, 2019). But in Taimyr, no work has yet been carried out on the genetic monitoring of reindeer, the creation of a genetic database. This work is yet to be done to study the genetic diversity of populations domesticated wild reindeer. Similar work on the creation of a genetic databank for the Nenets breed in domestic herds was carried out under experimental conditions in 2016 by the staff of the Naryan-Mar agricultural enterprise (Romanenko and Filippova., 2016). Creation of a genetic bank of the Taimyr reindeer breed will allow preserving the material, ensuring its availability and multipurpose use by specialists from different scientific fields.

Earlier, an opinion was expressed about the need to preserve the animals of the island population of wild reindeer inhabiting the Severnaya Zemlya archipelago (Belikov and Kupriyanov, 1985). The disappearance of this population will significantly impoverish the species gene pool, since this is the only wild reindeer population in the Eurasian Arctic adapted to live in the Arctic desert. Research on the genetic characteristics of this group of animals has yet to be carried out. The further development of reindeer husbandry, an increase in the productivity of animals both in Taimyr and in other territories engaged in reindeer husbandry, must be associated with the improvement of breeding programmes through modern methods of selection and breeding based on the use of molecular genetics. One way to genetically characterise reindeer is through the use of microsatellite markers. Such genetic studies will fill the gap in the study of Taimyr reindeer.

Maintaining the optimal degree of genetic variability and heterozygosity in populations is necessary to preserve the ability of animals to adapt to environmental conditions (climate change, negative effects of harmful substances). High genetic diversity ensures the evolutionary fitness of animals (Dubinin and Mashurov., 1986; Kuznetsova, 2010). The degree of genetic variation between and within populations correlates with their demographic history, as well as with environmental factors (Hamrick and Godt, 1990). Information on the genetic structure of animal populations not only makes it possible to assess the importance of elementary factors of evolution (selection, mutation, migration, genetic drift) under stressful conditions, but is also important for the recovery and

rational use of the species (Peakall and Smouse, 2012). Tissue samples (slices from the horns and skin) of 78 reindeer of the Taimyr population of the Nenets breed were used as a biological material for research. They were contained in the village of Tukhard (TUCH, n = 31) and the village of Khatanga (HAT, n = 47) of the Taimyr Dolgano-Nenets district of Krasnoyarsk Krai. For a comparative analysis of the populations of the Nenets reindeer breed, the studied sample included other breeding regions for the collection of tissue and DNA for the “Bank of genetic material of animals and birds” of the L.K. Ernst Federal Research Center for Animal Husbandry, including the Nenets Autonomous Okrug (NAO, n = 31) and the Yamalo-Nenets Autonomous Okrug (YNAO, n = 56), the Murmansk Oblast (MUR, n = 43) and the Komi Republic (n = 24). DNA isolation was performed using kits for isolation of genomic DNA “DNA-Extran” (CJSC Syntol, Russia), in accordance with the manufacturer's protocol. Analysis of polymorphism of nine STR-loci (NVHRT21, NVHRT24, NVHRT76, RT1, RT6, RT7, RT9, RT27, RT30) was carried out by the author's own methods (Kharzinova *et al.*, 2015). To characterise allelic and genetic diversity in the GenAlEx 6.5 software (Peakall and Smouse, 2012), the

average number of alleles (NA) and the effective number of alleles (NE) per locus were determined. Allelic diversity calculated using the ratification procedure (AR), observed (HO) and expected (HE) heterozygosity and inbreeding coefficient (FIS) were calculated in the “diveRsity” package of the R statistical environment, followed by visualisation in the “pophelper” package (Keenan *et al.*, 2013). Principal Component Analysis (PCA) was performed using the “adeget” package (Jombart, 2008) with visualisation “ggplot2” package (Wickham, 2009). The degree of genetic differentiation of the studied reindeer breeds was assessed based on pairwise D_{Jost} values (Jost, 2008). Matrix of pairwise values Jost's D was used to construct a phylogenetic tree using the NeighborNet algorithm in the SplitsTree 4.14.5 software (Huson and Bryant, 2005).

Based on the obtained microsatellite profiles of the entire studied sample of reindeer, the main parameters of allelic and genetic diversity were calculated. The results obtained are shown in Figure 1 and Table 1. All studied groups of reindeer were characterised by the predominance of expected heterozygosity over the observed one, which may indicate inbreeding in the studied animal samples.

