

THE STUDY OF GENETIC DIVERSITY OF GREY STEPPE CATTLE BREED BY ANALYZING THE CYTOCHROME B AND D-LOOP MITOCHONDRIAL MARKERS

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Abstract

The main purpose of this paper is to quantify the genetic variability of Grey Steppe cattle breed, by analysis of two mitochondrial markers (cytochrome b and mitochondrial control region d-loop), relevant for advanced studies of genetic diversity, phylogeny, molecular phylogeography and identification of taxonomic relationships of species representative of the Bovinae subfamily, using new molecular genetics techniques (isolation and purification of DNA by automatic extraction, quantification of total DNA by spectrophotometry technique, amplification of mitochondrial markers by PCR, validation of amplicons by electrophoresis technique, purification of DNA by columns, Sanger-type sequencing, nucleotide sequence analysis using complex statistical and molecular analysis programs). The results of this research, they come in addition to the existing information in the literature, with aspects regarding some uncertainties taxonomic, but also the highlighting of new information related to the evolutionary history of Grey Steppe, demonstrating at the same time the descent of this breed to *Bos taurus primigenius*.

Key words: *Bos taurus primigenius*, mtDNA, phylogeny, gene sequencing

INTRODUCTION

Since ancient times, humans were concerned about cattle breeding, first for meat production and work in agriculture and later for milk production [1]. Indigenous cattle breeds are considered socio-cultural heritage and valuable genetic resources due to their adaptation to local conditions, important aspects for reproduction and conservation programs [11]; [13]. Indigenous romanian cattle breeds are classified by many researchers into primitive breeds (Grey Steppe and Mocanita) and improved breeds (Romanian Spotted, Romanian Black Spotted, Brown, Pinzgauer) [5] [14]. Until 1850, we can discuss a majority proportion of two indigenous cattle breeds, existing in our country: Grey Steppe (found mainly in the steppe and hill areas) and Mocanita (widespread in mountainous areas). A favorable and decisive imprint in the

formation of these breeds was represented by the environmental conditions that contributed to the printing of the characteristic features of rusticity, adaptability to severe environmental conditions, increased resistance to disease and longevity [5] [14] [15]. Over time, it has been found the existence of four ecotypes of the Grey Steppe breed (Moldavian, Transylvanian, Dobrogean and Ialomițean) named according to the geographical area of the country [5]. The Romanian Grey Steppe belongs to the group of podolian breeds, still found today, in different areas of Europe. The common origin of this breed with other podolian breeds (Iskar Gray, Bulgarian Gray, Istrian, Slavonian Podolian, Katerini, Hungarian Gray, Maremma, Podolica, Turkish Gray, etc.) [17];[18] is the wild ancestor *Bos taurus primigenius*, which was declared extinct around the 16th century. In the literature are numerous studies based on mtDNA belonging to the *Bos taurus*, that have demonstrated the presence of a number of three haplogroups, geographically structured,

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respectively: T1 found in Africa, T2 originating in the Middle East and West of Asia and T3 widespread in Europe [8];[10]; [19]. Since 1892, with the import of new breeds, the Romanian Gray Steppe population has been facing a drastic decline in numbers, and since 2000, the Food and Agriculture Organization (F.A.O.) has decided to include the breed in risk status "endangered". In 2017, studies showed the existence of a number of approx. 150 heads in the area of Moldova and the Danube Delta [7], currently, the number of specimens of this breed has decreased considerably. Revised bibliographic references [3];[4];[9];[6];[15] regarding the endangered indigenous cattle breeds in Romania, confirm that they are carriers of a reservoir of valuable genes that must be preserved by applying different reproductive biotechnologies [12]. The main purpose of the project is to quantify the genetic variability of the Grey Steppe cattle breed, by analyzing two mitochondrial markers (cytochrome b and d-loop), relevant for advanced studies of genetic diversity, phylogeny, molecular phylogeography and identification of taxonomic relationships order of the representative species within the *Bovinae* subfamily. Thus, based on the results obtained, some recommendations will be formulated regarding the gene flow, increasing genetic variability and reduce the effects of inbreeding. At the same time, this study complements the existing information, with aspects regarding some taxonomic uncertainties, but also the highlighting of new information related to the evolutionary history of the two breeds.

