nature portfolio

Peer Review File

A prehistoric East-Asian Yersinia pestis genome and a ~5.3 ka trans Eurasian expansion of plague

Corresponding Author: Professor Yujun Cui

This manuscript has been previously reviewed at another journal. This document only contains information relating to versions considered at Nature Communications.

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
Thank you for the authors' detailed responses to my previous comments.
The authors have addressed my major concerns. In particular, the clarifications provided regarding key assumptions, as well as the additional explanations and contextualization, have significantly reduced the uncertainties in the original manuscript.
The responses improve the overall robustness and clarity of the manuscript.
I no longer have major concerns regarding the study, and I recommend the publication of the manuscript.

Minor comments :

Lines 314–316: Please avoid repeating verbatim the sentence already presented in lines 228–231; please rephrase accordingly.

Reviewer #3

(Remarks to the Author)
The authors provide a revised version of their article "A first prehistoric East Asian Yersinia pestis genome and a ~5.3 ka trans-Eurasian expansion of plague".
The manuscript has been improved significantly. The main results of the study are now presented more clearly. Also, the authors have added a section on the population genetic context of the individuals that have died of plague.

However, there are still key aspects related to the main conclusions of the article that require further clarification.

In the abstract it is stated that "Mapping variation onto the deep phylogeny further reveals a single concentrated phase of genomic remodeling on the basal LNBA$^+$ stem, including acquisition of ymt and multiple gene-disrupting indels and gene losses, highlighting an early episode of adaptation associated with later flea-borne lineages, whereas other deep phases show only background-like levels of change."

First, I still do not see how the presented study "reveals" these evolutionary changes as they have been described before (which the authors agree with in their response letter).
Second, it remains largely unclear what exactly the authors mean when stating "...whereas other deep phases show only background-like levels of change."
In the main text the following statement refers to this: "By contrast, mutation-type compositions on other deep phases did not differ significantly from the genome-wide background (Supplementary Data 5)."
The authors need to state more clearly what they want to say here. That the genomic changes that lead to flea adaptation do not occur convergently on other branches is undisputed. However, this is not a novel result.
At the same time there are other evolutionary changes in other parts of the tree (See for example Light-Maka et al. 2025 with respect to the LNBA- lineage). As experts on the genomic evolution of modern Y. pestis the authors will certainly have a

good overview with respect to the modern lineages of the tree.
Thus, while flea adaptation might be of highest relevance in Y. pestis evolution, evolutionary changes also happen in other parts of the tree.
Therefore, the authors should describe more clearly what is meant by "background-like levels of change". I am aware of the results described in section 4, but I am not sure what exactly the authors are suggesting here beyond the description of the already known adaptive changes.

There is one more formal point I would like to mention.
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Reviewer #4

(Remarks to the Author)
This study reports the first evidence of an ancient Yersinia pestis genome from East Asia, which seems like an exciting finding for the ancient pathogen community. I am a human population geneticist, and was asked to assess the human genetic analyses included in the study, therefore my review is focused exclusively on this aspect of the study.

While the identification of early Y. pestis in East Asia seems exciting, the human population genetic analyses implemented here are quite broad and feel disconnected from what is already known about human population history. In their current form, I don't think they strengthen the study.

Using approaches like PCA, f-statistics, and qpAdm, the authors detect broad ancestry signatures across Eurasia and argue that these represent an "active genetic corridor" linking these regions. However, genetic connections between populations from West Eurasia, Siberia, and East Asia are well-understood and have been documented in multiple studies. The finding of these connections is therefore unsurprising and reflects deep demographic history rather than providing direct evidence of unique plague transmission networks.

Rather than relying on these deep demographic connections to support specific transmission routes (or imply that they provide meaningful insights about the dynamics of specific transmission events), I recommend the authors use this genetic information to contextualize the populations involved. For instance, it would be valuable to more explicitly discuss how the divergence dates of the Y. pestis lineages align with the established human population histories of these regions, including Steppe migrations. Alternatively (or in addition), the authors could focus the human genetic analyses on demonstrating whether each newly identified individual appears to be a member of the local community or a genetic outlier, as they did for CY470.

