Genetic parameters of the Chukchi breed populations in generational change

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Abstract. The purpose of the work is to study the genetic characteristics of reindeer populations in the Chukotka Autonomous Region using DNA microsatellites. Genetic monitoring data for 2013 and 2018 are presented (the interval of one generation). In the genetic parameters of populations statistically significant differences were found. Samples are characterized by a large number of active alleles of breed diversity. The average number of alleles per locus ranged from 7.7 to 9.95. Indices of heterozygosity in the studied groups of deer are from 0.851 to 0.887 - indicate the prosperous condition of the populations of the Chukchi breed by genetic heterogeneity. The differentiation of livestock is influenced by inter-farm and inter-herd exchange of the allele pool. Criteria for the genetic similarity of the selective sample populations of 2013 and 2018 according to the frequencies of the ISSR markers were in the range from 0.852 to 0.993, which indicates the stability of the gene pools of the studied deer groups. Eco-geographical and economic conditions affect the frequency of ISSR markers with a force of $\eta^2 = 67-70.4\%$, being the main factor of intraspecific differentiation. According to the identified microsatellite loci, the populations of the Chukchi breed are in a state of genetic equilibrium. Factors which stabilize the genetic structure of the populations of the Chukchi breed are free crosses typical for reindeer, a large number of males involved in reproduction. Noting that the number of deer of the Chukchi breed is declining, constant monitoring of its gene pool is necessary, which provides the stability of populations.

1. Introduction

Reindeer husbandry in the Far North has an important economic and social significance, being the main employment and lifestyle sphere of the indigenous population. It allows to involve into the economic turnover the Arctic and subarctic tundra poor in forage resources, which are unsuitable for grazing other domestic animals [1,2,3].

In the Chukotka Autonomous Region, it is a key sector of agricultural production. Deer breeding is carried out by 14 municipal agricultural enterprises, peasant farms and personal subsistence farms of the population. By the 1-st of January, 2020, there were 132,454 of deer, including 63,505 hinds.

In the Far North-East of Russia, the Chukchi breed of reindeer (Rangifer tarandus L.) is the most numerous. It has a number of valuable economically significant qualities, such as high growth energy, good meat qualities and reproductive qualities, fitness for the area [4,5,6].

Because of the extreme ecological and geographical conditions of the Arctic, the specifics of keeping, biological features, incomplete domestication, selective breeding work with reindeer has no analogues among other branches of animal husbandry. Reindeer husbandry does not practice artificial
insemination and transplantation of zygotes. The selection efficiency is reduced because of a long interval between generations, limited period of using the producers, seasonality of reproduction, low preservation of the livestock and the business output of young animals, a free-group mating system. A significant influence of paratypical factors, low coefficient of heritability of economically useful features does not allow accelerated selection [7,8,9].

Now reindeer selective breeding work is carried out by traditional methods based on mass selection by phenotype. Its main elements are the selection of big producers, active during the rut, the selection of she-deers that raise calves well, the exchange of allele pool between herds and farms [10,11].

Under existing conditions, researches in studying and possible using in reindeer herding more effective techniques with using molecular-genetic methods that have successfully proven themselves in breeding farm animals are needed [12,13,14].

It is problematic to extrapolate the gained data of one number of individuals to the entire breed. For an objective assessment of the feature, it is desirable to have studies in the same herd in different years, taking into account the influence of the environment and the selection background on the dynamics of the indicator [15,16].

The purpose of the work is to study the genetic characteristics of reindeer populations in the territory of Chukotka Autonomous Region in the time interval of one generation.

2. Material and methods

Research-scientific work was carried out on the basis of the reindeer breeding agricultural enterprises, located in the Chukotka Autonomous Region (table 1).

