Genetic Characteristics of the Even Breed of Deer in Yakutia

V V Dodokhov¹, N I Pavlova¹², T D Rumyantseva¹, L A Kalashnikov³

¹Federal State Budgetary Educational Establishment of Higher Education “Arctic State Agrotechnological University” Sergelyakhskoye sh., 3, Yakutsk, 677007, Republic of Sakha (Yakutia), Russia
²Yakutsk Scientific Center for Complex Medical Problems, Sergelyakhskoye sh., 4, Yakutsk, Republic. Sakha (Yakutia), 677019
³Federal State Budget Scientific Institution “All Russian research institute of animal breeding” Lesnye Poliany, Pushkin district Moscow region, 141212

E-mail: dodoxv@mail.ru

Abstract. Microsatellite markers Rt6, BMS1788, Rt 30, Rt1, Rt9, FCB193, Rt7, BMS745, C143, Rt24, OheQ, C217, C32, NVHRT16, T40, C276 were used to study the genetic profile of the Even breed of deer cultivated in Yakutia. It was found that microsatellite markers in Even deer have a wide range of alleles. The total number of identified alleles reached 120 and varied depending on the locus from 3 (C32; C143) to 14 (BMS2088). The Chukchi deer breed is characterized by a high level of heterozygosity of microsatellite loci, the observed level of heterozygosity on average for all studied loci is 0.702. On average, 7.5 alleles (Na) were detected per locus, and the average number of effective alleles (Ne) was 3.9. The fixation index indicator has an average negative value of -0.024.

1. Introduction

The reindeer, Rangifer tarandus, is the most important element of the Northern ecosystems and an integral part of the life and culture of the indigenous peoples of the Republic of Sakha (Yakutia). This species is the only representative of the genus Rangifer, which belongs to the family of reindeer of the ruminant suborder, and one of the few animal species in which the wild form coexists with the domestic one.

During the years of comprehensive economic restructuring, the reindeer husbandry of the Republic of Sakha (Yakutia), as well as other regions of Russia, experienced a period of deep crisis, which resulted in the denationalization of large reindeer state farms and the termination of state support for the industry. During this period in the Republic, as a result of the reform of agricultural production and the destruction of the organizational and technological structure, in many ulus, deer were transferred to the ownership of small collective and peasant (tribal) farms.

The situation in the Republic’s reindeer husbandry began to change after the government took measures aimed at stabilizing the situation in reindeer husbandry. There are great prospects for the development of domestic reindeer husbandry in the Republic. But over the past ten years, the number of domestic reindeers has been steadily declining, and over this period of time, the number of reindeers has decreased by 24%.

The Even deer is bred in 11 ulus (districts) of the Republic of Sakha (Yakutia) tundra and mountain taiga zones. This is the most numerous of the existing breeds on the territory of the Republic, among
which researchers distinguish Even tundra and taiga deer. At the beginning of 2020, the number of heads is 91,803.

Assessment of genetic resources of reindeer husbandry, constant monitoring of population changes is a necessary condition for timely and effective actions aimed at preventing the disappearance of domestic reindeer breeds [1]. Measures to prevent genetic erosion and population extinction will be more effective if the factors contributing to their occurrence are clearly identified.

Different types of genetic markers are used to study the genetic structure of reindeer [2, 3, 4]. Currently, microsatellite DNA markers are most commonly used to identify evolutionary processes in populations of wild and domestic reindeer. Microsatellite loci are highly variable areas of DNA and consist of sequences repeating in tandems and ranging in length from one to six nucleotides. The use of microsatellites and polymerase chain reaction (PCR) are powerful tools for quantifying genetic variation both within and between breeds.

