Peer Review File

Manuscript Title: The genomic natural history of the aurochs

Reviewer Comments & Author Rebuttals

Reviewer Reports on the Initial Version:

Referee #1 (Remarks to the Author):

In the manuscript “The genomic natural history of the aurochs”, the authors present an impressive genomic data set obtained from fossil specimens and use these data to try and reveal the evolutionary history of this species. This effort in itself is laudable as very often, the aurochs is merely seen as the ancestor of domestic cattle and not as a species with an evolutionary history in its own right. The data are analysed by an equally impressive number of analyses. Unfortunately, this is where the problems with this manuscript begin. The multiple analyses and aspects addressed never come together into a meaningful story. It is like the authors are lost among all the analyses performed and therefore have put together paragraphs that are at best loosely connected. This is made worse by the attempt to write ina colourful language that often disguises rather than illuminates what the authors actually want to say (assuming they really know what they actually want to say). These problems are exacerbated by a number of factual errors that normally should not be in a submitted manuscript. I have detailed many of these issues below, but I should point out that my comments are not exhaustive as providing a fully comprehensive review of all issues is beyond the time I can spend. As I realize this may sound discouraging, I would like to emphasize the great potential I see in the dataset itself as well as in the results of the analyses performed. What the authors need to do in my mind is to take a more unpretentious approach to both interpretation and writing as well as put aside their preconceptions about what they believe or hope to find and simply interprete the results that are there (nothing more) in a clear and concise way and language. If they can achive this task, this manuscript clearly has the potential to become a seminal work regarding aurochs evolution.

Abstract: “….that revealed four distinct ancestries of aurochs….” I think this is a bit of an unfortunate phrasing, as the aurochs of course as any species has a single ancestry. What the authors mean is populations.
Also in the abstract, the authros are switiching between different tenses (which continues in the main text). I think it would be good if they could stay concistent. Generally, I found the abstract lacking in clarity. For example, it is not quite clear what the first half of the last sentence actually really means and whether southwest Asia refers to the same or a different region as “Near Eastern”.
I also would like to note that in my opinion (this is of course a matter of personal taset), a less pompous language than used by the authors is more suitable for communicating scientific results. As an example, I would clearly rephrase the follwing sentence: “Its fossil presence in Europe stretches over 650 thousand years ago (kya)1, but its earliest likely ancestors are detected in the Indomalayan biogeographic realm2, the centre of diversity for the tribe Bovini to which all wild cattle belong.”
This is true for many other sentences as well. Another example comes from pages 6/7. “The trajectories of the ancestries embedded in these clusters are dynamic and are contingent upon both climatic and cultural transitions.” Whatever this is supposed to mean.

I am not sure why the authors refer to the last hunted bull in 1620, since the aurochs as a wild species went exticnt in 1627 with the last cow dying.
I have to admit that I find figure 1 surprisingly poorly executed regarding how easy (or rather difficult) it is to extract the relevant information from this figure. I also diagree with some of the clusters shown, especially the Iberian and Italian ones, as some samples added to the Iberian cluster are genetically closer to some Italien specimens than to other Iberian ones. In that context, I would like to see more evidence that the following paragraph is really supported by the evidence "Accordingly, genomes from these three regions separate into different groups. Interestingly, the bulk of sampled European (central European, Scandinavian and British) genomes cluster tightly with the Iberian group (Figure 1). This pattern suggests genetic isolation between refugia, postglacial recolonisation of western Europe derived from the Iberian refugium, and the Alps formed a barrier to gene flow from the Italian refugium." For example a separate MDS with only the western European samples, some structure-like analysis or a qp-graph analysis. Even a simple distance matrix would help. In figures 2 and 3 the authors in fact present some such analyses, which, however, do not support the claims made. As the manuscript currently presents the evidence, it is in my opinion thus simply inadequate to support the claim made in this paragraph.
In my opinion, the manuscript would benefit from some adjustments here. I am not sure if the inclusion of domestic cattle in the map helps or rather hinders understanding of what is shown. There is also a factual error in the description of these findings in the text, since the cluster at the bootom left in the MDS-analysis does not consist of "two Near Eastern aurochsen" but rather includes one aurochs from the Near East, one aurochs from the west of North Africa and one from the Caucasus.
Generally, I have the impression that the authors do not interpret their results fully impartially, as another questionable interpretation follows already on page 7: "This Asian distinctiveness does not persist after the Holocene, when central Asian genomes shift towards the centre of the plot….." Looking at figure 1, I would argue that while the Holocene Central Aisan aurochsen are less distinct from the other regions than their Pleistocene counterparts, they still are distinct, meaning the distinctness persists, albeit in a weaker form.
A note on figure 2: the shading denoting domestic haplotypes is especially for haplogroup R only visible for readers who already know that it is there. Also, the authors should probably mention that for this figure, they also used a fair numer of published sequences. Moreover, panel c is rather crowded an getting an meaningful overview which haplotypes are pre-Holocene and which are Holocene is not really possible. I suggest to make two separate panels, one from Holocene and the other for pre-Holocene. It also does not become clear whether the major domestic haplotypes were recovered from aurochsen specimens or just added to the figure.
The following sentence on page 10 is phrased rather unfortunate: "However, a much more recent coalescence of all sampled Bos is estimated by Bayesian analysis of C14-dated mtDNA (implemented in BEAST; Figure 3) and genome-wide coalescence with cross-population analysis using the observed bovine mutation rate (implemented using MSMC2; Extended Data Figure 1)." First "….all samples Bos….." reads rather clumsily and is also unclear whether all members of the genus Bos sampled in this study or sampled so far in scientific studies is meant. Second, the tree the authors are referring to is found as figure 2B, not 3B. And third, the second part of the sentence is true only if one accepts a cross-coalescence rate of 50%. Part of the cross-coalescence between Bos primigenius and Bos namadicus extends beyond 1 million years, as by then, the plot has not yet reached 100%. I also would like to note that in extended data figure 1, the authors use Bos namadicus as designation,

while in the main text, they correctly state that they used Bos indicus as proxy for Bos namadicus – one of clearly too many small mistakes in this manuscript. Also, the cross-coalescent analysis by itself conceptually cannot reveal replacement, since occurrence in the fossil record by 650 kya does not imply that the coalescent time of the descendant population has to go back that far. The authors need to provide additional information, why replacment of the ancient European population (by which other population, btw) is a better explanation of the observed data than any other explanation.

Unfortunately, the arguments do not get better in the next section. It starts already with the statement "The estimated last common ancestry of Bos primigenius….." without qualification, which common ancestry the authros refer to – mitochondrial DNA (which seems to be the case given what the authors write later in the text), range of nuclear loci (and then up to which coalescence rate?) or population ancestry. And it does not get better in the second half of the sentence: "…….falls within Marine Isotope Stage 5 (129-71 kya), which had warm periods with ice retreat before the onset of the last glaciation19,20." MIS5 did not have "warm periods", it actually includes with the Eemian (from 130 – 115 kya) the previous interglacial when temperatures were in many places warmer than today and sea levels several meters higher. The "last Ice Age" also doe not refer to the period from 70-18kya, but instead to the Quaternary glaciation that started 2.58 mya.

The next paragraph reveals another problem. The authors state: "It is likely that the reduced gene flow in northern Eurasia was caused by range reduction with the onset of the glacial period21." For a start, something can only be reduced RELATIVE to something else. So the use of "reduced gene flow" without comparison to a case of higher gene flow makes no logical sense. Second, it is not clear what the authors mean with glacial period – the last glacial period started 115 kya, with the end of the Eemian interglacial. However, since their oldest sample is about 50 kya, the cannot make any statement about gene flow at the onset of the last glacial period. Third, it is unclear what they mean with "reduced gene flow in northern Eurasia" as it is really unclear, which set of samples this refers to. And last, Figure 1 shows a wide distribution of a genetic cluster of Pleistocene Aurochsen across Eurasia, contradicting the claim of a lack of gene flow between Pleistocene aurochsen from northern Eurasia.

Similar problems apply to the interpretation of admixture between different populations over time. In my opinin, the results simply do not support the conclusions drawn by the authors. They claim that "Admixture is evident in central Asian aurochsen from both ancestry profiles, as well as introgression of the western mtDNA haplotypes P and Q (figure 2C, 3B)." The first part of the claim is indeed true. However, for the Pleistocene, they have a single(!) sample from the region between Black and Caspian Sea that is indeed not admixed (the individual from east-central Asia falls outside the geographical range of the Holocene specimens). While I acknowledge that a single nuclear genome is representative of the genetic diversity of the population it originates from, I do not think that from a single specimen in the age range between 18 and 11.7 ky, one can make any claims to the causes of changes in genetic makeup of the population, when comparing it to a handful of specimens (three to be precise) dating to anywhere between 11.7-3.8 kya. Moreover, and this also applies to the additional specimens further east as well as to the mitochondrial DNA signal, they date to a time when admixture may have been caused by cross breeding with human-introduced domestic cattle, and not be a direct effect of climatic changes. I should also note that the middle Italien specimen from the Pleistocene does show evidence of admixture between the northern European and the Near Eastern population. A much more fine-scaled temporal separation of the specimens would be necessary to decide if any of the claims made are supported by the data. I have

the impression that the interpretations by the authors were to some extent driven by their expectations rather than by a cold, hard look at the factual evidence.

