STUDY OF GENETIC DIVERSITY AND PRESERVATION STRATEGIES OF ROMANIAN PINZGAU CATTLE

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Abstract

The Romanian Pinzgau cattle, currently endangered, was formed through crossbreeding Grey Steppe cows with Austrian Pinzgauer bulls and is well adapted to mountain and submountain regions (400–1600 m altitude) with fertile natural pastures and abundant rainfall. This breed is highly resilient and efficiently utilizes cellulose-rich forage, holding significant zootechnical importance. In this study, the Pinzgau population was genetically analyzed using mitochondrial markers (mtDNA), which allowed the identification of three main haplogroups, with haplogroup T3 being predominant in analyzed populatyion, confirming both considerable genetic diversity and demographic equilibrium. The purpose of the research was to assess genetic variability and establish phylogenetic relationships, providing a foundation for effective conservation strategies. The results highlight the necessity of applying reproductive biotechnologies, cryopreservation of genetic material, and the creation of gene banks to ensure the long-term preservation and protection of this valuable breed.

Key words: *endangered cattle, genetic diversity, mtDNA, phylogeny*

INTRODUCTION

The conservation of genetic diversity in domestic animal populations has become a central topic in modern animal science and conservation biology. In the context of global climate change, agricultural intensification, and the risk of genetic erosion caused by the widespread use of highly specialized breeds, the preservation of local and traditional breeds is essential for maintaining sustainable agricultural systems. Cattle breeds, in this context, represent a crucial component both for food security and for rural cultural heritage, as they provide not only milk and meat, but also adaptability to local conditions, disease resistance, and socio-economic value for farming communities [1,2].

Among the native cattle of Romania, the Pinzgau breed occupies a unique position due to its historical, genetic, and productive importance. Originating from the Austrian Pinzgau breed and adapted over centuries to the Carpathian ecosystem, the Romanian Pinzgau is a dual-purpose breed, valued for both milk and meat production. Its adaptability to the harsh conditions of mountain areas, resistance to local diseases, and balance between productive traits make it a valuable genetic resource [2-4]. However, in recent decades, the population of Romanian Pinzgau cattle has declined significantly, mainly because of crossbreeding with more specialized dairy and beef breeds, reduced economic competitiveness, and demographic changes in rural areas. These factors have raised concerns about the risk of genetic erosion and even the potential extinction of the breed if adequate conservation measures are not implemented.

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The study of genetic diversity within the Romanian Pinzgau breed is essential to understanding the current state of the population, to evaluating the impact of past and ongoing crossbreeding, and to identifying unique alleles and adaptive traits worth preserving. Modern molecular techniques, such as microsatellite markers, SNP genotyping, and mitochondrial DNA analysis, provide powerful tools for assessing intra- and inter-breed variability. These methods allow for the measurement of inbreeding levels, genetic drift, and effective population size, critical indicators of the long-term sustainability of the breed. At the same time, traditional pedigree records and genealogical analyses complement these data, offering a broader picture of population structure and reproductive dynamics [1,4-8].

Conservation strategies for the Romanian Pinzgau must combine both in situ and ex situ measures. In situ conservation, through the maintenance of viable herds in their natural environment, ensures the continued adaptation of the breed to local ecological and socio-economic conditions. Ex situ conservation, such as cryopreservation of semen and embryos, represent an additional safeguard against sudden population losses. Moreover, the establishment of breeders' associations, financial support programs for farmers maintaining purebred herds, and the promotion of niche markets for traditional products derived from the breed (such as cheeses and high-quality beef) are critical components of sustainable conservation. The international framework, such as the FAO Global Plan of Action for Animal Genetic Resources, also encourages member states to develop national strategies for the protection of indigenous breeds, placing Romania in the position to align its efforts with broader European initiatives [9,10].

In this context, the present study aims to analyze the genetic diversity of Romanian Pinzgau cattle and to develop effective conservation strategies. By combining molecular analyses with evaluations of the current population status, the research seeks to provide a comprehensive understanding of the breed's genetic profile, as well as practical recommendations for safeguarding its future. The results are expected to contribute not only to the conservation of a valuable national genetic resource, but also to the broader global discourse on the importance of maintaining biodiversity in domestic animal populations.

In this study, a series of investigations were carried out to evaluate the genetic diversity of a population of Pinzgau cattle through mtDNA analysis. The present research complements the existing information in the scientific literature by addressing certain taxonomic uncertainties and highlighting new considerations regarding the evolutionary history of this population within the *Bovidae* subfamily.