2. Research methods

The object of the study was deer of the Even breed (n=82) bred by the Federal State Unitary Enterprise Yuchyugeyskoe of the Oymyakon district. The material for genetic analysis of microsatellite loci was DNA samples isolated from blood leukocytes. Blood for DNA isolation was collected from the jugular vein in a volume of 6 ml in vacuum tubes for hematological studies with EDTA K3. DNA isolation was carried out in the educational research laboratory of Federal State Budgetary Educational Institution of Higher Education the Yakut State Agricultural Academy with a set of EXCELL BIOTECH reagents (Excel Biotech Corp., Yakutsk, Russia). Genotyping was performed using a set of reagents for multiplex analysis of 16 microsatellite markers of reindeer CorDis Rangifer (Gordiz LLC, Moscow, Russia). When processing experimental data, we used an add-in for Microsoft Excel GeneAlex 6.51.

Table 1. Description of microsatellite markers CorDis Rangifer.

| Loci   | Sequence and repeat motif | Allele range |
|--------|---------------------------|--------------|
| Rt6    | (CA)₁₉                   | 172-210      |
| BMS1788| (AC)₁₇                   | 142-174      |
| Rt30   | (AC)₁₅                   | 201-227      |
| Rt1    | (AC)₁₉                   | 239-267      |
| Rt9    | (AC)₂₁                   | 133-159      |
| FCB193 | (AC)₁₃                   | 120-150      |
| Rt7    | (AC)₁₁                   | 238-258      |
| BMS745 | (AC)₁₃                   | 124-142      |
| C143   | (ATGG)₁₇                 | 176-187      |
| Rt24   | (AC)₂₁                   | 234-272      |
| OheQ   | (TATC)₁₂ ATCTATCTATTTATC | 268-335      |
| C217   | CATC(CATG) (CATC)₁₅      | 215-219      |
| C32    | (ATCC)₃₄ (ACCT)₂ (ATCC)₁₇| 298-330      |
| Nvhrt16| (AC)₅ AT (AC)₄ ATGCGC (AC)₁₂| 184-226 |
| T40    | (ATCT)₄ ACCT ATCT (ATCT)₄ ACTG ACCT| 259-335 |
| C276   | (TSSA)₅ TSST TACG TCCA (TCCA)₃ TCCT TCCA TCTG (TCCA)₄ TCCG (TSSA)₅ TCCT TCCA TCCG (TSSA)₃ (TCCG)₂ TGCA (TCCA)₂ TCCG TCCA| 354-454 |
3. Results and discussion
The study found that microsatellite markers have a wide range of alleles. 120 alleles were found in Even deer. Depending on the locus, the number of alleles varied from 2 (C217) to 14 (BMS2088).

The highest number of alleles was found in loci BMS2088 (14) and OheQ (13). The number of alleles whose frequency exceeds 5% in these loci was 6 for each. In the BMS2088 locus, the most common allele was 154 BP with a frequency of 0.409. The OheQ locus was characterized by a predominance of alleles 306 BP and 311 BP. The same number of alleles (6 each) exceeding the frequency of occurrence above 5% had loci Rt30, Rt1, Rt7 and Rt24. In microsatellite loci FCB1533, NVHRT16, and C276, 5 alleles were identified, the frequency of which exceeds the level of 0.005.

