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Original Paper

Estimation of biodiversity and population structure of Russian reindeer breeds inhabiting Northeastern Siberia using microsatellite markers

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Three semi-domesticated reindeer breeds inhabiting the Republic of Sakha – Yakutia have been characterized using nine microsatellite markers. Genomic DNA was isolated from tissue samples of 123 individuals of the Chukotka (Khargin) (CHU, *n* = 47), the Evenk (EVK, *n* = 32) and the Even (EVN, *n* = 44) breeds, collected from different regions of Yakutia. Fragment analysis and sizing were run on ABI 3131xl genetic analyzer. Allele frequencies were calculated and used for the characterization of reindeer breeds and the evaluation of their genetic biodiversity. Nei's standard genetic distance was calculated and used for the construction of a Neighbor-Joining tree. Statistical analysis was conducted with GenAlEx 6.5.1, PAST 2.15 and STRUCTURE 2.3.4 software. The highest number of alleles, such as informative (with a frequency more than 5%), effective (Ne) and private (Pr), was detected in the CHU breed: Na ≥5% = 5.333 ±0.441, *Ne* = 4.517 ±0.393 and *Pr* = 1.111 ±0.389, while the EVN breed had the lowest number: 4.778 ±0.324, 4.315 ±0.488 and 0.444 ±0.242, respectively. The EVN breed occupied an intermediate position (5.000 ±0.373, 4.408 ±0.315 and 0.889 ±0.261). Among reindeer breeds, observed heterozygosity ranged from 0.729 ±0.026 to 0.608 ±0.050 with the lowest value found in CHU reindeer and the highest in EVK reindeer. A heterozygotes' deficiency was observed in all reindeer breeds. At K = 3, STRUCTURE analysis matches with the data of Nei's genetic distance dimension results, indicating the presence of a common consistent pattern. CHU and EVK reindeer breeds are characterized by a closer genetic relationship in comparison with the EVN breed, which formed a separate cluster.

Keywords: reindeer breeds, genetic diversity, population structure, microsatellites

1 Introduction

Reindeer is an essential element of Russian Northeast area ecosystem. This species is deeply integrated into life and culture of indigenous northern people and is known being suitable for meat and pelt production as well as a reliable means of transport (Davydov, 1997).

The Republic of Sakha (Yakutia) is one of the largest Russian regions inhabited by reindeer. Reindeer husbandry has always differed from other livestock sectors due to the preservation of a traditional breeding system.

In Russia there are four aborigine reindeer breeds which were officially recognized by a decree of the USSR Ministry of Agriculture: the Even, the Evenk, the Chukotka and the Nenets. All reindeer breeds are the result of selection by different northern communities and are characterized by their particular traits of behaviour and adaptability to respective environments (Stammier, 2005).

In the Republic of Sakha there are three reindeer breeds which are bred in different encampments: the Evenk, the Chukotka and the Even.

The Evenk breed was created by the Evenk people (or their ancestors) and is distributed in nine encampments of the taiga zone of the Republic. It is considered to be the oldest breed and to have been the basis for developing other breeds (Pomishin, 1981). Evenk reindeer are well adapted to taiga conditions. In winter they easily scrape away snow to get their food and can dig holes over one meter deep. In summer and autumn, the herds spread far away from the fenced enclosures (Zabrodin et al., 1989). The total stock of the Evenk breed amounts to about 52000 individuals (25.9% of the total reindeer herding of the Republic) (Robbek, 2012).

Chukotka reindeer inhabit two encampments of the tundra zone of Yakutia. These animals are less suited for long-distance migrations. Mukhachev (1990) identifies

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these traits with the Chukchi (indigenous people) practice of herding their animals on foot instead of driving sleds. They systematically slaughter reindeer with the highest mobility in order to maintain a less mobile reindeer population (Stammier, 2005). The total stock of these animals is about 21000 individuals (10.7% of the total reindeer herding of the Republic) (Robbek, 2012).

The Even breed is reared in 12 encampments of the mountain taiga, the tundra and the forest-tundra zones of Yakutia. They are well adapted to mountainous areas, occupying alpine pastures in summer and river valleys and depressions in winter (Zabrodin et al., 1989). The total stock of the Even breed is nearly 127000 individuals (63.4% of the total reindeer herding of the Republic) (Robbek, 2012).