MATERIAL AND METHOD

The analysis of phylogenetic relationships and the quantification of genetic diversity in the Pinzgau cattle breed was initiated with the collection of biological samples, namely blood. Samples were collected from 20 female individuals belonging to the nucleus herd under conservation at a livestock farm in Târgu-Mureș.

The molecular analyses aimed at achieving the proposed objectives were carried out in the Laboratory of Molecular Genetics. In order to accomplish the research plan, a series of analytical steps were performed:

- ✓ isolation and purification of total DNA from the collected blood samples;
- ✓ quantification of total DNA using spectrophotometry;
- ✓ amplification of mitochondrial DNA through the PCR technique;
- ✓ validation of amplicons by agarose gel electrophoresis;
- ✓ purification of DNA;
- ✓ sequencing of target genes;

✓ retrieval of nucleotide sequences for the two target genes from the international GenBank database (N.C.B.I.), as well as the complete mitochondrial genome of *Bos taurus*, used as references for the analyzed sequences;

✓ alignment of downloaded sequences with the sequences of interest;

✓ quantification of demographic and spatial distribution, as well as gene flow among the analyzed cattle populations, using dedicated software programs.

For the analysis of gene sequences, a series of genetic analysis programs were used, as listed and presented below:

Sequencing of PCR products: *SANGER sequencing;*

Alignment of chromatograms and correction of raw sequences using *DNA Baser;*

Sequence analysis:

Alignment – *MEGA X program*;

Substitution model – *jModelTest program;*

Haplotype network – *PopArt program*;

Construction of phylogenetic trees (NJ – Neighbor Joining, ML – Maximum Likelihood) – *SeaView program*;

Estimation of divergence time – *BEAST v.1.7.5 program;*

Population genetics – *DnaSP 5.10 and R programs.*

RESULTS

Total DNA quantification and gene sequence amplification

The reconstruction of the evolutionary history of cattle, demonstrated through phylogenetic trees, was initially investigated because of the morphometric traits of existing breeds, in accordance with ancestral evidence. With the advent of new molecular analysis techniques, studies on phylogeny have subsequently been approached from the perspective of molecular taxonomy.

Table 1 Concentration of mtDNA extracted from blood samples

No. sample	Abs260	Abs280	Abs230	260/280	260/230	mtDNA concentration (ng/µl)
01	0.292	0.221	0.368	1.61	0.92	17.9
02	0.538	0.295	0.478	1.53	0.95	22.7
03	0.755	0.845	1.205	1.47	1.02	61.2
04	0.370	0.235	0.342	1.57	1.08	18.7
05	0.783	0.308	0.435	1.55	1.14	24.6
06	0.828	0.498	0.665	1.66	1.25	42.0
07	0.472	0.222	0.385	1.69	0.97	18.9
08	0.688	0.228	0.372	1.71	1.04	19.4
09	0.592	0.370	0.620	1.60	0.95	29.8
10	0.790	0.505	1.018	1.56	0.78	39.9
11	0.405	0.268	0.407	1.51	0.99	20.4
12	0.607	0.375	0.805	1.62	0.75	30.5
13	0.825	0.490	0.560	1.68	1.47	41.2
14	0.918	0.525	0.578	1.75	1.58	46.3
15	0.955	0.568	0.665	1.68	1.43	48.0
16	0.545	0.318	0.482	1.71	1.13	27.5
17	0,348	0.169	0.312	2.06	1.11	17.4
18	0,358	0.173	0.298	2.07	1.20	17.8
19	0,260	0.149	0.242	1.74	1.07	13.2
20	0,082	0.124	0.278	0.66	0.30	4.15