The research material were tissue samples (muscle pieces, ear plucks) of deer of different sex and age groups. Samples were taken by the random method from clinically healthy animals during coral work (kind of sport) and planned slaughter for meat. In comparative molecular genetic studies, 570 samples were used, including 160 which were taken in 2013 and 410 in 2018.

| Household (population), designation | Livestock | Pasture-geographical zone |
|------------------------------------|-----------|----------------------------|
| UE AE «Peoneer», (PNR)             | 18081     | The Arctic Ocean coast Arctic tundra adjacent to the Chukchi Sea. |
| UE AE «Amguema», (AMG)             | 16608     | Arctic tundra in the northwest of the Chukchi Peninsula. |
| UE AE «Vozrozhdenie», (VZR)        | 8876      | Arctic tundra of the southwestern part of the Chukchi Peninsula on the coast of the Bering Sea and the Gulf of Cross. |

Samples were analyzed in the DNA technology laboratory of the All-Russian Research Institute of Pedigree under an agreement with the organization. Individual genotyping and identification of intraspecific genetic variation in deer populations was performed using the ISSR-PCR (Inter-Simple Sequence Repeats) method according to (AG) 9C primer. DNA and PCR separation were performed in accordance with generally accepted recommendations [17,18]. For the calculations, DNA fragments with a length of 180 to 1400 bp (base pair) were used, clearly distinguishable visually and forming distinct peaks during computer scanning of gels. Each amplicon was considered as a separate marker representing a nucleotide sequence which is between two inverted microsatellite repeats. Statistical processing of data was carried out using standard computer programs “Genepop” and known methods [19].

The dynamics of population genetic parameters was studied according to the data of the 2013 and 2018 samples. The interval between generations of reindeer is 5 years [20]. The reliability of the difference in the indicators of the sample sets was established by the Student’s t-test [21].

According to the frequencies of ISSR markers was calculated the average number of alleles per locus (Na), the number of effective alleles per locus (Ne), the homozygosity coefficient (Ca), the level
of theoretical or expected heterozygosity (He), genetic similarity (I), and the genetic distance between populations (D). To check whether the gene equilibrium was maintained in the population at any locus or if it was lost, was used the equality from the Hardy-Weinberg formula: $p_2q_2 = (2pq)^2$ / $2p^2q^2 = (2pq)^2$ [22, 23,24,25]. In the study of the influence of ecological, geographical, and economic conditions on the frequency of ISSR markers ($q^2$), a dispersive analysis of a one-time statistical complex was used [21]. The calculation of the PIC index (Polymorphic Information Contents) was performed according to the formula [26].

3. Results and its discussion
During the research, the genetic characteristics of the studied populations of the Chukchi breed data were got. All of them are typical for reindeer [13,27,28,29,30].

When comparing the parameters of the series of sample sets in 2013 and 2018, statistically significant differences were found in the frequencies of ISSR markers of the PNR population according to loci No. 1 (180-210 bp), No. 9 (700-770), No. 10 (850-980) and No. 11 (1100-1300), AMG populations – according to loci No. 2 (220-230 bp), No. 4 (330-350) and No. 8 (650-690), VZR populations – according to locus No. 9 (table 2).

Table 2. Frequency indices of ISSR-markers in reindeer populations of the Chukchi breed according to the data of 2013 and 2018, M ± m.