**Table 2.** Frequency of occurrence of alleles of microsatellite markers in Even deer.

| Locus  | Allele, bp | Freq. | Locus  | Allele, bp | Freq. | Locus  | Allele, bp | Freq. |
|--------|------------|-------|--------|------------|-------|--------|------------|-------|
| Rt6 1  | 182        | 0,061 | C143   | 176        | 0,329 |         | 298        | 0,128 |
|        | 202        | 0,439 |        | 180        | 0,665 | C32    | 306        | 0,433 |
|        | 204        | 0,201 |        | 238        | 0,061 |        | 322        | 0,439 |
|        | 206        | 0,238 |        | 242        | 0,293 |        | 132        | 0,579 |
|        | 144        | 0,140 | Rt7    | 244        | 0,165 | BMS745 | 134        | 0,195 |
|        | 145        | 0,055 |        | 250        | 0,110 |        | 136        | 0,177 |
|        | 146        | 0,116 |        | 252        | 0,140 |        | 184        | 0,116 |
|        | 151        | 0,055 |        | 254        | 0,165 |        | 206        | 0,146 |
|        | 154        | 0,409 |        | 284        | 0,079 | NVHRT16| 208        | 0,128 |
|        | 156        | 0,061 |        | 306        | 0,232 |        | 214        | 0,195 |
|        | 205        | 0,457 | OheQ   | 311        | 0,207 |        | 259        | 0,116 |
|        | 209        | 0,104 |        | 311        | 0,207 |        | 259        | 0,116 |
|        | 211        | 0,067 |        | 323        | 0,140 | T40    | 267        | 0,707 |
|        | 217        | 0,085 |        | 327        | 0,085 |        | 275        | 0,073 |
|        | 223        | 0,152 |        | 128        | 0,262 |        | 354        | 0,140 |
|        | 225        | 0,085 |        | 130        | 0,067 |        | 354        | 0,067 |
|        | 247        | 0,244 | FCB1533| 134        | 0,140 | C276   | 414        | 0,262 |
|        | 249        | 0,128 |        | 136        | 0,372 |        | 430        | 0,524 |
| Rt1    | 251        | 0,067 |        | 138        | 0,104 |        | 434        | 0,207 |
|        | 253        | 0,189 |        | 236        | 0,207 |        |            |       |
|        | 263        | 0,073 |        | 240        | 0,055 |        |            |       |
|        | 267        | 0,134 | Rt24   | 242        | 0,098 |        |            |       |
|        | 133        | 0,110 |        | 244        | 0,122 |        |            |       |
|        | 143        | 0,232 |        | 252        | 0,207 |        |            |       |
| Rt9    | 151        | 0,201 |        | 256        | 0,262 |        |            |       |
|        | 153        | 0,201 | C217   | 88         | 0,848 |        |            |       |
|        | 155        | 0,189 |        | 90         | 0,152 |        |            |       |
The highest number of alleles was found in loci BMS2088 (14) and OheQ (13). The number of alleles whose frequency exceeds 5% in these loci was 6 for each. In the BMS2088 locus, the most common allele was 154 BP with a frequency of 0.409. The OheQ locus was characterized by a predominance of alleles 306 BP and 311 BP. The same number of alleles (6 each) exceeding the frequency of occurrence above 5% had loci Rt30, Rt1, Rt7 and Rt24. In microsatellite loci FCB1533, NVHRT16, and C276, 5 alleles were identified, the frequency of which exceeds the level of 0.005.

The lowest number of alleles was found in loci C217 (2 alleles), C143 (3 alleles) and C32 (3 alleles). At the C217 locus, the 88-BP allele had a frequency of 0.848, and the 90-BP allele had a frequency of 0.152. In locus C143, 2 out of 3 alleles had a frequency of occurrence above 5%, an allele of 180 BP with a frequency of 0.665, and an allele of 176 BP with a frequency of 0.329. Locus C32 identified alleles 298 BP with a frequency of 0.128 and alleles 306 BP, 322 BP, which had a frequency of 0.433 and 0.439, respectively.

To characterize the allelofund of the Even breed, the average number of alleles (Na) and the number of effective alleles (Ne) per locus, the degree of observed (Ho) and expected heterozygosity (He), and the fixation index (Fis) were determined. The results of calculating the polymorphism of microsatellite loci in Chukchi deer are presented in table 3. According to the results obtained, the Even deer on the territory of Yakutia has a high genetic diversity. On average, there were 7.5 (Na) alleles per locus, and the number of effective alleles was 3.9 (Ne). The lowest number of effective alleles was found in the C217 locus (1,348 alleles), and the highest number was found in the Rt1 locus (6,858).

