

The Genomic Legacy of Human Management and sex-biased Aurochs hybridization in Iberian Cattle

Reviewed Preprint

Published from the original preprint after peer review and assessment by eLife.

About eLife's process

Reviewed preprint version 1

January 10, 2024 (this version)

Sent for peer review

October 9, 2023

Posted to preprint server

September 6, 2023

Torsten Günther , Jacob Chislausky, M. Ángeles Galindo-Pellicena, Eneko Iriarte, Oscar Cortes Gardyn, Paulina G. Eusebi, Rebeca García-González, Irene Urena, Marta Moreno, Alfonso Alday, Manuel Rojo, Amalia Pérez, Cristina Tejedor Rodríguez, Iñigo García Martínez de Lagrán, Juan Luis Arsuaga, José-Miguel Carretero, Anders Götherström, Colin Smith , Cristina Valdiosera 

Human Evolution, Department of Organismal Biology Uppsala University, Sweden • Centro Mixto UCM-ISCI de Evolución y Comportamiento Humanos, Spain • Laboratorio de Evolución Humana, Universidad de Burgos, Spain • Universidad Complutense Madrid, Spain • Centre for Palaeogenetics, Stockholm, Sweden • Instituto de Historia – CSIC, Spain • Área de Prehistoria, University of the Basque Country, Spain • Department of Prehistory and Archaeology, Valladolid University, Spain • Departamento de Prehistoria y Arqueología, UNED, Spain • Unidad Asociada de I+D+i al CSIC Vidrio y Materiales del Patrimonio Cultural (VIMPAC) • Dept. of Archaeology and History, La Trobe University, Australia

 https://en.wikipedia.org/wiki/Open_access

 Copyright information

Abstract

Cattle have been a valuable economic resource and cultural icon since prehistory. From the initial expansion of domestic cattle into Europe during the Neolithic period, taurine cattle (*Bos taurus*) and their wild ancestor, the aurochs (*B. primigenius*), had overlapping ranges leading to ample opportunities for intentional and unintentional hybridization. We performed a bioarchaeological analysis of 24 *Bos* remains from Iberia dating from the Mesolithic to the Roman period. The archaeogenomic dataset allows us to investigate the extent of domestic-wild hybridization over time, providing insight into the species' behavior and human management by aligning changes with cultural and genomic transitions in the archaeological record. Our results show frequent hybridization during the Neolithic and Chalcolithic, likely reflecting a mix of hunting and herding or relatively unmanaged herds, with mostly male aurochs and female domestic cattle involved in hybridization. This is supported by isotopic evidence of ecological niche sharing, with only a few domestic cattle possibly being managed. The proportion of aurochs ancestry in domestic cattle remains relatively constant from about 4000 years ago, probably due to herd management and selection against hybrids, coinciding with other cultural transitions. The constant level of wild ancestry (~20%) continues into modern western European breeds including the Spanish Lidia breed which is bred for its aggressiveness and fighting ability, but does not display elevated levels of aurochs ancestry. This study takes a genomic glance at the impact of human actions and wild introgression in the establishment of cattle as one of the most important domestic species today.

eLife assessment

Using genomic data from ancient and modern samples, this **important** study investigates the genomic history of cattle in Iberia, focusing on the admixture between domestic cattle and their wild ancestors, aurochs. The authors present **solid** evidence for interbreeding between domestic cattle and wild aurochs since the Neolithic period, although the extent, sex bias, and directionality of genetic flow over time remain highly unclear. The authors also show that the aurochs ancestry in cattle stabilized at ~20% since ~4000 years ago and continues into modern breeds, including the Lidia breed that is bred for aggressiveness and used in bullfighting. The work will be of interest to evolutionary biologists and quantitative geneticists who seek to understand the genomic history and genetic basis of trait variation of domesticated animals.

Introduction

Domestication has been the dominant and most enduring innovation of the transition from a hunter-gathering lifestyle to farming societies, representing the direct exploitation of genetic diversity of wild plants and animals for human benefit. Ancient DNA (aDNA) has proved crucial to understanding the domestication process and the interaction between domesticated species and their wild relatives both within domestication centers and throughout the regions that the domestics expanded into (1–13). The origins of the European domestic taurine, *Bos taurus*, are located in the Fertile Crescent (14, 15) and unlike dogs, pigs and goats, where the wild forms are still extant, the wild cow (the aurochs) went extinct in 1627. Aurochs, *B. primigenius*, was present throughout much of Eurasia and Africa before the expansion of domestic cattle from the Levant that accompanied the first farmers during the Neolithisation of Europe. Upon arrival, these early incoming domesticates inevitably coexisted with their wild counterparts in great parts of Europe facilitating gene flow in both directions. In general, taxa within the genus *Bos* can hybridize and produce fertile offspring (16) which may have facilitated and contributed to domestication, local adaptation and even speciation (5, 17–19). Mitochondrial DNA studies have previously indicated gene flow between domestic cattle and aurochs outside their domestication center (20–24) and more recently, genomic studies have shown the presence of European aurochs ancestry in modern taurine cattle breeds (25, 26). Although cattle have represented a significant economic resource and a prominent cultural icon for millennia, and have been studied through aDNA for more than a decade (20, 21, 27), to date only a few aurochs' genomes have been sequenced (5, 25). This limits our understanding of the interaction of early cattle herds and wild aurochs, the latter's local input into modern domestic breeds and human management of these animals in the past.

Aurochs have been widely exploited by humans since the European Palaeolithic, nevertheless, archaeological evidence indicates that the species survived in Europe until historical times. In Iberia, the most recent evidence is found at a Roman site in the Basque Country (28). Domestic cattle were introduced into Iberia with the Mediterranean Neolithic expansion and reached the northern coast of the peninsula around 7000 years cal BP (29). Consequently, aurochs and domestic cattle have coexisted in Iberia for about five millennia. Since then, cattle have played an important role in Iberian societies as a source of food and labour as well as cultural events such as bullfighting. Currently, there are more than 50 bovine breeds officially recognized in the Iberian Peninsula including the Lidia breed, a primitive, isolated population selected for centuries to develop agonistic-aggressive responses with the exclusive purpose of taking part in such socio-cultural events (30). Recently, it has been reported that Lidia breed individuals have the largest

brain size among a comprehensive data set of European domestic cattle breeds and are the most similar to wild Aurochs (31 [↗](#)). The combination of aggressiveness and larger brain size in the Lidia breed may suggest a higher proportion of aurochs ancestry compared to other cattle breeds.

Here, we present the genomes and stable isotope data of Iberian Bovine specimens ranging from the Mesolithic into Roman times from four archeological sites (Fig. 1A [↗](#)). We explore the extent of interbreeding between wild aurochs and domestic cattle over time and the correlation of genetic ancestry with metric identification and ecological niches. Finally, we compare the results to genomic data obtained from modern Iberian cattle breeds to estimate the genetic contribution of the now-extinct aurochs to the Iberian farming economy.

Results

Exploratory analysis

We successfully sequenced 24 Bovine specimens excavated at four prehistoric sites in Iberia (Fig. 1A [↗](#)). Nine of these individuals were inferred to or suspected to represent aurochs based on morphology or chronology. Direct radiocarbon dates and contextual dating placed the individuals between the Mesolithic (oldest sample moo001, 8641-8450 cal BP) and the Roman Age (youngest sample moo010p, 2260-2150 CalBP). Based on the number of reads mapping to the X chromosome, 13 individuals were identified as female and eleven as male for the samples with sufficient amounts of reads for this analysis. Sequencing coverage of the nuclear cattle genome was low to medium, reaching up to 4.7x with a mean of 0.38x (Dataset S1). The sequence data for non-UDG-treated libraries showed damage patterns characteristic of ancient DNA (Supplementary Information, Figure S1).

Nine individuals were assigned to the mitochondrial P1 haplogroup, one to haplogroup T1, one to haplogroup T4, and 13 to haplogroup T3 (Dataset S1). P haplogroups are dominant and thought to be endemic to European aurochs (32 [↗](#)). The prevalence of the T3 haplogroup in our samples is expected; this haplogroup is dominant among modern European *B. taurus* and is the most common haplogroup in ancient Western European domestic cattle. T3 was found in directly dated Neolithic samples from different sites providing direct evidence for the arrival of domestic cattle in northern Iberia during the Neolithic (Dataset S1). The specimen assigned to T1 (moo009a) is notable since this individual was previously used to argue for Bronze Age contact between Iberia and Africa, where the T1 haplogroup is thought to have originated (27 [↗](#), 33 [↗](#)). T4 is usually considered to be restricted to Asian breeds with rare finds in Europe, restricted to the Balkans (23 [↗](#)). The presence of T4 in Chalcolithic Iberia suggests that this haplogroup must have been distributed across Western Europe at low frequencies in prehistory. Furthermore, the fact that some specimens that were morphologically identified as aurochs carry domestic T haplogroups implies some level of interbreeding between the two groups.