| № locus | Fragment length, bp | VZR a | AMG a | PNR a |
|---------|---------------------|-------|-------|-------|
|         | 2013 n=60 | 2018 n=100 | Difference | 2013 n=61 | 2018 n=160 | Difference | 2013 n=49 | 2018 n=150 | Difference |
| 1       | 180-210       | 0.158 ±0.036 | 0.147 ±0.025 | 0.011 | 0.162 ±0.033 | 0.118 ±0.018 | 0.044 | 0.137 ±0.034 | 0.008 ±0.005 | 0.129 c |
| 2       | 220-230       | 0.003 ±0.005 | 0.024 ±0.011 | 0.021 | 0.000 ±0.003 | 0.113 ±0.017 | 0.113 c | 0.039 ±0.019 | 0.083 ±0.016 | 0.044 |
| 3       | 240-330       | 0.152 ±0.036 | 0.142 ±0.024 | 0.01 | 0.154 ±0.032 | 0.150 ±0.020 | 0.004 | 0.134 ±0.034 | 0.131 ±0.019 | 0.003 |
| 4       | 330-350       | 0.073 ±0.007 | 0.075 ±0.018 | 0.002 | 0.000 ±0.000 | 0.046 ±0.012 | 0.046 c | 0.076 ±0.007 | 0.055 ±0.013 | 0.021 |
| 5       | 350-430       | 0.165 ±0.037 | 0.150 ±0.025 | 0.015 | 0.165 ±0.033 | 0.144 ±0.020 | 0.021 | 0.137 ±0.035 | 0.131 ±0.019 | 0.006 |
| 6       | 440-520       | 0.162 ±0.036 | 0.148 ±0.025 | 0.014 | 0.162 ±0.033 | 0.141 ±0.019 | 0.021 | 0.137 ±0.035 | 0.136 ±0.020 | 0.001 |
| 7       | 520-570       | 0.145 ±0.035 | 0.141 ±0.024 | 0.004 | 0.162 ±0.033 | 0.141 ±0.019 | 0.021 | 0.137 ±0.035 | 0.136 ±0.020 | 0.001 |
| 8       | 650-690       | 0.135 ±0.034 | 0.136 ±0.024 | 0.001 | 0.130 ±0.030 | 0.061 ±0.013 | 0.069 b | 0.137 ±0.035 | 0.079 ±0.015 | 0.058 |
| 9       | 700-770       | 0.003 ±0.005 | 0.013 ±0.013 | 0.033 b | 0.046 ±0.019 | 0.085 ±0.015 | 0.039 | 0.064 ±0.025 | 0.131 ±0.019 | 0.067 b |
| 10      | 850-980       | 0.003 ±0.005 | 0.001 ±0.002 | 0.002 | 0.016 ±0.011 | 0.000 ±0.000 | 0.016 | 0.000 ±0.005 | 0.088 ±0.016 | 0.088 c |
| 11      | 1100-1300     | 0.000 ±0.000 | 0.000 ±0.000 | - | 0.000 ±0.000 | 0.000 ±0.000 | - | 0.000 ±0.000 | 0.020 ±0.008 | 0.020 b |

a VZR, AMG, PNR – identification of a population, watch in chapter materials and methods.

b $P \leq 0.05$.

c $P \leq 0.01; P \leq 0.001$. 

DOI: 10.1088/1755-1315/547/1/012003

IOP Conf. Series: Earth and Environmental Science 547 (2020) 012003
At $P<0.01$, the null hypothesis is rejected, and the revealed changes in the genome of animal populations of AMG according to loci No. 2, No. 4, and PNR according to loci No. 1 and No. 10 are accepted. The metamorphoses found in the deer genome were most likely due to the introduction of new genes as a result of incoming breeding material from other farms and various combinations when crossing unrelated groups of animals.

Identified changes in the long loci No. 10 and No. 11 of the deer genome of the PNR population could also be introduced by wild male migrants, who sometimes enter reindeer herds during the rut and mate with domestic females. Such a hypothesis is possible, as long loci are more characteristic for wild reindeer [27,28,29,30].

The statistical significance of the differences in the frequencies of the ISSR markers in the samples of 2013 and 2018 revealed according to loci No. 9 of the VZR population, No. 8 of the AMG population and No. 9 and No. 11 — the PNR population at $P<0.05$ does not allow rejecting the null hypothesis, as for this a significance level of $P<0.01$ is required ($H_0$ is rejected at $P<0.01$; $H_0$ is accepted at $P>0.05$). In this case, the probability is in the range from 0.01 to 0.05; so, the possibility of rejecting the null hypothesis is doubtful [21].

In the period from 2013 to 2018 there were no deer from the outside in the herds of the gene pool farm «Vozrozhdeniye» and there probably could not be the flow of new genes. On the contrary, in the agricultural establishments “Amguema” according to the plan of the breeding work in the Chukotka Autonomous Region, there were significant commings of breeding material. Through the buying and selling line of breeding animals, also in 2017, the «Pioner» agricultural establishments transferred 192 bulls from «Amguema». The exchange of allele pool, is obviously reflected in the frequency indices of the ISSR markers of the studied deer herds.