Below are more specific comments:

- In general, there are too many acronyms used, and not enough time is spent describing the background associated with the individuals being studied. It would be helpful to provide more information about the geographic and temporal context in which they were found, and what is already known about the population history of individuals from these regions and periods.
Several of the newly identified Y. pestis positive individuals have very low human genome-wide coverage <0.1x. The authors should be explicit about the coverage of the individuals and about the limitations associated with analyzing data of this quality.
- The PCA plots presented both in the main text and supplement are extremely broad. The plots show ancient individuals projected onto a continent-spanning background of present-day "Eurasian and Native American populations", so it is unsurprising that individuals sampled from closer geographic regions cluster together. Additionally, it would be good to provide more detailed information about how present-day populations are distributed in these plots, as they are currently all shown in grey with no annotations to help readers interpret their placement.
- The authors should spend more time setting up each qpWave, qpAdm, and f-statistic test, explicitly describing the specific population models they are designed to differentiate between. As currently devised, these tests seem primarily focused on detecting very broad differences in ancestry that mirror already well-understood patterns. If that is the goal, the authors should be explicit about this and about what those results mean in the context of the spread of Y. pestis.
- In Supplementary Data 4, the authors state: "The best feasible qpAdm model is highlighted in gray". However, the criteria for selecting the "best" model are unclear. It is not appropriate to assume that the highest p-value corresponds to the most accurate population genetic model (as established in Harney et al., 2021), yet I suspect this may be the metric used. The authors should clarify their selection criteria, and if multiple models have p-values over the threshold for plausibility, they should be considered equally plausible.
- A single paragraph is used to describe all of the human population genetic analyses, including PCA, f-statistics, and qpWave/qpAdm analyses. The descriptions of each tool (including considering each type of f-statistic separately) should be described separately, noting what parameters were used for each analysis.

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In this revised version of the manuscript all my comments were addressed sufficiently. I have no additional comments.

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The authors have made significant improvements to the methodological descriptions in the methods section of the manuscript, and I am happy to see that they shifted the focus of many of their analyses to assess whether each focal individual's ancestry matches the local ancestry observed among other individuals in the population or if they appear to be migrants.

However, I still think that the manuscript's framing will make it difficult for readers to understand what is already known about human population history and how that population history might relate to the spread of Y. pestis. Furthermore, the overall readability of the human population genetics section needs improvement, as the narrative is frequently interrupted by methodological details and a heavy use of acronyms.

Going forward, I suggest significantly reworking the text to avoid presenting well-established demographic clines as novel findings. The authors should move the heavy technical descriptions of each analysis from the results section into the methods or supplement. In their place, the results section should provide a more rounded explanation of the goals of each analysis, why the questions are relevant, and what the findings mean. This should be tied directly to what is already known about human population history in these areas.

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I no longer have major concerns regarding the study, and I recommend the publication of the manuscript.

Minor comments:

Q1. Lines 314–316: Please avoid repeating verbatim the sentence already presented in lines 228–231; please rephrase accordingly.

Response: We thank the reviewer for pointing this out and we have rephrased the text. The revised sentences (originally in Lines 228-231, now in Lines 245-249) read as follows:

"Taken together, the host genomic profiles from West Eurasia, East Eurasia, and the Minusinsk Basin support a demographic landscape shaped by both direct migrations and indirect regional interactions. This broad population connectivity provides demographic context for the staged, long-distance dispersal of *Y. pestis* during its early diversification."

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Therefore, the authors should describe more clearly what is meant by "background-like levels of change". I am aware of the results described in section 4, but I am not sure what exactly the authors are suggesting here beyond the description of the already known adaptive changes.

Response: We thank the reviewer for this important clarification and apologize that our previous wording caused confusion. We agree that several key evolutionary changes on the basal LNBA$^+$ stem, including acquisition of *ymt*, have been described previously. Our intention was therefore not to claim discovery of these individual events, but to place them within a phylogenetic and genome-wide mutational context. Among the seven deep phases, only P5$^+$ on the basal LNBA$^+$ stem shows significant enrichment of disruptive genomic remodeling relative to genome-wide background mutation patterns. This wording is also not intended to imply the absence of lineage-specific evolutionary changes elsewhere in the tree, but rather to distinguish the concentrated disruptive remodeling observed on P5$^+$ from the weaker or different patterns observed across the other deep branches examined here.