As mitochondrial genomes only reflect the maternal line of ancestry, they are not informative about the exact extent of interbreeding in our dataset. To avoid being constrained by the variation in modern domestic breeds as with common approaches such as projected PCA (Supplementary Information, Figure S2), we performed non-metric multi-dimensional scaling (NMDS) ordinations on a matrix of pairwise outgroup f_3 statistics to explore the genomic ancestry of the sequenced individuals. For reference, we included early cattle genomes from Anatolia and the Balkans as well as aurochs excavated from Morocco (Th7), Armenia (Gyu2), Anatolia (Ch22) and Britain (CPC98) (5 [↗](#), 25 [↗](#)), and calculated pairwise outgroup f_3 statistics. The NMDS ordination outcome (Fig. 1B [↗](#)) seems to represent a separation between domestic autosomal ancestry (to the left) and European aurochs ancestry (to the right). In contrast, aurochs from other regions (Th7 and Gyu2) seem genetically distinct. Many early domestic samples from Iberia fall close to early cattle from the Balkans and Anatolia as well as the Anatolian aurochs (Ch22). Notably, three of the Iberian

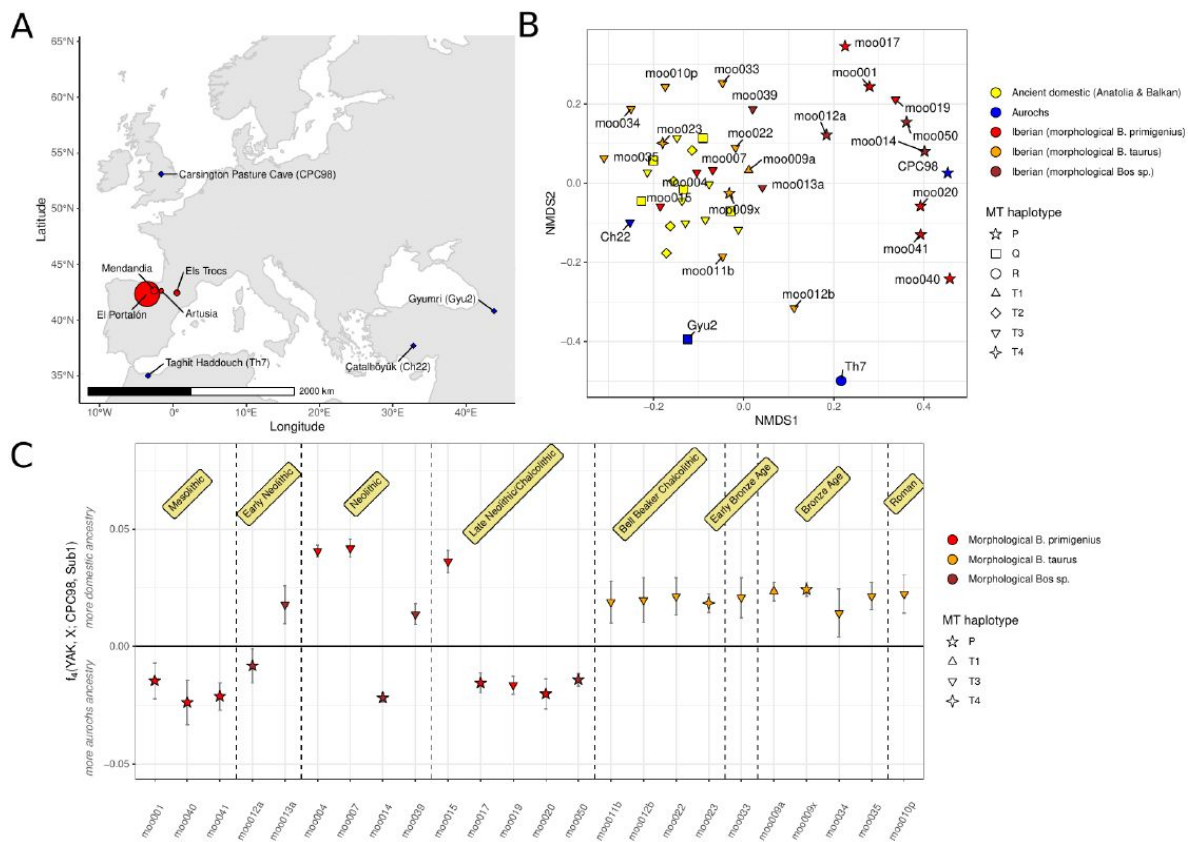


Figure 1.

(A) Map of Europe showing the Iberian sampling sites (red circles, size proportional to sample sizes) and the sites for published aurochs genomes used in the analysis (blue diamonds). (B) NMDS ordination of nuclear data of Iberian samples considered *B. taurus* samples (orange), Iberian samples that were morphologically considered *B. primigenius* (red), other Iberian *Bos* samples (brown), ancient domestic cattle from the Balkans and Anatolia (yellow), and aurochs (blue). Data point shape corresponds to mitochondrial haplogroups. (C) f_4 statistic measuring allele sharing of the Iberian samples with European aurochs (CPC98) or Anatolian Neolithic cattle (Sub1). The time periods displayed are contextual.

samples in this cluster (moo004, moo007, moo015) were morphologically identified as aurochs. Eight Iberian samples fall to the right or top-right in the plot, together with the aurochs from Britain (CPC98); only one of these (moo019) carried a domestic T3 mitochondrial genome, the others are all P. Out of nine samples that were presumed aurochs based on their morphological features, only six would be considered aurochs based on this analysis. Moreover, 2 of the 8 samples that fall to the right of this plot were not allocated a species. This highlights a substantial overlap between measurements or criteria that are used to distinguish wild and domestic *Bos* based on morphometrics.

This analysis suggests that one can use the British aurochs (CPC98) as a reference for Western European aurochs as it seems similar to our three low-coverage Mesolithic Iberian samples. Indeed, we do not see any evidence against them forming a clade to the exclusion of all other samples used for the NMDS (Supplementary Table S2). Using CPC98 and Sub1, a Neolithic Anatolian individual as references, we can perform f_4 statistics to measure which Iberian individuals share more alleles with one or the other (Fig 1C). Despite the relatively low coverage of some samples, the f_4 statistics are highly correlated with the first axis of the NMDS ($R^2=0.77$, $p=1.75 \times 10^{-8}$) implying that they detect the same pattern. Non-overlapping confidence intervals also confirm that the high genetic differentiation between Western European aurochs and domestic cattle allows confident assignment even with low coverage data. The three Mesolithic individuals as well as an additional six up until the Late Neolithic/Chalcolithic share most of their alleles with aurochs. Three individuals from the Neolithic and Late Neolithic/Chalcolithic share most of their alleles with domestic Anatolian cattle while two individuals (moo013a and moo039) are intermediate and their confidence intervals are not overlapping with the others, suggesting that they are the result of hybridization. More recent samples from the Bell Beaker period onwards all appear to have similar amounts of allele sharing with mostly domestic ancestry but some level of aurochs introgression.

Quantifying the extent of introgression

While f_4 statistics measure allele sharing but do not directly quantify the amount of introgression in the different specimens, we employed the qpAdm framework (34, 35) to model each Iberian individual from British aurochs (CPC98) and/or Anatolian Neolithic cattle (Sub1) as sources (Fig. 2). While the point estimates of qpAdm are noisier than the f_4 statistics, qpAdm also rejects ancestry models that would not be compatible with the data for each individual. For all 24 samples, models that fit the data were found. From these, seven individuals could be modeled from an aurochs source alone while eight individuals could be modeled with only domestic ancestry. For these individuals, a single source is the most parsimonious explanation but it should be noted that for eleven of these fifteen, a model of mixed aurochs/domestic ancestry would fit the data as well. However, it is noteworthy that nine of these eleven have very limited amounts of sequence data, making it possible that more data would lead to a rejection of the single source model for some of them. Seven samples could not be modeled from a single source and only fitted as a mixture between aurochs and domestic cattle implying that they carried substantial proportions of both ancestries. The ancestry estimates in the 2-source model vary substantially between individuals ranging from ~25% (moo009a) to ~90% (moo014) aurochs ancestry. These results are largely consistent with the f_4 statistics, although some individuals falling to the extremes of the f_4 range also show signals of admixture. For some Mesolithic individuals, the two-source model is consistent with the data but the depth of coverage for these individuals was generally low and proportions of domestic ancestry are not significantly different from zero. During the Neolithic, two individuals are exclusively of Anatolian domestic ancestry without aurochs introgression while others show ancestry from both sources with predominantly wild ancestry in most cases. All Chalcolithic individuals are consistent with the two-source model with aurochs ancestry estimates ranging from 7 to 84%. The wide range of different ancestry estimates in the Neolithic and Chalcolithic samples suggests that interbreeding between wild aurochs and domestic cattle was frequent in prehistoric Iberia. However, at some point, the herding practices must have changed since modern Iberian breeds show approximately 20-25% aurochs ancestry

(Supplementary Information, Table S2). In fact, we observe that after the Bell Beaker period the ancestry estimates change to predominantly domestic ancestry and aurochs proportions similar to those found in modern western European breeds (**Fig. 3A & C** [↗](#)).

A limitation of this analysis is the use of the British aurochs genome as a reference for the aurochs source population. There is a risk that this analysis will miss certain ancestries that are exclusive to Iberian aurochs while including some that are exclusive to the British aurochs, for example, contributions from glacial refugia other than Iberia. Our Mesolithic Iberian aurochs contained too little endogenous DNA to be used as a proxy aurochs reference and the three Neolithic and Chalcolithic samples estimated at >90% aurochs ancestry (including the 2.7x genome of moo014) already carry low levels of domestic ancestry (Supplementary Information, Figure S8).