Analysis of the average number of alleles per microsatellite locus shows that the studied populations are characterized by a high level of genetic diversity (table 3).

Table 3. Indicators of the genetic diversity of the populations of the Chukchi breed in generational change, $M \pm m$.

|                     | Population |            |            |            |            |            |
|---------------------|------------|------------|------------|------------|------------|------------|
|                     | VZR        | AMG        | PNR        |            |            |            |
| Rate                |            |            |            |            |            |            |
|                     | 2013       | 2018       | Difference | 2013       | 2018       | Difference |
| The average number  | 7.7        | 8.56       | 0.86       | 7.3        | 8.71       | 1.41\(^a\) |
| of alleles per locus| \pm 0.59   | \pm 0.35   | \pm 0.56   | \pm 0.125  | \pm 0.48   | \pm 0.263  |
| The number of        | 6.8        | 7.57       | 0.77       | 6.7        | 8.13       | 1.43\(^b\) |
| effective alleles    | \pm 0.34   | \pm 0.42   | \pm 0.25   | \pm 0.21   | \pm 0.31   | \pm 0.356  |
| Homozygosity         | 0.146      | 0.132      | 0.01       | 0.149      | 0.123      | 0.026      |
| coefficient          | \pm 0.04   | \pm 0.03   | 4          | \pm 0.04   | \pm 0.02   | \pm 0.047  |
| Expected             | 0.854      | 0.868      | 0.01       | 0.851      | 0.877      | 0.026      |
| homozygosity         | \pm 0.04   | \pm 0.03   | 4          | \pm 0.04   | \pm 0.02   | \pm 0.047  |

\(^a\) $P \leq 0.05$.
\(^b\) $P \leq 0.001$.

In 2013 – 2018 the average number of alleles per locus fluctuated with a general tendency to increase in the VZR population from 7.7 to 8.56 (11.1%, $P>0.05$), in the AMG from 7.3 to 8.71 (19.3%, $P \leq 0.05$), in the PNR - from 8.7 to 9.95 (14.3%, $P \leq 0.05$). A similar pattern was noted also for effective alleles. A large number of active alleles of breed diversity is observed in the samples.
In the studied massif of the Chukchi breed deer, there is a tendency towards an increase in heterozygosity and a decrease in the coefficient of homozygosity (table 3). The populations are characterized by a high level of heterozygosity of intermicrosatellite DNA - from 0.851 in the AMG population to 0.887 in the PNR population, which indicates the genetic diversity of the relevant loci of the deer genome. The differentiation of livestock is significantly affected by the inter-farm and inter-herd exchange of the allele pool conducted by agricultural enterprises. A high level of heterozygosity gives animals an advantage in adaptive traits and provides the stability of deer populations.

ISSR markers have a dominant manifestation character; so, the PIC (Polymorphic Information Content) index values were determined according to the formula Botstein et al. for diallelic loci: \( \text{PIC} = 2f(1-f) \), where \( f \) is the frequency of one of the two alleles [26]. In this case, \( f \) was calculated as \( \sqrt{R} \), where \( R \) is the frequency of variants in which there was no DNA fragment of a given length. So, the \( R \) value shows the proportion of homozygotes for the "recessive" allele - the absence of a DNA fragment of a certain length in the spectra of amplification products.

The dynamics of the polymorphic information content (PIC), which determines the average proportion of heterozygous individuals, by individual loci and on average in the studied populations of the Chukchi breed in 2013 and 2018, is presented in table 4.

All populations are characterized by the unequal contribution of deer genome loci into the indicator of polymorphic information content PIC.

In 2018, in the VZR at 6 loci, the PIC value decreased an increase was noted, at 4 loci, and on average compared to the level of 2013 value was 105.8%. In the AMG, the proportion of heterozygous individuals at 7 loci increased, at 3 loci on the contrary decreased, on average the 2013 level was exceeded by 57.5%. The highest value of PIC = 0.374 was found in this population. The herds of the “Amguema” agricultural enterprise in 2006-2016 years received 7670 deer from the “Vozrozhdenie”, “Pioneer” and “Kanchalansky” agricultural enterprises according to the plan of a breeding work in the Chukotka Autonomous Region.