This is also true for the preceding claim that “The earlier separation of Bos indicus (and by proxy its ancestor Bos namadicus) also endured through the glaciation, with no detectable admixture with other ancestries until human crossbreeding of domesticates from 4kya10 (Figure 3B).” Again, this claim is not really substantiated by the data, since the authors present no data on Bos namadicus and none of their aurochsen samples is geographically close to the centre of Bos indicus genetic diversity.

Just as a note on the section “Divergent demography in aurochs”: the authors test for introgression as a confounding factor in the demographic reconstructions of Bos indicus, but only for this population and also only for the PSMC analysis, disregarding the possibility that it could also affect other populations as well as the ROH anaylsis, includig the results of the imputation approach. And I would also note that an event cannot be both discrete and extended. This last paragraph also requires rephrasing to improve clarity.

I do not want to spend much more text on the Conclusions, since I addressed many of the critical points already above, but I would like to note that 90kya was not a particularly warm period during MIS5 as claimed by the authors, since 90kya falls into MIS5b, which is defined as a glacial sub-stage or stadial, i.e. one of the colder periods within MIS5.

Referee #2 (Remarks to the Author):

A: The key results in this manuscript are the assembly of 33 new aurochs' genomes and the subsequent analysis of 38 ancient aurochs' genomes that revealed four distinct ancestries of aurochs: European, Near Eastern, North Asian and South Asian, ranging in coverage from 0.06X to over 22X. The age span, as well as the geographical spread of these individuals are not only impressive but also necessary to investigate the rise and fall of the aurochs. Based on these analysis two key elements of the cattle domestication process are described; the initial capture of aurochsen by ~11 cal kya and the repeated secondary introgressions that helped shaped domesticated Bos populations. Looking at the mtDNA phylogeny in this study they demonstrates that considerable diversity existed in the wild, and that domestication only captured a limited subset of this genetic variation. The Y chromosome-based phylogeny, with its topology suggests at least six separate origins within Bos taurus, which is also a new discovery.
B: The data generated here is novel and is well suited to conduct the analysis described here. Which makes this study original with high significance as it tackles on of the keystone species. The results presented here are of value to a cross disciplinary audience, as it pertains to one of the main domesticates. As such these results has value for not only evolutionary biologist but archaeologist and the general audience.
C: I have no reason to question the data generated for this study. It is the presentation of the data that is problematic. Figure 1 is horrible; it is very hard to read/interpret, making the conclusions drawn from this in the text hard to follow. There is no explanation in the figure legend what all the different colors are, the lines drawn between some samples are not explained etc. As this is a key figure it needs to be easier to read and understand, it needs a revision. Maybe the LGM refugia could be marked on the map part of fig 1?
In the second paragraph on page 11, the authors mention a lower tolerance for colder conditions as an explanation, as seen in fig 3B, this is not what this figure deals with?
A standard set of methods has been used in this study all appropriate, the NGSadmix, MSMC2, imputations and ROH, and these are all well-established methods used in the field of ancient DNA and beyond. Based on the information given regarding the data and methodology, anyone should be able to reproduce the results.
D: The statistical analysis a predominately performed by using D statistics, BEAST and admixture and it has been used in a correct way to generate a set of figures (2). All of which are very schematic with a phylogeny that has been internally calibrated using 14C dates. It is not clear in figure 2b what the error bars are, so some clarification of this would make the figure stronger, as it stands it is however easy to read. Also, the * is unexplained.
What does the huge difference of converge do to the analysis and subsequent results? It is well-known that it is difficult to compare a 22X genome with a 0.06X, what allowances has been made to this work around this problem? On page 13, first paragraph only the high-coverage genomes are use, which are?
In the last paragraph on page 13, it says that 43 of the ancient genomes are above 0.5X, but the analysis only deals with 38 genomes (abstract)?
E: The conclusions and data interpretation are robust and valid even though the climate-based conclusions lack some support based on what is written in the manuscript.

F: If the climate is going to play a part in the description of the rise and fall of the aurochs, more scientific climate data should be presented. Mentioning the MSI5 and LGM is not enough or a colder or warmer climate. There are good climate proxies for the northern hemisphere that can be used to make this part stronger and more scientific.
The figures need to be clearer, more visually easy to interpret and with extensive legends that explains what all the data in them represents.
G: The references are appropriate albeit a bit sparse in the text.
H: The abstract is accessible and concise as is the introduction. Some more work could be done on the conclusions, based on comment under E.

In general, I find the writing to be choppy, whiteout proper segue way and it deters from the reading experience to some degree.

Referee #3 (Remarks to the Author):

This is an illuminating and timely paper. The authors analyzed 38 ancient genomes that documented four distinct ancestries of aurochs. A connection is also made to the domestication events and post-domestication admixture with ancestral strains in different regions.

In my view, the manuscript makes significant contributions on two fronts. First, it reveals long-term genomic history and divisions of ancestral aurochs that correspond to climatic events such as LGM and geographic barriers such as the Himalayas. Secondly, the genetic diversity of modern taurine cattle is best explained by globally dispersed introgressions from free-living oxen to domestic cattle. Both are interesting and significant contributions. I therefore support the publication of the manuscript.

Having suggested these patterns, the article could unpack more on the role of cattle-aurochs introgressions in facilitating environmental adaptations in each region. There has been some recent momentum in reconceptualizing domestication as a protracted and globally dispersed process rather than a localized event. Some of this literature focused on plant domestication (Liu & Jones 2024; Allaby et al. 2022); others have emphasized the role of gene flow in the domestication process itself (Marshall et al. 2014). The argument can be strengthened by connecting with this literature.

Allaby, R.G., C.J. Stevens, L. Kistler & D.Q. Fuller, 2022. Emerging evidence of plant domestication as a landscape-level process. Trends in Ecology and Evolution, 37(3), 268-97.
Liu, X. & M.K. Jones, 2024. Needs for a conceptual bridge between biological domestication and early food globalization. Proceedings of the National Academy of Sciences of the United States of America, 121, e2219055121.
Marshall, F., K. Dobney, T. Denham & J.M. Capriles, 2014. Evaluating the roles of dircted breeding and gene flow in animal domestication. Proc. Natl. Acad. Sci. U.S.A., 111(17), 6153-8.

Author Rebuttals to Initial Comments:

Referee #1 (Remarks to the Author):

In the manuscript "The genomic natural history of the aurochs", the authors present an impressive genomic data set obtained from fossil specimens and use these data to try and reveal the evolutionary history of this species. This effort in itself is laudable as very often, the aurochs is merely seen as the ancestor of domestic cattle and not as a species with an evolutionary history in its own right. The data are analysed by an equally impressive number of analyses. Unfortunately, this is where the problems with this manuscript begin. The multiple analyses and aspects addressed never come together into a meaningful story. It is like the authors are lost among all the analyses performed and therefore have put together paragraphs that are at best loosely connected. This is made worse by the attempt to write ina colourful language that often disguises rather than illuminates what the authors actually want to say (assuming they really know what they actually want to say). These problems are exacerbated by a number of factual errors that normally should not be in a submitted manuscript. I have detailed many of these issues below, but I should point out that my comments are not exhaustive as providing a fully comprehensive review of all issues is beyond the time I can spend. As I realize this may sound discouraging, I would like to emphasize the great potential I see in the dataset itself as well as in the results of the analyses performed. What the authors need to do in my mind is to take a more unpretentious approach to both interpretation and writing as well as put aside their preconceptions about what they believe or hope to find and simply interprete the results that are there (nothing more) in a clear and concise way and language. If they can achive this task, this manuscript clearly has the potential to become a seminal work regarding aurochs evolution.

We welcome the assessment that our results and analysis have the basis of a seminal work, and are encouraged that the referee agrees with us in the inherent value of aurochs-centred investigation. We appreciate the close reading of our text and detail our responses in line below. Briefly, we have extensively rewritten to clarify and make some of our inferences more conditional, focusing on presenting the results more clearly. This includes new analysis and figures and one new finding -a cline of admixture between European and Southwest Asian aurochs.

Abstract: "….that revealed four distinct ancestries of aurochs…." I think this is a bit of an unfortunate phrasing, as the aurochs of course as any species has a single ancestry. What the authors mean is populations.

1. We adjust and refer to population ancestries.

Also in the abstract, the authros are switiching between different tenses (which continues in the main text). I think it would be good if they could stay concistent. Generally, I found the abstract lacking in clarity. For example, it is not quite clear what the first half of the last sentence actually really means and whether southwest Asia refers to the same or a different region as "Near Eastern".

2. We have rewritten the abstract and now focus on the use of Southwest Asia throughout as our term for that region. We have also checked the text for tense consistency

I also would like to note that in my opinion (this is of course a matter of personal taset), a less pompous language than used by the authors is more suitable for communicating scientific results. As an example, I would clearly rephrase the follwing sentence: “Its fossil presence in Europe stretches over 650 thousand years ago (kya)1, but its earliest likely ancestors are detected in the Indomalayan biogeographic realm2, the centre of diversity for the tribe Bovini to which all wild cattle belong.”

