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STUDY OF PHENOTYPIC AND GENOTYPIC FEATURES OF REINDEER POPULATIONS OF THE NENETS BREED

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Abstract

Reindeer herding is the leading branch of animal husbandry in the Nenets Autonomous Okrug (NAO). Currently, reindeer herding is being conducted mainly by traditional methods, which are based on the assessment of the phenotype. For further development of reindeer herding and the increase of its productivity, it is necessary to improve the level of breeding work by using molecular genetic information. The aim of our study was to investigate the phenotypic and genetic features of the reindeer Nenets breed in the conditions of the NAO. To examine morphological features, the measurements of the exterior characteristics (height at withers, chest depth, chest width, chest girth, wrist girth, body length, loin width, head length, body weight) were made at three farms of the NAO: ERV (ERV, n = 28), Indiga (IND, n = 34) and Ilebts (ILB, n = 25). Based on the data obtained, the body built indices were calculated: massiveness, blockiness, bone, chest index, lengthiness and head. For genetic characteristics, the analysis of nine microsatellite (NVHRT76, RT9, NVHRT24, RT30, RT1, RT6, RT27, NV21, RT7) loci was carried out. Statistical processing of data was performed using the R software and R packages adegenet and diveRsity. For most of the exterior features, a significant advantage of IND and ILB over ERV was observed, respectively: height at withers — 99.2±0.42, 99.7±0.53 and 97.0±0.46 cm ($p < 0.01$); chest depth — 43.4±0.31, 42.6±0.32 and 43.7±0.46 cm; chest width — 27.7±0.35, 27.5±0.45 and 26.6±0.27 cm; chest girth — 125.6±0.69, 126.6±0.80 and 119.0±0.77 cm ($p < 0.01$); body length — 108.3±0.54, 107.7±0.81 and 102.2±0.32 cm ($p < 0.01$); wrist girth — 11.8±0.09, 11.9±0.11 and 11.21±0.05 cm; head length — 33.3±0.38, 33.9±0.51 and 32.1±0.18 cm; body weight is 104.6±1.17, 106.4±1.24 and 83.5±1.08 cm ($p < 0.01$). IND and ILB also exceeded ERV ($p < 0.01$) in massiveness, chest and lengthiness indices. As compared to the indicators of the 1970s, there was an increase in massiveness and chest index in IND and ILB, and bone and lengthiness in all the three groups studied. According to the results of principal component analysis (PCA), the similarity between IND and ILB was observed for both the morphological and genetic characteristics, which was manifested in the formation of overlapping arrays on the PCA-plot, while the ERV formed a relatively isolated cluster. Pairwise genetic distances confirmed the greater similarity between IND and ILB (F_{st} — 0.018 and D_{Jost} — 0.017). All the three populations were characterized by a deficit of heterozygotes ($F_{is} > 0$, CI 95 %). Allelic richness (A_r) varied from 6.17±0.499 (IND) to 6.78±0.494 (ILB). Thus, it was shown that the reindeer populations that are bred in the NAO have morphological and genetic differences. In addition, the populations with different morphological characteristics were not close genetically. Morphological features in the studied populations were within the breed standards, or exceeded them.

Keywords: reindeer breeding, morphological features, genetic diversity, microsatellites

Reindeer herding is the leading branch of animal husbandry in the northern regions of the Russian Federation. Indigenous peoples use reindeer for food, clothing and transport under harsh climatic conditions, to which other species of farm animals are not adopted, [1]. One of the leading places in the domestic reindeer population is the Nenets Autonomous Okrug (NAO). According to the pedigree records of January 1, 2015, about 170 thousand deer were bred in 23 farms [2] in the territory of the NAO. During economic reforms and intensive industrial development of the Far North territory, the Nenets saved reindeer husbandry [3]. By selective breeding, they created a large group of domestic reindeers with clearly expressed morphological and economically useful traits that animals steadily pass to their progeny. In 1985, the Nenets breed, among the other reindeer breeds was recorded in the State Register of livestock breeds, USSR. The deer of this breed are characterized by strong body built and medium size. This is the largest breed population of domestic reindeers on the North of European Russia and above the Urals, in the lower of Ob' and Yenisei rivers. Since the 1930s, massive zootechnical studies of Nenets deer in the Western Siberia resulted in improved animal productivity and morphometric parameters and exterior characteristics [4].