An important question that remains unexamined is the exact process that led to the bidirectional introgression since this would provide insight into human management practices. The fact that some individuals with predominantly aurochs ancestry carry T haplogroups (e.g. moo019) and that some individuals with predominantly domestic ancestry carry P haplogroups (e.g. moo009x) implies that females contributed in both directions. To assess whether the admixture process was sex-biased, we compared the allele-sharing patterns on the X chromosome and autosomes. Since females carry two X chromosomes and males only have one, we can assume that an excess of a certain ancestry on the X chromosome indicates more females from that particular source population. For the Mesolithic samples, as expected, we do not see any significant difference in the level of allele sharing between X chromosome and autosomes (**Fig. 3B** [↗](#)). Similarly, there is no evidence of sex bias for most individuals with predominantly aurochs ancestry (negative autosomal f_4 values), i.e. individuals that are most likely wild rather than domestic or managed. In contrast, all other individuals with feasible and non-rejected two-source models of ancestry show an excess of allele sharing with Anatolian domestic cattle than with aurochs on the X chromosome, when compared to the autosomes. This difference is significant for eight of those twelve individuals including an individual carrying a P mtDNA haplogroup (moo009x). Even one of the individuals for which only the single source domestic qpAdm model is accepted shows an excess of Anatolian domestic ancestry on the X chromosome (moo004), suggesting that sex-biased processes already took place prior to the arrival of cattle to Iberia. This general observation is consistent with the relatively clear (but not perfect) discrimination between wild and domestic based on mitochondrial haplogroups where modern breeds mostly carry Near Eastern haplogroups ([5](#) [↗](#), [21](#) [↗](#)). In the absence of aurochs Y chromosomal data, however, it is difficult to assess sex-biased processes from uniparental data alone. The comparison of X chromosomes and autosomes should generally have more power to detect such processes as they are less sensitive to genetic drift due to their recombining nature ([36](#) [↗](#)). Overall, this illustrates that the contribution of wild ancestry into domestic cattle was mostly through aurochs bulls while no clear trend can be seen for admixture into wild populations.

Aurochs ancestry in modern breeds and the Spanish Lidia cattle breed

We estimated aurochs ancestry in a set of Western European cattle breeds ([26](#) [↗](#)) as we performed for the prehistoric samples. Previous studies have used D statistics for pairwise comparisons between breeds ([25](#) [↗](#), [26](#) [↗](#), [37](#) [↗](#)). Such D statistics, however, are sensitive to biases including gene flow from populations not included in the analysis ([38](#) [↗](#)). Furthermore, qpAdm provides the possibility to reject scenarios not fitting the data. Our point estimates for the aurochs ancestry range between 20% and 25% across all breeds (**Fig. 3C** [↗](#)) and do not show an increase in aurochs ancestry in Iberian breeds ([37](#) [↗](#)). This result differs from the previous studies which suggested geographic differences in western and central Europe and we believe this could be due to ancestry from other, non-European groups in some commercial breeds (Supplementary Information). Importantly, not all tested breeds did fit the simple two-source model Anatolian Neolithic domestic

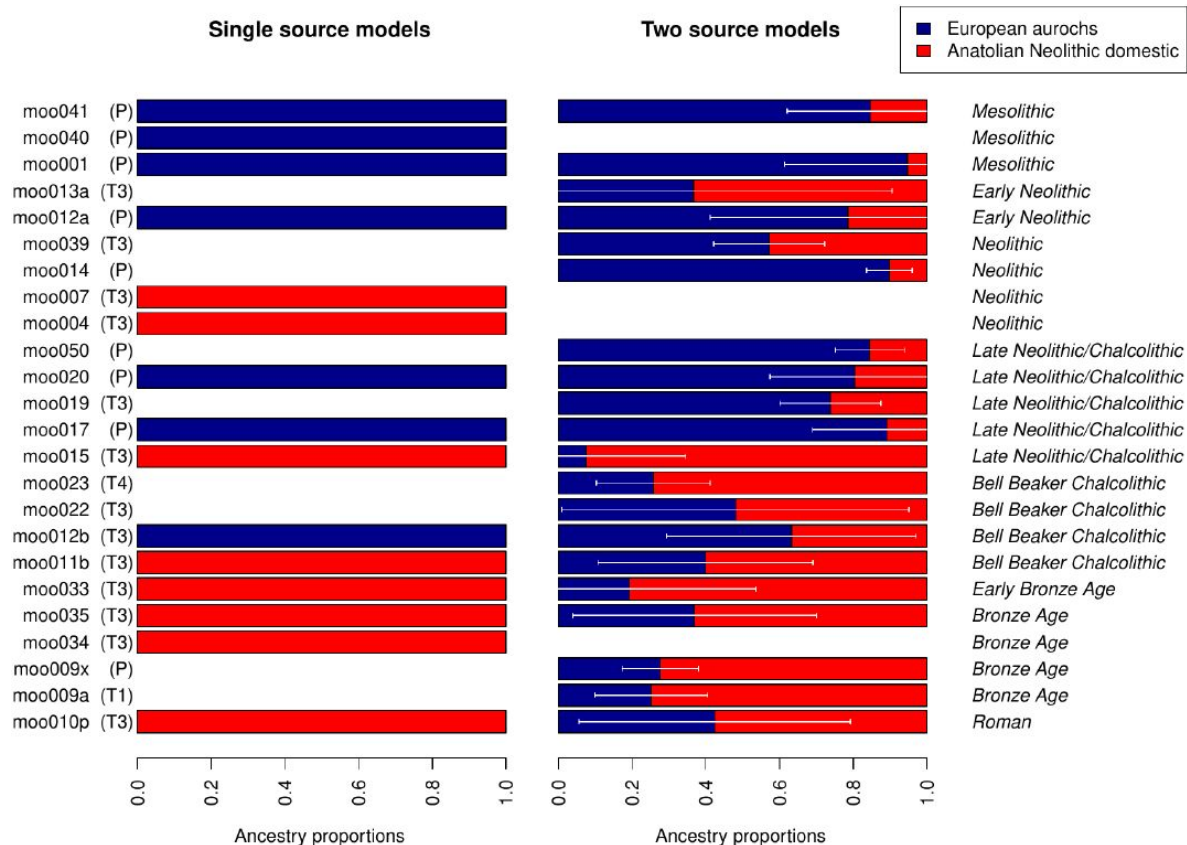


Figure 2.

Ancestry proportions in all sequenced Iberian *Bos* samples. For each sample, only feasible and not rejected ($p > 0.01$) models are displayed. For moo034 and moo010p, both single source models are consistent with the data, we display the single source model with the higher p-value. Error bars are showing block-jackknife estimates of the 95% confidence interval.

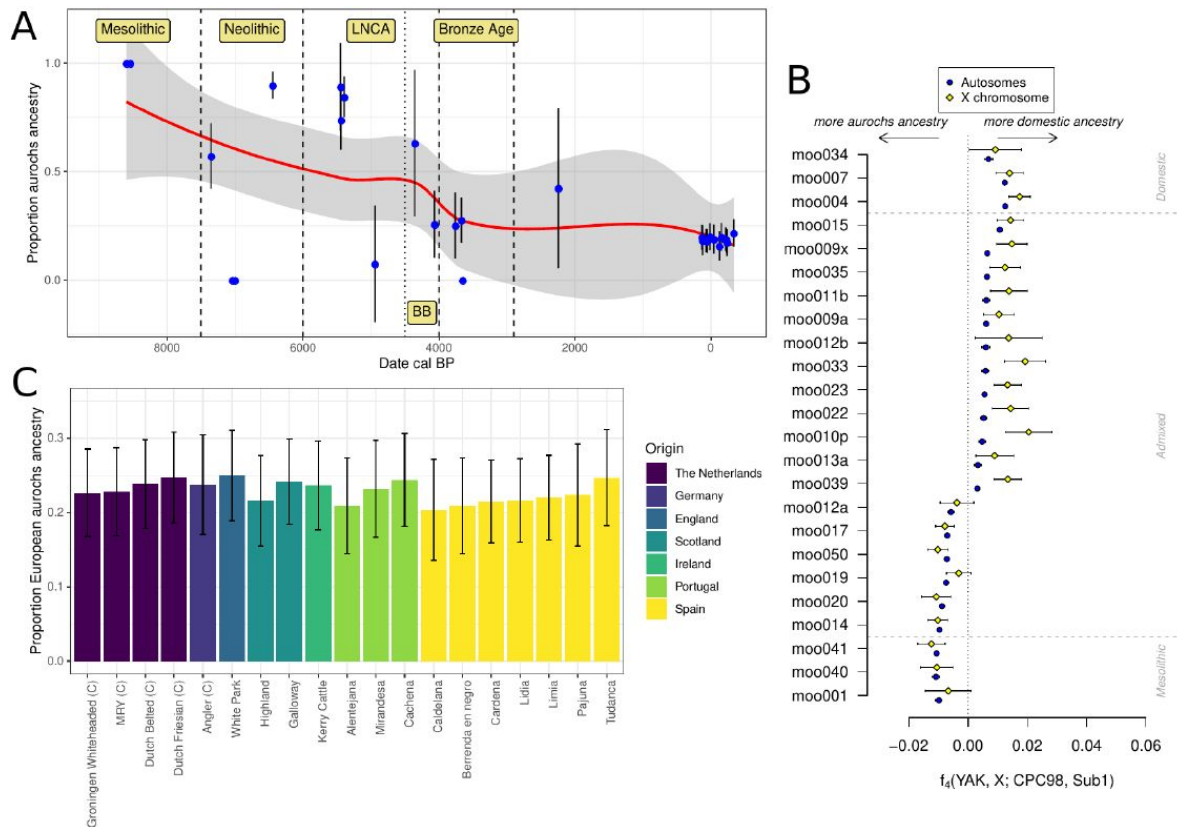


Figure 3.