MATERIAL AND METHOD

The analysis of phylogenetic relationships and the quantification of the genetic diversity of Grey Steppe cattle breed was initiated through the stage of collecting biological samples, respectively blood, for a number of 32 females. To achieve the goal plan, a series of genetic analysis methods were applied, starting from the isolation and purification of total DNA from blood samples, by automatic extraction, quantification of total DNA by

spectrophotometry technique, amplification of cytochrome b and d-loop by PCR, validation of amplicons by 1% agarose gel electrophoresis, purification of DNA through columns, sequencing of genes of interest and finally, crude analysis of nucleotide sequences.

RESULTS AND DISCUSSIONS

Numerous mtDNA studies in *Bovinae* species have identified a number of 5 major haplotypes, specific to the genus *Bos taurus*, the wild ancestor of domesticated cattle (T1, T2, T3, T4 and T5) and 2 haplotypes for *Bos indicus* (I1 and I2). Recent research has shown that almost all cattle belong to the macro-haplogroup T and the estimated time of divergence is ~ 16 thousand years, a time that indicates a narrow block in the evolutionary history of cattle in *Bos taurus*. The macro-haplogroup T is divided into 2 sister subclades (T5 and T1/T2/T3), the predominant subclade being represented by T1/T2/T3 [16]. Over time, T4 has been shown to be integrated into T3. Another haplogroup, P, was identified by analyzing the d-loop mitochondrial control region of cattle in northern and central Europe. Another study, aimed at analyzing the nucleotide sequences of d-loop, specific to an endangered breed of cattle in Italy, led to the identification of another haplogroup belonging to this breed, namely haplogroup Q. The same haplogroup Q, was also identified following the phylogenetic analysis of European ancestral cattle, with offspring in *Bos taurus primigenius* [15]. A study by Achilli et al. (2009) [2], on the origin of cattle based on mitochondrial genome analysis, demonstrated that not all cattle in Europe belong to haplogroup T. The author analyzed gene sequences of mtDNA for a number of 26 European cattle breeds (22 from Italy and 4 from other regions of Europe). Most breeds were in the T haplogroup and its subclades. 1.4% of the breeds analyzed were representative of haplogroups P and Q, specific to ancestral cattle in northern and central Europe, with *Bos taurus primigenius* as their common ancestor. Following the analysis of the

nucleotide sequences of the two mitochondrial markers, in the case of the Gray Steppe cattle breed, a number of 4 large haplotypes (T1, T2, T3 and P'QT) were identified, with specific connection networks. Each haplotype corresponds to a certain number of individuals (Fig. 1).

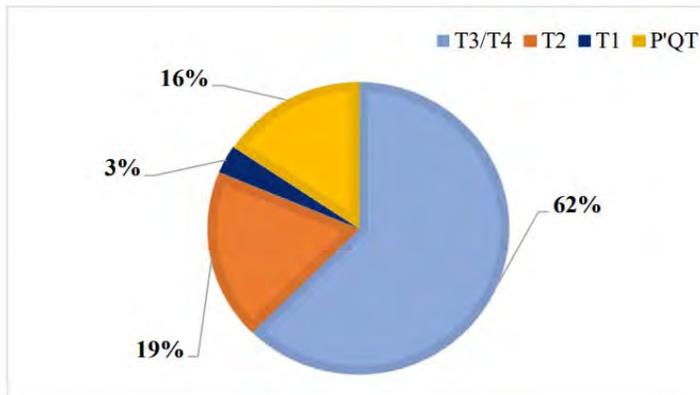


Fig. 1 Frequency of haplotypes in Grey Steppe cattle population

Regarding the distribution of haplotypes, it is found that the highest frequency is the T3/T4 haplotype, being identified in the case of a number of 20 individuals analyzed, totaling 62%, a number of 6 individuals were found in T2 haplotype, with a frequency of 19%, another identified haplotype, with a frequency of 16% was P'QT, representative for a number of 5 individuals (also identified in the analysis of nucleotide sequences from specimens of bulls of direct descent in *Bos taurus primigenius* and only one individual is included in the T1 haplotype, which has the lowest frequency, only 3%. The analysis of the nucleotide sequences based on the Network program resulted in the network of haplotypes represented graphically in figures 2.a and 2.b.

