

Mitogenome diversity of the Rendena cattle provides new clues on its maternal origin and the complex substructure of haplogroup T3

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ABSTRACT

The remote Alpine valley in northern Italy, Val Rendena, is home to an autochthonous dual-purpose cattle breed with unique historical and genetic traits preserved by their loyal human custodians despite geographic isolation and severe epidemics since the 1700s. To trace its evolutionary history and maternal origins, we assessed the mitochondrial DNA (mtDNA) variability and sequenced the complete mitogenome of 137 Rendena dairy cows, selected to represent most maternal lineages across the breed. We identified 86 distinct mtDNA haplotypes, indicating a high haplotype diversity ($H_d=0.986$). Phylogenetic analyses revealed that virtually all samples belong to the T macro-haplogroup ($T_3=91\%$; $T_2=7\%$; $T_5=1\%$), with only one falling within the Q1 lineage. The comparison with 31 new Alpine Grey mtDNAs (N haplotypes=26; $H_d=0.989$) revealed a strong genetic proximity of the two Alpine cattle populations, suggesting either a recent common ancestry or historical maternal gene flow. Notably, the analysis of Rendena mtDNA variation within a West-Eurasian context showed an ancestral link with the Balkans. However, the absence of haplotype sharing supports the uniqueness of the Rendena maternal gene pool. The high degree of mtDNA variation allowed us to refine the phylogeny within the T3 haplogroup, revealing a potential structure in the Rendena population. This study highlights the importance of preserving local cattle breeds as reservoirs of genetic diversity to support sustainable farming.

BACKGROUND

The Alps, one of the largest mountain ranges in Europe, represent an important reservoir of agricultural biodiversity and ancient pastoral traditions, with a wide variety of local cattle breeds. Most of these are dual-purpose and autochthonous, with limited distributions in marginal areas, including Rendena cattle [Guzzo et al. 2019], currently considered “vulnerable to extinction” (Figure 1). The earliest written records date back to the 18th century [Bonsembiante et al. 1988], when a plague decimated both humans and livestock, leading farmers to introduce Swiss cattle with similar traits. By the late 19th century, Rendena cattle had spread throughout northern Italy and became a reference dairy breed. However, during the two World Wars, government policies favored more productive breeds, causing a severe decline in Rendena population size. Its survival was ensured thanks to local farmers who continued to breed their native cattle, often in secrecy and against official directives. Despite the relatively confined geographical distribution, and several population bottlenecks caused by epidemics, Rendena cattle exhibit unique genetic features [Somenzi et al. 2024]. Genome-wide SNP analyses revealed selection signatures linked to both milk and meat production, as well as regions associated with immune response. While showing some genetic affinity with neighboring breeds such as Original Braunvieh and Murnau-Werdenfelser, Rendena has preserved distinct genetic and morphological characteristics. Mitochondrial DNA diversity in modern cattle breeds has been shaped by crossbreeding [Dorji et al. 2022]; however, breeding strategies usually focus on the selection of bulls to be crossbred with autochthonous cows [Mapel et al. 2024]. Hence, mtDNA preserves a deeper phylogenetic history and contributes to the maternal biodiversity [Di Lorenzo et al. 2018; Cubrik-Curic et al. 2021]. Most studies on maternal variation in West-Eurasian native cattle have focused on the mtDNA control region, although research has also been conducted on whole mitogenomes or on mitochondrial polymorphisms identified through genome-wide analyses. The present study aims to trace the evolutionary history and maternal origins of Rendena cattle, by assessing its mtDNA variability through the complete mitogenome sequencing. Then, the comparison with 31 new sequences from Alpine Grey cattle breed and with previously published mitogenomes of other West-Eurasian breeds, allowed us to refine the complex substructure of the taurine haplogroup T3.



Figure 1. A typical Rendena cow.

MATERIALS & METHODS

Whole blood was sampled from 137 Rendena (REN) dairy cows selected from official breeding records to represent the main maternal lines of the breed, and DNA was extracted as previously reported [Somenzi et al. 2024]. An additional set of samples from 31 Alpine Grey (GAL) cattle were analysed. The entire mtDNA molecule of each individual was amplified with Long-Range PCR as previously published in [Olivieri et al. 2015] and sequenced using Illumina MiSeq technology. Raw sequences were processed, and the obtained short reads were aligned to the bovine mitochondrial sequence V00654 [Anderson et al. 1982] with the specific mem algorithm, then the BAM files were filtered and sorted with SAMtools [Li et al. 2009]. All sequences were classified in haplotypes (HTs) and haplogroups (HGs). Then, most parsimonious (MP) trees were generated with MEGA and mtPhyl softwares and manually checked and adjusted. To refine the substructure of haplogroup T3, our final dataset (137 Rendena and 31 Alpine Grey mtDNAs) was compared with 431 complete mitogenomes of West-Eurasian cattle breeds retrieved in GenBank.