Several genetic surveys have been conducted for reindeer, using different genetic methods, such as, gel electrophoresis (Storset et al., 1978; Roed, 1985; Shubin, 1969), mitochondrial DNA markers (Flagstad and Røed, 2003; Cronin et al., 2005) and nuclear microsatellites (Ball et al., 2010; McDevitt et al., 2009; Zittlau et al., 2000; Wilson et al., 1997).

Microsatellite markers are commonly used in population genetic studies for analyses of gene flow, parentage verification, and studies on genetic diversity (Pfeiffer et al., 1997). Inasmuch as microsatellites are highly polymorphic, selectively neutral and co-dominant markers, they are best suited for the genetic diversity analysis (Cremer et al., 2006). We have recently studied the Russian reindeer genetic diversity using set of microsatellites (Kharzinova et al., 2015). However, this study included only part of breeds inhabiting the Sakha Republic and thus can give only particular representation of whole genetic diversity of Yakutian reindeer.

In the current study, we investigated microsatellite variability for three reindeer breeds collected throughout the Republic of Sakha-Yakutia to estimate the level of genetic biodiversity and the population structure of Russian reindeer.

2 Material and methods

We analyzed a total of 123 individuals of three reindeer breeds: the Chukotka (CHU, $n = 47$), the Evenk (EVK, $n = 32$) and the Even (EVN, $n = 44$) from different encampments of Yakutia.

DNA was extracted from tissue samples using the Nextech column (Agrobiogen Biotechnology GmbH, Munich, Germany) according to the recommendation of the manufacturer.

For studying the genetic diversity of reindeer breeds, nine microsatellite loci were chosen: NVHRT21, NVHRT24, NVHRT76, RT1, RT6, RT7, RT9, RT27, and RT30 (Røed and Midthjell, 1998; Wilson et al., 1997). Selected microsatellite markers were run in one multiplex PCR reaction. The PCR products were analyzed using ABI PRISM 3130xl (Applied Biosystems, USA) with Data Collection Software v3.0. The sizing of the fragments was performed with GeneMapper software v4.0 (Applied Biosystems, USA). For each locus and breed and across breeds, commonly derived statistics from the microsatellite genotypic data were obtained as follows: number of alleles per locus (Na), informative (Na ≥5%), effective (Ne) and private (Pr) number of alleles per locus, observed heterozygosity (Ho), expected heterozygosity, Shannon's information index (Hartl and Clark, 1997) and inbreeding coefficient (F_{is}).

The level of differentiation among populations (without recurring genotypes) was estimated with hierarchical analysis of molecular variance (AMOVA) using F_{ST} (IAM) value (Slatkin, 1995). The degree of genetic differentiation among populations was evaluated on the basis of F_{st} values (Weir and Cockerham, 1984) and Nei's genetic distances (Nei, 1977).

The calculations were performed using GenAlEx software (package version 6.5.1) (Peakall et al., 2012). Allele frequencies from a subset of nine markers were used to compute a matrix of genetic distances (Nei, 1977); this matrix was used to construct a phylogenetic tree of relationships among reindeer breeds. Genetic distances and the phylogenetic tree were computed using PAST software (ver. 2.15) (Ryan et al., 1995).

Additionally, we used STRUCTURE software Pritchard et al. (2000) to infer genetic population structure of all reindeer breeds. All individuals were combined into one dataset for analysis, without any a priori population assignments. The admixture was allowed with a single value of Δ inferred for all populations. We evaluated K values (the number of assumed populations) from 2 to 9 using a burn-in of 100,000 and 100,000 Markov chain Monte Carlo (MCMC) for each value of K. To identify the most probable groups (K) that would best fit the data, we used STRUCTURE HARVESTER (Earl and von Holdt, 2012), which implements the Evanno method (Evanno et al., 2005). Average values of similarity coefficient Q in the i-th cluster for the total number of clusters k (Qi/k) were calculated for each breed.

3 Results and discussion

In the present study, genetic polymorphisms in 123 individuals belonging to three reindeer breeds were analyzed with nine microsatellite loci. The allelic frequencies for each breed are shown in Figure 1.