3. We amend this sentence, and have extensively edited the manuscript for clearer language to help understanding.

This is true for many other sentences as well. Another example comes from pages 6/7. “The trajectories of the ancestries embedded in these clusters are dynamic and are contingent upon both climatic and cultural transitions.” Whatever this is supposed to mean.

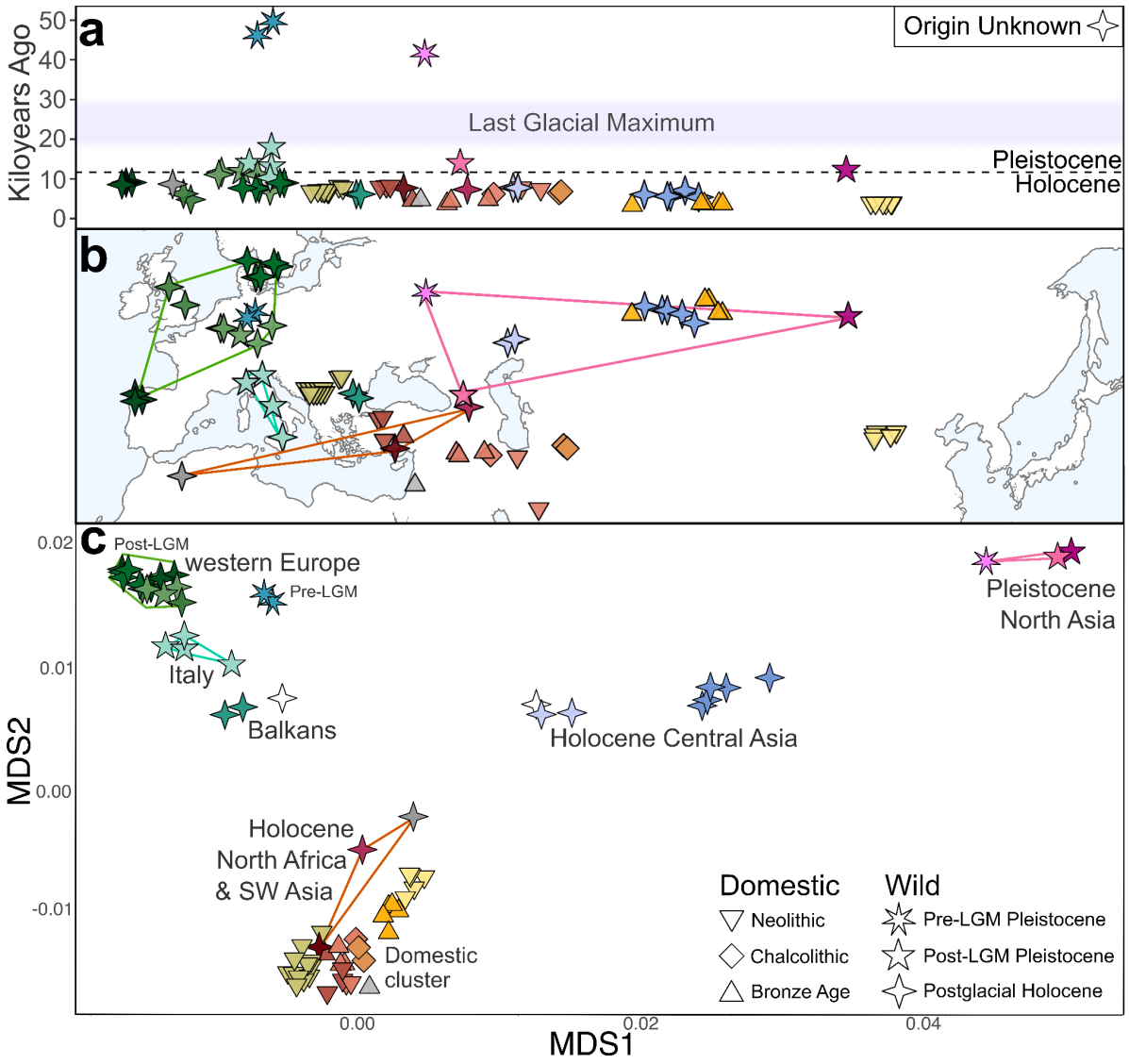
4. This attempted to refer to the theme that both climate and then human domestication and mobility shape genomes. However, we take this criticism and now try to put this point more clearly.

I am not sure why the authors refer to the last hunted bull in 1620, since the aurochs as a wild species went exticnt in 1627 with the last cow dying.

5. This was because the context was linking the bull to the cultural significance of its specific use as a drinking horn. The referee is correct about 1627 and we now amend and include mention of the last cow aurochs.

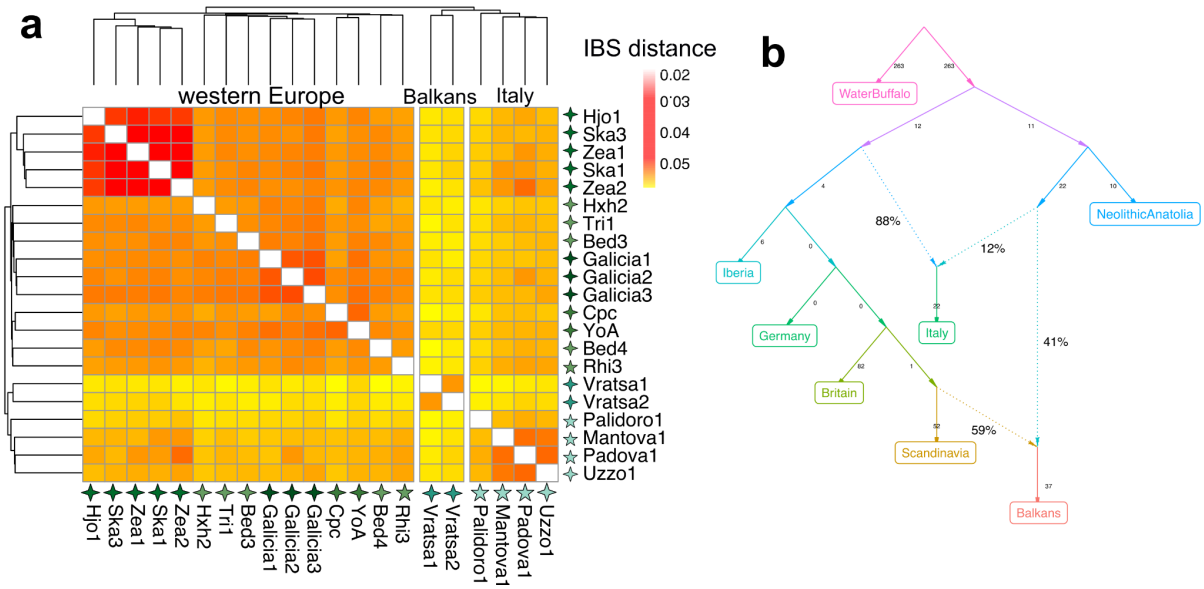
I have to admit that I find figure 1 surprisingly poorly executed regarding how easy (or rather difficult) it is to extract the relevant information from this figure. I also diagree with some of the clusters shown, especially the Iberian and Italian ones, as some samples added to the Iberian cluster are genetically closer to some Italien specimens than to other Iberian ones. In that context, I would like to see more evidence that the following paragraph is really supported by the evidence “Accordingly, genomes from these three regions separate into different groups. Interestingly, the bulk of sampled European (central European, Scandinavian and British) genomes cluster tightly with the Iberian group (Figure 1). This pattern suggests genetic isolation between refugia, postglacial recolonisation of western Europe derived from the Iberian refugium, and the Alps formed a barrier to gene flow from the Italian refugium.”

6. The figure is an attempt to communicate genetic location, geographical location and temporal time points for the data in a composite illustration within the space limitations of *Nature*. We note it is also criticised by reviewer 2. Therefore we amend Figure 1 for readability as follows: we reduce background colour to make both points and linking lines better stand out; we label the clusters more correctly; we standardise the symbol size throughout the three panels; we enlarge the dimensions of the most cramped top panel; we add more explanation to the legend.



For example a separate MDS with only the western European samples, some structure-like analysis or a qp-graph analysis. Even a simple distance matrix would help. In figures 2 and 3 the authors in fact present some such analyses, which, however, do not support the claims made. As the manuscript currently presents the evidence, it is in my opinion thus simply inadequate to support the claim made in this paragraph.

7. Thank you for this helpful criticism, it stimulated an additional analysis which we think has given interesting extra information about the Europeans. First, a heatmap using allele sharing distance and associated tree does show that the three groups cluster (see pasted figure below). Second, we modelled the European and Southwest Asian aurochs populations using AdmixtureGraph. This shows the shared evolutionary history among western European genomes (Iberia, Britain, Germany, Scandinavia). Italians (including the older 18kya sample) have ~ 12% admixture contribution of the Southwest Asian ancestry and Balkans genomes are modelled as having ~ 41%. This information is now collated in an Extended Figure in the manuscript, see below.