Specifically, we mapped variants onto the seven deep phases around the LNBA$^+$/LNBA$^-$ split and compared the composition of mutation types on each phase against genome-wide background mutation patterns (Supplementary Data 5). In this analysis, P5$^+$ on the basal LNBA$^+$ stem shows a significant excess of disruptive changes, particularly due to gene-disrupting indels (Fisher's exact test, $P = 4.1 \times 10^{-6}$). By contrast, P4 shows only a weaker shift limited to nonsynonymous substitutions, and no other deep phase shows a comparable enrichment of disruptive variants under the same framework. Revised Supplementary Data 5 now provides the corresponding Fisher's exact test results.

To improve clarity, we revised the Abstract and main text accordingly. In particular, we removed the phrase "background-like levels of change", and replaced "reveals" with wording that more accurately reflects our contribution. The revised text now states that

the basal LNBA^{+} stem shows significant enrichment of disruptive genomic remodeling, whereas no other deep phase shows comparable enrichment under the same analytical framework. These revisions are now reflected in the Abstract (Lines 37-43), the Section 4 title and main text (Lines 264-265 and 270-278), the Fig. 4 legend (Lines 305-307), and the Methods description of the phase-based comparison (Lines 667-668).

Q2. There is one more formal point I would like to mention.

In the Editorial Policy Check list it says "We affirm that archaeological, geological, and palaeontological materials samples were collected (and, where applicable, exported) in a responsible manner and in accordance with relevant permits and local laws, and that this information is detailed within the manuscript."

I cannot find where this information is detailed within the manuscript.

Please add this information and/or clarify where it can be found in the manuscript.

Response: Thank you for pointing this out. We have now added this part at the very beginning of the Methods section (under the subheading “Ethical statement and sample permissions”, Lines 391-399). The added text reads as follows:

" This study relies on previously excavated archaeological remains and does not involve new excavations or research on living humans or animals. We affirm that all archaeological materials were originally collected, handled, and sampled in a responsible manner, in strict accordance with relevant local laws, regulations, and ethical guidelines. All newly reported ancient samples were analyzed with explicit permissions from the sample managers—who are also co-authors of this study—ensuring that destructive ancient DNA analysis was scientifically and ethically appropriate."

Reviewer #4 (Remarks to the Author)

This study reports the first evidence of an ancient *Yersinia pestis* genome from East

Asia, which seems like an exciting finding for the ancient pathogen community. I am a human population geneticist, and was asked to assess the human genetic analyses included in the study, therefore my review is focused exclusively on this aspect of the study.

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Rather than relying on these deep demographic connections to support specific transmission routes (or imply that they provide meaningful insights about the dynamics of specific transmission events), I recommend the authors use this genetic information to contextualize the populations involved. For instance, it would be valuable to more explicitly discuss how the divergence dates of the *Y. pestis* lineages align with the established human population histories of these regions, including Steppe migrations. Alternatively (or in addition), the authors could focus the human genetic analyses on demonstrating whether each newly identified individual appears to be a member of the local community or a genetic outlier, as they did for CY470.

Response: We thank the reviewer for this careful and constructive assessment of the human population genetic analyses. We agree with the central point that these data

should primarily provide demographic and archaeological context for the *Y. pestis*-positive individuals, rather than be used to infer specific transmission routes directly. In revising the manuscript, we therefore restructured this part of the study with two aims: first, to place the infected individuals more clearly within the established population history of their respective regions; and second, to evaluate whether the newly reported hosts are consistent with local community membership or instead appear genetically unusual in their site-specific contexts. We have accordingly revised both the Results and Methods to clarify the purpose, scope, and limitations of the human genomic analyses, and to present these results more explicitly as contextual evidence for prehistoric plague dispersal.