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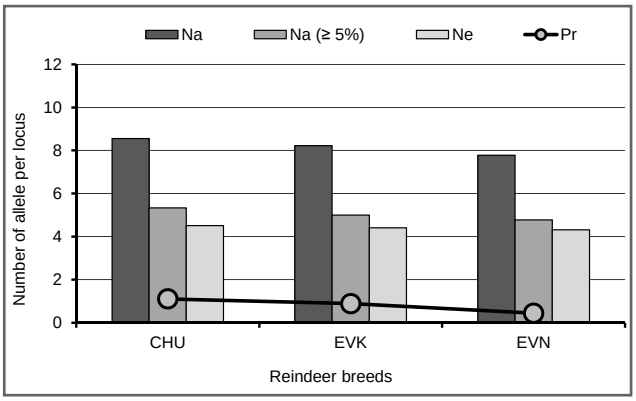

Figure 1 Allele frequencies for nine microsatellite loci in three reindeer
CHU, EVK, EVN – the Chukotka, the Evenk and the Even reindeer breeds (for description, see section Methods); the number of alleles per locus: Na – overall, Na (≥5%) – informative, Ne – effective, Pr – private alleles.

The CHU breed was characterized by relatively high number of alleles per locus (8.556 ±0.689) in comparison with 8.222 ±0.401 and 1.781 ±0.114 alleles in the EVK and the EVN breeds, respectively. The same trend is apparent for the average number of informative alleles (with a frequency more than 5%): the CHU = 5.333 ±0.441, the EVK = 5.000 ±00.373 and the EVN = 4.778 ±0.324. The mean effective number of alleles was 8.185 ±0.362 and ranged from 4.315 ±0.488 in the EVN to 4.517 ±0.393 in the CHU breeds. The number of private alleles was higher in the CHU (1.111 ±0.389), while in the EVK and the EVN this parameter was 0.889 ±0.261 and 0.444 ±0.242 respectively.

Additional genetic characteristics for each reindeer breed are shown in Table 1.

The EVK reindeer are characterized by a higher level of genetic diversity (*Ho* = 0.729 ±0.026 and *He* = 0.765 ±0.015) than the one relating to the CHU and the EVN reindeer breeds. The estimated value of expected heterozygosity was lower in the EVN reindeer breed. The greater value of unbiased expected heterozygosity was observed for the EVK (0.777 ±0.015) and the CHU (0.768 ±0.028) breeds, while for the EVN it was 0.752 ±0.008. The genetic diversity expressed as Shannon's information index value was lower in the EVN breed (1.608 ±0.108) in comparison with the CHU (1.711 ±0.083) and the EVK (1.692 ±0.060) reindeer breeds. The F_{IS} index was barely positive for all reindeer breeds on average confirming a relatively low heterozygote deficiency: the CHU = 0.200, the EVN = 0.043 and the EVN = 0.131.

The analysis of molecular variance (AMOVA) revealed that the greater part of the total genetic variability (96%) was due to differences between individuals within populations, while the remaining 4% was due to differences between breeds ($P < 0.001$).

Pairwise comparisons of genetic distance between all reindeer breeds based on F_{st} values and Nei's genetic distances are shown in Table 2.

Both measures of genetic distances indicated that the longest genetic distance (0.212 and 0.030) appeared between the CHU and the EVN reindeer breeds. The closest genetic distance (0.186 and 0.026) was between the CHU and the EVK reindeer breeds. CHU, EVK, EVN – the Chukotka, the Evenk and the Even reindeer breeds (for description, see section Methods).

Nei's standard genetic distances are graphically illustrated in Figure 2.

Table 1 Genetic variability within reindeer breeds

Reindeer breed	n	*HE* (±SD)	*HO* (±SD)	*uHe* (±SD)	*I* (±SD)	F_{is}
CHU	47	0.608 ±0.050	0.760 ±0.027	0.768 ±0.028	1.711 ±0.083	0.200
EVN	32	0.729 ±0.026	0.765 ±0.015	0.777 ±0.015	1.692 ±0.060	0.043
EVK	44	0.634 ±0.044	0.744 ±0.029	0.752 ±0.030	1.608 ±0.108	0.148
Over all	123	0.657 ±0.025	0.756 ±0.014	0.766 ±0.014	1.671 ±0.049	0.131

CHU, EVK, EVN – the Chukchi (Khargin), the Evenk and the Even reindeer breeds (for description, see section Methods); *n* – number of individuals sampled/breed, *HE* – mean expected heterozygosity, *HO* – mean observed heterozygosity, *uHe* – mean unbiased expected heterozygosity, I-Shannon's information index and F_{IS} – inbreeding coefficient averaged over 9 loci and 3 reindeer breeds

