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Origins of raw milk consumption in the Iberian Peninsula and Portuguese territory: archaeogenetics and zooarchaeology

As origens do consumo do leite cru na Península Ibérica e no território português: Arqueogenética e Zooarqueologia

GONÇALO GARCEZ FERNANDES

Faculdade das Ciências Humanas e Sociais,
Universidade do Algarve
Faculdade de Ciências e Tecnologia,
Universidade de Coimbra
goncalo.gz.fernandes@gmail.com
ORCID ID: 0009-0008-4974-5288

MARIA JOÃO VALENTE

Centro de Estudos de Arqueologia, Artes
e Ciências do Património (CEAACP)
Faculdade das Ciências Humanas e Sociais,
Universidade do Algarve
mvalente@ualg.pt
ORCID ID: 0000-0002-6137-5995

HUGO RAFAEL OLIVEIRA

Interdisciplinary Center for Archaeology
and Evolution of Human Behaviour (ICArEHB)
Faculdade das Ciências Humanas e Sociais,
Universidade do Algarve
hroliveira@ualg.pt
ORCID ID: 0000-0002-5038-073X

MARIA JOÃO FERNANDES MARTINS

Interdisciplinary Center for Archaeology
and Evolution of Human Behaviour (ICArEHB),
Universidade do Algarve
mjfmartins@ualg.pt
ORCID ID: 0000-0002-9118-7397

ABSTRACT: In Europe, lactose tolerance after weaning (lactase persistence – LP) is determined by a single mutation in the *MCM6* gene. The timing and mode of the emergence of raw milk consumption in Portuguese territory have not been addressed, despite its significance in Portuguese subsistence and culinary tradition. To identify the earliest instance of the LP mutation, we examined this locus in ancient DNA (aDNA) data from Iberian individuals spanning from the Palaeolithic to the Modern Age, available in the Allen Ancient DNA Resource database. Additionally, we reviewed zooarchaeological data on domesticated milk-producing species from bibliographic sources.

In Iberia, the earliest occurrence of the LP mutation dates to the Early Bronze Age. The individual also carried the Y-chromosome Haplogroup R1b, typically associated with the hypothesised Yamnaya movement during this period. In Portugal, the first individual with LP dates to the Roman Period. Zooarchaeological data suggest that milk was consumed in small quantities in Portugal until the Roman Period. Milk production increased more significantly during the Middle Ages, but its impact on LP remains to be determined.

KEYWORDS: Cattle; Caprine; Migration; Persistence of Lactase; Yamnaya.

RESUMO: Na Europa, a tolerância à lactose após o desmame (persistência da lactase – LP) depende de uma única mutação no gene *MCM6*. O tempo e o modo de emergência do consumo de leite cru em território português não foram investigados, apesar da sua importância na dieta e na cultura culinária portuguesas. Para identificar a ocorrência mais antiga da mutação causativa da LP, utilizámos o Allen Ancient DNA Resource para investigar os dados de ADN antigo (aDNA) para este locus em indivíduos ibéricos, do

Paleolítico à Idade Moderna. A partir de fontes bibliográficas, revimos dados zooarqueológicos sobre espécies produtoras de leite domesticadas.

Na Península Ibérica, a ocorrência mais antiga de LP ocorre em um indivíduo do início da Idade do Bronze. Este também apresentava o haplogrupo R1b do cromossoma Y, tipicamente associado ao hipotético movimento de pessoas associadas à cultura Yamnaya durante este período. Em Portugal, o primeiro indivíduo com LP data do Período Romano. Os dados zooarqueológicos sugerem que o leite era consumido em pequenas quantidades em Portugal até ao Período Romano. A produção de leite aumentou mais significativamente durante a Idade Média, mas o impacto desse aumento na LP ainda está por determinar.

PALAVRAS-CHAVE: Gado; Caprinos; Migração; Persistência da Lactase; Yamnaya.

1. INTRODUCTION

1.1. The ability to digest milk as adults in humans

Milk is a nutrient-rich food that can be transformed into various dairy products, such as cheese and yoghurt. It is composed of water, fats, proteins, lipids, minerals, amino acids, and sugars (Charlton *et al.* 2019; Roffet-Salque *et al.* 2018). Its main carbohydrate is lactose, a disaccharide formed by glucose and galactose, which serves as an energy source after digestion. Lactose represents between 3.8% and 5.3% of milk's total composition and contributes to the absorption of calcium, phosphate, magnesium, and vitamin D (Charlton *et al.* 2019; Roffet-Salque *et al.* 2018).

Lactase is the enzyme present in the small intestine that breaks down lactose into the assimilable sugars glucose and galactose (Roffet-Salque *et al.* 2018). Mammals, including humans, express lactase in early childhood when maternal milk is their main source of energy. After a weaning period, however, the lactase gene is typically deactivated (Curry 2013), a trait believed to be an adaptation to encourage young animals to move towards an adult diet and become independent from their mothers (Jobling – Hurles – Tyler-Smith 2019). In post-weaning humans, ingestion of even small quantities of milk leads to abdominal pain, flatulence and diarrhoea (Olds – Sibley 2003; Roffet-Salque *et al.* 2018).

After the domestication of animals, some human cultures learned that milk could be used as a food source after fermentation by bacteria of the genus *Lactobacillus*, *Lactococcus* or *Streptococcus*. These bacteria digest lactose into lactic acid, making dairy products edible to humans (Rosenstock – Ebert – Scheibner

2021). On the other hand, the ability to consume milk directly in adulthood depends on the persistence of the enzyme lactase after weaning (Lactase Persistence or LP).

In European populations (and elsewhere), LP is the result of a mutation, a single-nucleotide polymorphism (SNP) - **rs4988235**, located in an intron at position -13.910 of the *MCM6* gene of chromosome 2 (Curry 2013). This gene regulates the expression of the *LCT* gene that codes for lactase. The SNP can have two versions (alleles): a C or a T. An individual has two alleles, one inherited from the father and the other from the mother. Because the T allele is dominant, any individual who has received this allele from one or both parents (genotype C:T or T:T, respectively) will be able to digest milk as an adult. Individuals who have only the -13.910*C allele (genotype C:C) will be lactose intolerant.

The hypothesis is that this genetic selection arose after a long period of consumption of processed dairy products (Rocha 2012). Accordingly, in addition to the European continent, other human populations with an extensive history of milk consumption, such as those in the Middle East, West Africa, and East Africa, show high frequencies of the LP trait (Charlton *et al.* 2019). The LP trait emerged independently in these regions and is associated with SNPs in the *MCM6* gene; these SNPs are found in different regions within the gene (Liebert *et al.* 2017; Roffet-Salque *et al.* 2018).

Therefore, the -13.910*T allele is often indicated as representing one of the most intensively positively selected mutations documented in human evolutionary history (Evershed *et al.* 2022; Sabeti *et al.* 2006; Ségurel – Bon 2017). This mutation is often used as an example of gene-culture co-evolution, as its selective

advantage is intrinsically linked to the emergence and development of animal domestication and pastoralism (Beja-Pereira *et al.* 2003).

The nutritional benefits of milk consumption were particularly pronounced in northern European populations, where environmental constraints created selective pressures favouring LP. The high concentrations of vitamin D and calcium in milk provided essential nutrients that were otherwise difficult to obtain at high latitudes, where reduced solar radiation during winter months limited endogenous vitamin D synthesis through skin exposure to sunlight (Curry 2013). Additionally, lactose enhances calcium bioavailability and absorption, thereby reducing the incidence of rickets and osteomalacia in populations predisposed to these vitamin D-deficiency disorders (Rocha 2012).

However, LP is a relatively recent and still rare characteristic in most human populations. Lactase restriction is, in fact, the most common phenotype (characteristics or traits observable in an organism) worldwide (Rocha 2012), and most populations consume only fermented dairy products (Roffet-Salque *et al.* 2018). Furthermore, some pastoral populations consume only fermented dairy products and still exhibit high LP frequencies (e.g., Mongolian steppe nomadic peoples), as do the hunter-gatherer Hadza of Tanzania (Tishkoff *et al.* 2007). Consequently, the hypothesis that LP is a positively selected trait is not consensual, and the specific benefits of consuming fresh milk rather than processed dairy products in prehistoric contexts require further clarification.

Currently, the first occurrence of the -13.910*T allele was detected in an ancient DNA (aDNA) study in a Central European Bell Beaker culture individual who lived between approximately 2.300 and 2.200 BCE (Mathieson *et al.* 2015). From an archaeological point of view, the adaptation of tolerance to milk in Europe was acquired and spread rapidly, particularly in Northern and Central Europe (in Great Britain and Scandinavia, the frequency is >80% – Rocha 2012); in Southern Europe LP is less common (frequency of 35% in Portugal – Manco *et al.* 2013; estimated at 47% in Spain – Anguita-Ruiz – Aguilera – Gil 2020).