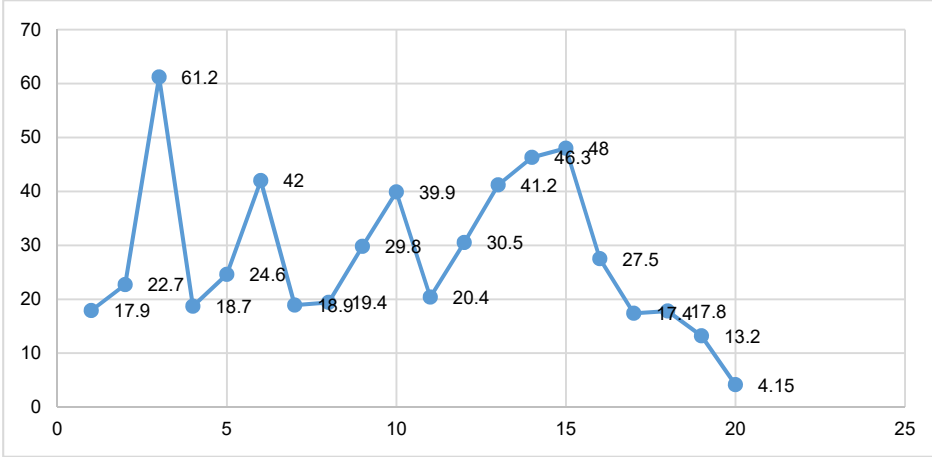


Fig. 1. Graphical representation of DNA concentration values in samples

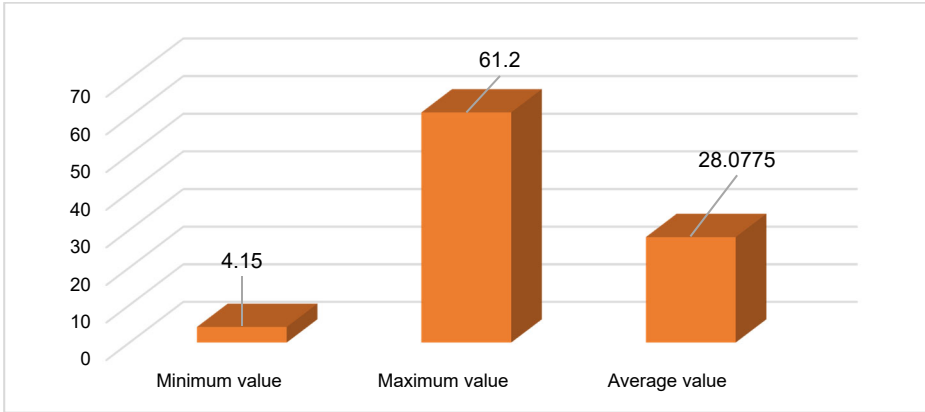


Fig. 2. Comparison between the average value of the DNA concentration, in relation to the minimum / maximum values (ng/µl)

The quantification of DNA samples from the 20 bovine females ranged from 4.15 to 61.2 ng/µl (Table 1 and Figure 1), with a mean of 28.07 ng/µl (Figure 2), while the ratio of the two absorbances, namely A260/A280, showed values between 0.66 and 2.13, with an average of 2.07.

Validation of PCR products

The isolated and purified DNA was amplified using the polymerase chain reaction (PCR), which enabled the exponential multiplication of specific sequences of genetic material.

The amplification of mtDNA was carried out using the primers BCYT-F and BCYT-R, in accordance with the specialized literature [11,12].

To assess the degree of purity, molecular size, or possible nonspecific contaminations, the amplicons resulting from the amplification of the gene sequences of interest were validated on a 1% agarose gel by migration at a voltage of 100 volts for a duration of 30 minutes. The size estimation of the amplified fragments was performed based on a 100 base pair molecular weight marker.

The image of the electrophoresis gel, after migration of the samples under the influence of the electric current, can be seen in Figure 3.