In my opinion, the manuscript would benefit from some adjustments here. I am not sure if the inclusion of domestic cattle in the map helps or rather hinders understanding of what is shown. There is also a factual error in the description of these findings in the text, since the cluster at the bootom left in the MDS-analysis does not consist of “two Near Eastern aurochsen” but rather includes one aurochs from the Near East, one aurochs from the west of North Africa and one from the Caucasus.

8. As pointed out by Referee3, the fate of aurochs after their domestication is a part of our manusctipt’s message, so we prefer to keep these points (many of which are also the presentation of new ancient genome data). What we hope to communicate here is the contrast between their wide geography and their tighter clustering close to one group of aurochs. We amend the mentioned label to a hybrid southwest Asia/north Africa term and no longer use the term ‘Near East’ as a category.

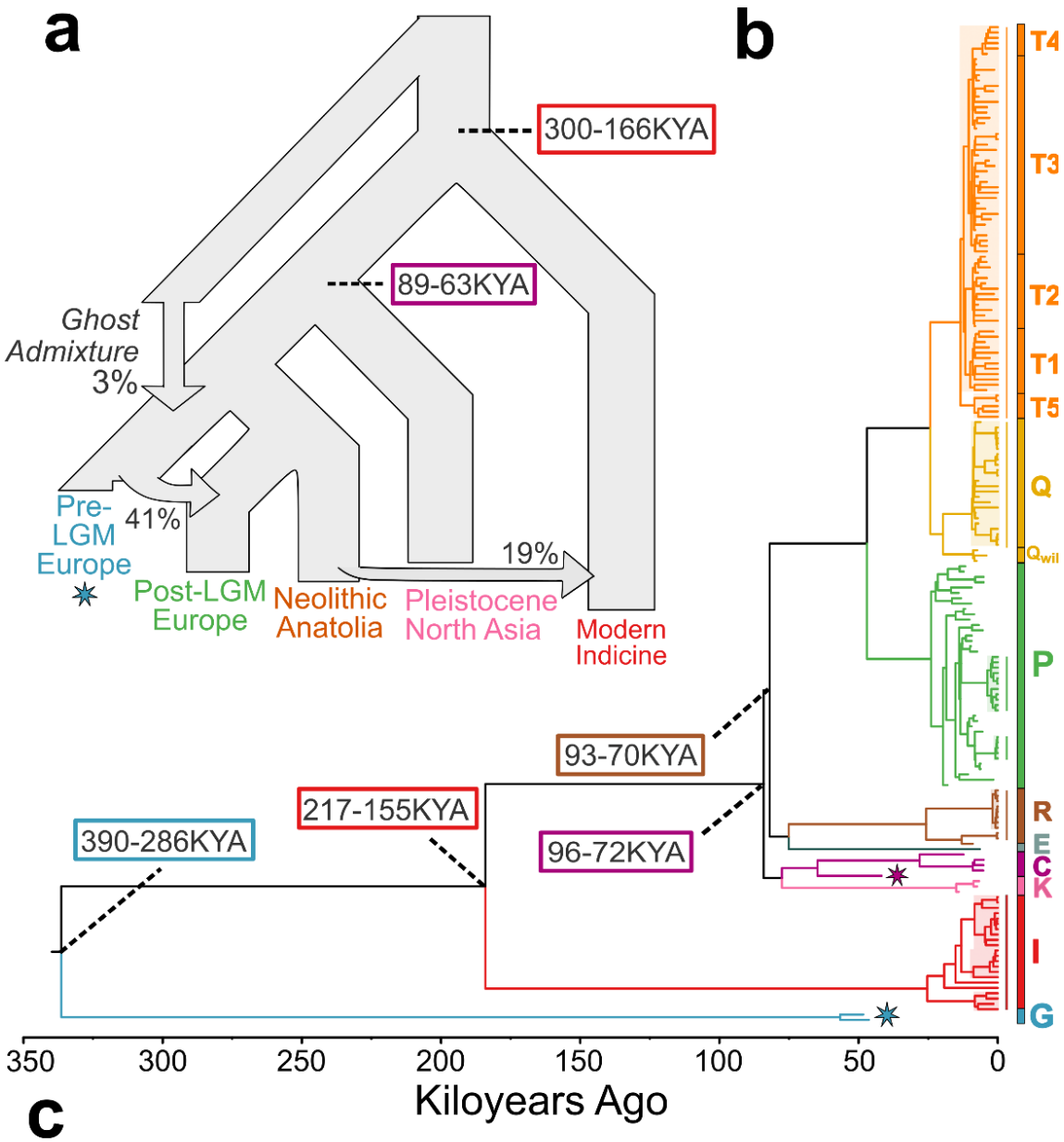
Generally, I have the impression that the authors do not interpret their results fully impartially, as another questionable interpretation follows already on page 7: “This Asian distinctiveness does not persist after the Holocene, when central Asian genomes shift towards the centre of the plot…..” Looking at figure 1, I would argue that while the Holocene Central Aisan aurochsen are less distinct from the other regions than their Pleistocene counterparts, they still are distinct, meaning the distinctness persists, albeit in a weaker form.

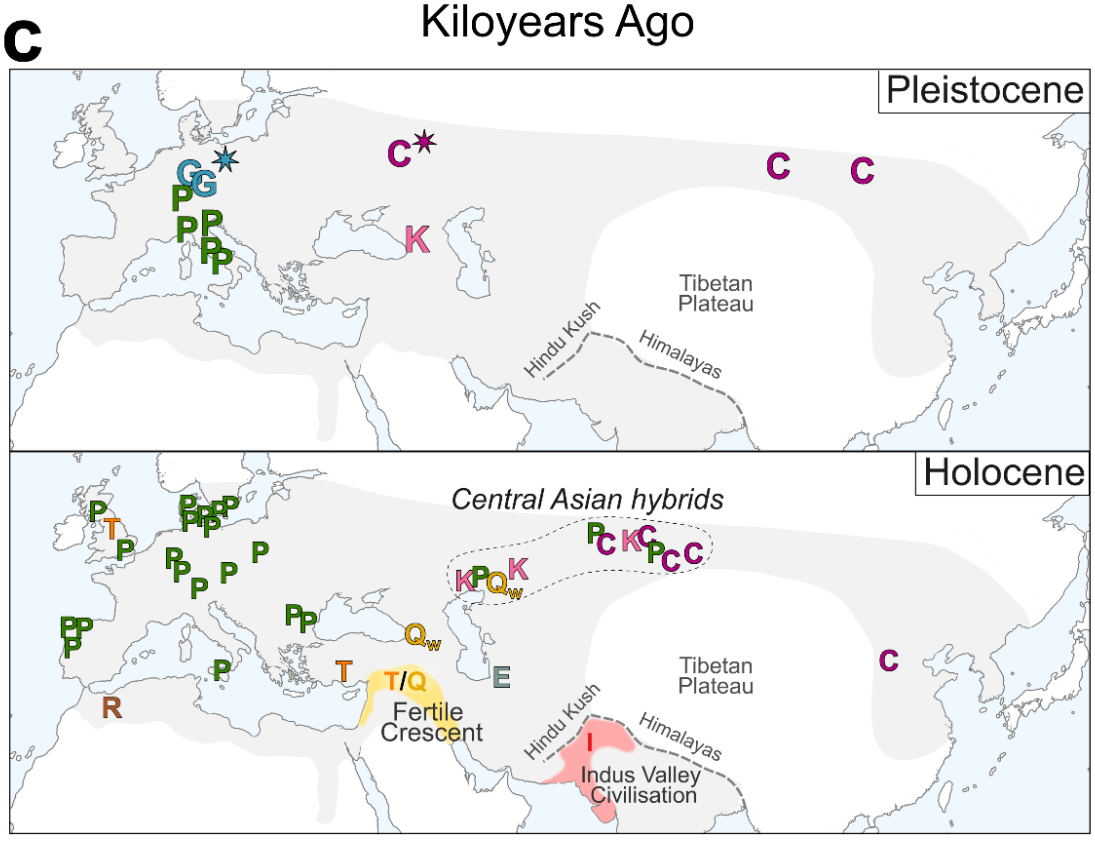
9. We agree that this is clumsy and now rephrase. Also, this raises a key point - can we assert that these are admixtures of the different aurochs population ancestries and we now explicitly test this with supervised ancestry modelling using qpAdm (Supplementary Section 3). We find that in all cases of Holocene Asian aurochs, the only acceptable model requires input from three ancestry sources (western Europe, Neolithic Anatolia and North Asia). This finding is reinforced by the existence of mtDNA haplotypes and Y chromosome haplotypes of European/South Asian origins.

We also tested if these aurochs could be modelled as admixtures of local domesticates and local aurochsen (*i.e.* Ancient Central Asian domesticates and North Asian genomes). In all cases, as above, the only accepted model was that Central Asian aurochs were modelled with three ancestries (including wild European). These analyses do not rule out some input from local domesticates but do rule in that aurochs admixture must have occurred.