Over the years, researchers have proposed several hypotheses to explain the origin and spread of LP. Recently, genetic studies have indicated that the spread of this genetic mutation to Europe may be

associated with the migration of the Yamnaya from the Pontic-Caspian steppes (Burger *et al.* 2020; Garnier – Sagart – Sagot 2017).

However, despite milk's important role in Portugal's past and current economy, interest in LP origin in the territory has not been extensively studied in the Portuguese archaeological record.

1.2. Beginning of milk use and production

Milk production is assumed to have begun with the exploitation of cattle and caprines in Southwest Asia and was later introduced into Europe with pastoralism (Greenfield 2010).

At this stage, the genetic adaptation of LP had not yet emerged, and milk had to be processed in containers. Thus, milk exploitation data are obtained by analysing organic residues from Neolithic ceramics and detecting dairy lipids. This is often the case with pots, where heating the food facilitates lipid absorption by the clay in the containers. However, understanding the specific processing practices undertaken is more complex (Evans *et al.* 2023).

Residue analysis of ceramic containers has demonstrated that milk adoption was an integral practice in some European Early Neolithic agriculture populations, specifically along the northern Mediterranean coast, in the Baltic region, and in Central Europe (Halstead 2024). The oldest evidence for milk processing is from ceramic vases of the Neolithic Linear Pottery Culture (Linearbandkeramik) from Central and Eastern Europe, dated to the 6th Millennium BCE (Casanova *et al.* 2022). A notable example is the analysis of 50 sieve fragments from the Kuyavia region (north-central Poland), which revealed evidence of milk lipids in most of the sieves (Roffet-Salque *et al.* 2018; Salque *et al.* 2013).

Prehistoric milk consumption can also be studied through zooarchaeological analyses of domesticated animals, particularly caprines (sheep and goats). Mortality profiles showing preferential culling of young males and prolonged retention of females suggest secondary product exploitation like dairy use (Halstead 2024; Valente – Carvalho 2019). This aligns with the “Secondary Products Revolution (SPR)” concept introduced by Andrew Sherratt in 1981, which proposed a 3rd–4th Millennium BCE shift in Southwest Asia and Europe toward utilising animal resources beyond meat, including milk, wool, and traction.

However, recent research indicates these practices likely emerged earlier than Sherratt’s original timeline (Halstead 2024; Vigne – Helmer 2007). Domestication and management of these animals, taking advantage of their fibres, fats, reproductive characteristics, and milk production, significantly transformed the daily lives of prehistoric communities (Gillis *et al.* 2022).

In Iberia, in sites such as El Portalón and the El Mirador cave in the Sierra de Atapuerca (northern Spain), milk production and consumption of dairy products have been detected in the Early Neolithic period (Galindo-Pellicena *et al.* 2020). This is assumed from the estimated age of slaughter of domestic animals (Galindo-Pellicena *et al.* 2020). For the present-day Portuguese territory, it is suggested that milk production was already a reality since the introduction of caprine and cattle in the Early Neolithic (Valente – Carvalho 2019).

During the Iberian Chalcolithic (3.000–2.200 BCE), archaeological evidence indicates a shift from predominantly local subsistence to more mobile strategies (Basílio 2020). This transition is marked by changes in the management and exploitation of domestic animals, aligning with the initial framework of the Secondary Products Revolution. In the Portuguese territory,

the use of secondary products from caprines and cattle – beyond milk – appears to have become fully established during the Final Neolithic to Early Chalcolithic (Valente – Carvalho 2019). Between the late 4th and early 3rd Millennium BCE, an agropastoral system emerged, catalysing significant social and cultural changes. This period saw reduced dependence on hunting and gathering, population growth, and increased sedentarisation (Cardoso 2007; Soares 2011).

1.3. Movements of people from the Pontic-Caspian Steppes in the 3rd Millennium BCE

People of the Yamnaya culture are thought to have domesticated the horse and introduced the wheel, enabling a more mobile way of life through ox-drawn carts (Anthony 2007; Silva *et al.* 2019). The Yamnaya had a pastoral economy centred on dairy products, complemented by hunting, fishing, and the collection of wild plants (Fernández-Domínguez 2023).

Ancient DNA studies (Haak *et al.* 2015; Allentoft *et al.* 2015) revealed a significant movement of people in the Early Bronze Age (3rd Millennium BCE), from the Pontic-Caspian steppes to Central and Western Europe, associated with the Pit-Grave (Yamnaya) and Corded Ware Cultures (CWC) (Fernández-Domínguez

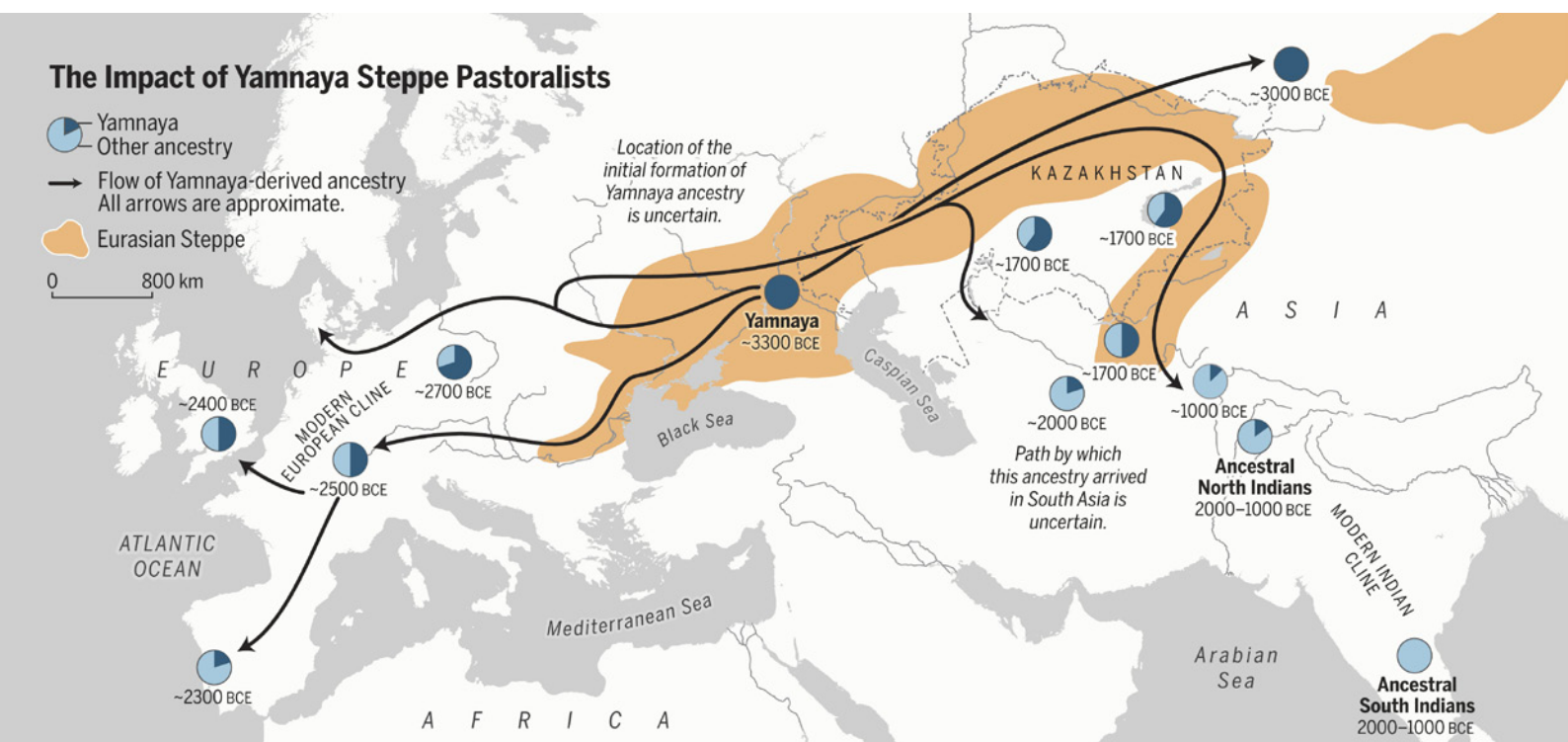


FIG. 1 Representation of migratory flow and proportion of Yamnaya ancestry depending on region (Source: Narasimhan *et al.* 2019).

2023) (Fig. 1). The chronology corresponds to substantial changes in material culture and the disappearance of Neolithic cultural expressions, including changes in funerary styles and pottery characteristic of that period (Callaway 2018).

aDNA studies suggest that the 3rd Millennium BCE movements of people associated with the Yamnaya culture also resulted in profound changes in the genetic composition of Central and Western Europe populations (Allentoft *et al.* 2024; Kristiansen 2022; Scorrano *et al.* 2021). Individuals from the Yamnaya archaeological material culture and CWC cultures share substantial genetic similarities. Moreover, the genetic influence of the steppes in certain regions is detected in skeletons related to the CWC and the Bell Beaker Culture, with approximately 75% genomic ancestry associated with Yamnaya (Silva *et al.* 2019). Studies have proposed that this genetic inheritance is greater in Northern Europe than in the South (Narasimhan *et al.* 2019).