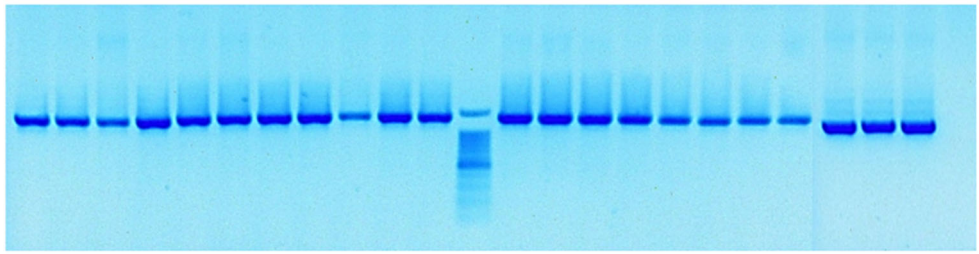
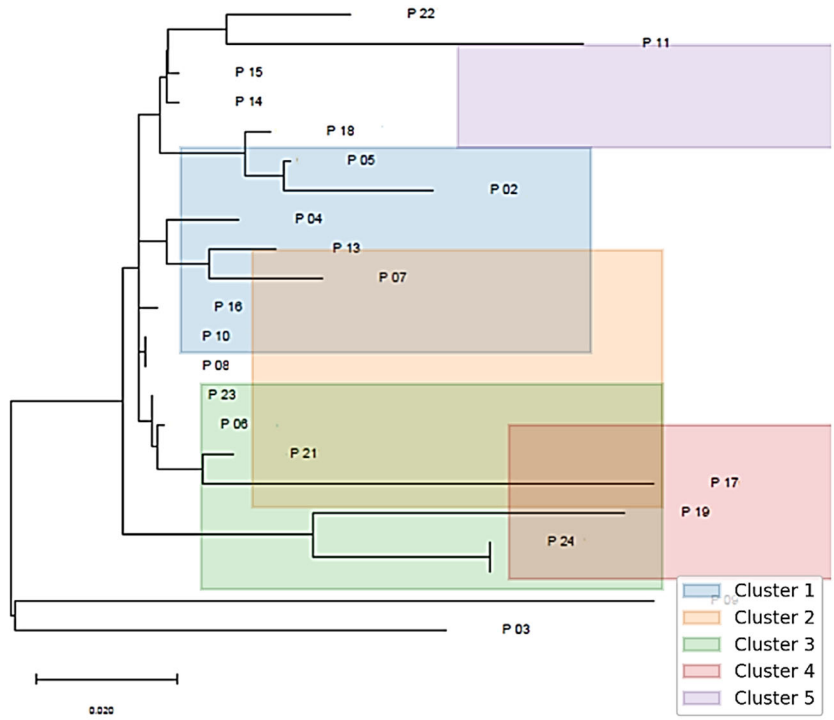


Fig. 3. Agarose gel electrophoresis of PCR amplicons

Following the electrophoretic validation of the amplicons, it was found that no nonspecific amplifications or other types of contamination occurred, and the primers used for amplification exhibited a high level of specificity for the analyzed gene sequence, resulting in a large number of copies.

Dynamics of the rate of evolution of the studied mitochondrial markers

In order to evaluate the phylogenetic relationships among individuals of the Pinzgau breed, based on the analysis of the nucleotide sequences of the mtDNA, a phylogenetic tree of the ML/NJ type was constructed using the SeaView software, graphically represented in Figure 4.



*P - abbreviation of the breed title, Pinzgau

Fig. 4. Phylogenetic tree based on mtDNA sequence analysis for Pinzgau breed

The results obtained from the genetic diversity analysis of the Pinzgau breed are considered to be an important tool in the development of conservation programs for the breed, which is currently in great danger of extinction.

DISCUSSIONS

The analysis of the genetic diversity of the Pinzgau breed, through mitochondrial DNA quantification, gene sequence amplification, and phylogenetic tree reconstruction, provides essential data for understanding intra-population variability and for establishing clear directions in the conservation of this genetic resource. The results obtained in the present study show DNA concentrations ranging from 4.15 ng/µl to 61.2 ng/µl, with a mean value of 28.07 ng/µl. These values fall within the ranges previously reported in cattle in similar research. For example, studies conducted by Achilli et al. (2008) and Achilli et al. (2009) on mitogenomic diversity in taurine cattle indicated average mitochondrial DNA concentrations between 20 and 50 ng/µl, suggesting that the results obtained for Pinzgau are comparable to those reported for other European breeds [13,14]. Moreover, the absorbance ratio A260/A280, used as an indicator of DNA purity, showed greater variability in the analyzed samples (0.66–2.13), but the mean obtained (2.07) is close to the values considered optimal in the literature (1.8–2.0), according to Bollongino et al. (2006). This indicates that most samples had a quality suitable for subsequent use in PCR reactions [15].

Regarding the validation of PCR products, the absence of nonspecific amplifications and the correct size of the obtained fragments demonstrate the high specificity of the primers used. Bradley et al. (2006) and Maretto et al. (2012), who showed that mitochondrial marker analysis through PCR represents a robust and reproducible method for investigating cattle phylogeny, reported similar results [16,17]. Furthermore, the successful amplification without contamination supports the assumption that the biological material collected and processed in this study was properly preserved and handled, an essential aspect in molecular genetic research.