A note on figure 2: the shading denoting domestic haplotypes is especially for haplogroup R only visible for readers who already know that it is there. Also, the authors should probably mention that for this figure, they also used a fair numer of published sequences. Moreover, panel c is rather crowded an getting an meaningful overview which haplotypes are pre-Holocene and which are Holocene is not really possible. I suggest to make two separate panels, one from Holocene and the other for pre-Holocene. It also does not become clear whether the major domestic haplotypes were recovered from aurochsen specimens or just added to the figure.

10. We alter this figure to make the domestic cattle clusters more visible. The reviewer is correct, we do use published sequences for these modern domestics and in the legend we now point the reader to the part of the supplement where details of the source of each sequence are given. Also, we now also reference an earlier source for nomenclature for several of these haplogroups. We also expand the map in panel C into two panels as suggested. T/Q and I are now only shown in the Holocene panel and the legend now mentions that within the shaded regions these are assumed origins.


a
300-166KYA
89-63KYA
Ghost
Admixture
3%
Pre-
LGM
Europe
41%
19%
Post-LGM
Europe
Neolithic
Anatolia
Pleistocene
North Asia
Modern
Indicine
b
T4
T3
T2
T1
T5
Q
Qwil
P
R
E
C
K
I
G
93-70KYA
217-155KYA
96-72KYA
390-286KYA
350
300
250
200
150
100
50
0
Kiloyears Ago


c
Pleistocene
Tibetan
Plateau
Hindu Kush
Himalayas
Holocene
Central Asian hybrids
Fertile
Crescent
Indus Valley
Civilisation

The following sentence on page 10 is phrased rather unfortunate: “However, a much more recent coalescence of all sampled Bos is estimated by Bayesian analysis of C14-dated mtDNA (implemented in BEAST; Figure 3) and genome-wide coalescence with cross-population analysis using the observed bovine mutation rate (implemented using MSMC2; Extended Data Figure 1).” First “….all samples Bos…..” reads rather clumsily and is also unclear whether all members of the genus Bos sampled in this study or sampled so far in scientific studies is meant.Second, the tree the authors are referring to is found as figure 2B, not 3B.

11. We have rewritten this paragraph - and succeeding ones. Apologies for this error in fig number.

And third, the second part of the sentence is true only if one accepts a cross-coalescence rate of 50%. Part of the cross-coalescence between Bos primigenius and Bos namadicus extends beyond 1 million years, as by then, the plot has not yet reached 100%.

12. This particular calculation is now not a major focus within our paper. Using MSMC it is correct to use the 50% interval as an estimate of separation. Eg quotation: “To get the relative cross coalescence rate (rCCR, see Fig. 3), you need to compute 2λ01⁄(λ00 + λ11), without any additional scaling. It can then be informative to compute the time point at which the relative CCR hits 0.5, to reflect an estimate of the split time between two populations” from Schiffels, S., Wang, K. (2020). MSMC and MSMC2: The Multiple Sequentially Markovian Coalescent. In: Dutheil, J.Y. (eds) Statistical Population Genomics. Methods in Molecular Biology, vol 2090. Humana, New York, NY. https://link.springer.com/protocol/10.1007/978-1-0716-0199-0_7
Many papers have employed this e.g. https://www.cell.com/ajhg/fulltext/S0002-9297(15)00156-1?cc=y%3D#gr3
https://academic.oup.com/gpb/article/20/6/1040/7274157

However, it is true that, in contrast to the comparison between east and west *Bos primigenius,* the *indicus-primigenius* coalescence has a long tail - this observation of more highly divergent segments may be contributed to by the exotic species introgression that we suggest later in the paper when discussing the PSMC demographic profiles. With this in mind it is important that the MSMC2 divergence estimates are supported by three other analyses which give overlapping intervals: a) mtDNA b) the times of divergence of PSMC demographic profiles and c) a pseudodiploid X chromosome specific analysis where we combine two haploid X chromosomes from divergent males and perform PSMC’. We now try to integrate these joint approaches better in the text (the X analysis shows a sharper division than the whole genome MSMC analysis for the *Bos indicus* divergence but was previously confined to the supplement).

I also would like to note that in extended data figure 1, the authors use Bos namadicus as designation, while in the main text, they correctly state that they used Bos indicus as proxy for Bos namadicus – one of clearly too many small mistakes in this manuscript.

13. This mistake is amended.

Also, the cross-coalescent analysis by itself conceptually cannot reveal replacement, since occurrence in the fossil record by 650 kya does not imply that the coalescent time of the descendant population has to go back that far. The authors need to provide additional information, why replacment of the ancient European population (by which other population, btw) is a better explanation of the observed data than any other explanation.

14. Replacement may be a clumsy description of what we see and we now phrase the phenomenon more in terms of the *Bos primigenius* coalescence during the last glacial period but with trace ancestry flowing through in Europe from earlier populations. We accept that coalescence should not be expected to track back to first colonisation. However, we do see a particular coalescent structure in our data which we think is interesting and worth teasing out. For example, it differs from that of another ancient lay sampled wild mammal - the wolf (Bergstrom et al Nature 2022) - which had genomic similarity within each 10ky timeslice implying high mobility and genetic exchange across Eurasia in the Pleistocene. We hope our rewrite has improved the discussion of our distinct patterns.

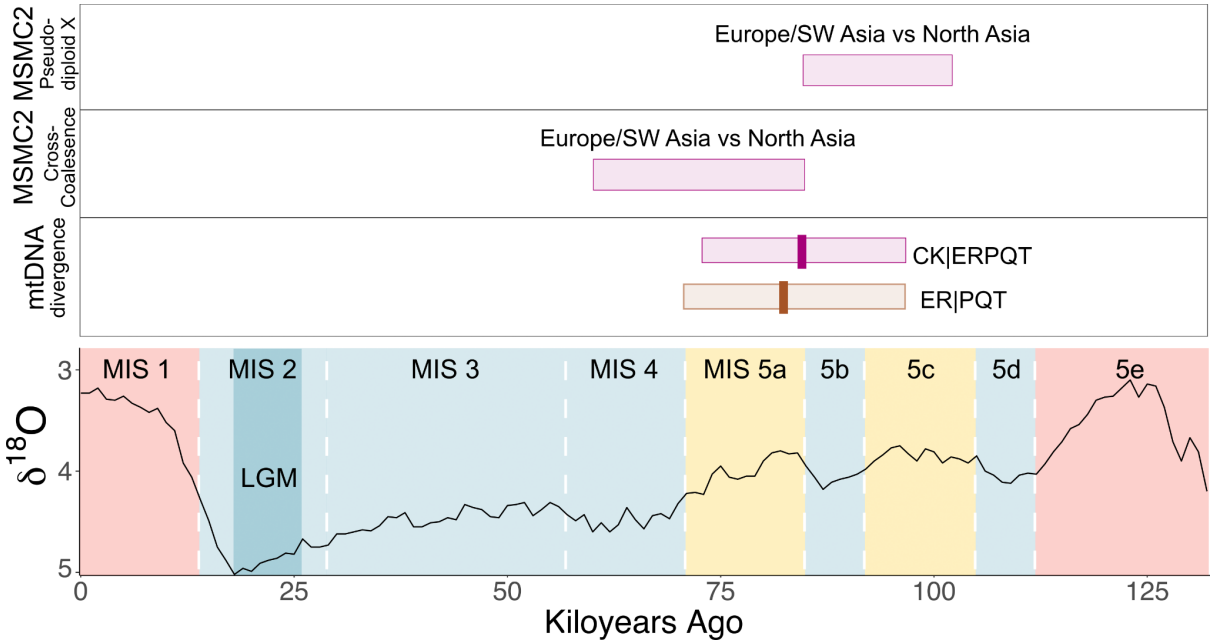
Unfortunately, the arguments do not get better in the next section. It starts already with the statement "The estimated last common ancestry of Bos primigenius….." without qualification, which common ancestry the authros refer to – mitochondrial DNA (which seems to be the case given what the authors write later in the text), range of nuclear loci (and then up to which coalescence rate?) or population ancestry.

15. This section is rewritten. Also we have added an Extended figure (pasted below the next point) that shows the overlap of the estimates from whole genome, X chromosome and mtDNA divergence estimates.

And it does not get better in the second half of the sentence: "…….falls within Marine Isotope Stage 5 (129-71 kya), which had warm periods with ice retreat before the onset of the last glaciation19,20." MIS5 did not have "warm periods", it actually includes with the Eemian (from 130 – 115 kya) the previous interglacial when temperatures were in many places warmer than today and sea levels several meters higher. The "last Ice Age" also doe not refer to the period from 70-18kya, but instead to the Quaternary glaciation that started 2.58 mya.

16. We agree we require more precise terminology when discussing the MIS-5 substages. We have replaced the term "warm stages" with the more accurate "interstadial stages" (as defined here:Otvos EG 2015 The Last Interglacial Stage: Definitions and marine highstand, North America and Eurasia Quaternary International 383:158-173) https://www.sciencedirect.com/science/article/abs/pii/S1040618214003061?via%3Dihub. Also, we have prepared an Extended Data Figure (pasted below) to better support our assertion that the European/SW Asian Vs North Asian *Bos primigenius* common ancestry falls

in MIS5 and this allows the reader to better judge a possible link with climatic change.



