EVOLUTION OF THE METHODS FOR ESTIMATION BIODIVERSITY IN REINDEER (Rangifer tarandus) (review)

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Abstract

Reindeer Rangifer tarandus, the only member of the genus Rangifer, is an important component of the food security of the indigenous people of the Russian North, and is an indispensable part of the Arctic ecosystems (A. Savchenko, 2014; V.G. Logino, 2014). To-date, due to a number of unfavorable natural and anthropogenic factors, population number of both domestic and wild reindeer is sharply decreasing. This leads to a loss of the genetic diversity, which is sufficient for survival in new habitats (Y.A. Stolpovsky, 2010). In this regard, it is significant to monitor the genetic diversity of resource breeds and wild reindeer populations with use of genetic markers. The review summarizes the results of the genetic diversity studies of reindeer using different molecular genetic analysis methods. The first genetic studies of reindeer began with the assessment of serum transferrin polymorphism in the 1960s (B. Gahne et al., 1961; M. Braend, 1964). Types of transferrin were distinguished from each other by the band position and mobility in gel electrophoresis (A.V. Soldal et al., 1979; K.H. Roed, 1985; P.N. Shubin et al., 1988). With the development of genetic technologies, DNA markers gained popularity (M. Caliskan, 2012). The so-called “anonymous” markers (initially RAPD and later ISSR) became the first DNA markers used to investigate the biodiversity of reindeer populations (V.V. Goncharov et al., 2009; N.V. Kol et al., 2006; T.M. Romanenko et al., 2014; G.Y. Bryzygalov, 2016). Since the publication of the complete nucleotide sequence of the control region of the mitochondrial genome of reindeer subspecies of Eurasia and North America, analysis of the polymorphism of mitochondrial DNA (mtDNA) has become widespread (M.A. Cronin, 1992; E. Randi et al., 2001; A.V. Davydov et al., 2007; M.V. Khodolova et al., 2009; A.N. Korolev et al., 2017). The method is a highly informative for revealing the phylogeny and origin of breeds and populations by the maternal line (O. Flagstad et al., 2003; N.A. Akopyan et al., 2016). Microsatellites have found great implementation in applied studies of genetics of reindeer (establishment of genetic structure, characteristic of allelic pool, identification and differentiation of individuals) (K.H. Roed et al., 1998; B.I. Jepsen et al., 2002; R. Courtois et al., 2003; M.A. Cronin et al., 2003; K.A. Zittlau, 2004; P.D. McLoughlin et al., 2004; A.D. McDevitt et al., 2009; A.I. Baranova et al., 2016). For Russian reindeer populations, a multiplex panel of nine microsatellites was developed (V.R. Kharzinova et al., 2015). It is successfully using in the routine testing of reindeer, including the detection of hybrids between wild and domestic forms (V.R. Kharzinova et al., 2016). However, with the development of new high-throughput technologies and new-generation analytical equipment (A. Vignal, 2002; E.K. Khlestikina, 2013), DNA chips based on genotyping of multiple SNPs come to the fore in genetic studies of farm animals (F.J. Steemers et al., 2007; S. Mastrangelo et al., 2014; T.E. Deniskova et al., 2015; B. Slim et al., 2015; N.A. Zinovieva et al., 2016; T.E. Deniskova et al., 2016, R. Yonesaka et al., 2016). To-date, despite the fact that there is no the specific DNA chip for reindeer, the use of the Bovine SNP50 BeadChip, designed for cattle, is the most effective and highly informative method for studying the reindeer genome (V.R. Kharzinova et al., 2015; V.R. Kharzinova et al., 2016; V.R. Kharzinova et al., 2017).

Keywords: Rangifer tarandus, reindeer, genetic diversity, genetic marker, SNP, DNA chip
Ecosystem stability stems from preservation, increase, and use of biodiversity. Information about genetic variability and processes involved in the origin and preservation of species play a critical role in understanding of the structure and evolution of populations [1]. Maintenance of the optimal genetic variability and heterozygosity in animal populations preserves their ability to adapt to natural environment (climate changes, negative impact of hazardous substances). High genetic diversity ensures evolutionary adaptiveness of the animals [2-4]. Levels of genetic variability within and among the populations correlate with their demographic history, as well as with environmental factors [5]. Information about genetic structure of the animal population not only enables to assess the importance of the fundamental evolutionary factors (selection, mutation, migration, genetic drift) under stress conditions, but is also important for restoration and rational use of the species [6]. Based on the aforesaid, it is important to develop methods and approaches providing a more objective overview of the genetic diversity of species and breeds. This is of particular importance due to the decreased number of wild and domestic animal breeds observed during the last 100 years [7]. According to the second report “The State of the World’s Animal Genetic Resources for Food and Agriculture” (Food and Agriculture Organization, FAO), nearly 17 % or 1458 breeds of farm animals in the world are on the verge of extinction, whereas the risk status of many others (58 %) is unknown due to the lack of information about the size and structure of populations [8].

