Polyloci genotyping by means of DNA fragments flanked by short inverted repeats in musk oxen genomes

Nikolay V. Bardukov, T.P. Sipko, A.V. Pheophilov, V.I. Glazko

Russian State Agrarian University – MAA named after K.A. Timiryazev,
Russia, Moscow, Timiryazevskaya str. 49, nikolay_bardukov@yahoo.com

Methods of polyloci genomic scanning which are based on nonspecific primers and amplification of anonymous DNA sequences are of great interest because of simplicity and low cost. Application of methods such as ISSR-PCR, IRAP-PCR and REMAP-PCR may be the only way to get good results rapidly in cases, when a new object which has not been studied previously and which genome has not been sequenced yet is chosen for research. However, all these methods suffer from a problem of results' reproducibility. This shortcoming was reduced when RAPD primers with random ten nucleotides sequence were replaced by ISSR and IRAP primers, which are composed from fragments of microsatellites and terminal flanks of mobile genetic elements respectively. Nonetheless the problem of spectra reproducibility was not solved completely.

In this study three reintroduced musk oxen populations were investigated. These are located in the west of Greenland, in the Taimyr Peninsula and in Wrangel Island. We carried out the DNA analysis of these animals in compare with ancient musk oxen from Yakutia (the end of the Pleistocene) and Taimyr Peninsula (middle of Holocene). There are a lot of musk oxen mitochondrial sequences in GenBank, but the nuclear genome of a musk ox has not been sequenced and SNP tests for this species were not created. Therefore we chose ISSR and IRAP methods of polyloci genotyping to study DNA of these animals.

Comparison of ancient animals' spectra acquired from degraded DNA with modern ones was carried out by means of 3 ISSR markers ($(GAG)_6C$, $(AG)_9C$ and $(GA)_9C$) and 2 long terminal repeat of endogenous retroviruses (IRAP markers). Thus we observed well reproduced conserved part and a variable part in every amplification specter. It is showed that amplification products loss not always takes place starting from long amplicons to short ones. Hence, in all spectra there were well reproduced "heavy" zones (more than 1000 bp) while some "light" amplicons were missing. We also found differences in reproducibility of spectra acquired with different primers. Thus, in musk oxen primer $(GAG)_6C$ produced much more conserved spectra compared to other ISSR markers. Amplicons of conserved part of spectra which show polymorphism in modern animals may be used for their reliable genotyping and constructing phylogenetic relationships. Two other amplicon types are also of special interest for investigations: the most reproducible ones in ancient spectra which are DNA regions of higher stability; and those which disappear in the first place as potentially the most unstable regions.

Observing features of locus presence in ancient and modern animals' spectra we may propose much higher allele diversity in ancient musk oxen populations. Thus, in spectra of 5 primers in sum modern animals lack 30 loci which are found in ancient ones while the latter lack only 14 loci which are observed in modern individuals. One should also take into account that the absence of some loci amplified may be connected with degradation of DNA extracted from bones of ancient individuals.

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