Mutations in the Y chromosome (inherited via the paternal route, present in males only) and in the mitochondrial genome (inherited via the maternal route) can be analysed together to define haplogroups (Brown – Brown 2011). The geographic and temporal distribution of these haplogroups can be particularly informative about people's movements, ancestry, and kinship in prehistory (Racimo *et al.* 2020). The Y-chromosome haplogroup R1b-M269 is associated with steppe ancestry, as this haplogroup has a very high frequency among Yamnaya men and was practically absent in Europe until their arrival (Haak *et al.* 2015; Mathieson *et al.* 2015).

In the Iberian Peninsula, it is postulated that a genetic replacement occurred between 2.500 and 2.000 BCE (Koch 2019; Narasimhan *et al.* 2019; Olalde *et al.* 2019). The R1b haplogroup and the steppe ancestry component became strongly present in the early Iberian Bronze Age (Villalba-Mouco *et al.* 2021). This prehistoric event had a drastic impact, replacing approximately 40% of the local population's total ancestry and about 90% of its Y-chromosome haplogroups. This genetic transformation mainly affected men, and no major changes in mitochondrial DNA were observed between the Neolithic and Bronze Age periods (Olalde *et al.* 2019). Assuming that the social structure of the Yamnaya was based on warrior elites,

it is plausible to consider that they established themselves as an elite without resorting to violence, as there is no evidence of conflict and, based on polygamous marriage patterns, had children with indigenous Iberian women. Moreover, genetic change occurred over 500 years. This Y-chromosome haplogroup would have gradually settled in the Iberian Peninsula throughout this period (Fernández-Domínguez 2023).

Despite the aDNA evidence supporting a Yamnaya migration with a genetic replacement in parts of Europe during the 3rd Millennium BCE, this hypothesis is far from consensual (Booth 2019; Furholt 2018; Hakenbeck 2019; Heyd 2017; Vander Linden 2016). An alternative hypothesis for the aDNA patterns observed is a population decline in Europe before or during the arrival of the Yamnaya people. This could have been particularly relevant in Iberia, where famine, internal wars over scarce agricultural land, the 4.2 Kya climatic event or the plague may have caused this population decline. Moreover, many archaeologists have pointed out that there is no evidence of a wave of violence or a "genocide" during this period (Basílio 2020) and that it is fallacious to identify the movement of material culture (or even some people) with a genetically homogeneous group of people (Heyd 2017).

Nevertheless, the first occurrences of the -13.910*T allele in Europe were found in skeletal remains associated with CWC and a genetic component attributed to the Yamnaya cultures (Saag *et al.* 2020). Therefore, this raises an important question: *Did individuals descending from Yamnaya groups bring LP to the Iberian Peninsula?*

1.4. Zooarchaeology of Domesticated Animals in the Portuguese Territory

Milk only became a significant component of the human diet following the domestication of animals, particularly cattle, sheep, and goats. While other species – such as horses, donkeys, camels, and yaks – also provide milk, their contribution to dairy production is far smaller.

Zooarchaeological research indicates that humpless cattle (*Bos taurus*), goats (*Capra hircus*) and sheep (*Ovis aries*) were among the earliest livestock to be domesticated in Southwest Asia (the Near East) (MacHugh – Larson – Orlando 2017). *Bos taurus*, domesticated

from its wild ancestor *Bos primigenius* (the aurochs), is thought to have undergone domestication around 8.500 BCE (Scheu *et al.* 2015). Goats, derived from the wild bezoar ibex (*Capra aegagrus*), are believed to have been domesticated during the 10th Millennium BCE, with the earliest domestic phenotypes identified in Anatolia (Daly *et al.* 2018). The wild ancestor of sheep is the Asian mouflon (*Ovis orientalis*); evidence for their management and herding practices dates to approximately 8.500 BCE, while morphologically domestic sheep appear by around 7.500 BCE (Kaptan *et al.* 2024). These domestic species were subsequently introduced to Europe through migration processes that paralleled human movements across the continent (Zeder 2008; Gillis *et al.* 2022).

Zooarchaeological analyses from Neolithic and Chalcolithic sites across southern Portugal confirm the presence of several domestic species, including cattle and caprines (sheep and goats), as early as the Early Neolithic (5.500–4.500 BCE) (Valente – Carvalho 2019). The Middle Neolithic (4.500–3.200 BCE) saw the establishment of goat farming as a recurring subsistence practice. By the Final Neolithic (3200–3000 BCE) and Chalcolithic (3.000–2.200 BCE), caprines became especially abundant in the region, while the presence of domestic cattle increased significantly alongside the growth of population clusters (Valente 2015). This period was marked by the development of a more complex agricultural economy, which enabled pastoralism, reliant on herds of sheep, goats, and cattle, to flourish and diversify subsistence strategies (Valente – Carvalho 2019).

1.5. This study

Building on this framework, we address two central questions: 1) *When did the genetic capacity for adult lactase persistence – specifically, the ability to digest raw milk – emerge in the Portuguese territory?* 2) *Did this development influence the management and exploitation of cattle, sheep, and goats?* To investigate these questions, we analysed data from an ancient DNA database for the presence of the -13.910*T allele in Iberian individuals. We hypothesise that this genetic trait was introduced during the Bronze Age, possibly by newcomers descending from the Yamnaya archaeological culture. Additionally, we conducted a comprehensive review of zooarchaeological evidence from Portuguese

sites to assess potential improvements in breeding milk-producing species and identify shifts in animal husbandry practices related to dairy exploitation.

2. METHODS

Ancient genomes were analysed for the presence of LP in human remains inhumed in the Iberian Peninsula, and the period in which this characteristic first appeared in Iberia, specifically in Portuguese territory, was determined. Ancient genomes of individuals from other regions of Europe were analysed to understand whether there was a significant disparity in the timing of the appearance of this genetic characteristic between the Iberian Peninsula and the rest of Europe.

Next, we investigated the possibility of a relationship between the appearance of this mutation and changes in the zooarchaeological record. The aim was to correlate patterns of milk production intensification in past populations in Portugal with the oldest lactose-tolerant individual(s) in the aDNA dataset or with an increase in the trait's frequency in Portuguese territory.

2.1. Use of Bioinformatic Tools for aDNA

Data Analysis

For the analysis of the aDNA data, we used the program R v. 4.3.2 (R Core Team 2022) and RStudio v. 2022.09.1 +394 (RStudio Team 2024) on a x86_64-w64-mingw32/x64 (64-bit) platform. The packages admixtools v. 2.0.4 (<https://github.com/uqrmaie1/admixtools>), and RTools 4.2 were also installed (<https://cran.r-project.org/bin/windows/Rtools/rtools42/rtools.html>).

The aDNA dataset used in our paper is part of the Allen Ancient DNA Resource (AADR, V54.1.p1) available on the David Reich Lab website (<https://reich.hms.harvard.edu/allen-ancient-dna-resource-aadr-downloadable-genotypes-present-day-and-ancient-dna-data>). This database compiles published genotypes obtained by Next Generation Sequencing (NGS) for thousands of archaeological and modern individuals, curated using the same bioinformatics protocol, allowing comparison between datasets. We downloaded the “1240k dataset” (4.8 GB) on 25-02-2024. On this date, the dataset contained information on 1,233,013 positions in the human genome for 16,389 individuals (9,990 from archaeological populations and 6,399 from present-day populations). We selected a subset

of individuals that referred to the Iberian Peninsula, excluding modern individuals. This subset comprised 583 individuals and 1,233,013 SNPs. A second subset was created containing only individuals from Central Europe. This separation of data was necessary because the RAM memory required to analyse all individuals exceeds the capacity of our local desktop.

The SNP rs4988235 coding was obtained for each individual, with the LP trait coded as either 0 (T:T genotype) or 1 (C:T genotype, heterozygous) in this dataset. Inversely, absence of LP is coded as 2 (C:C genotype). This coding requires a note on **allelic dropout**. Because of DNA degradation and fragmentation in an archaeological context, the probability that both chromosomes are preserved at the same position in the archaeological record is very low (Krüttli *et al.* 2014; Palomo-Díez *et al.* 2018). As such, heterozygotes are rarely represented in aDNA; specifically, for the SNP rs4988235 analysed here, the occurrence of code 1 is rare. Considering this, we are probably underestimating the LP mutation frequency. Specifically, individuals coding 2 may, in fact, represent a C:T genotype, a heterozygous individual whose T allele was not sequenced due to allelic dropout.