RESULTS & DISCUSSION

The mitochondrial variability of REN was evaluated through the analysis of the entire mitogenome (16638 bp) of 137 individuals and the comparison with 31 newly sequenced samples from GAL. In REN, we identified a total of 86 mtDNA HTs, with haplotype diversity (H_d) of 0.986, while in GAL, 27 HTs with a H_d of 0.989 were found. The MP tree of our 168 mitogenomes clearly highlights that all samples but two (one REN and one GAL, both from Q haplogroup), belong to the primarily domesticated T macro-haplogroup (Figure 2).

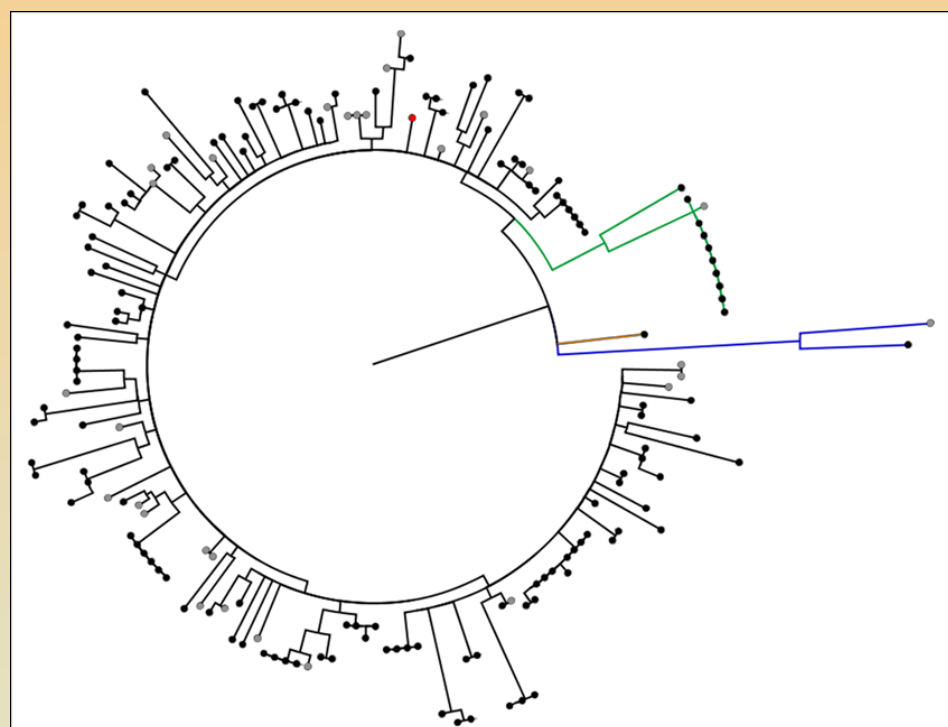


Figure 2. Most Parsimonious tree of our 168 (137 REN and 31 GAL) mitogenomes here analyzed, representing the relationships between the two Alpine cattle breeds. REN samples are in black, GAL in grey and BRS in red. The different HTs are indicated with one color for each branch: blue (Q), orange (T5), green (T2) and black (T3).

Within this clade, 128 REN (91%) and 27 GAL (94%) were T3, ten REN (7.5%) and one GAL (3%) were from T2 haplogroup, and one REN (0.75%) from T5. In general, REN and GAL show a high genetic variability, with many sub-branches defined by private mutations and the detection of rare haplogroups (i.e. T5 and Q1). The HG distribution was evaluated in a West-Eurasian context (with a total of 771 mtDNAs) and reported in Figure 3.

The presence of Q1 in REN, GAL and in few other local breeds from Italy, Egypt and Iberian Peninsula, and the uniqueness of our REN and GAL HTs could indicate an *in situ* differentiation from the founder domestic bovines arrived in the Alps. Considering the divergence time of T5 HG (16-18 ky BP) and its geographic distribution, our REN HT could derive from those mtDNAs present in the founding population involved in the domestication event occurred in Anatolia 10-11 ky ago. The absence of T1 HG in our samples indicates that, despite its widespread presence in southern Europe, the Alpine cattle were not subjected to the African influence. Since almost all our samples belong to the dominant maternal lineage in Europe, our T3 samples were compared with 431 complete mitogenomes of West-Eurasian cattle breeds retrieved in GenBank and belonging to the same haplogroup. The resulting MP tree (data not shown) highlighted that, even though a genetic proximity of REN, GAL and breeds from Alps, Balkans, Iberian and Italian Peninsulas could be observed, none of our 168 samples share HTs with the other European breeds. Due to this peculiarity, different mutations along the mitogenomes of REN and GAL allowed us to refine and detail the complex substructure of haplogroup T3, through the identification of different new sub-clades defined by novel diagnostic mutational motifs, in both coding and non-coding regions, shared with at least three samples presenting different HTs (Figure 4).