The next paragraph reveals another problem. The authors state: “It is likely that the reduced gene flow in northern Eurasia was caused by range reduction with the onset of the glacial period21.“ For a start, something can only be reduced RELATIVE to something else. So the use of “reduced gene flow” without comparison to a case of higher gene flow makes no logical sense.

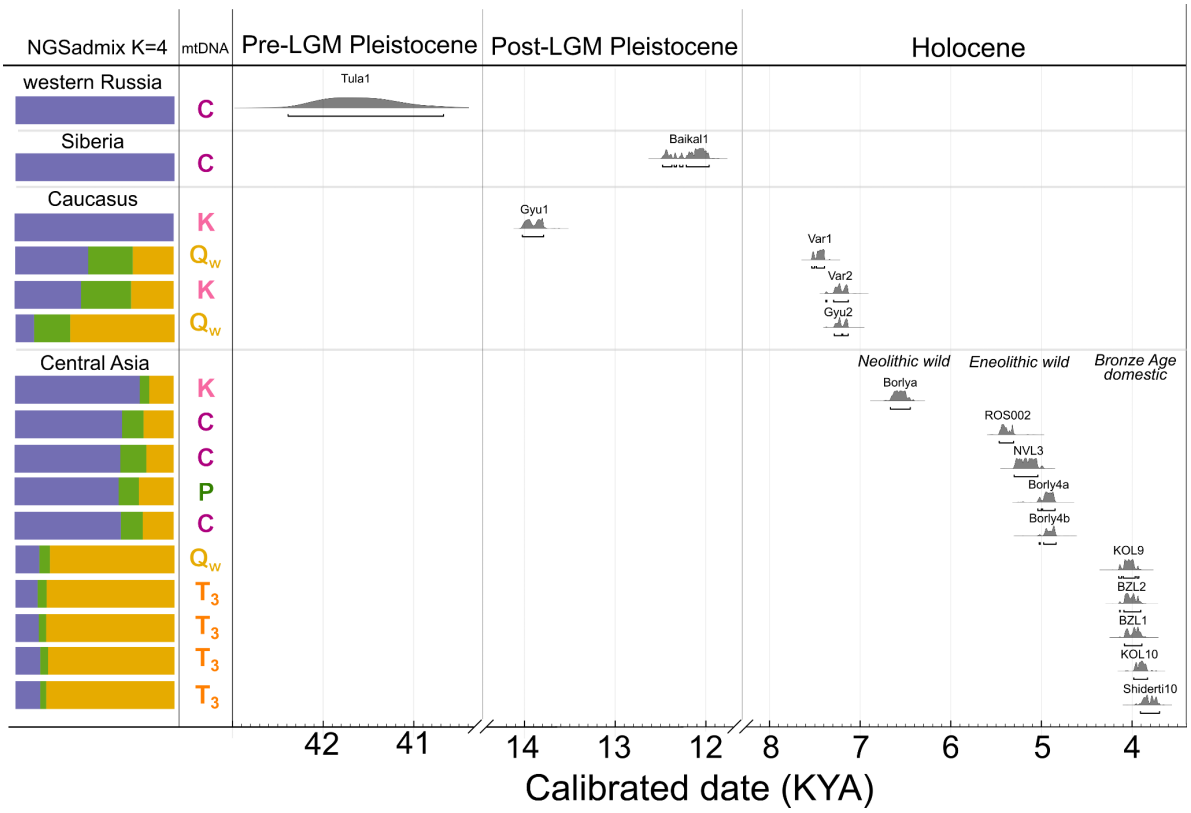
Second, it is not clear what the authors mean with glacial period – the last glacial period started 115 kya, with the end of the Eemian interglacial. However, since their oldest sample is about 50 kya, the cannot make any statement about gene flow at the onset of the last glacial period.

Third, it is unclear what they mean with “reduced gene flow in northern Eurasia“ as it is really unclear, which set of samples this refers to. And last, Figure 1 shows a wide distribution of a genetic cluster of Pleistocene Aurochsen across Eurasia, contradicting the claim of a lack of gene flow between Pleistocene aurochsen from northern Eurasia.

17. We have completely rewritten this section (we submit a version of the manuscript with tracked changes for clarity); the term “reduced gene flow” is no longer used.

Similar problems apply to the interpretation of admixture between different populations over time. In my opinin, the results simply do not support the conclusions drawn by the authors. They claim that “Admixture is evident in central Asian aurochsen from both ancestry profiles, as well as introgression of the western mtDNA haplotypes P and Q (figure 2C, 3B).” The first part of the claim is indeed true. However, for the Pleistocene, they have a single(!) sample from the region between Black and Caspian Sea that is indeed not admixed (the individual from east-central Asia falls outside the geographical range of the Holocene specimens). While I acknowledge that a single nuclear genome is representative of the genetic diversity of the population it originates from, I do not think that from a single specimen in the age range between 18 and 11.7 ky, one can make any claims to the causes of changes in genetic makeup of the population, when comparing it to a handful of specimens (three to be precise) dating to anywhere between 11.7-3.8 kya.

18. We think that a more transparent display of the ancestries sampled in the key regions with respect to C14 dates will help the reader interpret changes - we now include the diagram below as an extended figure. Our text is rewritten. We consider it is worth pointing out that the Central Asian/Caucasus admixtures appear in the Holocene but it is true (and now clearer to the reader) that the unadmixed status just prior to this features a single individual and that there is a sampling gap which could be profitably closed by future sampling.



Moreover, and this also applies to the additional specimens further east as well as to the mitochondrial DNA signal, they date to a time when admixture may have been caused by cross breeding with human-introduced domestic cattle, and not be a direct effect of climatic changes. I should also note that the middle Italien specimen from the Pleistocene does show evidence of admixture between the northern European and the Near Eastern population. A much more fine-scaled temporal separation of the specimens would be necessary to decide if any of the claims made are supported by the data. I have the impression that the interpretations by the authors were to some extent driven by their expectations rather than by a cold, hard look at the factual evidence.

19. We agree on the desirability of further temporal sampling - such material is not currently available to us. We have investigated if the autosomal signal of admixture might be explained by local domesticate input but this model does not give an acceptable fit (see point 9 above). Also, several of the aurochsen sampled in the region precede the arrival of domestic cattle, as discussed in Supplemental Information 6. Lastly, the domestic-like

mitotypes in Central Asian hybrids (both Q) are actually an outgroup to the highly numerous Q haplotype in ancient and modern cattle - we now label these as Qw in the phylogeny and maps of Figure 3 for clarity. Regarding Italian/SW Asian admixture, please see point 7 above.

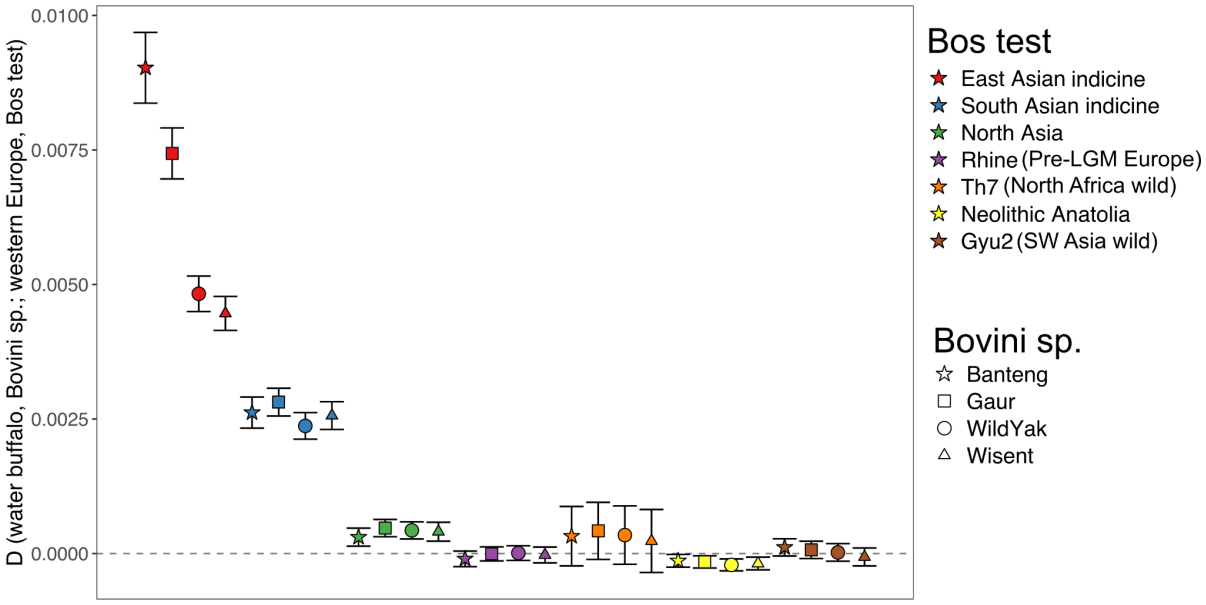
This is also true for the preceding claim that "The earlier separation of Bos indicus (and by proxy its ancestor Bos namadicus) also endured through the glaciation, with no detectable admixture with other ancestries until human crossbreeding of domesticates from 4kya10 (Figure 3B)." Again, this claim is not really substantiated by the data, since the authors present no data on Bos namadicus and none of their aurochsen samples is geographically close to the centre of Bos indicus genetic diversity.