On the other hand, genotype 0 is more accurately understood as “only allele T is detected”, although it is impossible to say if the individual had the genotype T:T or C:T. As such, because the allele T is dominant, we can be certain that individuals coded as 0 or as 1 could digest milk, but we cannot be certain that individuals coded as 2 were indeed lactose intolerant.

Lastly, the NA value indicates the absence of data for this SNP in the individual in question (*i.e.*, it cannot be determined whether the individual had LP because the section of the genome containing the MCM6 gene was not sequenced for that individual).

The command lines used, annotations and a detailed method description are available in Supplementary Material 1 and should allow for independent replication.

Next, information on individuals with LP was obtained from the annotation file in the “1240k dataset” (the file with extension .anno, containing the radiocarbon dating of the individual, origin, Y and mitochondrial haplogroup if available, sex, etc.). The objective was to verify the frequency of individuals with LP in each period.

2.2. Prehistoric Archaeological Sites with Evidence of Cattle and Caprine Husbandry

We reviewed the available archaeological and zooarchaeological references concerning cattle, sheep and goat remains associated with evidence of milk production in Portugal. Indicators of dairying include lipid residues in ceramic vessels, the presence of cheese-making implements, and the age profiles of slaughtered animals. Regarding the latter method, only cases suggesting a tendency to keep goats alive until an advanced age (4–6 years) were considered. This practice can be interpreted as evidence for the exploitation of secondary products, such as milk or even wool (in the case of sheep) (Charlton *et al.* 2019). The data are presented as Presence/Absence, without consideration of the specific archaeological context for each site.

Due to their morphological similarities, most studies do not distinguish between sheep (*Ovis aries*) and goats (*Capra hircus*); as a result, the generic classification *Ovis/Capra* is commonly used in zooarchaeological reports (Aleixo – Valente 2020), which complicates efforts to address species-specific management and exploitation strategies. Recent methodological advances will make it easier to differentiate between sheep and goats in the future. Techniques such as dental and bone morphometrics, as well as ZooMS (Zooarchaeology by Mass Spectrometry), have shown promise in this area (Jeanjean *et al.* 2023).

Forty-eight archaeological sites were selected for this study, each providing evidence of domestic cattle and caprine husbandry and featuring faunal assemblages that have been thoroughly analysed in scientific publications and other bibliographic sources. The georeferencing (latitude and longitude) of these sites was obtained from the online platform *Portal do Arqueólogo*. This spatial data is presented in Supplementary Material 2 and the figures were produced using *R* v. 4.4.1 (R Core Team 2022) and *RStudio* v. 2022.09.1 +394 (RStudio Team 2024), with *R* packages *ggplot2* v. 3.5.1 (Wickham 2016), *sf* v. 1.0-21 (Pebesma – Bivand 2023), *ggspatial* v. 1.1.10 (Dunnington 2025) and *rnat-uralearth* v. 1.1.0 (Massicotte – South 2025). The chronological framework adopted for the study is outlined in Table 1.

TABLE 1 CHRONOLOGY OF THE IBERIAN PENINSULA AND THE PORTUGUESE TERRITORY

PERIOD	IP	BIBLIOGRAPHY	PORTUGAL	BIBLIOGRAPHY
Neolithic	5.500-3.000 BCE	Szécsényi-Nagy <i>et al.</i> 2017	5.500-3.000 cal BCE	Valente - Carvalho 2019
Early Neolithic	5.500-4.500 BCE	Szécsényi-Nagy <i>et al.</i> 2017	5.500-4.500 cal BCE	Valente - Carvalho 2019
Middle Neolithic	4.500-3.000 BCE	Szécsényi-Nagy <i>et al.</i> 2017	4.500-3.200 cal BCE	Valente - Carvalho 2019
Late Neolithic			3.200-3.000 cal BCE	Valente - Carvalho 2019
Chalcolithic	3.000-2.200 BCE	Basílio 2020	3.000-2.000 cal BCE	Valente - Carvalho 2019
Bronze Age	2.200-900 cal BCE	Fokkens - Harding 2013	2.000-800 BCE	Cardoso 2007
Early Bronze Age	2.200-1.550 cal BCE	Fokkens - Harding 2013	2.000-1.200 cal BCE	Valente - Carvalho 2019
Medium Bronze Age	1.550-1.300 cal BCE	Fokkens - Harding 2013	—	—
Late Bronze Age	1.300-900 cal BCE	Fokkens - Harding 2013	—	—
Iron Age I	—	—	800-600 BCE	Cardoso 2007
Iron Age II	—	—	500-200 BCE	Cardoso 2007
Roman	—	—	200 BC-476 CE	Cardoso 2007
Middle Ages	476 CE-15 th Century	Banniard 1989	476 CE-15 th Century	Banniard 1989

3. RESULTS AND DISCUSSION

3.1. Genomic Data on Lactase Persistence

The Iberian Peninsula subset includes 583 individuals, 53 of whom lived in regions that are currently Portuguese territory. The Iberian subset extends from the Neolithic to the Middle Ages.

In total, 147 individuals had no data for the targeted SNP (25.2% of the individuals analysed). For the two genotypes conferring the LP trait, we observed Genotype 0 in 11 individuals (1.8% of the total number of individuals; 2.5% if considering individuals with available data for SNP rs4988235), and Genotype 1 was not observed (*i.e.*, heterozygous individuals). Genotype 2 was thus observed in 425 individuals.

As discussed previously, the number of lactose-tolerant individuals in the dataset is probably underestimated because of the effect of allele dropout in aDNA. A study of SNPs associated with eye and hair colour in Bronze Age individuals found 10% allelic dropout across all sequenced SNPs (Schmidt *et al.* 2020), confirming this is a recurring problem in aDNA studies.

The analysed genomic data indicate that the oldest individual with LP in the Iberian Peninsula, individual (genetic ID - "ZAP002"), was found in Lorca, in the Murcia region of Spain. He lived during the Early Bronze Age, around 2.200-1.550 BCE. He was male

with haplogroup R1b, the most common in the Yamnaya populations (Tab. 2). It is possible, therefore, that this individual had steppe (Yamnaya) ancestry. Fifty other individuals from regions that are now in Spain shared the R1b Y-chromosome haplogroup, all dating back to the Bronze Age (of which 40 from the beginning of that period).

In the Spanish territory, of the individuals with the LP allele, two were males who lived in the transition from the Iron Age to the Roman Period, one female and one man who lived around 416-700 CE (Late-Ancient) and, finally, two females and two males, who lived during the Islamic Medieval Period (Tab. 2).

Only two male individuals with LP were identified in locations currently within Portuguese territory, and these lived during the Roman Period (Tab. 2). The first, with the genetic ID - "R10503.SG", was recovered from an archaeological site dated to 250 - 450 CE in Condeixa-a-Velha, in the District of Coimbra. The second, "R10488.SG," lived at the end of the Roman Period (archaeological site dated to approximately 441-593 CE) in a site now located in São Domingos de Rana, in the Municipality of Cascais (Fig. 2; Tab. 2).

The rarity of the LP allele throughout Prehistory up to the Medieval period is surprising, considering the present-day prevalence of LP in the Iberian Population. In Portugal, ~35% of the population has the

TABLE 2 GENETIC DATA OF INDIVIDUALS FROM THE IBERIAN PENINSULA WITH LACTASE PERSISTENCE.

ID	G	CHRONOLOGY	PERIOD	LOCALITY	COUNTRY	LAT.	LONG.	S	HY
I8206	0	340-57 CAL BCE	Iron Age-Roman	Girona	Spain	42.1333	3.1083	M	—
I3320	0	300-100 BCE	Iron Age-Roman	Valência	Spain	40.138	-0.07	M	—
I12515	0	1100-1300 CE	Middle Ages	Valência	Spain	39.9333	-0.2	M	—
I7497	0	1000-1100 CE	Middle Ages	Granada	Spain	37.8071083	-2.543041667	F	—
I7499	0	1000-1100 CE	Middle Ages	Granada	Spain	37.8071083	-2.543041667	F	—
I10853	0	993-1155 CAL CE	Middle Ages	Girona	Spain	42.0507	2.867	M	—
I12034	0	416-700 CE	Roman-Middle Ages	Girona	Spain	42.01656	2.814897	F	—
I12162	0	416-700 CE	Roman-Middle Ages	Girona	Spain	42.01656	2.814897	M	—
ZAP002	0	2200-1550 BCE	Bronze Age	Múrcia	Spain	37.674387	-1.697	M	R1b
R10503.SG	0	441-593 CAL CE	Roman	Cascais	Portugal	38.7261	-9.3653	M	—
R10488.SG	0	250-450 CE	Roman	Coimbra	Portugal	40.099444	-8.490556	M	—

Abbreviations: ID, Genetic ID of individuals; G, Genotype; Lat., Latitude; Long., Longitude; S, Sex; HY, Haplogroup Y.