Decrease in abundance has affected all species, including the reindeer (*Rangifer tarandus*), playing a key role in life of peoples of the Far North. Reindeers are not only the priority element of the Arctic societies, but also the crucial component of food security for the peoples of northern Siberian territories [9]. For the peoples of the North, deer breeding forms the basis of way of living; it is a continuous process with regular alternation of used pasture grounds. Domestic reindeer population of four breeds provides indigenous community with food and necessary materials for houses and cloths [10]. Wildlife reindeer species are under serious threat since ecological changes in the environmental conditions (i.e. melting of glaciers due to climate warming) along with destabilize the population size. Decrease in population size means decrease of biological diversity and may result in loss of the unique and valuable cultures of small indigenous groups and in ethnic disaster [11, 12]. Among the objects in agricultural sphere, reindeer is one of the least genetically studied species.

Present overview summarizes genetic diversity findings obtained with the use of various methodologies for the reindeer *Rangifer tarandus* Linnaeus, 1758, the only species of genus *Rangifer* Smith H., 1827 and a member of subspecies *Odocoileinae*. Reindeer studies with the use of DNA-micro-matrices based on BeadArray platform are described for the first time. This approach based on genotyping multiple SNP markers enables to assess biological diversity of such unique member of natural fauna of the Northern Russia across the entire genome and not merely at the level of a certain gene.

First method used to describe biological diversity and genetic differences within and among reindeer populations was gel electrophoresis, and the first marker was serum protein transferrin (Tf) polymorphism of which was identified yet in 1959 [13]. Various Tf types differed from each other by location of bands in the gel due to different electrophoretic mobility. Tf locus remains the most frequently used protein marker for assessment of reindeer biodiversity during the period preceding discovery of DNA polymorphism [14-17]. Six various Tf types in reindeers, in one of which 3 alleles were detected, have been reported in 1961 [14]. In 1964, 8 Tf alleles were detected in three domestic, one semi-domesticated and one wildlife population of reindeer in Norway [13]. In further-
ance, number of Tf alleles identified in the Norwegian population had increased up to 12 [17]. Among 9 proteins studied in reindeer of Spitsbergen Island only transferrin was polymorphic and, accordingly, was suitable for assessment of biodiversity [15]. Two alleles not found in the Norwegian animals were found in reindeer population of Spitsbergen Island, based on which they were separated into the specific phylogenetic group, *R. tarandus plutyrhynchus* [15, 16].

Specific attention was paid to studying of Tf locus in the Russian population of reindeer. P.N. Shubin [18] had described 5 alleles of Tf locus, number of which had increased up to 13 in further studies [19]. Upon comparative study of wildlife and domestic populations in Tyymr, the most number of Tf alleles was identified in wildlife reindeer leading to the conclusion about low identity of two populations [20]. Ten genetic variants of Tf were identified in Nenets species [21]. High polymorphism of Tf locus had defined wide use of Tf electrophoretic screening for identification of the events precedent to the evolution of reindeer populations, including assessment of their passing through the bottleneck [22].

Discovery of DNA structure and development of the methods for determination of its variability had resulted in stepwise replacement of protein polymorphism study by DNA sequence analysis. DNA markers are relatively easy to find, they are located along the entire genome, are fully independent of environmental conditions, and, in fact, can detected at any stage of body development [1].

There is an abundance of genetic locus, polymorphism of which may be used in molecular marking. The most conservative ones may be used for global classifications [23], and the most variable ones allow assessment of diversity within a population [24]. Discovery of various polymorphism types is based on modification of the Polymerase Chain Reaction (PCR) technique which enabled to use various types of DNA markers in many science domains and agricultural sectors [25]. Evolution of molecular markers had resulted in emergence of the new knowledge about biodiversity of reindeer populations, including estimates of genetic variability, genetic structure, extent of differentiation and phylogenetic relationship of breeds and populations of this species.

Thus, with the use of the earliest marking techniques, RAPD (random amplified polymorphic DNA), it was found that Nenets breed of reindeer is characterized by higher levels of polymorphism and heterozygosity than Ewenki breed [26]. DNA inter simple sequence repeats (ISSR) test had enabled to establish genetic structure of reindeer population on the Kolguev Island and to study genetic diversity of artificially created and maintained population of domestic reindeer at natural territorial habitats on the Kaninsko-Timanskaya tundra of Nenets Autonomous Okrug [27, 28]. Average pairwise indices of similarity and average heterozygosity values of the analyzed population were calculated during study of ISSR polymorphism in Tuvin population of reindeer [29]. ISSR technique formed the basis for description of the genetic structure of the Chukot breed [30]. Heterozygosity level of intermicrosatellite DNA comprised 0.851-0.876, which is illustrative of the genetic diversity of relevant locus.