**Table 4. The value of the PIC index (polymorphic information content) in the Chukchi breed populations.**

| № locus | Fragment length, bp | Population |
|---------|---------------------|-------------|
|         | VZR 2013 | VZR 2018 | AMG 2013 | AMG 2018 | PNR 2013 | PNR 2018 |
| 1       | 180-210   | 0.3200   | 0.2325   | 0.2233   | 0.4981   | 0.0000   | 0.0577   |
| 2       | 220-230   | 0.0199   | 0.1533   | 0.0000   | 0.4999   | 0.2617   | 0.4694   |
| 3       | 240-330   | 0.4057   | 0.3507   | 0.3810   | 0.1723   | 0.2449   | 0.3116   |
| 4       | 330-350   | 0.3766   | 0.4148   | 0.0000   | 0.2791   | 0.4421   | 0.3513   |
| 5       | 350-430   | 0.0000   | 0.1274   | 0.0000   | 0.3439   | 0.0000   | 0.3206   |
| 6       | 440-520   | 0.2428   | 0.1904   | 0.2233   | 0.3865   | 0.0000   | 0.0494   |
| 7       | 520-570   | 0.4528   | 0.3659   | 0.2232   | 0.3865   | 0.0000   | 0.0494   |
| 8       | 650-690   | 0.4885   | 0.4175   | 0.4971   | 0.3513   | 0.0000   | 0.4556   |
| 9       | 700-770   | 0.0199   | 0.2239   | 0.2560   | 0.4474   | 0.3956   | 0.3206   |
| 10      | 850-980   | 0.0199   | 0.0066   | 0.0958   | 0.0000   | 0.0000   | 0.4815   |
| 11      | 1100-1300 | 0.0000   | 0.0000   | 0.0000   | 0.0000   | 0.0000   | 0.1407   |

The maximum increase in the average proportion of heterozygous individuals was detected in the PNR population, where PIC increased at almost all loci, and on average in 2018 was close to the level of 2013 - 183%. In the “Pioneer” agricultural enterprise the inter-team deer exchange in 2016 was...
2303 individuals, in 2017 - 2458, in 2018 - 1480, which provided a significant increase of heterozygotes.

The degree of genetic similarity according to loci and alleles of polymorphic systems between animals of different groups within a population and between different populations is judged by the criterion of genetic similarity (I). The larger its value (in the range from 0 to 1), the greater the similarity between the groups of animals according to the loci. All alleles of the locus were included in the processing, both those alleles that were found in animals of both compared groups and alleles that were found in animals of one sample population, but not those which individuals of another population have. The criterion for genetic similarity of the 2013 and 2018 sample populations calculated by Mayal-Lindstrom [25] was equal in the VZR 0.993 ± 0.007; the AMG - 0.913 ± 0.018 and the PNR - 0.852 ± 0.025 deer populations, which indicates the stability of the gene pools of populations. Coefficients of genetic similarity of frequencies of ISSR markers according to L.A. Zhivotovsky [21], were close in value: in the population VZR - 0.985, the AMG - 0.898, the PNR - 0.890.

Genetic similarity between the VZR and the PNR populations during 2013-2018 decreased, and the genetic distance increased (table 5). Allele pool exchange between these groups of deer in this period did not occur.

| Compared populations | Genetic similarity index | Genetic distance |
|----------------------|--------------------------|------------------|
|                      | 2013         | 2018         | 2013 | 2018 |
| VZR / PNR            | 0.915        | 0.823        | 0.085 | 0.177 |
| VZR / AMG            | 0.913        | 0.931        | 0.087 | 0.069 |
| AMG / PNR            | 0.896        | 0.898        | 0.104 | 0.102 |

In contrast, between the VZR and the AMG populations, genetic similarities increased and genetic distance decreased. Obviously, this is due to the movement of the animal population and the corresponding gene flow from the VZR to the AMG. The genetic similarity and genetic distance between the AMG and the PNR remained virtually unchanged.