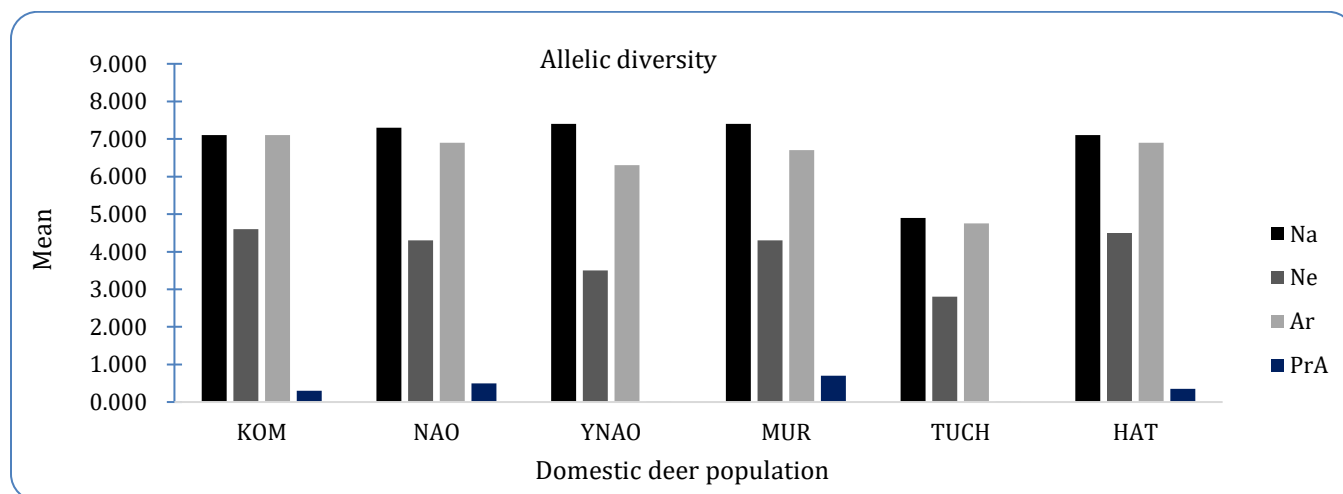


Figure 1. Allelic diversity of the Taimyr populations of domesticated reindeer in comparison with other regional populations of the Nenets breed.

Note: NA – the average number of alleles per locus; NE – the average number of effective alleles per locus; AR – allelic diversity, PR – the number of private alleles per locus; For decoding of abbreviations of populations see materials and research methods.

Principal Component Analysis (PCA) was used to determine sample variability. Its feature is the ability to project samples onto orthogonal coordination axes, each

of which consists of a linear combination of allelic or genotypic values (Novembre *et al.*, 2008). The results of the PCA analysis, which made it possible to assess the

range of variability and to trace the main patterns of population genetic differentiation of individuals of the

studied Taimyr populations of reindeer from the comparison groups as given in Figure 2 (Baskin, 2000).

Table 1. Parameters of genetic diversity of the Taimyr populations of domesticated reindeer with other regional populations of the Nenets breed.

Population	n	HO	HE	FIS (FIS 95% CI > 0)
KOM	24	0.560±0.057	0.751±0.036	0.256[0.14;0.372]
NAO	31	0.570±0.05	0.741±0.027	0.229[0.112;0.346]
YNAO	56	0.522±0.1	0.623±0.08	0.135[-0.082;0.352]
MUR	43	0.556±0.079	0.681±0.088	0.181[0.065;0.295]
TUCH	31	0.437±0.046	0.577±0.051	0.211[0.022;0.398]
HAT	47	0.510±0.073	0.751±0.024	0.330[0.162;0.498]

Note: n=animal units; HO – observed heterozygosity; HE – expected heterozygosity; FIS – inbreeding coefficient with a 95% confidence interval.

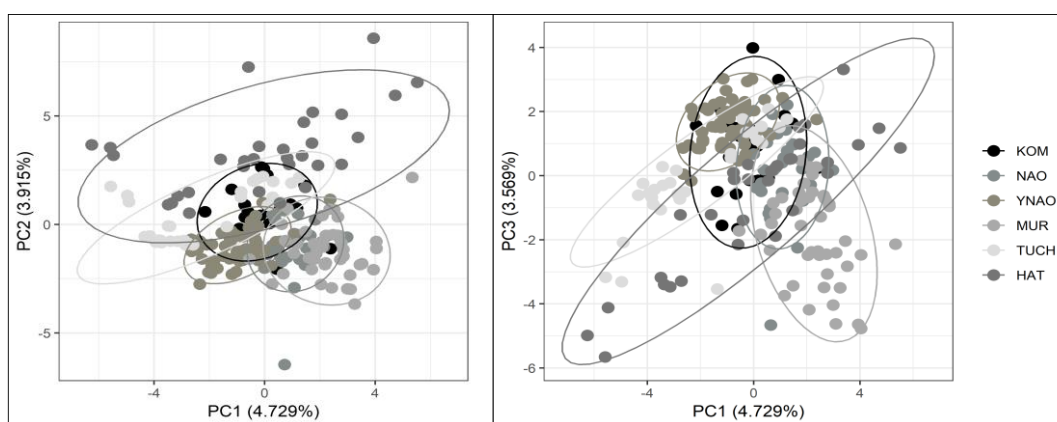


Figure 2. Projection of individuals of Taimyr populations of domesticated reindeer with other regional populations of the Nenets breed on the plane of two coordinates according to PCA analysis.

Note: For decoding of abbreviations of populations, see materials and methods.