Unlike the other branches of livestock husbandry, pedigree breeding of reindeers is carried out mainly by traditional methods, which are based on visual estimates of productive, primarily meat, qualities and allow indirect assessment of the reproductive system. For a long time, the estimated of deer variety within and between populations was based on a phenotypic evaluation of animal morphological features characterizing their shape and appearance [5]. However, it is not enough for studing a population.

Development of reindeer herding and an increase in animal productivity are associated with the improvement of breeding work through modern selection and breeding methods. One of them is the use of microsatellite sequences, also known as short tandem repeat (STR), for genetic characterization of reindeers. Microsatellites are highly polymorphic, evenly dispersed throughout a genome and characterized by Mendelian inheritance. They are widely used in validation and control of animal origin, in estimation of the degree of inbreeding, breed purity, biodiversity, genetic differentiation and genetic structure of breeds of the main species of farm animals [6-10]. Microsatellite markers have been successfully used in studying genetic diversity [11, 12] and introgression of domestic and wild reindeer populations [13].

This is the first principal component analysis of Nenets breed reindeers in the territory of the NAO based on morphological and genetic data. The findings showed the presence of at least two groups in Nenets breed, which was also confirmed by comparing pairwise genetic distances among research populations.

The objective of our study was to investigate the phenotypic and genetic features of the reindeer (*Rangifer tarandus*) Nenets breed in the conditions of the Nenets Autonomous Okrug.

Techniques. Exterior features of the Nenets does reindeer aged 3.5-8.5 years were recorded in three farms of the NAO in 2016, i.e. APK Cooperative Farm ERV (ERV population, n = 28), Indiga (IND, n = 34), Ilebts (ILB, n = 25). Height at withers, chest depth and chest width were assessed using a Leidten measuring stick; chest girth, wrist girth, body length were estimated using measuring tape; loin width and head length were measured with the Wilkins' compass; body weight was recorded by weighting using a dynamometer DPU-5-2 for 500 kg (LLC Plant Testing Devices, Russia). Body mass index were calculated including massiveness, blockiness, boniness, chest index, lengthiness, big-headedness. The data obtained during inspection of three reindeer herding farms were summed up by age groups and the average values were calculated. For comparison, body indexes of the Nenets breed recorded in the 1970s, were used [14].

For genetic characterization, DNA was isolated for ear tissue samples using Nexttec™ 1-step DNA Isolation columns (Nexttec Biotechnologie GmbH, Germany) as per manufacturer's recommendations. Polymorphism of 9 microsatellite loci (NVHRT76, RT9, NVHRT24, RT30, RT1, RT6, RT27, NV21, RT7) was assessed on DNA analyzer ABI3130xl (Applied Biosystems, USA) according to the previously developed protocol [15]. Allele sizes were determined in GeneMapper 4.0 software (Applied Biosystems, USA) followed by converting to numerical expressions, based of which the genotype matrix was formed in Microsoft Excel format. As control group for access of genetic diversity, wild reindeer samples (n = 32) collected in the course of expeditions to Western Taimyr were used.

Statistical data processing was carried out using R 3.3.3 software [16]. The calculation of indicators for Principal Component Analysis (PCA) based on microsatellite sequences was carried out with R adegenet software [17]. To visualize the results, the R ggplot2 package was used [18]. The expected (H_e) and observed (H_o) heterozygosity, inbreeding coefficient (F_{is}), allelic diversity (A_r) and genetic distances were calculated using R diveRsity [19]. Also, using this package, the degree of genetic populations differentiation was estimated based on the pairwise values of F_{st} [20] and D_{Jost} [21]. In the tables and text, the arithmetical mean values (M), standard errors (±SEM), and coefficients of variation (Cv) are given.