Table 2 Genetic differentiation of three reindeer breeds

Reindeer breed	CHU	EVK	EVN
CHU	*	0.186	0.212
EVK	0.026	*	0.206
EVN	0.030	0.029	*

CHU, EVK, EVN – the Chukotka, the Evenk and the Even reindeer breeds (for description, see section Methods); above the diagonal the genetic distances according to M. Nei are shown; below the diagonal there are the pairwise values of F_{st}

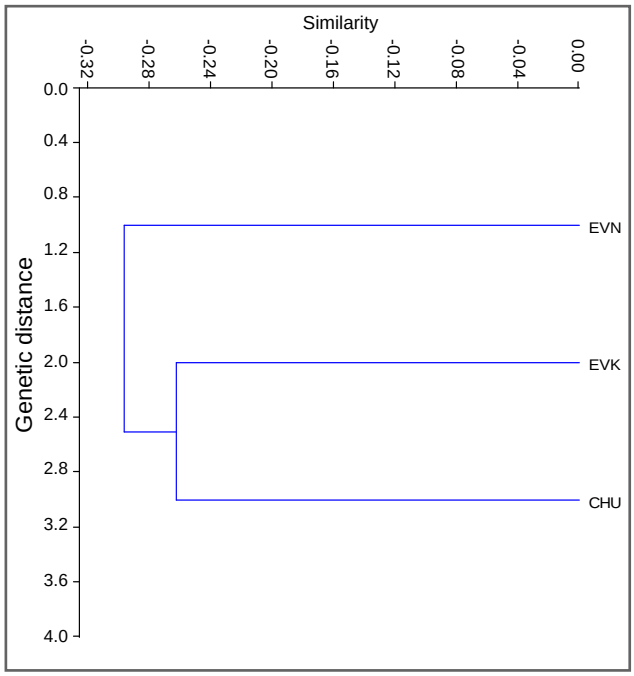

Figure 2 UPGMA dendrogram based on the Nei's genetic distances between reindeer breeds

The dendrogram was produced with the UPGMA clustering technique, using PAST software. The selected dendrogram indicates a separation into two distinct clusters. The first cluster was formed by the CHU and the EVK reindeer breeds. Another cluster was formed by the EVN reindeer breed. This might be explained by their geographical origin from different encampments of Yakutia.

The population structure of 123 reindeer individuals was analyzed with a Bayesian based approach. The highest delta *K* value was calculated as previously described (Evanno et al., 2005). The optimum ΔK value (ΔK = 3.68) was found at $K = 3$ (Figure 3).

EVN reindeer individuals were represented by one cluster with low levels of admixture and had the highest value of membership coefficient (with $Q > 0.85$) for the majority of animals ($Q_{3/3}$ = 0.867 ±0.03). However, six individuals showed admixed ancestry with CHU and EVK clusters at $Q > 0.8$. CHU and EVK reindeer individuals formed two clusters with a high level of admixture. The average values of the membership coefficient were respectively $Q_{1/3}$ = 0.456 ±0.071 and $Q_{1/2}$ = 0.481 ±0.057.

The study of genetic diversity of the sole representative of the genus *Rangifer* using genetic markers has always interested scientists worldwide.

The first studies of the genetic structure of the unique representative of the *Cervidae Gray* family began in the 60 s of the last century following the wide application of the gel electrophoresis method. Extremely important data were obtained from the study of blood serum using this method. Thus, polyacrylamide gel electrophoresis was used to analyze transferrin variation in reindeer, which was described by Storset et al. (1978), Baccus et al. (1983), Roed (1985), Cronin (1995). Shubin (1969) found out that in Russia a transferrin locus in reindeer is represented by 13 genetic variants. While gel electrophoresis has provided geneticists with the most up to date genetic data, this technique shows certain limitations (Grant and Utter, 1980; Grant, 1984).