LP-13,910*T allele (rarer in the South (27%) compared with the North (38%) and Centre (39%) (Manco *et al.* 2013). In Spain, the frequency of LP is estimated at 47% (Anguita-Ruiz – Aguilera – Gil 2020). Our analysis of the dataset suggests that up until the Roman period/Middle Ages, this allele (and thus LP) was rare. Even discounting allelic dropout and missing data, if the allele was frequent, probability suggests that it should show up more frequently in the data.

If LP was indeed rare until the Roman period, why did its frequency increase so much afterwards? Interestingly, the pattern we report here for Iberia has been reported in other European regions. The earliest identification of the LP allele in present-day Ukraine is from an individual with Yamnaya ancestry, dated to 3.960 BCE; however, the frequency of this allele remained very low in Europe (1.2%) between 3.000 and 2.000 BCE (Ségurel *et al.* 2020; Burger *et al.* 2020). In Central Europe, the LP allele was rare throughout the Neolithic and Bronze Age, reaching a frequency of 72% in the populations from this area by the 13th century (Krüttli *et al.* 2014). In the Alps, aDNA shows that the LP allele became frequent only in the last 3.000 years (Burger *et al.* 2020), with a low frequency in local Iron Age populations (Warnberg *et al.* 2023). Except for the Baltic Region and Scandinavia (where it rises early on), the frequency of the LP allele increased

only around the end of the 1st Millennium BCE, and it only took off during the Middle Ages (Evershed *et al.* 2022). The rarity of the LP allele in the aDNA data contrasts with the data of Plantinga *et al.* (2012) using the classic PCR method (different from the NGS used in the AADR data), which is more susceptible to contamination but less susceptible to allelic dropout. Studying graves in the Basque Country dated to between 3.000-2.500 BCE, they detected the LP allele in 7 out of 26 individuals (26.9%)

One hypothesis for this pattern in the Iberian Peninsula is that the mutation conferring LP was indeed introduced during the Bronze Age. Despite conferring selective advantages, it took centuries for a significant frequency increase due to genetic drift (Nielsen – Slatkin 2013). Moreover, the fact that only one individual with LP was identified among the 50 associated with the Bronze Age with haplogroup R1b might be due to allelic dropout, combined with the absence of Y haplotype information.

Given the information available from our aDNA dataset, the simultaneous emergence of haplogroup R1b and the LP allele strengthens the hypothesis that the LP trait was introduced in the Iberian Peninsula by people with steppe ancestry. The 50 individuals from the Iberian Bronze Age with haplogroup R1b support the hypothesis of gradual genetic change in the

eastern zone of the peninsular territory between 2.500 and 2.000 BCE (Koch 2019), which corresponds with the interpretation proposed by Villalba-Mouco *et al.* (2021).

Alternatively, this mutation may have developed and intensified independently in distinct European regions; that is, the mutation conferring LP was already present in low frequencies in Europe before the putative Yamnaya arrival, or even in a small part of the Iberian population. Migration would have facilitated the spread of the mutation across the territory. However, there is no genetic evidence to support this hypothesis, other than the Plantinga *et al.* (2012) high frequency of LP early in the Basque Country. Moreover, it is unlikely that two mutations emerged simultaneously in the exact genomic location and with the same phenotype. At least three additional different LP alleles are known for the same genomic region as the -13910C>T mutation, the most common in Europe. However, these SNPs do not occur precisely in the same genomic position: -13907C>G is present in pastoral groups in Sudan, -14010G>C in East Africa, and -13915T>G in the Arabian Peninsula. It is assumed these emerged much more recently and were never detected in past European populations (Strachan – Read 2019; Tishkoff *et al.* 2007). Therefore, the data indicate that the most likely hypothesis is that LP emerged in the steppes.

To compare the Iberia dataset with Central Europe, we used the same method and the AADR to analyse individuals from regions where the Yamnaya Migration is assumed to have been more intense and associated with the CWC and Bell Beaker archaeological cultures (Kristiansen 2022). We selected individuals from Germany, Armenia, Bulgaria, Czechia, Slovakia, France, Hungary, Moldova, the Netherlands, Poland, the United Kingdom, Romania and Ukraine. A total of 401 ancient individuals with the ability to tolerate milk were identified. Notably, 239 individuals with this characteristic were registered in the United Kingdom, followed by Germany with 69 and Ukraine with 57. The LP allele in North and Central Europe became more frequent in the Roman Period and the Middle Ages (Fig. 3), agreeing with the study by Krüttli *et al.* (2014) for Medieval Germany. In contrast to the Iberian Peninsula, however, the LP allele was highly frequent in the Iron Age in North and Central Europe, becoming rarer afterwards.

From the Central and Northern European subset, three individuals with LP stood out. These would have

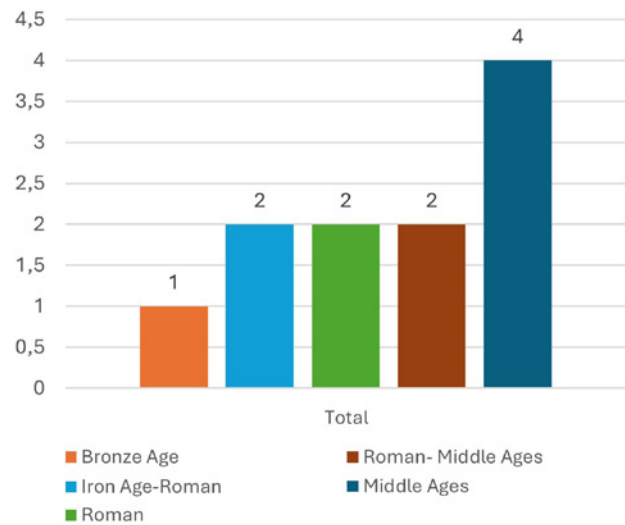


FIG. 2 Total number of individuals with LP in the Iberian Peninsula.

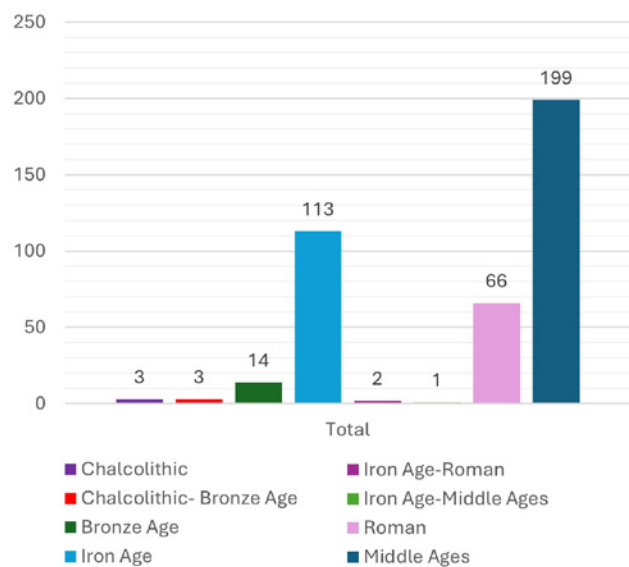


FIG. 3 Total number of individuals with LP from Central and Northern Europe.

lived in the Chalcolithic or Early Bronze Age around 2.800-1.800 BCE (Tab. 3). A female (Genetic ID -“I7201”) and a male (“I7202”) were identified in present-day Czechia, while another female (“I5531”) was recovered from an archaeological site in present-day German territory. The Y-chromosome haplogroup was unavailable for the male, so it is unknown whether he carried the R1b haplogroup common among steppe people. However, if we assume that the LP allele was introduced by migrating Yamnaya, the presence of women with LP supports the idea that the movement involved both men and women. On the other hand, we cannot dismiss the possibility that these individuals were the

TABLE 3 GENETIC DATA FROM CHALCOLITHIC-ASSOCIATED INDIVIDUALS FROM EUROPE WITH LACTASE PERSISTENCE

ID	G	CHRONOLOGY	PERIOD	REGION	LAT.	LONG.	S	HY
I27380	0	2453-2146 cal BCE	Chalcolithic	United Kingdom	50.872397	0.89596028	M	R1b
KON004	0	2567-2344 cal BCE	Chalcolithic	Czech Republic	50.555735	13.660563	F	—
ALT_4_noUDG	0	2573-2356 cal BCE	Chalcolithic	Germany	49.468332	9.744072	M	R1b
I7201	0	2800-1800 BCE	Chalcolithic-Bronze Age	Czech Republic	50.0504153	14.3667267	F	—
I7202	0	2800-1800 BCE	Chalcolithic-Bronze Age	Czech Republic	50.0504153	14.3667267	M	—
I5531	0	2800-1800 BCE	Chalcolithic-Bronze Age	Germany	48.705913	11.325375	F	—

Abbreviations: ID, Genetic ID of individuals; G, Genotype; Lat., Latitude; Long., Longitude; S, Sex; HY, Haplogroup Y.

daughters of local women and Yamnaya men who transmitted the LP allele to their offspring.