(A) Estimates of aurochs ancestry (estimated using the 2 source model, if working and except for Mesolithic individuals) in directly dated Iberian samples over time. Error bars indicate one standard error for the 2 source models. Modern Iberian breeds with working 2 source models are added around date 0 with some vertical jitter. Approximate boundaries for the main sampling periods are indicated by dashed vertical lines. (B) Comparison of allele sharing of Iberian samples with aurochs and Anatolian domestic on the autosomes versus X chromosomes. Individuals were separated into three separate groups: Mesolithic samples, samples with admixed ancestry (sorted by autosomal f_4 estimate), and individuals for which only the single source, domestic model fit the data. (C) qpAdm estimates of Aurochs ancestry in modern western European cattle breeds from the (26) dataset. Commercial breeds are marked with a "C". The figure is only showing breeds with feasible and non-rejected two source models, all results are shown in Supplementary Information, Table S2. Error bars are showing block-jackknife estimates of the 95% confidence interval.

+ British aurochs, likely representing low levels of contributions from other groups, e.g. indicine cattle (26). For instance, the two-source model was rejected for four of the ten commercial breeds in the dataset.

Cattle have played an important role in Iberian culture during the last centuries as they have been part of numerous traditional popular events including bullfighting. The Lidia breed, a heterogeneous group of Iberian cattle that is mainly bred for aggressive behavior, has commonly been used for such popular festivities (30). Even though Lidia cattle has only been actively bred for agonistic behavior for about 200 years, some people attribute their aggressiveness and appearance as an indication of high levels of aurochs ancestry (31). We additionally use medium coverage genomes of six Lidia individuals (39) to estimate their proportion of aurochs ancestry. Lidia cattle in the (26) data set had a point estimate of 21.6% (95% confidence interval: [16.1, 27.1]) aurochs ancestry and estimates in the individual genomes ranged from 15.8% [8.3, 23.4] to 26.1% [18.8, 33.5] (Supplementary Information, Figure S5) – all overlapping with the observed range for other western European breeds. Despite some variation between individuals, which might be attributable to noise due to low coverage sequencing data in the reference populations, we do not observe a systematically elevated level of aurochs ancestry compared to other modern breeds or ancient samples since the Bronze Age. While these results reject the idea that the specifics of Lidia cattle can be attributed to a substantially increased genome-wide aurochs ancestry, it does not rule out the possibility that the roots of their aggressiveness and appearance are indeed due to aurochs variants at key loci responsible for those traits. An in-depth investigation of such questions would require a larger dataset of aurochs genomes as well as a more comprehensive Lidia sampling due to their fragmentation in highly distant genetic lineages (30).

Stable Isotope Analysis

In addition to their ancestry, we studied the ecology of the bovids through stable isotope analysis of bone collagen. Lynch et al. (40) suggested that stable isotope data could be used to infer niche separation between the species in Britain, with domestic cattle in more open settings, while aurochs (about 1‰ more depleted in $\delta^{13}\text{C}$) were habitually in more forested areas, or wet ground. Most likely facilitated through human management of the domestic cattle, separating them from their wild counterparts. In contrast, Noe-Nygaard et al. (41) failed to observe such an effect in samples from Denmark and northern Germany.

Considering our dataset (using genetically defined categories; *B. primigenius* and predominantly *B. primigenius* against *B. taurus* and predominantly *B. taurus* - to distinguish the two categories of *Bos*) and other data published on Iberian cattle (categorised on morphology/date) (42–51) the nitrogen isotope means are statistically different (see Dataset S1 and Supplementary Information). This is mostly due to some domestic cattle with $\delta^{15}\text{N}$ values greater than 6.5‰. This could be explained by some taurine cattle having exclusive habitual access to high nitrogen isotope ratio resources, for example, human management such as corralling on manured ground, or feeding with manured crops, would produce this effect. Nevertheless, there is generally a large amount of overlap in the isotope values for the two groups.

Discussion

We generated and analyzed biomolecular data from *B. primigenius* and *B. taurus* spanning more than 9000 years in the same region. Cattle are important livestock in the Iberian Peninsula today, and our results illustrate the interaction between domestic cattle and their wild relatives in the past. The two groups show signs of frequent hybridization starting soon after the arrival of cattle to the peninsula, as evident in our oldest directly dated Neolithic individual (moo039, 7426-7280 CalBP) where signals of carrying both ancestries are clear as qpadm only considers the admixture

model as fitting the data. Throughout the Neolithic, we observed large variations in the wild versus domestic ancestry per individual, but this pattern later stabilized (to 20-25% aurochs ancestry) from the Chalcolithic/Bronze Age onwards. This could reflect a transition from hunting and herding to predominantly herding and it is possible that systematic herd management led to the nearly constant levels of aurochs ancestry over the last 4000 years. This period also coincides with several other societal changes; including the Bell Beaker complex, and the introduction of human ancestry from the Pontic steppe and domestic horses into the Iberian Peninsula (9, 52–54). Around this time, humans also started processing a significantly higher amount of dairy products connected with the “secondary product revolution” (55, 56). Aurochs were probably present in Iberia until Roman times (28) leaving possibilities for interbreeding but we cannot exclude that various factors such as hunting or changing vegetation had led to a substantial decline in the wild aurochs population around the early Bronze Age. A previous study on cattle morphology from the site of El Portalón described a decrease in size from the Neolithic to the Chalcolithic and a further significant size decrease from the Chalcolithic to the Bronze Age (57) and associated this change in size to the aridification of the area at this time (58). Indeed, this climatic change could also be related to a reduction of the aurochs population contributing to the stabilization of the levels of ancestry in domestic cattle from the Bronze Age to the present. Nonetheless, our stable isotope results suggest that wild and domesticated groups often did not occupy substantially different niches on the Iberian Peninsula. Material excavated from Denmark suggested that aurochs changed their niches over time (41) demonstrating some flexibility depending on local vegetation and the possibility of aurochs adapting to changing environments.

The reduced level of aurochs ancestry on the X chromosome (compared to the autosomes) in admixed individuals suggests that it was mostly aurochs males who contributed wild ancestry to domestic herds. Consequently, the offspring of wild bulls and domestic cows could be born into and integrated within managed herds. It is unclear how much of this process was intentional but the possibility of a wild bull inseminating a domestic cow without becoming part of the herd suggests that some level of incidental interbreeding was possible. Modern breeders are still mostly exchanging bulls or sperm to improve their stock which manifests in a lower between-breed differentiation on the X chromosome (37).

The lack of correlation between genomic, stable isotope and morphological data highlights the difficulties of identifying and defining aurochs to the exclusion of domestic cattle. All of these data measure different aspects of an individual: their ancestry, ecology or appearance, respectively. While they can give some indication, none of them are a direct measurement of how these cattle were recognised by prehistoric humans, whether they were herded or hunted. It remains unclear whether our ancestry inferences had any correlation to how prehistoric herds were managed and how much intentional breeding is behind the observed pattern of hybridization. It is even possible that all hybrids identified in this study were part of domestic herds.

Even though wild aurochs populations went extinct, European aurochs ancestry survived into modern cattle with a relatively uniform distribution across western European breeds. Isolated Iberian Lidia, bred for their aggressiveness, appears to be no exception to this pattern. This rejects the notion that an overall increased proportion of aurochs ancestry causes the distinctiveness of certain breeds, but considering the functional relevance of archaic introgression into modern humans (59), it is possible that aurochs variants at functional loci may have a substantial influence on the characteristics of modern cattle breeds. Our low coverage sequencing data did not allow us to investigate this but future bioarchaeological studies combining different types of data will have the possibility to clarify the role of the extinct aurochs ancestry in modern domestic cattle.

Conclusions

Using a bioarchaeological approach we have demonstrated that since cattle arrived in Iberia there has been bidirectional introgression with the local aurochs population, but the wild to domestic direction is sex biased with mainly aurochs bulls contributing to domestic herds. Admixture proportions vary for the first few millennia but stabilize during the Early Bronze Age at approximately 20% of wild ancestry in domestic herds, a level that is still observed in modern Iberian breeds, including the more aggressive Lidia breed. This is likely to be the result of loose management of herds, becoming more controlled over time.

The amount of hybridisation observed in the ancient cattle makes it difficult to define what a domestic or wild *Bos* is, bringing into doubt the validity of such categorisations. Our interpretation is made more difficult by the overlap in morphological and metric data, creating further difficulties in species determination (especially in hybrids) and niche sharing as revealed by stable isotopes. To some extent, our interpretation is moot, as the salient matter is, how did prehistoric humans interact with cattle? What was their sense of wild and domestic and hybridisation? While we have recognised individual hybrids, to what extent these were part of domestic herds or intentionally bred and managed is uncertain.