Below are more specific comments:

Q1. In general, there are too many acronyms used, and not enough time is spent describing the background associated with the individuals being studied. It would be helpful to provide more information about the geographic and temporal context in which they were found, and what is already known about the population history of individuals from these regions and periods.

Several of the newly identified *Y. pestis* positive individuals have very low human genome-wide coverage <0.1x. The authors should be explicit about the coverage of the individuals and about the limitations associated with analyzing data of this quality.

Response: We sincerely thank the reviewer for the helpful suggestions regarding the archaeological background and the transparency of our data quality. To improve readability and provide clearer context, we have revised the main text (Lines 203, and 235-236) to minimize the use of acronyms and explicitly state the site names and cultural affiliations for all studied individuals. These updates offer a more detailed geographic and temporal framework, helping readers better understand the population history and the relationships between these early communities.

Regarding the human genomic data, we now reported the mean coverage for each new individual in the main text (Lines 209 and 232). We agree with the reviewer that low-

coverage data (<0.1×) can introduce statistical uncertainty at the individual level. To address this and improve the reliability of our findings, we adopted a group-based analytical strategy. Instead of focusing on single individuals, we merged samples from the same site or the same cultural (e.g., the Zhengjiagou and Okunevo culture groups). This approach increases the genetic information available for analysis, which helps to reduce noise and provides more robust results for PCA and ancestry modeling. While we acknowledge the limitations of low-coverage data, we believe this grouping strategy provides a more robust basis for characterizing the broad ancestral profiles of these populations.

Q2. The PCA plots presented both in the main text and supplement are extremely broad. The plots show ancient individuals projected onto a continent-spanning background of present-day "Eurasian and Native American populations", so it is unsurprising that individuals sampled from closer geographic regions cluster together. Additionally, it would be good to provide more detailed information about how present-day populations are distributed in these plots, as they are currently all shown in grey with no annotations to help readers interpret their placement.

Response: We appreciate this point and agree that the original PCA presentation was too broad to be maximally informative. In the revised manuscript, we therefore reorganized the PCA analyses by constructing three distinct background datasets: (1) a broad Eurasian and Native American background to highlight the affinity of YP_preLNBA_RV2039 toward ANE-related gene pools (represented by MA1 and AG3), as shown in Supplementary Fig. 6a and b; (2) a Eurasian-focused background to provide higher resolution for observing the tight clustering of individuals from the Frälsegården, Warburg, and Zhengjiagou sites, as well as the genetic affinities between our *Y. pestis*-positive samples and corresponding period published individuals, as presented in the revised Fig. 3a and b; and (3) an East Asian-focused background to clarify the genetic relationships between the newly reported individuals from the Zhengjiagou site and other samples from the same site, as shown in Supplementary Fig.

6c and d.

Furthermore, we have updated the PCA plots in the Supplementary Information (Supplementary Fig. 7) to improve clarity: the generic grey circles have been replaced with color-coded population abbreviations, and we have provided a comprehensive table in the Supplementary Tables (Supplementary Data 13) that details the full names and linguistic affiliations corresponding to these abbreviations. These changes make the PCA results easier to read and better aligned with the narrower contextual questions addressed in the revised manuscript.

Q3. The authors should spend more time setting up each qpWave, qpAdm, and f-statistic test, explicitly describing the specific population models they are designed to differentiate between. As currently devised, these tests seem primarily focused on detecting very broad differences in ancestry that mirror already well-understood patterns. If that is the goal, the authors should be explicit about this and about what those results mean in the context of the spread of *Y. pestis*.

Response: We agree with the reviewer that the purpose of each population genetic analysis needed to be stated more clearly. In the revised manuscript, we have rewritten the relevant Results and Methods sections to clarify that these analyses are not intended to identify specific plague transmission routes or to present broad ancestry signals as novel discoveries in themselves. Rather, they are used to place the *Y. pestis*-positive individuals within the known demographic structure of Eurasia during the relevant time periods and to assess what kinds of population connections formed the broader context in which plague dispersal occurred.

