# Analysis of the Genetic Diversity of Endangered Cattle Breeds Based on Studies of Genetic Markers

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**Abstract**

In the last 15 years, about 300 breeds out of 6,000 belonging to different species of animals have been identified by the Food and Agriculture Organization of the United Nations (FAO) as extinct. Among the cattle breeds seriously threatened with extinction are the podolian cattle from different parts of Europe. These belong to a group of very old European breeds, having ancestors in Bos primigenius. By studying molecular markers, can be obtained information on the genetic structure of endangered cattle breeds and through identification of some sections of the genome that influence certain traits, can be assess the conservation value of genetic resources of animal origin. The aim of this paper is to identify the main molecular markers used in the analysis of endangered cattle breeds.

**Keywords**: conservation, molecular markers, phylogeny, podolian cattle.

## 1. Introduction

Molecular markers are used in gene mapping to mark spots in a genome and make linkage maps. Molecular markers that flank the quantitative and qualitative trait loci show high polymorphism and are another source of discrimination even between related individuals. They help us locate and visualize them on the chromosome, in each individual used in parental forms, for repeated crosses and backcrosses, so that we actually retain only those individuals who have the genes of interest. For the genotyping of animals, it is necessary to identify and map on the chromosome a sufficiently large number of genetic markers with high polymorphism, but covering the entire genome by their distribution. After their location on the chromosome, the respective markers will serve as areas for marking genes or quantitative locus. Genetic markers are fragments of DNA, usually non-informational (hypervariable regions of DNA or introns), sometimes even informational (functional genes), used to detect chromosomal segments involved in the manifestation of a character and the genes underlying their manifestation. The ideal genetic markers are those with high polymorphism, characterized by the possession of two or more differentiable alleles, codominant and which can be easily highlighted [1]. Genetic analysis techniques have evolved considerably over time, allowing genome sequencing in different species of interest. The phylogeny of many individuals has been studied by researchers since 1980. Another type of genetic research has focused on the study of genetic markers associated with the characteristics of milk / meat production, especially in endangered cattle breeds, which is useful for appreciation of the conservation value of genetic resources of animal origin as well as for establishing the degree of uniformity of the breed [2]. Many other molecular

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markers associated with the place of formation and domestication of the breed have been analyzed over time by many researchers in the field. This paper is supplemented by new information from the literature on molecular markers studied for the analysis of genetic diversity of endangered cattle breeds.

## 2. Materials and methods

This paper is a review of the most relevant research in the literature in the country and abroad, on molecular markers used in studies of genetic diversity of cattle breeds. For the realization of the paper were consulted a number of 14 bibliographical references in the field, of which 2 books and 12 specialized articles, but also information from different international databases.