Sequence analysis of mitochondrial DNA (mtDNA) is widely used for assessment of the level of genetic diversity, phylogenetic relationship within and between reindeer populations. High polymorphism, matriliney and lack of mtDNA recombination also enable the effective application of such approach for determination of the historical origin of breeds and populations [31]. The most popular is use of the control region (CR) of mtDNA and its hyper variable part — D-loop. Full nucleotide sequence of CR in Eurasian and North American reindeer subspecies was published in 2003 [32].

Polymorphism analysis of D-loop fragment had enabled to describe di-
versity of reindeer population found in the Tojinsky region of the Republic of Tyva [33]. Prevalence of a single haplotype was established regardless of quite high level of genetic diversity. It is assumed that species with such variant of mtDNA come of one doe. Significant differences between the Chukot breeds and breeds from the Siberian tundra and North American caribou were found based on mtDNA haplotype analysis, including hyper variable part, left domain of the control region of D-loop [34]. Authors explain this by temporal gap in the origin of forms and unequal genetic exchanges between populations. High haplotype diversity as compared to other island forms was found based on mtDNA analysis in animals from Novaya Zemlya Archipelago, Kolguev Island, and from other places. Two haplotypes characteristic of the Kolguev Island were found in animals from Novaya Zemlya. Nowadays, such population involves 7 haplotypes, i.e. almost the same amount as that typical for other groups occupying much larger continental areas. Presence of 1-3 haplotypes is usually typical for island forms [35].

Study of the control region of mtDNA in wildlife reindeer inhabiting the European part of Russia highlighted quite high level of haplotype diversity of such population (0.914). Phylogenetic analysis had demonstrated close relationships between the European reindeer and wildlife Siberian reindeer. Common haplotype of the reindeer from the Murmansk region with wildlife reindeer inhabiting South-Western Norway had been described. The assumption was made that in the near past wildlife reindeer of the European North of Russia formed common population with reindeer inhabiting northern region of the Asian part of Eurasia [36]. Study of D-loop of mtDNA in reindeer from the continental part of the European North-East of Russia (eastern regions of Archangelsk area, the Republic of Komi, and Nenets Autonomous Okrug) revealed relatively high genetic diversity values. Phylogenetic analysis had enabled to found close relationship of such reindeer with animals from the Siberian tundra. Influence of domestic reindeer on formation of the genetic diversity in wildlife reindeer was generally acknowledged to be insignificant. Genetic lines of the extinct group of forest reindeer in Nizhny Novgorod area were found among the recent groups of reindeer from the European North-East of Russia [37]. During study of the polymorphism of mitochondrial DNA in Cervidae species, including 51 reindeer populations inhabiting the Europe and Asia, it was found that red deer had originated from the territory between the Kyrgyzstan and North India [38]. P. Gravlund et al. [39] had demonstrated polygenetic origin of three high arctic species: R. t. pearyi (Canadian Archipelago) and R. t. eogroenlandicus (Eastern Greenland, extinct since 1900 A.D.) are closely interrelated and, possibly, came from the North Arctic; R.t. platyrhynchus (Spitsbergen) came of forest Eurasian deer. Analysis of mtDNA variability in Cervidae species was reported in a series of studies [40-42].

Studies show that mtDNA polymorphism analysis in reindeer serves high informative tool for description of the genetic diversity, clarification of phylogeny and differentiation of breeds and populations within the species.

Microsatellites are also widely used for molecular and genetic analysis of diversity of the animal breeds and populations. This is the class of short tandem repeats (STR) of DNA [43-45] present both in non-coding and coding genome regions, as well as in chloroplast [46] and mitochondrial genomes [47]. These are widely used in assessment of the genetic structure of reindeer breeds and populations. Applied significance of STR is shown in publications of the scientists from America [48-50], Norway [51, 52], Canada [53-55], Great Britain [56], Denmark [57], Ireland [58], and Russia [59-61]. Use of 13 STRs had enabled to describe genetic diversity of various reindeer subspecies inhabiting Norway, Canada, Western Greenland, Spitsbergen, Alaska, and Finland [62]. Due
to the isolated geographical habitat, population of reindeer from Spitsbergen had the least values by the average number of allele per locus and heterozygosity level, and was less than other populations by number of polymorphic STRs (5 of 13). Based on analysis of 11 microsatellites, A. Mcdevitt et al. [58] had studied structure of two reindeer populations from the North America. Use of 14 STRs had enabled to identify genetic differentiation between two continental reindeer populations in Spitsbergen with habitat at distance nearly 45 km from each other [56]. M. Ball et al. [63] had analyzed polymorphism of 11 microsatellite loci in forest reindeer population (R. t. caribou) of the Central Canada. J. Kushny and J. Coffin [64] studied genetic diversity of three reindeer populations in Canada (R. t. groenlandicus, R. t. pearyi, R. t. caribou) with the use of 4 microsatellite loci. Using 16 microsatellite loci, A.I. Baranova et al. [65] reported clear separation of reindeer from the continental part of the Eurasian area and Arctic islands. Reindeers from the Asian and European regions of Russia were genetically more close to each other than to Kamchatka reindeer breeds. Moreover, they found quite poor separation of reindeer from eastern Eurasia in some habitats (Tomsk region, Khanty-Mansy Autonomous Okrug, Taymyr, Yakutia, and Chukotka), that evidences on their close genetic relationship.