Results. Comparing the exteriors (Table 1), it can be noted that the ILB and IND reindeer-doe exceed the ERV reindeer-doe in height at withers by 2,75 and 2,27 cm ($p < 0.05$), respectively. The difference between IND and ILB reindeer-does was unreliable. All values comply with the breed standard of 94.5-100.5 cm [4]. In the studied reindeer-doe groups, the chest depth did not have significant differences. The chest width of IND was 1.06 cm more than that of ERV ($p < 0.05$). The IND and ILB reindeer-doe chest girth behind the shoulder was larger than that in ERV by 6.61 and 7.62 cm ($p < 0.01$), respectively. A similar trend was observed in the body length value, which was higher for IND and ILB than for ERV by 6.11 and 5.53 cm ($p < 0.01$), respectively. The last two indices for ERV were within the norm, while IND and ILB exceeded the breed standards for the chest width (114.5-124.0 cm) and the body length (99.0-107.0 cm) [4]. Loin width estimates were available only for IND and ILB and differed not reliably. In three farms, the wrist girth values ranged from 11.21±0.05 to 11.88±0.11 cm that complied with the breed standard (10.0-12.0 cm). Differences between IND and ERV ($p < 0.01$), and also between ILB and ERV ($p < 0.01$) were statistically significant. The head length of reindeer does in ERV population was less than that of IND and ILB ($p < 0.01$). ILB and IND does were 22.94 and 21.16 kg heavier ($p < 0.01$), respectively, compared to ERV animals thus showed superiority in body weight. The difference in body weight of IND and ILB animals (1.78 kg) was not statistically significant.

Body built indexes gave a better understanding of the exterior (Table 2). A higher animal massiveness in IND and ILB populations compared to ERV ($p < 0.01$) testified for the better torso development. This parameter of ERV animals was comparable to NAO data of the 1970s (NEN'70) (see Table 2). Blockiness is a convenient indicator for body weight assessment. Its differences among reindeer does from surveyed farms were not statistically significant ($p < 0.05$) and did not differ from the average for NEN'70. Boniness in ERV population was lower ($p < 0.05$) compared to IND and ILB, that pointed at more coarse skeleton. It should be noted that this index exceeded the value of NEN'70 in all tested breeding population. The chest index, reflecting a degree of animal chest development was higher in IND and ILB than in ERV, but significant differences were found only between ILB and ERV ($p < 0.05$). It should be noted that this index in

ERV was lower than that of NEN'70. The stretch index of IND and ILB also exceeded that of ERV (at $p < 0.01$ and $p < 0.05$, respectively). The head length indexes of studied populations differed not significantly.

1. Comparative estimates of exterior and body weight of reindeer (*Rangifer tarandus*) Nenets does aged 3.5-8.5 years (Nenets Autonomous Okrug, 2016)

Population	*n*	*M*±SEM	*Cv*, %	min-max	Range of variability
			Height at withers, cm		
ERV	28	96.97±0.46	2.5	93.0-104.0	11.0
ILB	25	99.72±0.53	2.7	95.0-106.0	11.0
IND	34	99.24±0.42	2.5	95.0-106.0	11.0
			Chest depth, cm		
ERV	28	43.66±0.46	2.6	41.7-46.3	4.6
ILB	25	42.64±0.32	3.8	38.0-46.5	8.5
IND	34	43.35±0.31	4.2	39.0-47.0	8.0
			Chest width, cm		
ERV	28	26.59±0.27	5.3	24.0-29.6	5.6
ILB	25	27.54±0.45	8.1	22.0-34.0	12.0
IND	34	27.65±0.35	7.4	22.0-31.0	9.0
			Chest girth, cm		
ERV	28	118.96±0.77	3.4	110.5-126.0	15.5
ILB	25	126.58±0.80	3.2	120.0-134.0	14.0
IND	34	125.57±0.69	3.2	118.0-134.0	16.0
			Body length, cm		
ERV	28	102.21±0.32	1.7	99.0-106.0	7.0
ILB	25	107.74±0.81	3.8	102.0-117.0	15.0
IND	34	108.32±0.54	2.9	102.0-114.0	12.0
			Loin width, cm		
ERV	–	–	–	–	–
ILB	25	11.88±0.20	8.4	10.0-15.0	5.0
IND	34	12.07±0.20	10.0	9.0-14.0	5.0
			Wrist girth, cm		
ERV	28	11.21±0.05	2.6	11.0-12.0	1.0
ILB	25	11.88±0.11	4.6	11.0-13.0	2.0
IND	34	11.75±0.09	4.2	11.0-13.0	2.0
			Head length, cm		
ERV	28	32.12±0.18	2.9	31.0-34.0	3.0
ILB	25	33.86±0.51	7.5	30.0-39.0	9.0
IND	34	33.25±0.38	6.6	28.0-39.0	11.0
			Bogy weight, kg		
ERV	28	83.46±1.08	6.8	75.0-107.0	32.0
ILB	25	106.40±1.24	5.8	96.0-121.0	25.0
IND	34	104.62±1.17	6.5	88.0-121.0	33.0