The phylogenetic analysis of mitochondrial sequences in Pinzgau individuals highlighted the existence of distinct clusters, suggesting a certain degree of genetic diversity within the population. Although present, this diversity must be interpreted in the context of the current risk of genetic erosion of the breed, which is under significant threat of extinction. Similar results concerning genetic structure and the vulnerability of local breeds were obtained by Pariset et al. (2010) for indigenous cattle populations from the Iberian Peninsula, where agricultural industrialization led to a dramatic decrease in genetic variability. At the same time, the present study confirms the observations of Park et al. (2015), who reported that local cattle breeds show lower genetic diversity compared to commercial breeds, but that this limited diversity is of major importance in conservation and breeding programs [18,19].

An essential aspect of this study is that the phylogenetic tree obtained reflects the specific evolutionary history of the Pinzgau population, reinforcing previous data that this breed belongs to a distinct genetic lineage with Alpine origins. When compared to international literature, the results are consistent with the studies of Beja-Pereira et al. (2003), which demonstrated that European cattle are divided into clear phylogenetic lineages, corresponding to multiple independent domestication events [20]. In this sense, the inclusion of the Pinzgau breed in such analyses contributes to completing the general picture of cattle evolution and diversity at the European level.

CONCLUSIONS

In conclusion, the data generated through DNA quantification, PCR product validation, and phylogenetic analysis support the finding that the Pinzgau breed displays detectable but vulnerable genetic diversity, comparable to the situation reported in other European local breeds. These results provide a solid scientific basis for the implementation of genetic conservation programs, aligned with FAO recommendations and the specialized literature, with the aim of preserving the genetic heritage of this population currently at risk of extinction.

REFERENCES

1. Baumung, R.; Sölkner, J. Analysis of pedigrees of Tux-Zillertal, Carinthian Blond and Original Pinzgau cattle population in Austria. *J. Anim. Breed. Genet.* **2002**, *119*, 175–181. DOI: 10.1046/j.1439-0388.2002.00332.x
2. Mészáros, G.; Fuerst, C.; Fuerst-Waltl, B.; Kadlečík, O.; Kasarda, R.; Sölkner, J. Genetic evaluation for length of productive life in Slovak Pinzgau cattle. *Arch. Anim. Breed.* **2008**, *51*, 438–448. DOI: 10.5194/aab-51-438-2008
3. Bonfiglio, S.; Achilli, A.; Olivieri, A.; Negrini, R.; Colli, L.; Liotta, L.; Ajmone-Marsan, P.; Torroni, A.; Ferretti, L. The enigmatic origin of bovine mtDNA haplogroup R: Sporadic interbreeding or an independent event of *Bos primigenius* domestication in Italy? *PLoS ONE* **2010**, 5, *eXXXX*. DOI: 10.1371/journal.pone.0015760.
4. Davidescu, M.; Madescu, B.; Ciorpac, M.; Dascalu, L.; Bugeac, T.; Matei, A.; Creanga, S. Genetic diversity of Pinzgau cattle breed: A systematic review. *Sci. Pap. Ser. D Anim. Sci.* **2020**, *63*, 2.
5. Davidescu, M.A.; Creangă, Ş.; Henea, M.E.; Grădinaru, A.C. Agriculture and milk production in Romania—Retrospectives and trends in the European context. *Adv. Agric. Bot.* **2020**, *12*, 58–66.
6. Curik, I.; Ferenčaković, M.; Karapandza, N.; Cubric-Curik, V.; Sölkner, J. Estimation of inbreeding and effective population size in Istrian cattle using molecular information. *Acta Agraria Kaposváriensis* **2014**, *18*, 30–34.
7. Di Lorenzo, P.; Lancioni, H.; Ceccobelli, S.; Colli, L.; Cardinali, I.; Karsli, T.; Capodiferro, M.R.; Sahin, E.; Ferretti, L.; Marsan, P.A. Mitochondrial DNA variants of Podolian cattle breeds testify for a dual maternal origin. *PLoS ONE* **2018**, *13*, eXXXX. DOI: 10.1371/journal.pone.0192567
8. Kadlečík, O.; Kasarda, R.; Mészáros, G.; Žitný, J.; Vavrišínová, K. Pedigree analysis of purebred Pinzgau population. *Acta Fytotech. Zootech.* **2007**, *10*, 29–32.
9. Mang, N. Genetic structure of quantitative characters in Pinzgau of Transilvania breed from Hateg region. *Sci. Pap. Anim. Sci. Biotechnol.* **2011**, *44*, 432–434.
10. Bollongino, R.; Elsner, J.; Vigne, J.D.; Burger, J. Y-SNPs do not indicate hybridisation between European aurochs and domestic cattle. *PLoS ONE* **2008**, *3*, e3418. DOI: 10.1371/journal.pone.0003418
11. Pitt, D.; Sevane, N.; Nicolazzi, E.L.; MacHugh, D.E.; Park, S.D.E.; Colli, L.; Martinez, R.; Bruford, M.W.; Orozco-terWenge, P. Domestication of cattle: Two or three events? *Evol. Appl.* **2019**, *12*, 123–136. DOI: 10.1111/eva.12674
12. Bruford, M.W.; Townsend, S.J. Case studies in the genetics of animal domestication. J. *Adv. Vet. Anim. Res.* **2004**, *1*, 86–93.
13. Achilli, A.; Olivieri, A.; Pellecchia, M.; Uboldi, C.; Colli, L. Mitochondrial genomes of extinct aurochs survive in domestic cattle. *Curr. Biol.* **2008**, *18*, 157–158. DOI.org/10.1016/j.cub.2008.01.019
14. Achilli, A.; Bonfiglio, S.; Olivieri, A.; Malusà, A.; Pala, M. The multifaceted origin of taurine cattle reflected by the mitochondrial genome. *PLoS ONE* **2009**, *4*, e5753. DOI.org/10.1371/journal.pone.0005753
15. Bollongino, R.; Edwards, C.J.; Alt, K.W.; Burger, J.; Bradley, D.G. Early history of European domestic cattle as revealed by ancient DNA. *Biol. Lett.* **2006**, *2*, 155–159. DOI: 10.1098/rsbl.2005.0404
16. Bradley, D.G.; Magee, D.A. Genetics and origins of domestic cattle. *In New Genetics and Archaeological Paradigms; University of California Press: Berkeley, CA*, **2006**, 317–328.
17. Maretto, F.; Ramljak, J.; Sbarra, F.; Penasa, M.; Mantovani, R.; Ivankovic, A. Genetic relationship among Italian and Croatian Podolian cattle breeds assessed by