Thus, as geneticists have encountered an increasing number of questions that cannot be resolved with gel electrophoresis, DNA methods have increasingly generated more interest in the application of suitable molecular markers. Microsatellites are one of the most popular genetic markers for a wide range of applications in population genetics, conservation biology, and evolutionary biology (Khidr et al., 2014). Due to the high degree of polymorphism, type of Mendelian inheritance

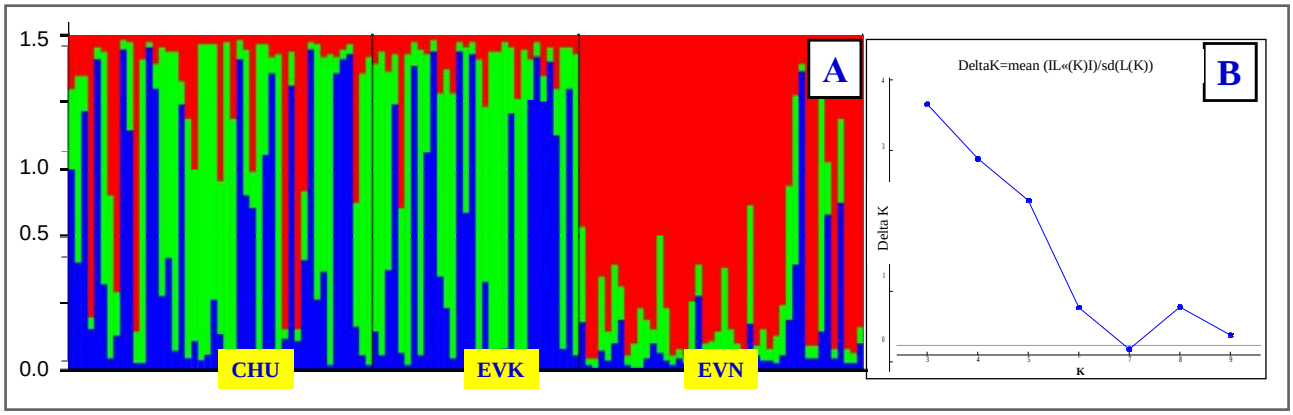

Figure 3 Population structure of analyzed three reindeer breeds using a model-based clustering method implemented in STRUCTURE for $K = 3$ (A); Estimates of the most likely number of clusters in the STRUCTURE analysis derived from the log likelihood associated with each *K*-value
CHU, EVK, EVN – the Chukotka, the Evenk and the Even reindeer breeds (for description, see section Methods); Delta K (blue line) is the rate of change of the likelihood

and the uniform distribution over the whole genome, the sets based on microsatellites have been already developed and applied to all main species of farm animals (Zinovieva et al., 2011).

Nowadays, many publications illustrate the applied significance of STR for the characterization of populations of reindeer. For example, Cote et al. (2002) showed the relatively low levels of genetic diversity between two populations of Svalbard reindeer, which were estimated by data from 14 microsatellites. To study *Cervidae* from Scandinavia Roed et al. (1998) used sets of 75 microsatellite primers of bovine. As a result, it revealed 21 polymorphic loci and high degree of heterozygosity of moose, red deer, reindeer and roe deer. The genetic structure of populations of reindeer (*R. tarandus groenlandicus* and *R. tarandus caribou*) in North America has been described by McDevitt et al. (2009) using 11 STR. The level of genetic diversity in these species was moderately high, and these data are consistent with previous studies. Courtois and Bernatchez. (2002) used the molecular genetic methods, based on analysis of eight STR, for researching seven reindeer populations in eastern North America. They found out that three of them were geographically isolated from the rest and the level of genetic diversity of them was lower. The new molecular genetic technique such as whole genome SNP scanning was recently shown to be suitable for reindeer analysis (Kharzinova et al., 2015).

Although *Rangifer tarandus* has been investigated by many scientists from different countries using microsatellite markers, only little information about different Russian reindeer populations is currently available. This is the first study in which there is an attempt to understand the genetic diversity of three native reindeer breeds from different regions of the Sakha Republic.

4 Conclusions

Our study provides a consistent genetic overview of the genetic biodiversity and population structure of three reindeer breeds inhabiting one of the largest regions in Russia.

Reindeer herding on this territory is not only connected to agriculture sector, but also represents an important cultural factor in the life of reindeer herders. The data obtained in our study might be useful for the preservation and management of these breeds and clearly demonstrate that these reindeer breeds harbour a rich reservoir of genetic diversity. Further studies on the genetic structure of Russian reindeer breeds from Yakutia region are necessary in order to characterize reindeer genetic diversity in this area.

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