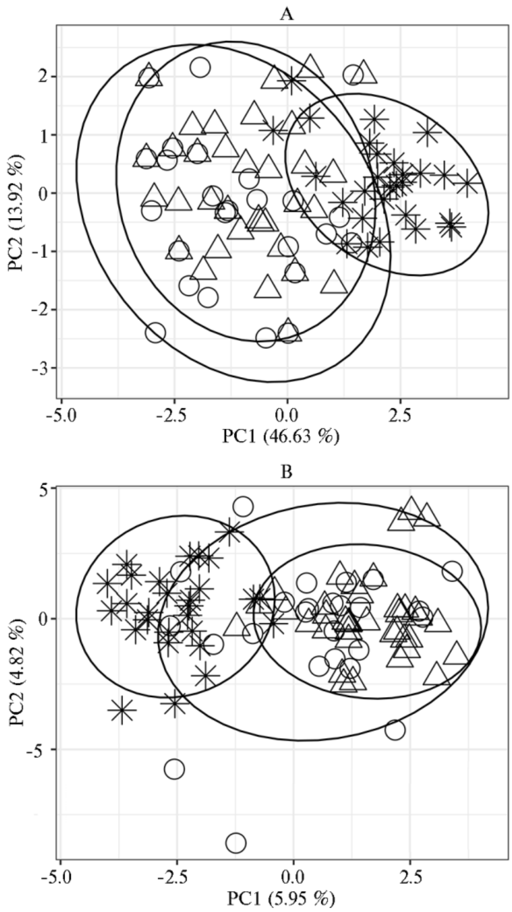
Note. ERV, IND and ILB — populations of farms ERV, Indiga and Ilbets; *n* — sample size, *M* — arithmetic mean, SEM — standard error, *Cv* — coefficient of variability, min-max — min and max values. Dash means data absence.

2. A comparison of exterior and body weight in current populations of reindeer (*Rangifer tarandus*) Nenets does aged 3.5-8.5 years and those of 1970th (Nenets Autonomous Okrug, 2016)

Body index	ERV (*M*±SEM)	ILB (*M*±SEM)	IND (*M*±SEM)	NEN'70 (*M*)	Breed standard [4], %
Massiveness	122.74±0.91	126.97±0.73	126.57±0.67	123.0	118.7-123.8
Blockness	116.40±0.73	117.59±0.88	115.99±0.67	117.7	–
Boneness	11.57±0.06	11.91±0.10	11.84±0.08	10.8	10.8-12.1
Chest index	60.96±0.77	64.67±1.12	63.86±0.88	62.8	59.4-65.5
Lengthiness	105.46±0.50	108.08±0.83	109.20±0.60	103.9	–
Head length	33.14±0.21	33.98±0.53	33.51±0.38	34.4	–

Note. ERV, IND and ILB — current populations of farms ERV, Indiga and Ilbets; NEN'70 — average data for NAO in the 1970th [4]. *M* — arithmetic mean, SEM — standard error. Dash means data absence

Based on the comparative analysis of morphological variability of the does from three farms (Fig. 1, A), it was established that the IND and ILB animals were in the same cluster, that is, had similar exteriors. The most of ERV does were clustered separately of the above-mentioned populations, indicating a different morphological type. Genetic analysis by microsatellites (see Fig. 1, B) showed similar results. The IND and ILB animals formed a common array, while the ERV does formed a relatively isolated cluster. PC1 and PC2 were responsible for 5.95 % and 4.82 % of the genotypic variability, respectively. In general, genetic analysis revealed a greater difference of ERV from other groups than morphological analysis.



Principal component analyses (PCA) **of morphological** (A) **and genetic** (B) parameters of **raindeer** (***Rangifer tarandus***) **Nenets does aged 3.5-8.5 years:** △ — population of Indiga farm (IND), ○ — **population of Ilbets farm** (ILB), ✳ — **population of ERV farm** (ERV). Circles mark 95 % confidence interval of clussterisation (Nenets Autonomous Okrug, 2016)

Calculation of pairwise F_{st} values (Table 3) showed the lowest genetic distance of 0.018 ($p > 0.05$) between IND and ILB. The difference between ERV and the other two populations was more significant, 0.101 for IND and 0.056 for ILB. When comparing farm populations to wild animals, ERV (0.041) and ILB (0.042) populations were the closest to the latter. IND was characterized by a greater remoteness from the wild form (0.078).