Another source of uncertainty in our determinations is the limited knowledge about the genetic diversity in European aurochs, forcing us to use a British aurochs as a proxy for unadmixed Iberian aurochs. Further regional (and temporally longitudinal) aurochs genomes would aid future genomic studies defining the genetic variation in the European aurochs population.

Materials and Methods

Data generation

We attempted DNA extractions of 50 archaeological remains from which we successfully extracted DNA from 24 individuals identified as domestic cattle and aurochs excavated from four prehistoric sites in Iberia: El Portalón de Cueva Mayor (n=18), Artusia (n=1), Els Trocs (n=2) and Mendandia (n=3). Teeth and bones were UV irradiated (6 J/cm² at 254 nm) and the first millimeter of bone/tooth surface abraded using a Dremel™ tool. DNA was extracted in a dedicated ancient DNA facility using a silica-based DNA extraction protocol (60 [60](#)). For each sample, 100-200mg of bone or tooth powder were incubated for 24 h at 37°C, using the MinElute column Zymo extender assembly replaced by the High Pure Extender Assembly (Roche High Pure Viral Nucleic Acid Large Vol. Kit) and performed twice for each sample. DNA extracts were subjected to UDG treatment for the removal of deaminated cytosines and were further converted into blunt-end double stranded Illumina multiplex sequencing libraries (61 [61](#)). Between seven and fifteen qPCR cycles were performed to amplify the DNA libraries using indexed primers (61 [61](#)). These were subsequently pooled at equimolar concentrations and shotgun sequenced on Illumina HiSeq and Novaseq sequencing platforms.

Radiocarbon dates

Eight Bone and teeth were directly radiocarbon dated (AMS) at Waikato University in New Zealand and two teeth at Beta Analytics in the United States. Radiocarbon dates were calibrated using the OXcal 4.4 program (62 [62](#)) and the IntCal20 calibration curve (63 [63](#)). Three samples from the site of Mendandia were conventionally radiocarbon dated at Groningen (Netherlands) radiocarbon laboratory and calibrated as above.

Stable Isotopes Analysis

Many of the samples analysed here were radiocarbon dated and stable isotope data (via IRMS) were generated in this process, to augment this data we also produced stable isotope data for some additional samples in this dataset, where they were available. The additional samples underwent bone collagen or tooth dentine collagen extraction at the Laboratorio de Evolución Humana (Universidad de Burgos) following the protocol of (64). In brief, this is a cold acid demineralization, followed by Milli Q water rinsing, gelatinization at pH3 (24 hrs at 70°C), Eze filtering and lyophilization. Collagen yields (as % mass of starting bone) were recorded. Stable isotope values ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) and %C, %N were measured in duplicate at the Universitat Autònoma de Barcelona, unless only one sample was successful in the analysis. Collagen samples (approx. 0.4 mg) were analysed using a Flash IRMS elemental analyser (EA) coupled to a Delta V Advantage isotope ratio mass spectrometer (IRMS), both from Thermo Scientific (Bremen, Germany) at the Institute of Environmental Science and Technology of the Universitat Autònoma de Barcelona (ICTA-UAB). International laboratory standard IAEA-600 was used, with measurements made relative to Vienna PeeDee Belemnite (V-PDB) for $\delta^{13}\text{C}$, and air N_2 (AIR) for $\delta^{15}\text{N}$. The average analytical error was $<0.2\text{‰}$ (1σ) as determined from the duplicate analyses of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. In house standards used was dog hair collected and homogenized for interlaboratory comparisons.

Data processing

HiSeq X10 reads have been trimmed and merged using AdapterRemoval (65) while adapters for NovaSeq 6000 reads have been trimmed with cutadapt (66) and merging was performed with FLASH (67) requiring a minimum overlap of 11bp. Single-end reads of at least 35bp length were then mapped to the cattle reference genomes UMD3.1 (68) and Btau5 (69) using bwa (70) with the non-default parameters: -l 16500, -n 0.01, and -o 2. Different sequencing runs per sample were merged with samtools (71) and consensus sequences were called for duplicate sequences with identical start and end coordinates (72). Finally, reads with more than 10% mismatches to the reference genome were removed. Biological sex was assigned to the samples mapped to the Btau_5 reference genome (as UMD3.1 does not contain a Y chromosome assembly) using the R_x method (73) modified for 29 autosomes.

For comparative purposes, we also processed published data from (5, 17, 18, 25) using the same bioinformatic pipeline. Furthermore, we downloaded sequence data for six Spanish Lidia cattle (39), a single modern water buffalo (*Bubalus bubalis*, Jaffrabadi-0845) (74) and a single zebu cattle individual (Sha_3b) (75) and processed them with our ancient DNA mapping pipeline. To obtain a pseudohaploid Yak (*Bos grunniens*) sequence, we followed the approach by (26) splitting the Yak reference genome (76) contigs into 100bp fragments and mapping them to the UMD3.1 reference genome.

Data Analysis

Mitochondrial consensus sequences were called using ANGSD (77) and the options-doFasta 2-doCounts 1-minQ 30-minMapQ 30. Mitochondrial haplogroups were then assigned to the whole mitogenome sequences using the Python script MitoToolPy (78).

For population genomic analysis, we used the panel of modern European breeds presented by (26) which were genotyped at ~770,000 SNPs. Prior to genotype calling, all ancient BAM files were modified such that Ts in the first 5 bases of each fragment and As at the last 5 base pairs of each fragment have a base quality of 2. To generate pseudohaploid representations of each individual, we randomly draw a single read with mapping and base quality of at least 30 at each SNP position. If the allele carried by the ancient individual was not one of the two known alleles, we removed the site from the panel.

To conduct an ordination of the nuclear data, sequences of 43 ancient Eurasian cattle and two aurochs were obtained from (25) and (5). Outgroup f_3 statistics were calculated for all pairs of our Iberian *Bos* samples, using a Yak (*Bos grunniens*) genome as an outgroup, and a distance matrix for all samples was calculated as $1-f_3$. All f -statistics were calculated in R version 4.1.2 (79) package ‘admixtools2’ (80). The distance matrix was used to compute scores for non-metric multi-dimensional scaling (NMDS) ordinations using the metaMDS function in the ‘vegan’ R package and 10000 random starts (81).

We used admixtools2 (80) and qpAdm (34, 35) to model the ancestry proportions in the samples. CPC98 was used as a source for European aurochs ancestry while the domestic Anatolian Neolithic Sub1 was used as a source for domesticated cattle ancestry. As “right” populations, we used Bes2, Gyu2, *B. indicus*, Yak and *Bison bonasus bonasus* PLANTA. qpAdm was run with auto_only=FALSE and allsnps=TRUE. The same setup was used for modern western European breeds from (26) excluding breeds from Italy and the Balkan as non-aurine ancestry (26) in them would lead to a rejection of the models. We also used admixtools2 to reconstruct admixture graphs with the find_graphs() function. We first generated an f_2 matrix from the genotype data with maxmiss=0.15 and auto_only=FALSE and then ran find_graphs with stop_gen = 10000, stop_gen2 = 30 and plusminus_generations = 10 as suggested in (80). For between 0 and 5 migration events, we started the graph search with 1000 different seeds to select the model with the best likelihood for each number of migrations. Graph fits for different numbers of migrations were then compared using qpgraph_resample_multi() and compare_fits() using 100 bootstraps.

To obtain unbiased genotype calls for autosomes and the X chromosome for the analysis of sex bias, we called SNPs across all ancient and modern BAM files using ANGSD (77) and the parameters -GL 1 -doHaploCall 1 -doMajorMinor 1 -SNP_pval 1e-7 -doMaf 1 -minMaf 0.05 -minQ 30 -minMapQ 30 -remove_bads 1 -uniqueOnly 1 -rmTrans 1 -doCounts 1 -doGeno -4 -doPost 2 -doPlink 2 -minInd 3. f_4 statistics were then calculated using admixtools2 (80).

Acknowledgements

We are incredibly grateful to the excavation teams at the four archaeological sites. Sequencing was performed at The National Genomics Infrastructure (NGI) Stockholm. The computations and data handling were enabled by resources provided by the National Academic Infrastructure for Supercomputing in Sweden (NAISS) and the Swedish National Infrastructure for Computing (SNIC) at Uppmax, partially funded by the Swedish Research Council through grant agreements no. 2022-06725 and no. 2018-05973. This project was supported by grants from the Royal Physiographic Society of Lund (Nilsson-Ehle Endowments) to T.G. and C.V., Vetenskapsrådet (2017-05267) to T.G., Ramón y Cajal (RYC2018-025223-I) to C.V. and a Beatriz Galindo Fellowship (BGS220-461AA-69201) to C.S.