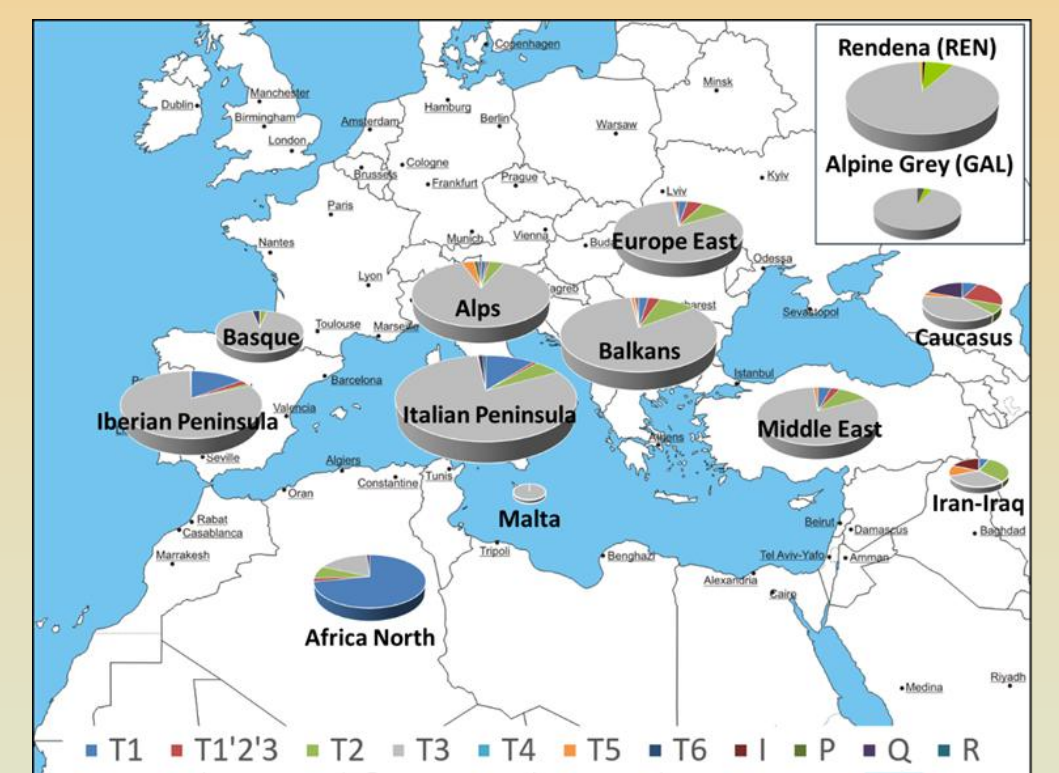


Figure 3. Geographic distribution and frequencies of cattle mtDNA haplogroups in a West-Eurasian context.

The new sub-structure includes mitogenomes belonging to REN, GAL and other cattle especially from Alps, Balkans, Iberian and Italian Peninsulas. Among the 23 sub-branches here identified for the first time, seven (T3b1, T3b2, T3b2a, T3b3, T3b4, T3b5 and T3b6) fall within the sub-HG T3b, while 16 (T3d, T3i, T3j1, T3j2, T3l, T3m, T3n, T3o, T3p1, T3p2, T3p3, T3p4, T3p5, T3p6, T3p7, T3p8, T3p9, T3p10, T3p11, T3p12, T3p13, T3p14, T3p15, T3p16, T3p17, T3p18, T3p19, T3p20, T3p21, T3p22, T3p23, T3p24, T3p25, T3p26, T3p27, T3p28, T3p29, T3p30, T3p31, T3p32, T3p33, T3p34, T3p35, T3p36, T3p37, T3p38, T3p39, T3p40, T3p41, T3p42, T3p43, T3p44, T3p45, T3p46, T3p47, T3p48, T3p49, T3p50, T3p51, T3p52, T3p53, T3p54, T3p55, T3p56, T3p57, T3p58, T3p59, T3p60, T3p61, T3p62, T3p63, T3p64, T3p65, T3p66, T3p67, T3p68, T3p69, T3p70, T3p71, T3p72, T3p73, T3p74, T3p75, T3p76, T3p77, T3p78, T3p79, T3p80, T3p81, T3p82, T3p83, T3p84, T3p85, T3p86, T3p87, T3p88, T3p89, T3p90, T3p91, T3p92, T3p93, T3p94, T3p95, T3p96, T3p97, T3p98, T3p99, T3p100) present a combination of the diagnostic mutations with the transition at the nucleotide position (np) 169, thus falling out of the T3b sub-clade.