20. We now delete this sentence, and now refer in a later paragraph to the expansion of Bos indicus "This mirrors the later spread of *Bos indicus* domestic ancestry beyond its south Asian region of origin, which is also inferred to have been male-mediated[10]."

Just as a note on the section "Divergent demography in aurochs": the authors test for introgression as a confounding factor in the demographic reconstructions of Bos indicus, but only for this population and also only for the PSMC analysis, disregarding the possibility that it could also affect other populations as well as the ROH anaylsis, includig the results of the imputation approach. And I would also note that an event cannot be both discrete and extended. This last paragraph also requires rephrasing to improve clarity.

21. We have computed these tests for introgression and amend Extended data figure 5 (pasted below), focusing on the four Eurasian wild cattle species as possible introgressors for each of the sub populations of aurochs). Also with edits to Supplementary Data 5 and text in Supplementary Information Section 4. These *D* tests ask if any of these tested aurochs have an excess of exotic allele sharing relative to western European aurochs. Both South Asian and East Asian *Bos indicus* show this emphatically, consistent with some introgression of divergent Bovini with their ancestors. As the article text mentions, this accords with their increased estimated Ne in PSMC in the deep past, and as discussed in point 12 above, a tail of divergence in MSMC cross coalescence. *D* statistic values for *Bos primigenius* are an order of magnitude smaller. Of the latter, those involving North Asian aurochs may indicate some excess allele sharing (possibly low level input from local aurochs into Bovini species or *vice versa*). However, we do not think this skews our demography results because: a) there is no elevation of North Asian Ne in the PSMC curve in deeper time when divergent species input would exhibit, as it does in *Bos indicus* - in fact before 50kya they have the lowest, or joint lowest Ne. b) contrasting ROH profiles (which we see up to 4Mb) are governed by recombination and are therefore more recent in origin, depending on demographic processes within thousands, not hundreds of thousands of years. Also and importantly, the main inferences we draw (higher ROH in post Vs preLGM Europe, higher ROH in domestic Neolithic Vs Southwest Asian, lower ROH in later cattle) are not dependent on North Asians. c) Imputation effectiveness is tested by the downsampling of high coverage genomes, imputing these and comparing ROH profiles (Supplementary information Section 5). This shows that our imputation of aurochs, including North Asians, gives equivalent results to

direct observation of variants using higher quality data. We have rewritten the "discrete and extended" phrase.



Extended Data Figure 5. Unequal Bovini allele-sharing in *Bos*. We explicitly tested allele-sharing of *Bos* populations with wild Eurasian Bovini relative to western European aurochs using *D* (water buffalo, Bovini sp.; western Europe, *Bos* test) using SNPs called on Bovini outgroups. We repeatedly observed significant excess allele sharing between indicine and outgroup Bovini species compared to western European populations. All other *Bos primigenius/Bos taurus* populations suggest clade integrity, except Pleistocene North Asia which displayed slightly higher allele sharing with Bovini outgroups, but at an order of magnitude lower than that shown by *Bos indicus*.

I do not want to spend much more text on the Conclusions, since I addressed many of the critical points already above, but I would like to note that 90kya was not a particularly warm period during MIS5 as claimed by the authors, since 90kya falls into MIS5b, which is defined as a glacial sub-stage or stadial, i.e. one of the colder periods within MIS5.

22. We have rewritten the Conclusions section. Now it is structured by the different population ancestries, rather than by different climate effects.

Referee #2 (Remarks to the Author):

A: The key results in this manuscript are the assembly of 33 new aurochs' genomes and the subsequent analysis of 38 ancient aurochs' genomes that revealed four distinct ancestries of aurochs: European, Near Eastern, North Asian and South Asian, ranging in coverage from 0.06X to over 22X. The age span, as well as the geographical spread of these individuals are not only impressive but also necessary to investigate the rise and fall of the aurochs. Based on these analysis two key elements of the cattle domestication process are described; the initial capture of aurochsen by ~11 cal kya and the repeated secondary introgressions that helped shaped domesticated Bos populations. Looking at the mtDNA phylogeny in this study they demonstrates that considerable diversity existed in the wild, and that domestication only captured a limited subset of this genetic variation. The Y chromosome-based phylogeny, with its topology suggests at least six separate origins within Bos taurus, which is also a new discovery.

B: The data generated here is novel and is well suited to conduct the analysis described here. Which makes this study original with high significance as it tackles on of the keystone species. The results presented here are of value to a cross disciplinary audience, as it pertains to one of the main domesticates. As such these results has value for not only evolutionary biologist but archaeologist and the general audience.

23. We appreciate these positive comments.

C: I have no reason to question the data generated for this study. It is the presentation of the data that is problematic. Figure 1 is horrible; it is very hard to read/interpret, making the conclusions drawn from this in the text hard to follow. There is no explanation in the figure legend what all the different colors are, the lines drawn between some samples are not explained etc. As this is a key figure it needs to be easier to read and understand, it needs a revision. Maybe the LGM refugia could be marked on the map part of fig 1?

24. We have reworked Figure 1 (see point 6 above). We think there will be too much clutter to mark refugia and these are not so well defined geographically in any case. We have modified the legend to be more explanatory.

In the second paragraph on page 11, the authors mention a lower tolerance for colder conditions as an explanation, as seen in fig 3B, this is not what this figure deals with?

25. This sentence has been omitted in the revision. Please see point 20 above.

A standard set of methods has been used in this study all appropriate, the NGSadmix, MSMC2, imputations and ROH, and these are all well-established methods used in the field of ancient DNA and beyond. Based on the information given regarding the data and methodology, anyone should be able to reproduce the results.

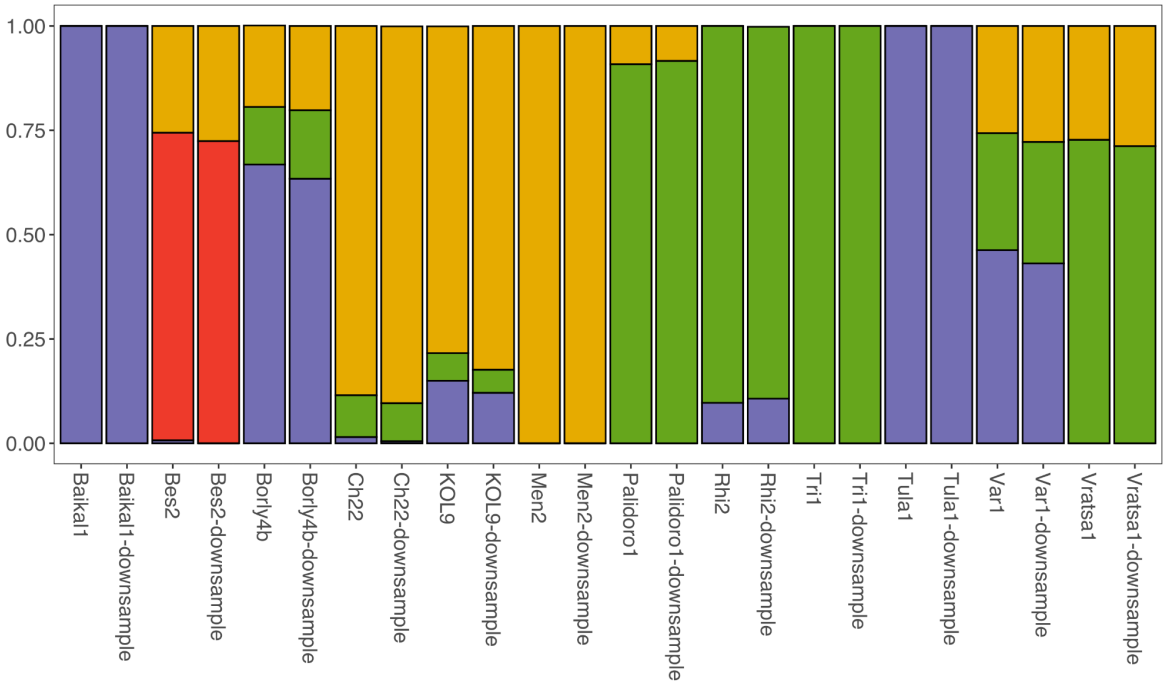
D: The statistical analysis a predominately performed by using D statistics, BEAST and admixture and it has been used in a correct way to generate a set of figures (2). All of which are very schematic with a phylogeny that has been internally calibrated using 14C dates. It is not clear in figure 2b what the

error bars are, so some clarification of this would make the figure stronger, as it stands it is however easy to read. Also, the * is unexplained.