Additionally, three other individuals were identified with the LP trait, dated to the end of the Chalcolithic, whose data are equally relevant: a male (Genetic ID - "ALT_4_noUDG") from the current region of Germany and another male ("I27380") from the current territory of the United Kingdom, both with LP and Haplogroup R1b, plus a female ("KON004") from the Czech territory (Tab. 3).

3.2. Cattle, Caprines and Milk Production in the Zooarchaeological Prehistory Record in Portugal (Presence/Absence Data)

When the LP trait became more common in the Iberian Peninsula, dietary practices may have shifted, with raw milk becoming a regular component of the diet. If this were the case, we would expect changes in animal management during this period, with a greater emphasis on milk production. *Does the zooarchaeological record reflect such a shift?*

In the Portuguese territory, several archaeological assemblages from the Neolithic, Chalcolithic, Bronze Age and Iron Age yielded remains of cattle and caprines. However, only a few of these assemblages currently include the considered indicators (lipid residues, cheese-making implements, animal age profiles) (Supplementary Material 2).

The Central and Southern regions of Portugal have the highest concentration of prehistoric mammal remains, with Estremadura standing out as the area with the most extensive archaeofaunal research. In

contrast, evidence for archaeofauna is limited in Central-Northern and Northern Portugal, where zooarchaeological studies remain underdeveloped. In some of these northern areas, the scarcity of studies reflects the limited availability of bone remains, partly the outcome of low preservation in acidic soils (Cardoso 1996; Senra 2018) (Fig. 4).

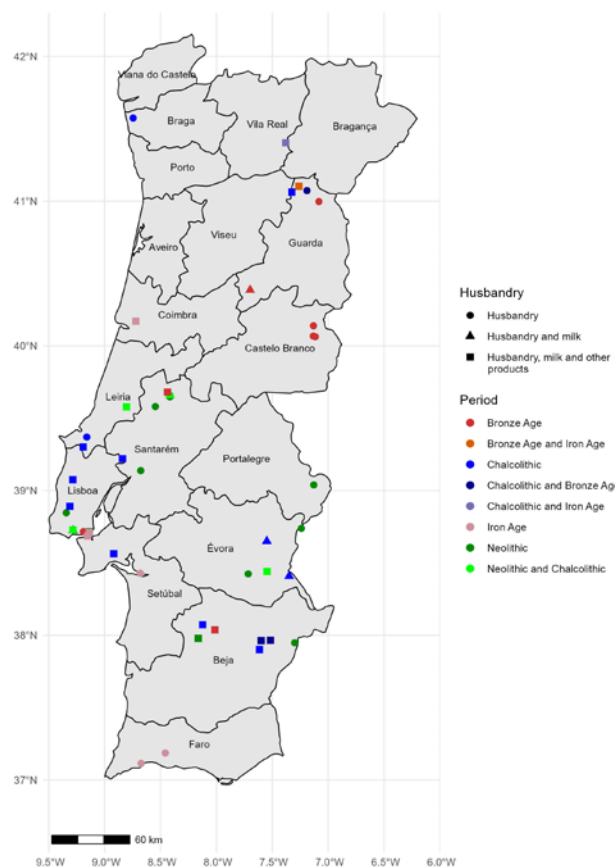


FIG. 4 Map of prehistoric archaeological sites with evidence of caprine and cattle husbandry. Some sites contain evidence from multiple periods.

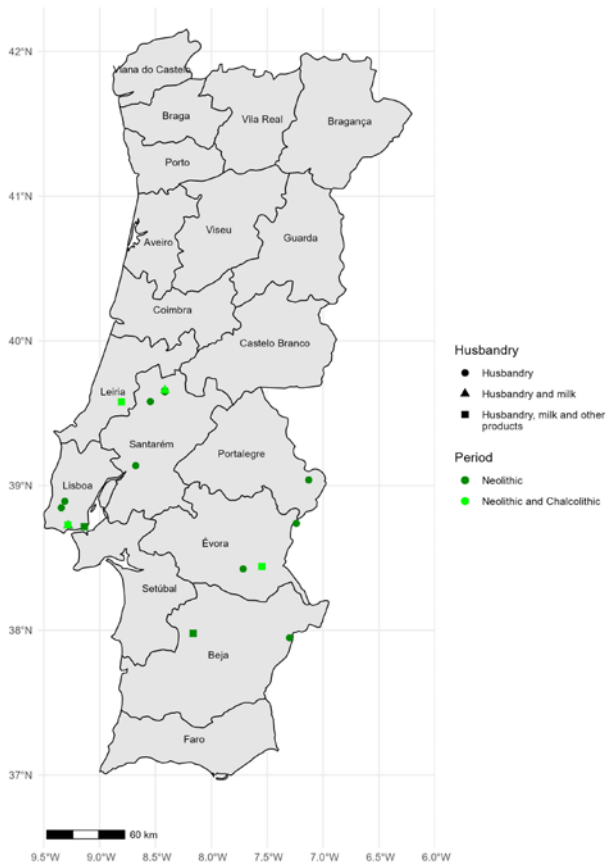


FIG. 5 Neolithic archaeological sites with evidence of cattle, milk production, and other secondary products.

Based on the available data on prehistoric dairy practices in Portugal, we propose that dairy consumption was already established in the southern region by the Early Neolithic (Supplementary Material 2). This conclusion is supported by evidence from the age profiles of slaughtered animals and lipid residue analyses from ceramic vessels (Valente – Carvalho 2019). Among the sites with Neolithic occupations, seven have been identified as showing evidence of milk production (Fig. 5).

During the Chalcolithic, evidence for milk use has been identified at 11 sites. However, the available data suggests that milk was not a major resource, as there is little indication of its intensive exploitation. Nonetheless, the discovery of fragments of cheese-making equipment at Castro da Columbeira, Castro da Vila Nova de São Pedro, Castro de Chibanes, and Povoado do Mercador confirms that some dairy processing did occur. If the LP allele was present in Portuguese populations during this period, it would likely have been found in only a small proportion of individuals (Fig. 6).

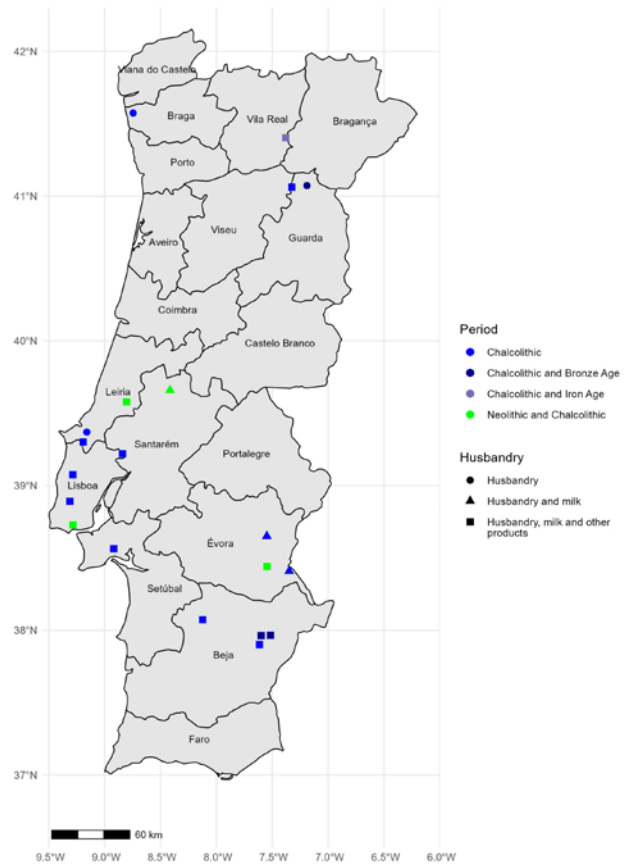


FIG. 6 Chalcolithic archaeological sites with evidence of cattle, milk production, and other secondary products.

The analysis of most faunal assemblages from Bronze Age and Iron Age sites remains extremely limited (Figs. 7 and 8). Although data are scarce or lacking, it can be proposed that during the Bronze Age, dairy and other secondary animal products likely became a consistent feature of the daily lives of prehistoric communities across both southern and northern Portugal.

3.3. Discussion

The development of dairy production and consumption in the Iberian Peninsula represents a complex interplay among cultural practices, livestock management strategies, and genetic adaptations spanning from prehistoric times to the medieval period.