The identification of the P'QT haplogroup following the analysis of the nucleotide sequences of the Grey Steppe cattle breed, indicates that this haplogroup is of ancestral type, being specific for *Bos taurus primigenius*, from which this breed of cattle evolved. Within this haplogroup, a number of 5 individuals were identified (SS_22; SS_25; SS_26; SS_30; SS_34), representing a

percentage of 16% of the total analyzed specimens. The genetic distance between T1, T2 and T3 haplotypes varies between 1 and 4 sites. The presence of T-derived haplotypes suggests a demographic expansion of this population.

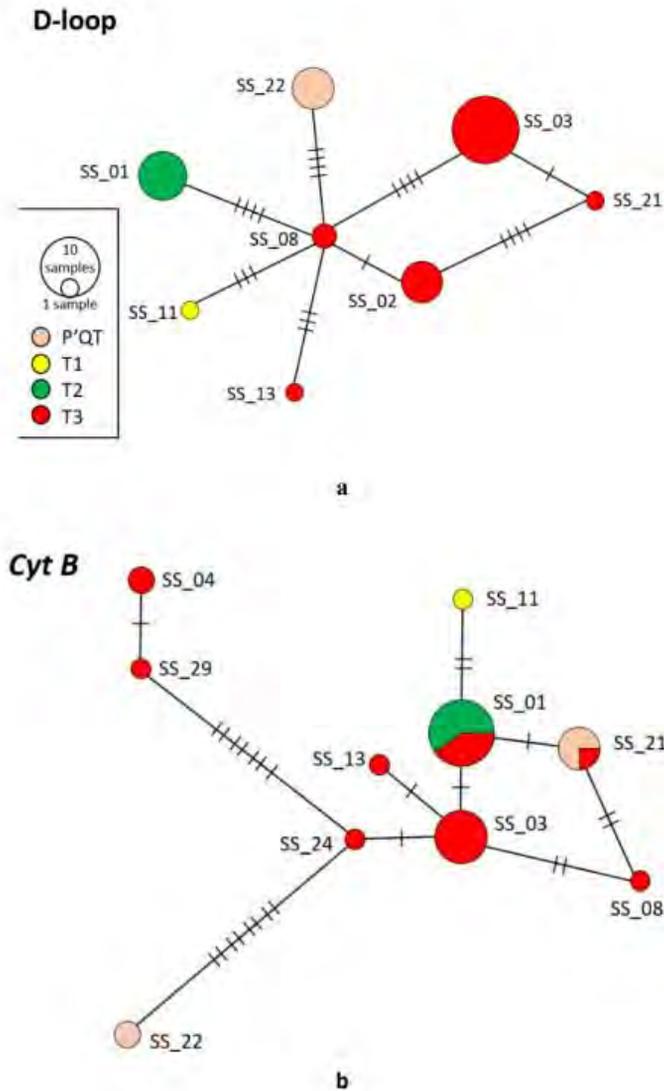


Fig. 2 The network haplotypes of Grey Steppe cattle population a. cytochrome b; b. d-loop

CONCLUSIONS

Given that a number of 5 individuals were identified in the P'QT haplogroup, representing 16% of the total number of specimens analyzed, it can be concluded that these individuals could be used in the selection process for crossbreeding, in order to preserve the breed in its pure state. The results of this research can help to improve

the conservation program of this breed and current information on the genetic diversity of the population, as a valuable tool for efforts to conserve this endangered gene pool.

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