Following the reviewer's suggestion, we now place greater emphasis on whether the *Y. pestis*-positive individuals appear genetically consistent with their local communities. Specifically, we employed extensive pairwise qpWave tests to assess the genetic affinities between individuals, which confirmed that the *Y. pestis*-positive individuals were not genetic outsiders but were well-integrated locals (Lines 191–196). Additionally, we utilized multiple analytical approaches to verify the high degree of

genetic similarity between previously published Okunevo individuals and our newly obtained samples (Lines 233–237). We have also revised the wording in the main text to avoid implying that these human genomic results directly reconstruct transmission events. Instead, we present them as demographic context showing that the infected hosts were embedded within broader patterns of regional connectivity, local continuity, and, in some cases, population turnover.

Q4. In Supplementary Data 4, the authors state: “The best feasible qpAdm model is highlighted in gray”. However, the criteria for selecting the "best" model are unclear. It is not appropriate to assume that the highest p-value corresponds to the most accurate population genetic model (as established in Harney et al., 2021), yet I suspect this may be the metric used. The authors should clarify their selection criteria, and if multiple models have p-values over the threshold for plausibility, they should be considered equally plausible.

Response: We thank the reviewer for this comment regarding the criteria for selecting the most feasible qpAdm model. In our analysis, we define a model as feasible only if it satisfies four specific statistical criteria: (1) a p-value > 0.05; (2) all ancestry coefficients larger than 0; (3) all ancestry coefficients greater than their standard errors; and (4) a p-nested value < 0.01. We emphasize that all models meeting these thresholds are considered statistically plausible, and we do not select models based solely on the p-values. To identify the most biologically and historically meaningful model among these plausible candidates, we further evaluated the chronological and geographical context of the source populations, generally requiring that source populations predate the target population unless a later source is justified by specific genetic structure. Additionally, we employed a rotation strategy to distinguish between genetically similar source populations and to ensure the robustness of our results. We have revised the Methods section (Lines 637-643) to clarify these selection criteria and to state that all models passing the aforementioned thresholds are considered equally plausible.

Q5. A single paragraph is used to describe all of the human population genetic analyses, including PCA, f-statistics, and qpWave/qpAdm analyses. The descriptions of each tool (including considering each type of f-statistic separately) should be described separately, noting what parameters were used for each analysis.

Response: We thank the reviewer for this suggestion. In the revised manuscript, we have revised the Methods section (Lines 595-643) to describe each analytical tool, including PCA, f-statistics, qpWave, and qpAdm, in separate subsections. In this updated version, we have provided specific details for each method, including the parameters used for PCA, the specific configurations and interpretation of the f-statistics calculated, and the specific settings used for qpWave and qpAdm. These revisions were made to improve clarity and reproducibility and to make the role of each method in the study more explicit.

Reviewer's comment:

Reviewer #4 (Remarks to the Author):

The authors have made significant improvements to the methodological descriptions in the methods section of the manuscript, and I am happy to see that they shifted the focus of many of their analyses to assess whether each focal individual's ancestry matches the local ancestry observed among other individuals in the population or if they appear to be migrants.

However, I still think that the manuscript's framing will make it difficult for readers to understand what is already known about human population history and how that population history might relate to the spread of Y. pestis. Furthermore, the overall readability of the human population genetics section needs improvement, as the narrative is frequently interrupted by methodological details and a heavy use of acronyms.

Going forward, I suggest significantly reworking the text to avoid presenting well-established demographic clines as novel findings. The authors should move the heavy technical descriptions of each analysis from the results section into the methods or supplement. In their place, the results section should provide a more rounded explanation of the goals of each analysis, why the questions are relevant, and what the findings mean. This should be tied directly to what is already known about human population history in these areas.

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- Lines 196–197: There appear to be a few typos here (such as a rogue closing parenthesis and missing spaces) that make this sentence difficult to follow. Please review and correct.

- Figure 3: Figure 3 should be amended to avoid implying that the clines being shown are specific to the transmission of plague, rather than reflecting broader, well-established population history.

Response to Reviewer #4

We thank the reviewer for clearly identifying these issues. In accordance with the editor's instruction, we have completely removed all host human genomic data and associated analyses from the manuscript; details of these revisions are provided in our response to the editor above.