## 3. Results and discussion

Piera Di Lorenzo et al., 2018 studied the genetic diversity of podolian cattle breeds and aimed to reconstruct their origin. Therefore, 18 podolian breeds have been studied phylogenetically (Piemontese, Bianca di Val Padana, Romagnola, Mucco Pisano, Calvana, Chianina, Maremmana, Marchigiana, Italian Podolian, Ukrainian Grey, Romanian Grey, Hungarian Grey, Slavonian Syrmian Pod., Istrian Cattle, Podolsko, Bulgarian Grey, Katerini, Turkish Grey), by researching mitochondrial DNA (mtDNA). To interpret the results, nine other cattle breeds were compared (Valdostana, Gray Alpine, Italian Brown, Italian Red Pied, Cabannina, Reggiana, Agerolese, Cinisara, Modicana). The global analysis clearly highlighted some peculiarities of some genes in the mtDNA group. The analysis of the main components indicated a genetic proximity between five breeds (Chianina, Marchigiana, Maremmana, Podolica Italiana and Romagnola). A suggestive hypothesis shows the ancestral double contribution to the current genealogical background of podolian breeds [3]. In Romania, the study of genetic polymorphisms of major milk proteins (casein, lactalbumin and lactoglobulin) in various native cattle and the possibility of using them as genetic markers to improve milk quality or identify the authenticity / origin of milk and other dairy products, were performed by researcher Bâlteanu V.A., through his research in his doctoral thesis, identified a new allele of αS1-CN in bulls from the Grey Steppe breed, being completely characterized using a combined methodology of protein, DNA and DNA sequencing. Isoelectric focusing (IEF) and PCR techniques have been successfully tested in Romania to characterize milk protein polymorphisms. The αS1-casein allele, discovered in the Grey Steppe breed has not been reported in any other European cattle breed, being an ancestral allele that comes directly from the wild ancestors of the breed, providing the first molecular evidence of its phylogenetic position, information whose knowledge is necessary in the context of conservation of genetic resources [4]. Although it is not found in a Romanian population, it seems that the Pinzgau breed is also a carrier of a certain allele. The investigation of milk samples by the IEF technique of Pinzgau animals, located in Austria, Bavaria and Germany by Erhardt in 1996, revealed a new variant of casein (κ-casein G) with a frequency of 0.003, an allele that it was not found in milk samples collected from Limpurger, another endangered breed [5]. However, the family of milk proteins is quite large, with a significant influence on the composition of milk and its physicochemical properties, characteristics that have been well documented in recent years for various alleles and genotypes at each locus. In an overview of all caseins, in most cases, the results of different associations are contradictory due to their location in the structure of the 6th pair of chromosomes. In the study of caseins, many scientists take into account the genetic structure of κ-casein. Therefore, the κ-casein allele is well known to be associated with an increased yield of milk production [6] the B allele, with a higher casein concentration [7] increased protein and fat yield [8]. Genotype AA is associated with high milk production and genotype BB is associated with high-fat milk and casein and the B allele of k-casein leads to a significant increase in the amount of protein in milk, BB homozygous animals produce less milk, but with a higher protein content. On the other hand, AA homozygotes on the β-lactoglobulin gene are associated with both an increased milk production and an increased protein content in milk. These researchers recommend the selection of Jersey cows with the homozygous BB genotype of k-casein when the aim is to obtain a milk with a good quality protein, which is used in cheese production. Taking into account the two cattle breeds, the characterization of the milk protein polymorphism resulted in a higher frequency of the B allele at the k-casein locus in Grey Steppe cattle and a higher frequency of the A allele in the case of the Pinzgau breed. Romanian. Allele A was found in Pinzgau cattle in a unique combination for milk protein loci located on the 6th chromosome (C-A2-BA, which encodes the following proteins: αS1-, β-, αS2- and k-casein, respectively). In the case of whey proteins, β-lactoglobulin is evaluated mostly due to its polymorphism, with type B and BB genotypes being observed in associations with desirable traits for the dairy industry, such as fat percentage, shorter coagulation time and a higher thermal stability of proteins. There are many other genes associated with milk production, for example, the pituitary transcription factor and the growth hormone gene. The genes of Pituitary Factor 1 polymorphisms (POU1F1 or PIT1- Pituitary Factor 1) and growth hormone receptors (GHR - Growth Hormone Receptor) were researched on Romanian Grey Steppe [9]. Following the investigation of 60 blood samples, two alleles were found at the PIT1 locus, the B allele being predominant in variant A and the Podolica breed, although allele A was found to be associated with milk production and body conformation [10]. Given the GHR gene, allele A has been reported in several breeds of cattle, being associated with higher percentages of fat and protein [9]. In the case of meat production and its quality, an influence of different alleles of some genes has been established, for example Leptin (LEP) and Calpastatin (CAST). At the same time, leptin also plays an important role in glycogen synthesis and glucose transport [11]. A series of investigations on different varieties within the Grey Steppe breed considered the study of hemoglobin (Hb), transferrin (Tf) and potassium (K), in erythrocyte polymorphisms [12]. Three variants of Hb were discovered in Grey Steppe cattle by Soysal MI, Kök S., (2008), with the highest mean frequency for type A (more than 90%), followed by B and C alleles [13]. Based on the interpretation of vertical electrophoresis using polyacrylamide gel as a migration support, a single type corresponding to type A was observed in the Romanian Grey Steppe [14]. In addition to the usual variants of the Hb gene, an unusual polymorphism of hemoglobin has been recognized in podolian cattle breeds in Italy, involving AY and Azebu variants that were not detected in other breeds studied [12]. At the serum transferrin locus, seven alleles were reported in Grey Steppe cattle (A, B, D, D1, D2, E and F), of which variants A and B were reported at a frequency between 0.21 and 0.43 and 0.02 to 0.05, the mean frequencies for other reported alleles being in the following descending order: D>D1 = D2>E>F. In the same breed, three alleles were detected at the serum transferrin locus, variant D being reported in more than half of the investigated population, allele E being encountered with a lower incidence than allele A. The genotype EE was found to be associated with a yield of milk production, and any of the E alleles present in the genotype seems to be associated with good milk production and a higher percentage of fat. The lowest performances in terms of milk quantity and fat percentage were reported for cows with AD genotype and for cattle with DD genotype, low performances were found in terms of fat content. Potassium polymorphism in erythrocytes of Turkish Grey was investigated by Soysal M.I. et al. (2008), who identified two alleles, KH and KL, as a function of red cell potassium concentrations, LK and HK. The frequencies reported following the investigation of 39 individuals of the Turkish Grey, showed a higher incidence of KL alleles in over ¾ of the population [13], agreeing the results of researchers Gonzales and Vallego (1983) who studied the Sayaguesa, Morucha, Cordena Andokza and Blanca Cocerona breeds, also highlighting a high frequency of KL alleles. Therefore, molecular markers have proven to be indicators associated with milk/meat production traits, especially in the case of endangered cattle breeds, useful information for assessing the conservation value of animal genetic resources and for analyzing diversity genetics of the breed. Numerous researchers have found that there are genes associated with milk production, such as the growth hormone gene, the gene that causes prolactin synthesis, the genes that cause the synthesis of caseins and lactoglobulins, or the pituitary transcription factor (Pit 1) [12].

## 4. Conclusions

Molecular analysis techniques have evolved considerably over time, allowing the investigation of genome diversity by sequencing it to different species of zootechnical interest, while identifying numerous single nucleotide polymorphisms (SNPs). Thus, the phylogeny of many animal species has been studied by researchers since 1980. Research in the past has been mainly based on the study of genetic markers associated with characters in individuals of different species of interest, as well as research on microsatellites due to the very high polymorphism and the large amount of genetic information. By studying molecular markers, information can be obtained on the genetic structure of cattle breeds and by identifying sections within the genome, sections that influence certain traits, we can assess the conservation value of a genetic resource of animal origin. The aim is to preserve these breeds, due to their special biological properties, increased resistance and adaptability to diseases and severe environmental conditions, as well as an extraordinary ability to recover fodder residues or poor quality fodder. The use and testing of new molecular marker research techniques will help clarify issues regarding the genetic diversity of endangered cattle breeds. At the same time, it will be possible to establish associations between gene polymorphism and morpho-productive features. The validation of molecular analysis methods will allow the use of genetic information in genetic conservation programs both nationally and internationally.

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