The development of an agricultural-pastoral economy in southern Portugal, documented from the Late Neolithic through the Chalcolithic, reveals a progressive increase in sheep/goat and cattle livestock (Valente 2015; Valente – Carvalho 2019). Zooarchaeological studies suggest that milk production occurred as early as the Early Neolithic in Portuguese territory. However, these data cannot definitively determine whether the

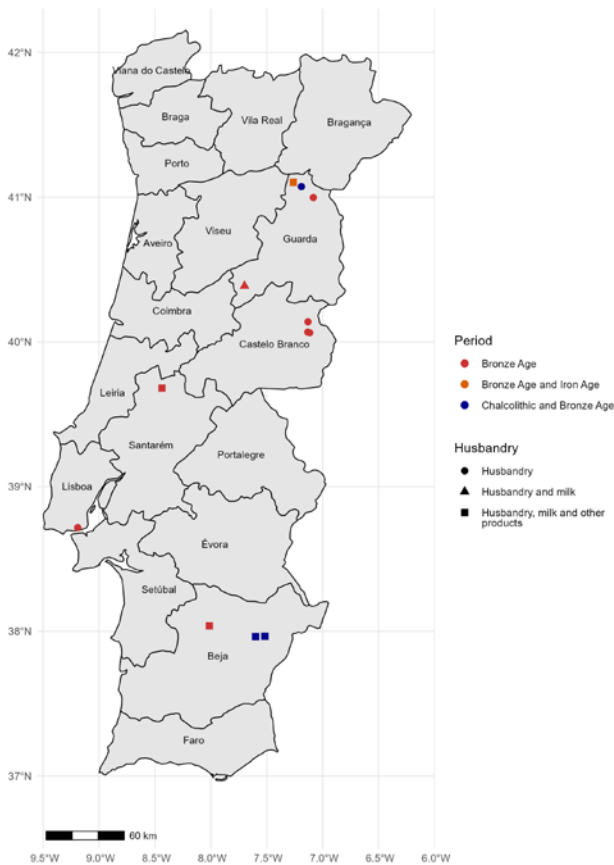


FIG. 7 Bronze Age archaeological sites with evidence of cattle, milk and other secondary products.

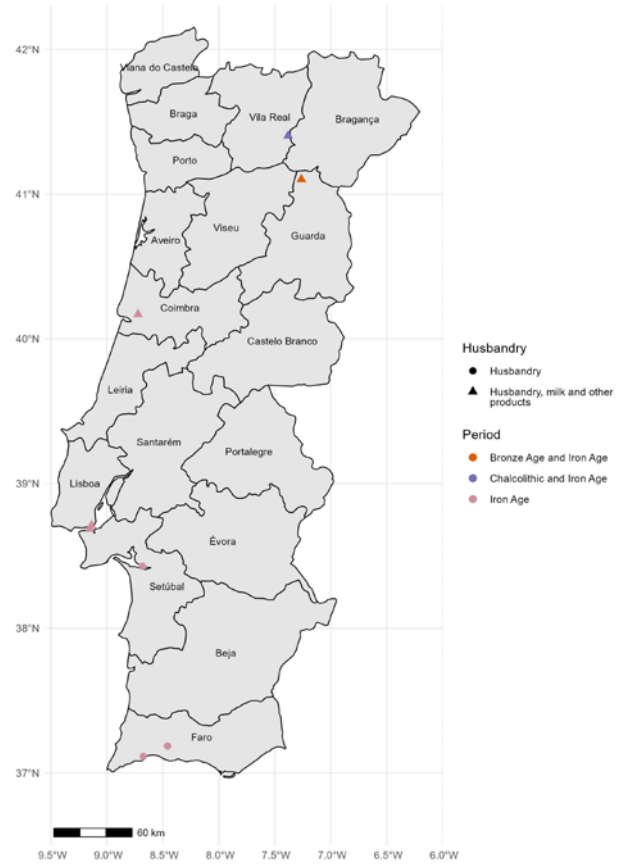


FIG. 8 Iron Age archaeological sites with evidence of cattle, milk and other secondary products.

milk was processed into dairy products or consumed directly unprocessed. Evidence from other European regions supports the former scenario, indicating that early milk exploitation primarily involved processing rather than direct consumption (Evershed *et al.* 2022). If milk was indeed being produced during these early periods, it was most likely transformed into dairy products rather than consumed raw (Charlton *et al.* 2019).

In Iberia, based on the aDNA genetic data analysed here, the allele conferring LP was apparently so rare in prehistoric populations that it remained undetected in aDNA data until the Roman period, with the LP allele first detected only from the 3rd Century CE onward. This genetic evidence supports the interpretation that raw milk consumption was a relatively late development in the region.

Historical sources and zooarchaeology also suggest such late consumption of raw milk. For the Roman period, evidence concerning milk consumption derives chiefly from classical literary sources. These texts indicate that milk was not a favoured beverage among Romans in the Italian Peninsula, although

it was commonly consumed on farms and less frequently in urban environments (Déry 2000). When milk was consumed, it appears that goat and sheep milk were preferred to that of cattle (Vergil, *Georgics* 3.308–310; Columella, *On Agriculture* 7.6.4), reflecting the general Roman perception of cattle primarily as draught or labour animals, although certain “breeds” may have been managed for dual purposes (Toynbee 1973: 323).

Zooarchaeological evidence from Portugal remains limited in this respect. In Odemira, data suggest a distinct shift in livestock management strategies between the Iron Age and the Roman period. Davis and Vilhena (2017) report that cattle during Roman times were slaughtered at significantly older ages than in the Iron Age – a pattern commonly interpreted as indicative of their primary use as draught animals. As no sex profiling was undertaken, either morphologically or genetically, it can only be hypothesised that some of these older individuals were females maintained for milk production throughout adulthood. Nevertheless, it seems probable that when milk was produced

in larger quantities, it was largely converted into fermented products, particularly cheese, whether of the hard, longer-lasting varieties or soft forms intended for more immediate consumption.

Archaeological evidence from the Middle Ages indicates a steady expansion of cattle and caprine herding across Portugal (Davis 2008). This trend is supported by a range of medieval written sources that describe the deliberate management of goats, sheep, and cattle into advanced age to maximise secondary products such as wool, manure, draught and transport services, and milk. Both Islamic (García-Sánchez 1983; Morales 1992; Rosenberger 1999) and Christian (Gonçalves 2004; Marques 2017) records document these livestock and dairy practices, highlighting their continuity and the significant economic and cultural role of dairy production irrespective of political or religious context.

Recently, Tente and Valente (in print) additionally note that documentary and iconographic evidence suggests late medieval dairy consumption was socially stratified, being more prevalent in urban and elite contexts (namely by incorporating milk in cooking recipes, as shown in the 16th century Cookery Book of Infanta D. Maria; (Manuppella – Arnaut 1967), while ethnoarchaeological studies in Serra da Estrela show that transhumant sheep flocks underpinned local wool and cheese industries. Nevertheless, most milk appears to have been processed into dairy products such as cheese and butter or used in cooking, as the direct consumption of raw milk entailed health risks (Braga 2003). Overall, medieval Portuguese dairying was neither uniform nor static but adapted to local ecological conditions, market needs, and cultural norms. The extent to which this growing reliance on dairy contributed to the emergence of lactose tolerance observed in medieval genetic datasets remains to be fully determined.

The rapid prevalence of the LP allele in the Portuguese population is especially notable and reflects the growing incorporation of raw milk consumption into local dietary practices. That said, it is important to recognise that this process is probably recent, as the direct consumption of raw milk in Portugal without cooking only became established from the 18th century onward, and even then in lesser quantities than in other Southern European countries such as Spain or Italy (Braga 2003).

4. CONCLUSION

This study examined the emergence and dissemination of lactase persistence in the Iberian Peninsula, with a particular focus on the current Portuguese territory. By integrating archaeogenetic analysis with a review of the available zooarchaeological evidence, we propose a complex, gradual process of cultural and biological transformation. LP was likely introduced by descendants of groups associated with the Yamnaya archaeological culture who carried the -13.910*T allele. This is supported by the fact that the first individual with this allele (“ZAP002”, a male dating from the Early Bronze Age) also carried the Y-chromosome haplogroup R1b.

The prevalence of the LP causative allele remained low until the Roman Period, based on the aDNA data available (*i.e.*, low frequency in the dataset). In Portugal, the LP causative allele was detected only in individuals from the Roman period onwards. In Iberia, the frequency of the LP mutation increased during the Middle Ages, following a pattern observed elsewhere in Europe.

The zooarchaeological record in Portuguese territory supports the notion that milk was already being processed and consumed in the Neolithic in southern Portugal. However, this early milk consumption would have involved fermented products (dairy), which are digestible by lactose-intolerant individuals.

The overlapping lines of evidence support a model in which dairy consumption predates the genetic adaptation for LP; raw milk became a widespread dietary component once the allele became frequent in the population. In the Portuguese case, this seems to have happened as recently as the Middle Ages, when documents mention milk usage in cooking. More aDNA data in the future can help determine when the allele responsible for LP reached frequencies similar to those of present-day populations.

Finally, we describe a straightforward and replicable method for using the freely available ancient human DNA dataset (AADR) for archaeogenetic research. The accompanying code, provided as Supplementary Material, can be adapted to investigate the occurrence of specific traits in past populations, provided that the relevant SNPs are known.