For a visual representation of the genetic relationship of domesticated deer in the village Tukhard and Khatanga, with other regional populations of the Nenets breed, the pairwise genetic distances of the D index was calculated

(Jost, 2008), which takes into account the proportion of allelic variations in the populations. Visualisation of this numerical matrix using the NeighborNet algorithm is shown in Figure 3.

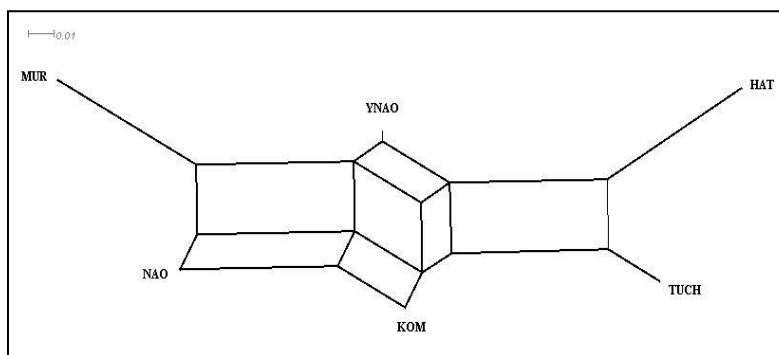


Figure 3. Phylogenetic dendrogram of the genetic relationships of the Taimyr populations of domesticated reindeer with other regional populations of the Nenets breed, built on the basis of the matrix of pairwise genetic distances of Jost's D using the NeighborNet algorithm

Note: For decoding of abbreviations of populations, see materials and methods.

Figure 1 shows that the Taimyr population of Tukhard village had the minimum values of all parameters of allelic diversity. Reindeer from Khatanga village had similar values in the average number of alleles and the number of effective alleles per locus. At the same time, this group surpassed the YNAO, MUR, and NAO reindeer populations in allelic diversity calculated using the ratification procedure ($AR=0.333\pm 0.167$ versus 6.158 ± 0.768 , 6.711 ± 0.928 , and 6.931 ± 0.472 , respectively). Private alleles were detected only in the Khatanga population, which averaged 0.333 ± 0.167 . According to this indicator, HAT exceeded KOM ($PR=0.222\pm 0.147$) and was inferior to NAO (0.556 ± 0.242) and MUR (0.778 ± 0.324). According to the parameters of genetic diversity (Table 1), Tukhard population had minimal values of both observed and expected heterozygosity. In turn, the Khatanga reindeer, in terms of the observed heterozygosity, had values similar to all other groups of the Nenets breed (Sheng *et al.*, 2005).

Despite the fact that Figure 2 clearly demonstrates the presence of strongly overlapping arrays of the studied samples of domesticated reindeer, which indicates a high degree of similarity in the genetic structure of all the samples studied, due to their belonging to the same breed, the first component (PC1) revealed two clusters formed by groups of reindeer of the Nenets Autonomous Okrug and the Murmansk Oblast, and deer of all other groups. At the same time, the reindeer populations of Tukhard and Khatangi villages were located most closely to each other (Altukhov, 1989). In turn, the second component (PC2) separated the Khatanga reindeer, and the third component (PC3) the Yamal reindeer from the rest of the animal population. Figure 3. Shows the results of the phylogenetic dendrogram of the genetic relationships of the Taimyr populations of domesticated reindeer. They are consistent with the projection of the studied individuals on the plane of two coordinates according to the PCA analysis: representatives of the Taimyr deer settled down on one side of the net and formed one cluster. At the same time, the Khatanga deer were located more distant from the general cluster of the studied animals.

CONCLUSIONS

Thus, as a result of the analysis of the polymorphism of nine microsatellites, a characteristic of the genetic diversity of two Taimyr populations of the Nenets

domestic reindeer bred in the Taimyr Dolgano-Nenets Okrug of the Krasnoyarsk Krai was given, as well as a comparative analysis with the populations of the Nenets breed from other breeding regions (Nenets and Yamalo Nenets Autonomous Okrug, Murmansk Oblast and the Komi Republic). The nature of the identified genetic links is most likely due to the geographical localisation of the studied individuals of the Taimyr populations, revealing their more similar genetic structure. The reindeer of Tukhard village are characterised by a minimum level of both allelic and genetic diversity, a further decrease in which can destroy local adaptations and coadapted gene complexes, which ultimately will affect the population's resistance to adverse environmental conditions. Particular attention should be paid to the maximum positive values of the inbreeding coefficient in the sample of deer from the Khatangi village. Low genetic variability does not allow to adapt to habitat conditions, which are constantly deteriorating. Therefore, comprehensive measures are needed to improve the environmental situation, covering different areas of activity: ecology, production, economics, etc. The information obtained is recommended to be used to implement programs to improve selection and breeding work in the reindeer husbandry industry in the Tukhard and Khatanga villages. Also take into account when conducting genetic monitoring and compiling a DNA genetic bank of the Taimyr population of domestic and wild reindeer.

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