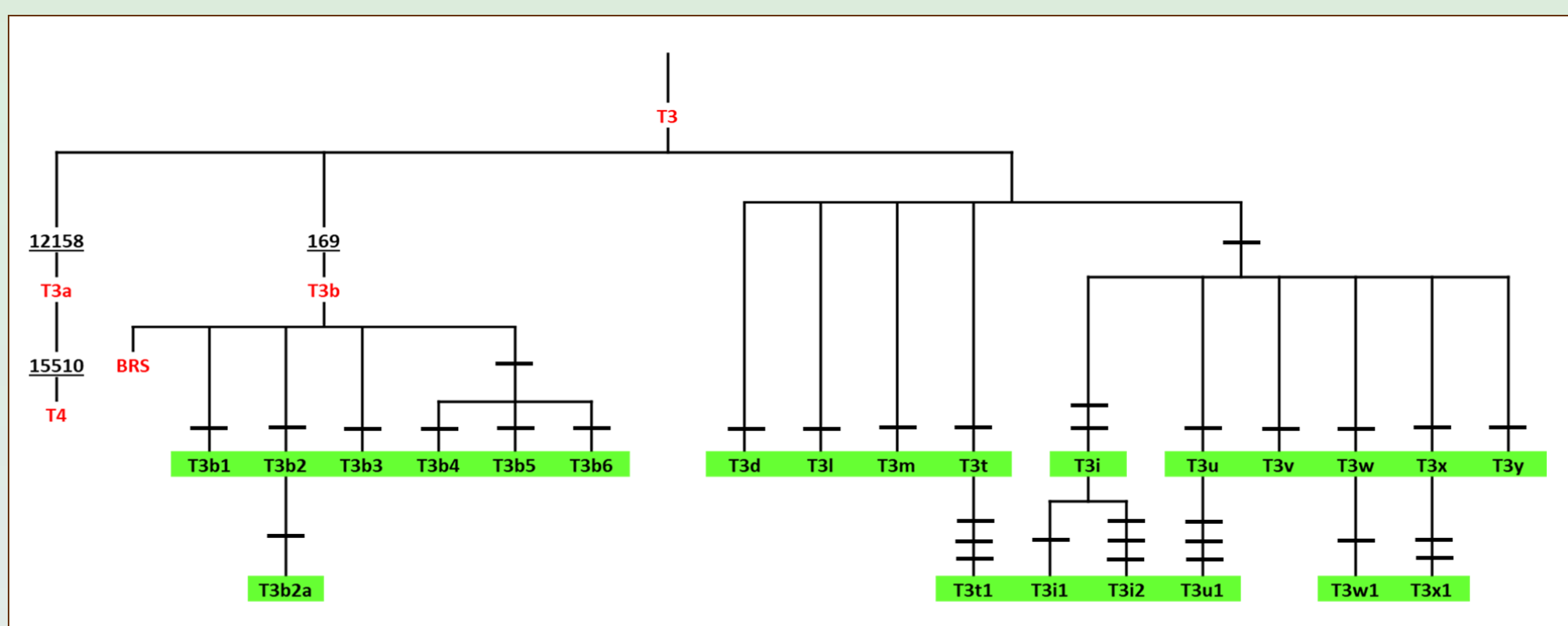


Figure 4. Schematic tree showing the substructure of T3 haplogroup. The new sub-branches here identified for the first time are highlighted in green. Dashes indicate the novel mutational motifs.

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

Local breeds represent an important reservoir of genetic diversity useful for current and future support for traditional and sustainable farming, agrifood products, and resilience in the face of global change. Our findings showed a genetic input from the Balkans into Rendena Valley, whose mtDNAs did not seem to be affected by the importation of cows from Swiss after the population decline that underwent the REN. The genetic peculiarities of REN and GAL and their great variability along the maternal lines allowed for a refinement of the complex substructure of T3 HG, through the identification of several new sub-branches defined by novel mutational motifs. Further Bayesian analysis will be performed in order to define the REN and GAL effective population size changes at coalescent events through the demographic model (Bayesian skyline plot, BSP), to calculate the age estimates of each sub-branch, and, probably to re-calibrate the cattle molecular clock. Few published data of complete mitogenomes, with respect to control-region sequences, the difficulty in identifying certain haplogroups based only on D-loop haplotypes and the lack of ancient data from Alps could represent a limitation for the comparison analysis, thus further analyses, especially on bone samples, shed light on the mtDNA peculiarities of Rendena cattle.

ACKNOWLEDGEMENTS

REFERENCES