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POLÍTICA EDITORIAL

Objectivos

A Ophiussa – Revista do Centro de Arqueologia da Universidade de Lisboa foi iniciada sob a direcção de Victor S. Gonçalves em 1996, tendo sido editado o volume 0. A partir do volume 1 (2017), a Revista Ophiussa converteu-se numa edição impressa e digital da UNIARQ – Centro de Arqueologia da Universidade de Lisboa (ISSN 1645-653X / E-ISSN 2184-173X). Em 2025, a revista deixou de ser publicada em formato impresso, passando a disponibilizar-se exclusivamente em versão digital, em acesso aberto, em <https://ophiussa.letras.ulisboa.pt>

O principal objectivo desta revista é a publicação e divulgação de trabalhos com manifesto interesse, qualidade e rigor científico sobre temas de Pré-História e Arqueologia, sobretudo do território europeu e da bacia do Mediterrâneo.

Periodicidade

A Ophiussa – Revista do Centro de Arqueologia da Universidade de Lisboa publicará um volume anual. O período de submissão de trabalhos decorrerá sempre no primeiro semestre e a edição ocorrerá no último trimestre de cada ano.

Secções da revista

A revista divide-se em duas secções: artigos científicos e resenhas bibliográficas. Excepcionalmente poderão ser aceites textos de carácter introdutório, no âmbito de homenagens ou divulgações específicas, que não serão submetidos à avaliação por pares. Isentas desta avaliação estão também as resenhas bibliográficas.

Os autores / editores que pretendam apresentar uma obra para resenha devem enviar dois exemplares para a direcção da Revista Ophiussa: um para o autor/autora da resenha que será convidado para o efeito e outro para a Biblioteca da Faculdade de Letras da Universidade de Lisboa. Aceita-se igualmente a apresentação de propostas de resenhas espontâneas.

Aceitam-se trabalhos redigidos em português, inglês, espanhol, italiano e francês.

Processo de avaliação por pares

Os artigos submetidos são sujeitos a um processo de avaliação por parte de revisores externos (double blind peer review).

Todas as submissões (artigos e resenhas) serão avaliadas, em primeira instância, pela Coordenação Editorial, no que respeita ao seu conteúdo formal e à sua adequação face à política editorial e às normas de edição da revista. Os artigos que cumprirem estes requisitos serão posteriormente submetidos a um processo de avaliação por pares cega / double blind peer review (mínimo de dois revisores). O Conselho Científico, constituído pela direcção da UNIARQ e por investigadores externos, acompanhará o processo de edição.

Esta etapa será concretizada por investigadores externos qualificados, sendo os respectivos pareceres entregues num período não superior a três meses. Os revisores procederão à avaliação de forma objectiva, tendo em vista a qualidade do conteúdo da revista; as suas críticas, sugestões e comentários serão, na medida do possível, construtivos, respeitando as capacidades intelectuais do(s) autor(es). Após a recepção

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Serão considerados os seguintes princípios éticos:

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EDITORIAL POLICY

Objectives

Ophiussa – Revista do Centro de Arqueologia da Universidade de Lisboa started under the direction of Victor S. Gonçalves in 1996, with the edition of volume 0. After Volume 1 (2017) it became a printed and digital edition of UNIARQ – Centro de Arqueologia da Universidade de Lisboa (ISSN 1645-653X / E-ISSN 2184-173X). In 2025, the journal ceased to be published in print format and became available exclusively in digital, open-access form at <https://ophiussa.letras.ulisboa.pt>

The main objective of this journal is the publication and dissemination of papers of interest, quality and scientific rigor concerning Prehistory and Archeology, mostly from Europe and the Mediterranean basin.

Periodicity

Ophiussa – Revista do Centro de Arqueologia da Universidade de Lisboa will publish an annual volume. The submission period will always occur in the first quarter of each year and the edition will occur in the last quarter.

Journal sections

The journal is divided into two sections: scientific articles and bibliographic reviews. Exceptionally, texts of an introductory nature may be accepted, in the context of specific tributes or divulgations, which will not be submitted to peer-review evaluation. Exemptions from this evaluation are also the bibliographic reviews.

Authors / editors wishing to submit a book for review should send two copies to the direction of Revista Ophiussa: one to the author of the review who will be invited for the purpose and another to the Library of the School of Arts and Humanities of the University of Lisbon. Spontaneous proposals are also accepted.

Papers written in Portuguese, English, Spanish, Italian and French are accepted.

Peer review process

Submitted articles are subject to a double blind peer-review evaluation process.

All submissions (articles and reviews) will be considered, in the first instance, by the Editorial Board, regarding its formal content and adequacy in face of the editorial policy and the journal editing standards. Articles that meet these requirements will subsequently be submitted to a blind peer-review process (minimum of two reviewers). The Scientific Council, constituted by UNIARQ direction and external researchers, will follow the editing process.

This stage will be carried out by qualified researchers, and their feedback will be delivered within a period of no more than two months. The reviewers will carry out the evaluation in an objective manner, in view of the quality and content of the journal; their criticisms, suggestions and comments will be, as far as possible, constructive, respecting the intellectual abilities of the author(s). After receiving the feedback, the author(s) has a maximum period of one month to make the necessary changes and resubmit the work.

Acceptance or refusal of articles will have as sole factors of consideration their originality and scientific quality.

The review process is confidential, with the anonymity of the evaluators and authors of the works being ensured, in the latter case, up to the date of its publication.

Papers will only be accepted for publication as soon as the peer review process is completed. Texts that are not accepted will be returned to their authors.

The list of reviewers will be published in 3-year cycles, indicated at the end of *Ophiussa* (printed and digital version).

Publication ethics

The Journal *Ophiussa* follows the guidelines established by the Committee on Publication Ethics (COPE, the Ethics Committee Publications): <https://publicationethics.org/>

Only original papers will be published. For the purpose of detecting plagiarism or duplicity, the URKUNDU platform (<https://www.orkund.com/pt-br/>) will be used. Practices such as the deformation or invention of data will be rejected. Authors are responsible for ensuring that the works are original and unpublished, the result of the consensus of all authors, and comply with current legality, having all necessary authorizations. Articles that do not comply with these ethical standards will be rejected.

Contributions submitted for publication must be unpublished. Article submissions can not include any problem of forgery or plagiarism. Illustrations that are not from the author(s) must indicate their origin. The Scientific Council and Editorial Board assume that the authors have requested and received permission to reproduce these illustrations and, as such, reject the responsibility for the unauthorized use of the illustrations and legal consequences for infringement of intellectual property rights.

It is assumed that all Authors have made a relevant contribution to the reported research and agree with the manuscript submitted. Authors must clearly state any conflicts of interest. Collaborations submitted that directly or indirectly had the financial support of third parties must clearly state these sources of funding.

Texts proposed for publication must be unpublished and should not have been submitted to any other journal or electronic edition.

The content of the works is entirely the responsibility of the author(s) and does not express the position or opinion of the Scientific Council or Editorial Board.

The editorial process will be conducted objectively, impartially and anonymously. Errors or problems detected after publication will be investigated and, if proven, corrections, retractions and / or responses will be published.

The following ethical principles will be considered:

1) RESPONSIBILITY:

Ophiussa through its editors and authors has the absolute responsibility for approval, condemning all bad practices of scientific publication.

2) SCIENTIFIC FRAUD

Ophiussa will seek to detect manipulation and falsification of data, plagiarism or duplicity, with the appropriate detection mechanisms.

3) Editorial policy and procedures:

a) Authors must have participated in the research process and in the review process, and must ensure that the data included is real and authentic and are obliged to issue retractions and corrections of errors of published articles;

b) Reviewers must carry out an objective and confidential review and have no conflicts of interest (research, authors or funders), and must indicate relevant published works that were not cited;

c) In the detection of fraud or malpractice in the evaluation phase, it must be indicated by the reviewers and in the post-publication phase by any reader.

d) In case of detection of bad practices in the evaluation phase or of detection of previously published articles, the Editorial Board will send the occurrence to the author, establishing a period of 7 days for clarification, which will be subsequently evaluated by the Editorial Board. In the post-publication phase, the Editorial Board may file or determine the retraction in a subsequent issue, indicating the previous procedures.

Digital file preservation policy

The journal guarantees the permanent accessibility of digital objects through backup copies and use of DOI, integrating the Public Knowledge Project's Private LOCKSS Network (PKP-PLN), which generates a decentralized file system.

Regarding the self-archiving, the magazine also includes Sherpa/Romeu

(<https://v2.sherpa.ac.uk/id/publication/41841>).

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This edition immediately and freely provides all of its content, in open access, in order to promote global circulation and exchange of scientific research and knowledge. It follows Creative Commons guidelines (license CC/BY/NC/ND 4.0).

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For more information contact:

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