References

1. Frantz L. A. F., et al. (2019) **Ancient pigs reveal a near-complete genomic turnover following their introduction to Europe** *PNAS* **116**:17231–17238
2. Frantz L. A. F., Bradley D. G., Larson G., Orlando L. (2020) **Animal domestication in the era of ancient genomics** *Nat Rev Genet* :1–12
3. Botigué L. R., et al. (2017) **Ancient European dog genomes reveal continuity since the Early Neolithic** *Nat Commun* **8**
4. Irving-Pease E., et al., Lindqvist C., Rajora O. P. (2018) **Paleogenomics of Animal Domestication** in *Paleogenomics* https://doi.org/10.1007/13836_2018_55
5. Verdugo M. P., et al. (2019) **Ancient cattle genomics, origins, and rapid turnover in the Fertile Crescent** *Science* **365**:173–176
6. Daly K. G., et al. (2018) **Ancient goat genomes reveal mosaic domestication in the Fertile Crescent** *Science* **361**:85–88
7. Daly K. G., et al. (2021) **Herded and hunted goat genomes from the dawn of domestication in the Zagros Mountains** *Proceedings of the National Academy of Sciences* **118**
8. Fages A., et al. (2019) **Tracking Five Millennia of Horse Management with Extensive Ancient Genome Time Series** *Cell* **177**:1419–1435
9. Librado P., et al. (2021) **The origins and spread of domestic horses from the Western Eurasian steppes** *Nature* **598**:634–640
10. Yurtman E., et al. (2021) **Archaeogenetic analysis of Neolithic sheep from Anatolia suggests a complex demographic history since domestication** *Commun Biol* **4**:1–11
11. Bergström A., et al. (2020) **Origins and genetic legacy of prehistoric dogs** *Science* **370**:557–564
12. Larsson M. N., et al. (2023) **Ancient Sheep Genomes reveal four Millennia of North European Short-Tailed Sheep in the Baltic Sea region** *bioRxiv*
13. Ginja C., et al. (2023) **Iron age genomic data from Althiburos – Tunisia renew the debate on the origins of African taurine cattle** *iScience* **26**
14. Peters J., Helmer D., Von den Driesch A., Segui M. Sana (1999) **Early Animal Husbandry in the Northern Levant** *Paléorient* **25**:27–48
15. Helmer D., Gourichon L., Monchot H., Peters J., Segui M., Vigne J.-D., Peters J., Helmer D. (2005) **Identifying early domestic cattle from Pre-Pottery Neolithic sites on the Middle Euphrates using sexual dimorphism** in *The First Steps of Animal Domestication: New Archaeozoological Approaches* :86–96
16. Zhang K., Lenstra J. A., Zhang S., Liu W., Liu J. (2020) **Oxbow Books Evolution and domestication of the Bovini species** *Animal Genetics* **51**:637–657

17. Palacio P., et al. (2017) **Genome data on the extinct *Bison schoetensacki* establish it as a sister species of the extant European bison (*Bison bonasus*)** *BMC Evol Biol* **17**
18. Węcek K., et al. (2017) **Complex Admixture Preceded and Followed the Extinction of Wisent in the Wild** *Molecular Biology and Evolution* **34**:598–612
19. Ward J. A., et al. (2022) **Genome-wide local ancestry and direct evidence for mitonuclear coadaptation in African hybrid cattle populations (*Bos taurus/indicus*)**
20. Beja-Pereira A., et al. (2006) **The origin of European cattle: Evidence from modern and ancient DNA** *Proceedings of the National Academy of Sciences* **103**:8113–8118
21. Achilli A., et al. (2008) **Mitochondrial genomes of extinct aurochs survive in domestic cattle** *Current Biology* **18**:R157–R158
22. Schibler J., Elsner J., Schlumbaum A. (2014) **Oxbow Books Incorporation of aurochs into a cattle herd in Neolithic Europe: single event or breeding?** *Sci Rep* **4**
23. Cubric-Curik V., et al. (2022) **Large-scale mitogenome sequencing reveals consecutive expansions of domestic taurine cattle and supports sporadic aurochs introgression** *Evolutionary Applications* **15**:663–678
24. Bro-Jørgensen M. H., et al. (2018) **Ancient DNA analysis of Scandinavian medieval drinking horns and the horn of the last aurochs bull** *Journal of Archaeological Science* **99**:47–54
25. Park S. D., et al. (2015) **Genome sequencing of the extinct Eurasian wild aurochs, *Bos primigenius*, illuminates the phylogeography and evolution of cattle** *Genome biology* **16**
26. Upadhyay M., et al. (2016) **Genetic origin, admixture and population history of aurochs (*Bos primigenius*) and primitive European cattle** *Heredity*
27. Anderung C., et al. (2005) **Prehistoric contacts over the Straits of Gibraltar indicated by genetic analysis of Iberian Bronze Age cattle** *Proceedings of the National Academy of Sciences of the United States of America* **102**:8431–8435
28. Altuna J., Mariezkurrena K. (2002) **El problema de la domesticación de bovinos en el País Vasco y resto de la Región Cantábrica** *Congresos de Estudios Vascos* :123–128
29. Cubas M., et al. (2016) **Re-evaluating the Neolithic: The Impact and the Consolidation of Farming Practices in the Cantabrian Region (Northern Spain)** *J World Prehist* **29**:79–116
30. Cañón J., et al. (2008) **Genetic variation within the Lidia bovine breed** *Animal Genetics* **39**:439–445
31. Balcarcel A. M., Veitschegger K., Clauss M., Sánchez-Villagra M. R. (2021) **Intensive human contact correlates with smaller brains: differential brain size reduction in cattle types** *Proceedings of the Royal Society B: Biological Sciences* **288**
32. Sinding M.-H. S., Gilbert M. T. P. (2016) **The Draft Genome of Extinct European Aurochs and its Implications for De-Extinction** *Open Quaternary* **2**
33. Colominas L., et al. (2015) **Detecting the T1 cattle haplogroup in the Iberian Peninsula from Neolithic to medieval times: new clues to continuous cattle migration through time** *Journal of Archaeological Science* **59**:110–117

34. Haak W., et al. (2015) **Massive migration from the steppe was a source for Indo-European languages in Europe** *Nature* <https://doi.org/10.1038/nature14317>
35. Harney E., Patterson N., Reich D., Wakeley J. (2021) **Assessing the performance of qpAdm: a statistical tool for studying population admixture** *Genetics* **217**
36. Keinan A., Mullikin J. C., Patterson N., Reich D. (2009) **Accelerated genetic drift on chromosome X during the human dispersal out of Africa** *Nat Genet* **41**:66–70
37. da Fonseca R. R., et al. (2019) **Consequences of breed formation on patterns of genomic diversity and differentiation: the case of highly diverse peripheral Iberian cattle** *BMC Genomics* **20**
38. Rogers A. R., Bohlender R. J. (2015) **Bias in estimators of archaic admixture** *Theoretical population biology* **100**:63–78
39. Eusebi P. G., et al. (2022) **A novel missense variant in endothelin-2 (EDN2) causes a growth and respiratory lethal syndrome in bovine** *Animal Genetics* **53**:583–591
40. Lynch A. H., Hamilton J., Hedges R. E. M. (2008) **Where the wild things are: aurochs and cattle in England** *Antiquity* **82**:1025–1039
41. Noe-Nygaard N., Price T. D., Hede S. U. (2005) **Diet of aurochs and early cattle in southern Scandinavia: evidence from 15N and 13C stable isotopes** *Journal of Archaeological Science* **32**:855–871
42. Fernández-Crespo T., Schulting R. J. (2017) **Living different lives: Early social differentiation identified through linking mortuary and isotopic variability in Late Neolithic/ Early Chalcolithic north-central Spain** *PLOS ONE* **12**
43. Fontanals-Coll M., Subirà M. E., Bonilla M. D.-Z., Duboscq S., Gibaja J. F. (2015) **Investigating palaeodietary and social differences between two differentiated sectors of a Neolithic community, La Bòbila Madurell-Can Gambús (north-east Iberian Peninsula)** *Journal of Archaeological Science: Reports* **3**:160–170
44. GarciaGuix E., Richards Michael P., Subir M. E. (2006) **Palaeodiets of Humans and Fauna at the Spanish Mesolithic Site of El Collado** *Current Anthropology* **47**:549–556
45. Jones J. R., et al. (2019) **Investigating prehistoric diet and lifeways of early farmers in central northern Spain (3000–1500 CAL BC) using stable isotope techniques** *Archaeol Anthropol Sci* **11**:3979–3994
46. Navarrete V., et al. (2017) **Feeding Management Strategies among the Early Neolithic Pigs in the NE of the Iberian Peninsula** *International Journal of Osteoarchaeology* **27**:839–852
47. Salazar-García D. C. (2009) **Estudio de la dieta en la población neolítica de Costamar: Resultados preliminares de análisis de isótopos estables de Carbono y Nitrógeno** *Torre la Sal (Ribera de Cabanes, Castellón): evolución del paisaje antrópico desde la Prehistoria hasta el Medioevo* :411–418
48. Salazar-García D. (2011) **Aproximación a la dieta de la población calcolítica de La Vital a través del análisis de isótopos estables del carbono y del nitrógeno sobre restos óseos** *La Vital (Gandia, Valencia). Vida y Muerte En La Desembocadura Del Serpis Durante El III y El I Milenio a.C* :139–143