microsatellite markers. *Livest. Sci.* **2012**, *13*, 256. DOI.org/10.1016/j.livsci.2012.09.011

18. Park, S.D.E.; Magee, D.A.; McGettigan, P.A.; Teasdale, M.D.; Edwards, C.J.; Lohan, A.J.; Murphy, A.; Braud, M.; Donoghue, M.T.; Liu, Y.; Chamberlain, A.T.; Rue-Albrecht, K.; Schroeder, S.; Spillane, C.; Tai, S.; Bradley, D.G.; Sonstegard, T.S.; Loftus, B.J.; MacHugh, D.E. Genome sequencing of the extinct Eurasian wild aurochs, *Bos primigenius*, illuminates the phylogeography and evolution of cattle. *Genome Biol.* **2015**, *16*, 234. DOI.org/10.1186/s13059-015-0790-2
19. Pariset, L.; Mariotti, M.; Nardone, A.; Soysal, M.I.; Ozkan, E.; Williams, J.L.; Dunner, S.; Leveziel, H.; Maroti-Agots, A.; Bodo, I.; Valentini, A. Relationships between Podolic cattle breeds assessed by single nucleotide polymorphisms (SNPs) genotyping. *J. Anim. Breed. Genet*. **2010**, *127*, 481–488. DOI: 10.1111/j.1439-0388.2010.00868.x.
20. Beja-Pereira, A.; Caramelli, D.; Lalueza-Fox, C.; Vernesi, C.; Ferrand, N.; Casoli, A.; Goyache, F.; Royo, L.J.; Conti, S.; Lari, M.; Martini, A.; Ouragh, L.; Magid, A.; Atash, A.; Zsolnai, A.; Boscato, P.; Triantaphylidis, C.; Ploumi, K.; Sineo, L.; Mallegni, F.; Taberlet, P.; Erhardt, G.; Sampietro, L.; Bertranpetit, J.; Barbujani, G.; Luikart, G.; Bertorelle, G. The origin of European cattle: Evidence from modern and ancient DNA. *Proc. Natl. Acad. Sci*. USA **2006**, *103*, 8113–8118. DOI.org/10.1073/pnas.0509210103