The F_{st} values are widely used and applicable for comparing findings reported by other researchers. However, recent studies have shown a limited use of this indicator to assess the degree of differentiation between populations with high intrapopulation heterozygosity [23], including reindeer populations [24]. Calculation of D_{Jost} pairwise values is the most suitable method for such purposes [21].

For domestic populations, the D_{Jost} values were similar to the pairwise F_{st} values, but for the wild population D_{Jost} was higher. While F_{st} indicated that genetically IND and ILB are closer to the wild population than to ERV, the D_{Jost} showed that the distance between ERV and other domestic populations is less than that of wild animals.

The indices of the observed heterozygosity of three populations did not differ significantly from each other ($p < 0.05$) and were in the range from 0.551±0.046 for ILB to 0.567±0.043 for IND (Table 4). Also, significant differences for the expected heterozygosity were not observed. The inbreeding rate (F_{is}) in all studied groups was positive ($p < 0.05$), which indicates a lack of heterozygotes in the populations. Perhaps

3. Indicators of pairwise genetic distances F_{st} and D_{Jos} for domestic reindeer (*Rangifer tarandus*) Nenets does (Nenets Autonomous Okrug, 2016)

Population	ERV	ILB	IND	WLD
ERV	0,000	0,054	0,107	0,060
ILB	0,056	0,000	0,017	0,116
IND	0,101	0,018	0,000	0,187
WLD	0,041	0,042	0,078	0,000

N o t e. ERV, IND and ILB — current populations of farms ERV, Indiga and Ilbets, WLD — wild population. F_{st} is given in the low table part. D_{Jost} is in the upper part.

this is the result of target selection and limited exchange of genetic material with other herds. The indices of allelic diversity varied from 6.173±0.499 for IND to 6.788±0.494 for ILB and did not differ significantly. Compared to the wild population, domestic reindeer were characterized by lower heterozygosity and allelic diversity.

4. Indicators of genetic diversity of reindeer (*Rangifer tarandus*) Nenets does breed (Nenets Autonomous Okrug, 2016)

Population	n	H_o (M±SEM)	H_e (M±SEM)	F_{is} (95 % CI)	A_r (M±SEM)
ERV	28	0.563±0.058	0.706±0.047	0.193 (0.061-0.323)	6.430±0.650
ILB	25	0.551±0.046	0.742±0.027	0.257 (0.163-0.358)	6.778±0.494
IND	34	0.567±0.043	0.718±0.030	0.201 (0.088-0.322)	6.173±0.499
WLD	32	0.622±0.056	0.796±0.023	0.223 (0.102-0.330)	8.280±0.666

Note. ERV, IND and ILB — current populations of farms ERV, Indiga and Ilbets; n — sample size, H_o — observed heterozygosity, H_e — expected heterozygosity, F_{is} — coefficient of inbreeding, A_r — allelic diversity; M — arithmetic mean, SEM — standard error, CI — confidence interval.

Thus, reindeer does, bred in the Nenets Autonomous Okrug, had morphological and genetic differences. Animals from Indiga farm (IND) and Ilebts farm (ILB) for number of basic body parameters (height at withers, chest width, chest girth, wrist girth, body length, head length) and body weight exceeded the does of ERV farm (ERV). The coefficient of variation for all studied parameters were within 10 %, which shows a high degree of population consolidation, and by some indicators (chest depth, chest width, body length, wrist girth and head length) the ERV reindeer does were the most homogeneous. Exterior measurements in all populations either were within the breed standard, or exceeded standards (for example, for body length, chest girth in the IND and ILB populations). On boniness and extension, there was an increase in indices compared to those of 1970th (NEN'70). There was an increase in the massiveness in ILB and IND compared tp the breed standard, while in ERV the values were within the norm and did not differ from NEN'70. Microsatellite analysis revealed that the IND and ILB animals formed a common massif, while ERV animals clustered as an isolated group. It is shown that genetically different groups were characterized by different phenotypic features. Our findings are indicative of the need for a complex morphological and genetic analysis of reindeer populations. The in-depth information about the reindeer population structure will allow development of a strategy for management and further monitoring of the species.

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