49. Salazar-García D. C., et al. (2014) **Isotope evidence for the use of marine resources in the Eastern Iberian Mesolithic** *Journal of Archaeological Science* **42**:231–240
50. Sarasketa-Gartzia I., Villalba-Mouco V., le Roux P., Arrizabalaga Á., Salazar-García D. C. (2018) **Late Neolithic-Chalcolithic socio-economical dynamics in Northern Iberia. A multi-isotope study on diet and provenance from Santimamiñe and Pico Ramos archaeological sites (Basque Country, Spain)** *Quaternary International* **481**:14–27
51. Villalba-Mouco V., et al. (2018) **Reconstruction of human subsistence and husbandry strategies from the Iberian Early Neolithic: A stable isotope approach** *American Journal of Physical Anthropology* **167**:257–271
52. Olalde I., et al. (2018) **The Beaker phenomenon and the genomic transformation of northwest Europe** *Nature* **555**:190–196
53. Valdiosera C., et al. (2018) **Four millennia of Iberian biomolecular prehistory illustrate the impact of prehistoric migrations at the far end of Eurasia** *PNAS*
54. Olalde I., et al. (2019) **The genomic history of the Iberian Peninsula over the past 8000 years** *Science* **363**:1230–1234
55. Francés-Negro M., et al. (2021) **Neolithic to Bronze Age economy and animal management revealed using analyses lipid residues of pottery vessels and faunal remains at El Portalón de Cueva Mayor (Sierra de Atapuerca, Spain)** *Journal of Archaeological Science* **131**
56. Evershed R. P., et al. (2022) **Dairying, diseases and the evolution of lactase persistence in Europe** *Nature* **608**:336–345
57. Galindo-Pellicena M. A., et al. (2020) **Metrical analysis of bovine bone remains from the Neolithic to the Bronze Age at the El Portalón site (Atapuerca, Burgos) in the Iberian context** *Quaternary International* **566**:211–223
58. Martínez-Pillado V., et al. (2014) **Upper Pleistocene and Holocene palaeoenvironmental records in Cueva Mayor karst (Atapuerca, Spain) from different proxies: speleothem crystal fabrics, palynology and archaeology** *International Journal of Speleology* **43**
59. Reilly P. F., Tjahjadi A., Miller S. L., Akey J. M., Tucci S. (2022) **The contribution of Neanderthal introgression to modern human traits** *Current Biology* **32**:R970–R983
60. Dabney J., et al. (2013) **Complete mitochondrial genome sequence of a Middle Pleistocene cave bear reconstructed from ultrashort DNA fragments** *Proc. Natl. Acad. Sci. U.S.A* **110**:15758–15763
61. Meyer M., Kircher M. (2010) **Illumina Sequencing Library Preparation for Highly Multiplexed Target Capture and Sequencing** *Cold Spring Harbor Protocols* **2010**
62. Ramsey C. B. (2009) **Bayesian analysis of radiocarbon dates** *Radiocarbon* **51**:337–360
63. Reimer P. J., et al. (2020) **The IntCal20 Northern Hemisphere radiocarbon age calibration curve (0–55 cal kBP)** *Radiocarbon* **62**:725–757
64. Richards M. P., Hedges R. E. M. (1999) **Stable Isotope Evidence for Similarities in the Types of Marine Foods Used by Late Mesolithic Humans at Sites Along the Atlantic Coast of Europe** *Journal of Archaeological Science* **26**:717–722

65. Schubert M., Lindgreen S., Orlando L. (2016) **AdapterRemoval v2: rapid adapter trimming, identification, and read merging** *BMC Research Notes* **9**
66. Martin M. (2011) **Cutadapt removes adapter sequences from high-throughput sequencing reads** *EMBnet.journal* **17**:10–12
67. Magoč T., Salzberg S. L. (2011) **FLASH: fast length adjustment of short reads to improve genome assemblies** *Bioinformatics* **27**:2957–2963
68. Zimin A. V., et al. (2009) **A whole-genome assembly of the domestic cow, *Bos taurus*** *Genome Biology* **10**
69. (2022) , **Btau_5.0.1 -Genome -Assembly -NCBI** (June 23, 2022).
70. Li H., Durbin R. (2009) **Fast and accurate short read alignment with Burrows-Wheeler transform** *Bioinformatics* **25**:1754–1760
71. Li H., et al. (2009) **The Sequence Alignment/Map format and SAMtools** *Bioinformatics* **25**:2078–2079
72. Kircher M. (2012) **Analysis of high-throughput ancient DNA sequencing data** in *Methods in Molecular Biology* (Clifton, N.J) :197–228
73. Mitnik A., Wang C.-C., Svoboda J., Krause J. (2016) **A Molecular Approach to the Sexing of the Triple Burial at the Upper Paleolithic Site of Dolní Věstonice** *PLOS ONE* **11**
74. Dutta P., et al. (2020) **Whole genome analysis of water buffalo and global cattle breeds highlights convergent signatures of domestication** *Nat Commun* **11**
75. Chen N., et al. (2018) **Whole-genome resequencing reveals world-wide ancestry and adaptive introgression events of domesticated cattle in East Asia** *Nat Commun* **9**
76. Hu Q., et al. (2012) **The Yak genome database: an integrative database for studying yak biology and high-altitude adaption** *BMC Genomics* **13**
77. Korneliussen T. S., Albrechtsen A., Nielsen R. (2014) **ANGSD: Analysis of Next Generation Sequencing Data** *BMC Bioinformatics* **15**
78. Peng M.-S., et al. (2015) **DomeTree: a canonical toolkit for mitochondrial DNA analyses in domesticated animals** *Molecular Ecology Resources* **15**:1238–1242
79. R Core Team (2016) **R: A Language and Environment for Statistical Computing** (*R Foundation for Statistical Computing*)
80. Maier R., et al. (2023) **On the limits of fitting complex models of population history to f-statistics** *eLife* **12**
81. Oksanen J., et al. (2020) **vegan: Community Ecology Package**

Article and author information

Torsten Günther

Human Evolution, Department of Organismal Biology Uppsala University, Sweden

For correspondence: torsten.gunther@ebc.uu.se

ORCID iD: [0000-0001-9460-390X](https://orcid.org/0000-0001-9460-390X)

Jacob Chisauksy

Human Evolution, Department of Organismal Biology Uppsala University, Sweden

M. Ángeles Galindo-Pellicena

Centro Mixto UCM-ISCIH de Evolución y Comportamiento Humanos, Spain

Eneko Iriarte

Laboratorio de Evolución Humana, Universidad de Burgos, Spain

ORCID iD: [0000-0001-8365-5616](https://orcid.org/0000-0001-8365-5616)

Oscar Cortes Gardyn

Universidad Complutense Madrid, Spain

ORCID iD: [0000-0001-7685-3980](https://orcid.org/0000-0001-7685-3980)

Paulina G. Eusebi

Universidad Complutense Madrid, Spain

ORCID iD: [0000-0002-0682-038X](https://orcid.org/0000-0002-0682-038X)

Rebeca García-González

Laboratorio de Evolución Humana, Universidad de Burgos, Spain

Irene Urena

Centre for Palaeogenetics, Stockholm, Sweden

Marta Moreno

Instituto de Historia – CSIC, Spain

Alfonso Alday

Área de Prehistoria, University of the Basque Country, Spain

ORCID iD: [0000-0002-0024-3462](https://orcid.org/0000-0002-0024-3462)

Manuel Rojo

Department of Prehistory and Archaeology, Valladolid University, Spain

ORCID iD: [0000-0002-9317-8654](https://orcid.org/0000-0002-9317-8654)

Amalia Pérez

Laboratorio de Evolución Humana, Universidad de Burgos, Spain

Cristina Tejedor Rodríguez

Department of Prehistory and Archaeology, Valladolid University, Spain

Iñigo García Martínez de Lagrán

Departamento de Prehistoria y Arqueología, UNED, Spain

Juan Luis Arsuaga

Universidad Complutense Madrid, Spain
ORCID iD: [0000-0001-5361-2295](https://orcid.org/0000-0001-5361-2295)

José-Miguel Carretero

Laboratorio de Evolución Humana, Universidad de Burgos, Spain, Unidad Asociada de I+D+i al CSIC Vidrio y Materiales del Patrimonio Cultural (VIMPAC)

Anders Götherström

Centre for Palaeogenetics, Stockholm, Sweden

Colin Smith

Laboratorio de Evolución Humana, Universidad de Burgos, Spain, Dept. of Archaeology and History, La Trobe University, Australia
For correspondence: cismith@ubu.es
ORCID iD: [0000-0001-5901-8780](https://orcid.org/0000-0001-5901-8780)

Cristina Valdiosera

Laboratorio de Evolución Humana, Universidad de Burgos, Spain, Dept. of Archaeology and History, La Trobe University, Australia
For correspondence: cevaldiosera@ubu.es
ORCID iD: [0000-0003-4948-2226](https://orcid.org/0000-0003-4948-2226)

Copyright

© 2024, Günther et al.

This article is distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use and redistribution provided that the original author and source are credited.