The observed heterozygosity was (Ho) 0.702, the expected (He) - 0.685. The proximity of the observed and expected heterozygosity values indicates the presence of a moderate level of inbreeding when using a random crossing system in a closed population of Chukchi deer. Low indicators of observed heterozygosity were found in loci C143 (0.451), T40 (0.512), and the minimum value of this indicator was taken in locus C2188 (0.256), and the maximum value was Rt7 (0.890).

Table 3. Population genetic characteristics of Even deer.

| Locus  | Na  | Ne   | Ho  | He  | F    |
|--------|-----|------|-----|-----|------|
| Rt6    | 1   | 3.387| 0.756| 0.705| -0.073|
| BMS2088|14  | 4.669| 0.817| 0.786| -0.040|
| Rt30   | 8   | 3.787| 0.732| 0.736| 0.006|
| Rt1    | 12  | 6.858| 0.866| 0.854| -0.014|
| Rt9    | 9   | 5.431| 0.841| 0.816| -0.031|
| C143   | 3   | 1.818| 0.451| 0.450| -0.003|
| Rt7    | 8   | 5.627| 0.890| 0.822| -0.083|
| OheQ   | 13  | 7.001| 0.878| 0.857| -0.024|
| FCB1533|8   | 4.114| 0.707| 0.757| 0.066|
| C217   | 2   | 1.348| 0.256| 0.258| 0.009|
| Rt24   | 9   | 5.458| 0.817| 0.817| 0.000|
| C32    | 3   | 2.522| 0.622| 0.603| -0.031|
| BMS745 | 6   | 2.465| 0.659| 0.594| -0.108|
| NVHRT16| 7   | 4.876| 0.829| 0.795| -0.043|
| T40    | 6   | 1.913| 0.512| 0.477| -0.073|
| C276   | 5   | 2.718| 0.598| 0.632| 0.055|
| Mean   | 7.5 | 3.999| 0.702| 0.685| -0.024|

Heterozygote deficiency was detected in 4 loci out of 16 studied. Accordingly, the fixation index is positive in 4 loci and negative in 12 loci out of the 16 studied. The average value of the fixation index
for the Chukchi breed is -0.024 with fluctuations from -0.108 in the BMS745 locus to 0.066 in the FCB1533 locus.

4. Conclusion
Thus, the results of genetic testing showed that microsatellite markers have a wide range of alleles and have a high informative value. The Even deer bred in the Federal State Unitary Enterprise Yuchyugeyskoe is characterized by a high level of heterozygosity. When using a random crossing system, there is a moderate level of inbreeding. In recent years, the number of Even deer has been declining, which may lead to a loss of diversity. The preservation of Even deer in Yakutia is one of the most important tasks for the Republic, since this breed has good meat qualities, high viability and is adapted to the unfavorable conditions of Yakutia. A promising method for maintaining the level of genetic diversity is genetic monitoring of the Even deer breed by polymorphism of microsatellite DNA loci. The results of the study can contribute to obtaining information about the genetic structure of Even reindeer and be used in programs to improve and preserve domestic reindeer.

5. References
[1] Ulimbashev M B 2018 Rational Use Of The Gene Pool Of Valuable Animal Breeds In Order To Preserve Biological Diversity South Of Russia: Ecology, Development 2(13) pp 165-183
[2] Shubin P N 1988 Genetics Transferrin Of European Reindeer And Moose Genetics 5(1) 37-41
[3] Mitrofanova O V 2015 Evaluation Of The Possibility Of Using Microsatellite Markers In The Rangifer Tarandus Reindeer Materials Of The All-Russian Scientific-Practical Conference "Problems Of Development And Conservation Of The Arctic" (St. Petersburg) pp 117-118
[4] Kharzinova V R, Dotsev A V, Solovyov A D, Fedorov V I, Okhlopkov I M, Wimmers K, Reyer H, Brem G, Zinovieva N A 2017 Population-Genetic Characteristics Of Domesticated Reindeer In The Republic Of Yakutia Based On Genome-Wide SNP Analysis Agricultural Biology Vol 52 4 pp 669-678 Doi: 10.15389 Agrobiology.2017.4.669rus