26. We think that error bars will be too cluttered in this already busy display. However, we include a figure (S9) in the supplement that shows all BEAST error bars. We have enlarged the legend and explain the asterisk (highlights the three very early pre-LGM samples).

What does the huge difference of converge do to the analysis and subsequent results? It is well-known that it is difficult to compare a 22X genome with a 0.06X, what allowances has been made to this work around this problem? On page 13, first paragraph only the high-coverage genomes are use, which are?

27. In the study as a whole we take careful consideration to the effect of lower coverage data, using different minimum coverage thresholds for the different analyses.
    -Only high-coverage genomes (8X+) were used for MSMC2 analyses, with strict filters for minimum coverage depths applied.
    -Only genomes of 0.5X and above were used for imputation and ROH analysis; we also tested the accuracy of imputation at this coverage by downsampling high-coverage samples and imputing (Supplementary Information Section 4).
    -Only mitochondrial genomes of average coverage of 10X and above were used in Bayesian analyses.
    -Low coverage genomes (0.06X) are co-analysed with high coverage genomes in NGSadmix. We opted to use a Genotype Likelihood method to account for uncertainty in lower coverage samples. As a test, we downsampled 12 higher coverage samples (representative of different aurochs/cattle population) to 0.06X, re-calculated genotype likelihoods and re-ran NGSadmix at K=4. We observe minimal fluctuation in predicted ancestral components (side-by-side comparison is pasted below).



-We also co-analysed high and low coverage genomes in dimensionality reduction analyses (e.g. MDS plots of pseudohaploidised genomes). We iteratively downsampled each sample above 0.06X to 0.06X and re-ran this analysis and replotted the results. Once again, minimal flux in sample orientation was observed, with all downsampled genomes occupying the same cluster observed in Figure 1C. All downsampled plots are available to view at: https://osf.io/8haux/

In the last paragraph on page 13, it says that 43 of the ancient genomes are above 0.5X, but the analysis only deals with 38 genomes (abstract)?

28. These 43 ancient genomes include both wild (25) and domestic (18) genomes above 0.5X. We have amended the text to clarify this.

E: The conclusions and data interpretation are robust and valid even though the climate-based conclusions lack some support based on what is written in the manuscript.

29. We have tried to make the support more visually available -see points 16, 18 above. Also, the manuscript is largely rewritten with the climate conclusions phrased more conditionally.

F: If the climate is going to play a part in the description of the rise and fall of the aurochs, more scientific climate data should be presented. Mentioning the MSI5 and LGM is not enough or a colder or warmer climate. There are good climate proxies for the northern hemisphere that can be used to make this part stronger and more scientific.

30. We have added an extended figure (point 16 above) with climate data to address this issue. More granular climate proxy data could be availed of but would not be appropriate. As we hope is now more clear to the reader, our error margins for the divergence of continental populations are too wide to assign a specific interstadial stage.

The figures need to be clearer, more visually easy to interpret and with extensive legends that explains what all the data in them represents.

31. We have reworked two main text figures, added extended figures and expanded the main legends.

G: The references are appropriate albeit a bit sparse in the text.

H: The abstract is accessible and concise as is the introduction. Some more work could be done on the conclusions, based on comment under E.

32. We have rewritten the Conclusions.

In general, I find the writing to be choppy, whiteout proper segue way and it deters from the reading experience to some degree.

33. We have substantially rewritten the text, please see the track changes pdf, and hope it is now improved.

Referee #3 (Remarks to the Author):

This is an illuminating and timely paper. The authors analyzed 38 ancient genomes that documented four distinct ancestries of aurochs. A connection is also made to the domestication events and post-domestication admixture with ancestral strains in different regions.

In my view, the manuscript makes significant contributions on two fronts. First, it reveals long-term genomic history and divisions of ancestral aurochs that correspond to climatic events such as LGM and geographic barriers such as the Himalayas. Secondly, the genetic diversity of modern taurine cattle is best explained by globally dispersed introgressions from free-living oxen to domestic cattle. Both are interesting and significant contributions. I therefore support the publication of the manuscript.

Having suggested these patterns, the article could unpack more on the role of cattle-aurochs introgressions in facilitating environmental adaptations in each region. There has been some recent momentum in reconceptualizing domestication as a protracted and globally dispersed process rather than a localized event. Some of this literature focused on plant domestication (Liu & Jones 2024; Allaby et al. 2022); others have emphasized the role of gene flow in the domestication process itself (Marshall et al. 2014). The argument can be strengthened by connecting with this literature.

Allaby, R.G., C.J. Stevens, L. Kistler & D.Q. Fuller, 2022. Emerging evidence of plant domestication as a landscape-level process. Trends in Ecology and Evolution, 37(3), 268-97.

Liu, X. & M.K. Jones, 2024. Needs for a conceptual bridge between biological domestication and early food globalization. Proceedings of the National Academy of Sciences of the United States of America, 121, e2219055121.

Marshall, F., K. Dobney, T. Denham & J.M. Capriles, 2014. Evaluating the roles of dircted breeding and gene flow in animal domestication. Proc. Natl. Acad. Sci. U.S.A., 111(17), 6153-8.

34. Thanks for the positive commentary and this suggestion. We did perform an analysis searching for specific genomic adaptations linked to aurochs introgression within European cattle, but without interpretable signals emerging (not shown). We think more ancient aurochs data would be required to power such an analysis in other regions and possibly in this one. Nevertheless, we take the point that these introgressions may have had adaptive consequence and add a comment to this effect and reference the Marshall et al paper regarding the importance of wild gene flow in an extended domestication process.

Reviewer Reports on the First Revision:

Referee #1 (Remarks to the Author):

Since the current manuscript is the revised version of a previous submission and I am not a friend of multiple rounds of revision, I will be short: the authors have extensively revised the manuscript and taken substantial effort to address the comments by both me and the other reviews. IN my opinion the revised version is indeed much improved. I congratulate the authors on their efforts and achievements and have no further comments.

Referee #2 (Remarks to the Author):

The manuscript, The genomic natural history of the aurochs, has improved a lot, taking into account mine and the other reviewers comments (the third reviewer didn't really have anything to comment on), so thank you for all the hard work doing this. The figures have improved, and they are now much easier to interpret. Having said this, I still have a few comments, and questions for the authors.

Why did the Bos indicus not get plotted in figure 1 (Line 144), it is mentioned throughout the rest of the manuscript? It would have been nice to see it on the plot in figure 1c.

One line 260, it would be kind to the reader to give a few examples of mammals that have been studied prior to this.

One lines 261-262, how many genomes do you have, further down number of >0.5X genomes are mentioned (line 281), be consistent.

It would be interesting to have some discussion about the sample size and the problem this might cause. Drawing conclusions of a pre LGM population based on two individuals sees slightly problematic? Also, the number of post-LGM close to the LGM are also low, so this too might be an issue? Maybe adding a couple of sentences with some contemplation on this, would be useful.

Make the text under heading Methods, uniform in font and size.

Author Rebuttals to First Revision:

Referee #1 (Remarks to the Author):

Since the current manuscript is the revised version of a previous submission and I am not a friend of multiple rounds of revision, I will be short: the authors have extensively revised the manuscript and taken substantial effort to address the comments by both me and the other reviews. IN my opinion the revised version is indeed much improved. I congratulate the authors on their efforts and achievements and have no further comments.

We thank the reviewer for their constructive feedback, which has improved the paper.

Referee #2 (Remarks to the Author):

The manuscript, The genomic natural history of the aurochs, has improved a lot, taking into account mine and the other reviewers comments (the third reviewer didn't really have anything to comment on), so thank you for all the hard work doing this. The figures have improved, and they are now much easier to interpret. Having said this, I still have a few comments, and questions for the authors.

We thank the author for the positive feedback and respond to the last comments in turn below.

Why did the Bos indicus not get plotted in figure 1 (Line 144), it is mentioned throughout the rest of the manuscript? It would have been nice to see it on the plot in figure 1c.

We do not include *Bos indicus* in this figure as its much greater divergence would dominate the axes of variation, swamping the interesting signals in this figure of within-*Bos primigenius* patterns of divergence. The indicine divergence is well known and is not our focus (we do not sequence any ancient *indicus*) and is adequately communicated by other figures in the manuscript.

One line 260, it would be kind to the reader to give a few examples of mammals that have been studied prior to this.

We have added examples of mammals.

One lines 261-262, how many genomes do you have, further down number of >0.5X genomes are mentioned (line 281), be consistent.

We have added this information.

It would be interesting to have some discussion about the sample size and the problem this might cause. Drawing conclusions of a pre LGM population based on two individuals sees slightly problematic? Also, the number of post-LGM close to the LGM are also low, so this too might be an issue? Maybe adding a couple of sentences with some contemplation on this, would be useful.

We have added text in our conclusions:

"Interestingly, much of Europe was recolonised from the former, although denser temporal sampling of European aurochsen during the last 50kya may provide higher resolution of refugial dynamics."

Make the text under heading Methods, uniform in font and size.

We have fixed these issues.