We have developed multiple locus panel with 9 STRs for better performance and informativity of STR-analysis to control validity of origin and assessment of biodiversity of the Russian reindeer populations (Even, Ewenki, and Nenets breeds, and Tuvin population) [59]. It enabled to study sample allele pools of two great reindeer populations, domestic Nenets breeds and Taimyr wild reindeer population, and to identify the extent of genetic introgression between them. Although cluster analysis had shown high genetic segregation of both forms, several individuals of mixed genetic origin were found [66]. According to S.A. Kotovaya et al. [67], STR markers remain the most valuable genetic tool for i) studying population variability, ii) identification of possible substructures of animal populations inhabiting either the same territory, or geographically isolated or distant territories, iii) identification of the population structure of wildlife and domestic animals of the same species; iv) gene mapping and assessment of the gene flow between the groups of animals, and v) establishing the paternity and identification of species.

Alongside, obtainment of new information about the animal genome, improvement of the methodological approaches, development of high-performance genome analysis technologies, and creation of advanced analytical equipment had enabled the use of various genetic markers for allele pool studies [68]. Nowadays, single nucleotide polymorphism (SNP) analysis is mostly demanded for these purposes. There are many SNP detection techniques, starting from the analysis of restriction fragment length polymorphism (RFLP) (RFLP analysis) and ending by pyrosequencing [69]. However implementation of projects for determination of entire nucleotide genome sequences in most species of the farm animals had resulted in creation of DNA micromatrices (DNA-chips), which enabled SNP markers to take the leading position in animal genome studies. DNA chips are sets of the large number of oligonucleotides at miniature solid substrates designated for analysis of DNA sequences [70]. BeadArray-based technique of parallel full-genome genotyping of multiple SNPs (up to hundreds thousands) is the most popular [71]. Information on biodiversity [72-74], evolutionary relationships, extent of introgression and variability of animal breeds and populations had been obtained based on full-genome SNP screening with the use of DNA chips of various density [75, 76].

Recently, DNA chips for the most popular species of the farm animals (cattle, sheep, goats, pigs, and horses) and chicken are developed by Illumina Inc.
(USA) (http://www.illumina.com) and Affymetrix Inc. (USA) (http://www.affymetrix.com). Nevertheless, study of biodiversity in members of Cervidae genus with the use of native DNA chip seems to be an impossible task due to the lack of information on entire genome. However, several authors had demonstrated possibility of using commercial DNA chips developed for related domestic animals to study biodiversity and structure of subspecies population [82-85], as well as to differentiate horned ungulates (Bovidae) from deer family (Cervidae) [80]. For R. tarandus species, whole-genome genotyping started from testing of two commercial average density chips, Bovine and OvineSNP50 BeadChip [81]. It was established that Bovine SNP50 BeadDeveloped for cattle is more effective for genome reindeer scanning since its use enables to detect larger number of polymorphic SNPs as compared to the chip created for domestic sheep. One of the last publications regarding reindeer genetics study with the use of multiple SNP markers had provided for population and genetic description of three breeds raised at the territory of the Republic of Sakha—Yakutia (Even, Ewenki, Chukot, and Khargin breeds) [82]. It was shown that individuals of the Chukot breed leave behinds their congeners of two other breeds by extent of the genetic diversity, but, however is characterized by less number of the unique polymorphisms. Besides, regardless of the identified clear segregation of each breed, Even and Ewenki breeds are closer genetically.

Therefore, we have provided detailed description of how approaches to study genetic diversity of the reindeer evolved from the simplest (transferrin) to modern high-performance (DNA chips). Regardless of the successful application of the later, wide implementation of the new techniques (e.g. next generation sequencing, NGS) and cheapening of the existing sequencing techniques (Sanger method) allow studying the entire nucleotide sequence of genome of the unique species, Rangifer tarandus, thus opening new perspectives for its studies.

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