Agricultural populations of reindeer exist under the constant influence of natural and economic factors that have a definite effect on their phenotype and genetic structure [7, 8, 11]. Using the dispersive analysis of a one-factor statistical complex, the dependence of the frequencies of DNA ISSR markers on the influence of a complex of causal factors nominated as the “ecology and economy” factor was studied. The current factor includes the system of natural components of the pasture territory (range) - geography, climate, agrometeorological conditions, phenology, flora conditions, chemical composition of feeding plants, water, soil. This includes production and household features – ways of keeping and grazing deer, the condition of zootechnic and breeding work, veterinary prophylaxis, the organizational level of an agricultural enterprise, etc. [7, 11, 12].

In reindeer husbandry there is a constant movement of livestock. To prevent inbreeding and introductions of new genes, a deer exchange between herds, inter-farm buying and selling of breeding animals, receiving litter, planned slaughter of deer for meat is carried out. A certain proportion of individuals is eliminated from reindeer herds as a result of culling animals by age, veterinary requirements, bonitation indicators, and other reasons. The unproductive waste of the deer population annually amounts up to 20% or more of the herd's turnover as a result of the death of animals from diseases, extreme natural phenomena, predatory animals and birds. Migrating wild reindeer enter herds during the rut and mate with domestic deer. These factors have a definite effect on the gene pools of agricultural reindeer populations.

According to the data of 2013 and 2018, the indicator of the influence force \( \eta \times 2 \) of the current factor “ecology and economy” on the frequency of ISSR markers in populations was 0.704 ± 0.0040 and 0.670 ± 0.0021, respectively. The value \( F_{exp} = 5.22 \) and 4.47 exceeds the level of theoretical value \( F_{theor} = 3.3 \) (P≤0.01), i.e. goes beyond random deviations. Therefore, F criterion allows to reject the zero hypothesis and recognize the statistically significant effect of the studied factor on the frequency
of DNA fragments in populations of the Chukchi breed with an average influence force of $\eta x^2 = 70.4\%$ and 67%. Correlation ratio index: $\eta x = 0.839 \pm 0.0433$ and $0.819 \pm 0.0268$ also indicates a significant causal link between the signs, the influence of ecological-geographical and economic conditions on the microevolution processes in reindeer husbandry [10,11, 27, 28, 29, 30, 31].

The performed calculations found out that the studied populations of the Chukchi breed according to the identified microsatellite loci are in a state of genetic equilibrium. Free mating, typical for herd animals, including reindeer, a large number of males involved in reproduction, are factors that stabilize the structure of the populations of the Chukchi breed.

4. Conclusion
According to 2013 and 2018 years (interval of one generation) in the Chukchi breed populations statistically significant differences according to the frequencies of ISSR DNA markers were revealed.

The average number of alleles per locus ranged from 7.3 to 9.95, a similar pattern was also marked for effective alleles. The level of heterozygosity - from 0.851 to 0.887 - indicates a quite high genetic diversity of the corresponding loci of the deer genome, a significant adaptive potential that provides the stability of populations. The genetic differentiation of populations is significantly affected by inter-farm and inter-herd exchange of allele pool.

Eco-geographical and economic conditions determine the frequency of ISSR markers in populations with the value $\eta x^2 = 67\text{-}70.4\%$ thereby being the main factor of intraspecific differentiation.

The genetic similarity of the sample populations criteria of 2013 and 2018 according to the frequencies of ISSR markers ranged from 0.852 to 0.993, which indicates the stability of the genetic structure of populations.

The Chukchi breed populations according to the identified microsatellite loci are in a state of genetic equilibrium. Free mating, typical of reindeer, a large number of males involved in reproduction are factors that stabilize the structure of populations.

Taking into account that the number of deer of the Chukchi breed is declining, constant monitoring of its gene pool which provides the stability of populations, is necessary.

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