Editors

Reviewing Editor

Ziyue Gao

University of Pennsylvania, Philadelphia, United States of America

Senior Editor

George Perry

Pennsylvania State University, University Park, United States of America

Reviewer #1 (Public Review):

My main concern is the use of the 700K SNP dataset. This set of SNPs suffers from a heavy ascertainment bias, which can be seen in the PCA in the supplementary material where all the aurochs cluster in the center within the variation of cattle. Given the coverage of some of the samples, multiple individuals would have less than 10K SNP covered. The majority of these are unlikely to be informative here given that they would just represent fixed positions between taurine and indicine or SNPs mostly variable in milk cattle breeds. The authors would get a much better resolution (i.e. many more SNPs to work with their very low genome coverage data) using the 1000 bull genome project VCF data set: <https://www.ebi.ac.uk/ena/browser/view/PRJEB42783> which based on whole genome resequencing data from many cattle. This will certainly help with improving the resolution of qpAdm and f4 analysis, which

have huge confidence intervals in most cases. Right now some individuals have huge confidence intervals ranging from 0 to 80% auroch ancestry...

I agree with the authors that qpAdm is likely to give quite a noisy estimate of ancestry here (likely explain part of the issue I mentioned above). Although qpAdm is good for model testing here for ancestry proportion the authors instead could use an explicit f4 ratio - this would allow them to specify a model which would make the result easier to interpret.

The interpretation of the different levels of allele sharing on X vs autosome being the result of sex-bias admixture is not very convincing. Could these differences simply be due to a low recombination rate on the X chromosome and/or lower effective population size, which would lead to less efficient purifying selection?

The authors suggest that 2 pop model rejection in some domestic population might be due to indicine ancestry, this seems relatively straightforward to test.

The first sentence of the paper is a bit long-winded, also dogs were domesticated before the emergence of farming societies.

It would be good to be specific about the number of genomes and coverage info in the last paragraph of the intro.

- <https://doi.org/10.7554/eLife.93076.1.sa2>

Reviewer #2 (Public Review):

Summary:

In this paper, the authors investigated the admixture history of domestic cattle since they were introduced into Iberia, by studying genomic data from 24 ancient samples dated to ~2000-8000 years ago and comparing them to modern breeds. They aimed to (1) test for introgression from (local) wild aurochs into domestic cattle; (2) characterize the pattern of admixture (frequency, extent, sex bias, directionality) over time; (3) test for correlation between genetic ancestry and stable isotope levels (which are indicative of ecological niche); and (4) test for the hypothesized higher aurochs ancestry in a modern breed of fighting bulls.

Strengths:

Overall, this study collects valuable new data that are useful for testing interesting hypotheses, such as admixture between domestic and wild populations, and correlation between genome-wide aurochs ancestry and aggressiveness.

Weaknesses:

Most conclusions are partially supported by the data presented. The presence of admixed individuals in prehistorical periods supports the hypothesized introgression, although this conclusion needs to be strengthened with an analysis of potential contamination. The frequency, sex-bias, and directionality of admixture remain highly uncertain due to limitations of the data or issues with the analysis. There is considerable overlap in stable isotope values between domestic and wild groups, indicating a shared ecological niche, but variation in classification criteria for domestic vs wild groups and in skeletal elements sampled for measurements significantly weakens this claim. Lastly, the authors presented convincing evidence for relatively constant aurochs ancestry across all modern breeds, including the Lidia breed which has been bred for aggressiveness for centuries. My specific concerns are outlined below.

Contamination is a common concern for all ancient DNA studies. Contamination by modern samples is perhaps unlikely for this specific study of ancient cattle, but there is still the possibility of cross-sample contamination. The authors should estimate and report

contamination estimates for each sample (based on coverage of autosomes and sex chromosomes, or heterozygosity of Y or MT DNA). Such contamination estimates are particularly important to support the presence of individuals with admixed ancestry, as a domestic sample contaminated with a wild sample (or vice versa) could appear as an admixed individual.

A major limitation of this study is uncertainty in the "population identity" for most sampled individuals (i.e., whether an individual belonged to the domesticated or wild herd when they were alive). Based on chronology, morphology, and genetic data, it is clear the Mesolithic samples from the Artusia and Mendandia sites are bona fide aurochs, but the identities of individuals from the other two sites are much less certain. Indeed, archeological and morphological evidence from El Portalon supports the presence of both domestic animals and wild aurochs, which is echoed by the inter-individual heterogeneity in genetic ancestry. Based on results shown in Fig 1C and Fig 2 it seems that individuals moo017, moo020, and possibly moo012a are likely wild aurochs that had been hunted and brought back to the site by humans. Although the presence of individuals (e.g., moo050, moo019) that can only be explained by two-source models strongly supports that interbreeding happened (if cross-contamination is ruled out), it is unclear whether these admixed individuals were raised in the domestic population or lived in the wild population and hunted.

Such uncertainty in "population identity" limits the authors' ability to make conclusions regarding the frequency, sex bias, and directionality of gene flow between domestic and wild populations. For instance, the wide range of ancestry estimates in Neolithic and Chalcolithic samples could be interpreted as evidence of (1) frequent recent gene flow or (2) mixed practices of herding and hunting and less frequent gene flow. Similarly, the statement about "bidirection introgression" (on pages 8 and 11) is not directly supported by data. As the genomic, morphological, and isotope data cannot confidently classify an individual as belonging to the domesticated or wild population, it seems impossible to conclude the direction of gene flow (if by "bidirection introgression" the authors mean something other than "bidirectional gene flow", they need to clearly explain this before reaching the conclusion.)

The f_4 statistics shown in Fig 3B are insufficient to support the claim regarding sex-biased hybridization, as the f_4 statistic values are not directly comparable between the X chromosome and autosomes. Because the effective population size is different for the X chromosome and autosomes (roughly 3:4 for populations with equal numbers of males and females), the expected amount of drift is different, hence the fraction of allele sharing (f_4) is expected to be different. In fact, the observation that moo004 whose autosomal genome can be modeled as 100% domestic ancestry still shows a higher f_4 value for the X chromosome than autosomes hints at this issue. A more robust metric to test for sex-biased admixture is the admixture proportion itself, which can be estimated by qpAdm or f_4 -ratio (see Patterson et al 2012). However, even with this method, criticism has been raised (e.g., Lazaridis and Reich 2017; Pfennig and Lachance, 2023). In general, detecting sex-bias admixture is a tough problem.

In general, the stable isotope analysis seems to be very underpowered, due to the issues of variation in classification criteria and skeletal sampling location discussed by the authors in supplementary material. The authors claimed a significant difference in stable nitrogen isotope between (inconsistently defined) domestic cattle and wild aurochs, but no figures or statistics are presented to support this claim. Please describe the statistical method used and the corresponding p-values. The authors can consider including a figure to better show the stable isotope results.

- <https://doi.org/10.7554/eLife.93076.1.sa1>

Reviewer #3 (Public Review):**Summary:**

Günther and colleagues leverage ancient DNA data to track the genomic history of one of the most important farm animals (cattle) in Iberia, a region showing peculiarities both in terms of cultural practices as well as a climatic refugium during the LGM, the latter of which could have allowed the survival of endemic lineages. They document interesting trends of hybridisation with wild aurochs over the last 8-9 millennia, including a stabilisation of auroch ancestry ~4000 years ago, at ~20%, a time coincidental with the arrival of domestic horses from the Pontic steppe. Modern breeds such as the iconic Lidia used in bullfighting or bull running retain a comparable level of auroch ancestry.

Strengths:

The generation of ancient DNA data has been proven crucial to unravel the domestication history of traditional livestock, and this is challenging due to the environmental conditions of the Iberian peninsula, less favourable to DNA preservation. The authors leverage samples unearthed from key archaeological sites in Spain, including the karstic system of Atapuerca. Their results provide fresher insights into past management practices, and permit characterisation of significant shifts in hybridization with wild aurochs.

Weaknesses:

- Treatment of post-mortem damage: the base quality of nucleotide transitions was recalibrated down to a quality score of 2, but for 5bp from the read termini only. In some specimens (e.g. moo022), the damage seems to extend further. Why not use dedicated tools (e.g. mapDamage), or check the robustness by conditioning on nucleotide transversions?
- Their more solid analyses are based on qpAdm, but rely on two single-sample donor populations. As the authors openly discuss, it is unclear whether CPC98 is a good proxy for Iberian aurochs despite possibly forming a monophyletic clade (the number of analysed sites is simply too low to assess this monophyly; Supplementary Table S2). Additionally, it is also unclear whether Sub1 was a fully unadmixed domestic specimen, depleted of auroch ancestry. The authors seem to suggest themselves that sex-biased introgression may have already taken place in Anatolia ("suggesting that sex-biased processes already took place prior to the arrival of cattle to Iberia").

Alternatively, I recommend using Struct-f4 as it can model the ancestry of all individuals together based on their f4 permutations, including outgroups and modern data, and without the need to define pure "right" and "left" populations such as CPC98 and Sub1. It should work with low-coverage data, and allows us to do f4-based MDS plots as well as to estimate ancestry proportions (including from ghost populations).

- In the admixture graph analyses (supplementary results), the authors use population groups based on a single sample. If these samples are pseudohaploidised (or if coverage is insufficient to estimate heterozygosity - and it is at least for moo004 and moo014), f3 values are biased, implying that the fitted graph may be wrong. The graph shown in Fig S7 is in fact hard to interpret. For example, the auroch Gyu2 from Anatolia but not the auroch CPC98 also from Anatolia received 62% of ancestry from North Africa? The Neolithic samples moo004 and moo014 also show the same shocking disparity. I would consider re-doing this analysis with more than a sample per population group.

- <https://doi.org/10.7554/eLife.93076.1.sa0>