Plague in Denmark (1000-1800)

**A longitudinal study of *Yersinia pestis***

## Authors

* **Katherine Eaton**\* [0000-0001-6862-7756](https://orcid.org/0000-0001-6862-7756)  
   Department of Anthropology, McMaster University; McMaster Ancient DNA Center
* **Ravneet Sidhu**  
   Department of Anthropology, McMaster University; McMaster Ancient DNA Center
* **Jennifer Klunk** [0000-0002-6521-8516](https://orcid.org/0000-0002-6521-8516)  
   McMaster Ancient DNA Center; Daicel Arbor Biosciences
* **Julia Gamble** [0000-0001-7486-757X](https://orcid.org/0000-0001-7486-757X)  
   Department of Anthropology, University of Manitoba
* **Jesper Boldsen** [0000-0002-2850-0934](https://orcid.org/0000-0002-2850-0934)  
   ADBOU, University of Southern Denmark
* **Ann Carmichael**  
   Department of History, Indiana University Bloomington
* **Nükhet Varlık** [0000-0001-6870-5945](https://orcid.org/0000-0001-6870-5945)  
   Department of History, Rutgers University-Newark
* **Sebastian Duchene** [0000-0002-2863-0907](https://orcid.org/0000-0002-2863-0907)  
   The Peter Doherty Institute for Infection and Immunity, University of Melbourne
* **Leo Featherstone** [0000-0002-8878-1758](https://orcid.org/0000-0002-8878-1758)  
   The Peter Doherty Institute for Infection and Immunity, University of Melbourne
* **Vaughan Grimes** [0000-0002-2177-3147](https://orcid.org/0000-0002-2177-3147)  
   Department of Archaeology, Memorial University
* **Brian Golding** [0000-0002-7575-0282](https://orcid.org/0000-0002-7575-0282)  
   Department of Biology, McMaster University
* **Sharon DeWitte** [0000-0003-0754-8485](https://orcid.org/0000-0003-0754-8485)  
   Department of Anthropology, University of South Carolina
* **Hendrik Poinar** [0000-0002-0314-4160](https://orcid.org/0000-0002-0314-4160)  
   Department of Anthropology, McMaster University; McMaster Ancient DNA Center

## Abstract

The epidemiology of plague in the past is highly controversial, owing to the scarcity and ambiguity of historical evidence. A frequent source of debate is the re-emergence and continuity of plague in Europe during the 14th to 18th centuries CE. Scandinavia is particularly underrepresented in the historical archives, despite having a uniquely long history of plague (5000 years) as revealed through ancient DNA analysis. To better understand the evolutionarhistorical evolutionary history and historical epidemiology of plague in this region, we performed in-depth (N=298), longitudinal screening (800 years) for the plague bacterium, *Yersinia pestis*, across 13 archaeological sites in Denmark. We captured the emergence and continuity of *Y. pestis* in this region over a period of 400 years (14th - 17th century CE), for which the plague-positivity rate was 8.3% (3.3% - 14.3% by site). These results deepen the epidemiological link between the plague bacterium and the unknown *pestilence* that afflicted medieval and early modern Europe. Furthermore, this study paves the way for the next generation of historical disease research, in which hypotheses concerning mortality can be tested using population-scale, genomic evidence from ancient pathogens.

## Introduction

Europe endured a 500-year long pandemic of plague from the 14th to 18th centuries CE [[1](#ref-1CPIgshmC)]. During this period, the Second Pandemic of Plague, outbreaks reoccurred approximately every 10 years with mortality estimates as high as 50% during the infamouswhat is now often referred to as the Black Death (1346-1353) [[2](#ref-NS5uCsyk)]. Paleogeneticists have identified the plague bacterium *Yersinia pestis* as the most likely agent, although the epidemiology of this pandemic remains controversial [[3](#ref-pePeAsdw)], where the major source of debate remains: mortality and spread. The ecology of *Y. pestis* is highly complex, and involves both zoonotic “spillover” from rodent populations as well as inter-human transmission [[4](#ref-uM6Rh5Fu)]. As a result, both disease exposure and spread are known to vary between regions and over time [[3](#ref-pePeAsdw)]. These differences are challenging to reconcile, and have led to significant controversy concerning the location of plague reservoirs in Europe during the pandemics long reign [[5](#ref-q03vv4Sd)].

Recent studies have explored this question by synthesizing genetic evidence [[5](#ref-q03vv4Sd)] and historical records [[6](#ref-Fm9dbzGl),[7](#ref-1093vihdz)] across Europe. These sources have significant geographic gaps, such as the complete lack of evidence from Scandinavia in digitized databases [[8](#ref-d3V1G36x)], attributed to the sparseness of historical sources and ambiguity with regards to disease terminology during the medieval period [[2](#ref-NS5uCsyk)]. Recent ancient DNA research [[9](#ref-AQa9Tn4j)] revealed that the history of plague in Scandinavia is among the oldest in the world, and established the presence of a sister strain of pandemic *Y. pestis* in Sweden 5000 years ago. This detection of *Y. pestis* in a Bronze Age individual raises the intriguing possibility of long-term persistence of plague in Scandinavia, with pandemic *Y. pestis* re-emerging as a local, endemic disease.

To evaluate the evolutionary history of the plague bacterium in Denmark and the possibility of undocumented plague persistence, we screened for the presence of *Y. pestis* in the Anthropological DataBase Odense University (ADBOU) collection. This extraordinary collection includes preserved and curated skeletal remains from over 16,000 Danish individuals, dated from the Viking Age to the Early Modern period. To ensure a wide variety of locations were represented, we sampled 298 individuals across 13 archaeological sites from the mainland (Jutland), as well as two islands (Funen and Lolland). Based on the skeletal and radiocarbon (?) dates, these individuals represent 800 years of population history (1000-1800 CE) which includes both the historically associated pandemic period in Denmark (1350-1657) as well as the so-called quiescent periods (1000-1350 CE, 1658-1800) for which no outbreaks of plague were historically documented [[3](#ref-pePeAsdw)].

## Results and Discussion

We detected *Y. pestis* in 7 archaeological sites using PCR assays and targeted capture coupled sequencing (Figure 1A). Across the 7 sites, 8.3% of individuals (13/157) tested positive for *Y. pestis*, ranging from 3.3% at Ribe Lindegärden to 14.3% at Hågerup. This positivity rate is likely an underestimate of the ‘true’ prevalence of *Y. pestis* in Danish populations, due to variable DNA preservation. On the other hand, it may be an overestimate due to the effects of selective mortality (disproportionate deaths of frail indidivduals) that likely make assemblages of deceased individuals unrepresentative of the living, i.e., the osteological paradox [[10](#ref-a0Rr24xp)]. While the exact extrapolation is unclear, our *Y. pestis* positivity rate (3.3 - 14.3%) does align with mortality estimates (5 - 20%) during the later epidemics of the medieval and early modern period, at least in some contexts [[11](#ref-SDiEWfMf),[12](#ref-13NOJLbvF)].

* **Citation recommendations for plague mortality ranges?**

Of the 13 plague-positive individuals, 9 had sufficient sequencing depth (>3X) of the *Y. pestis* chromosome for phylogenetic analysis (Figure 2C). To estimate a time-scaled phylogeny and tip dates for these 9 samples, we fit a relaxed molecular clock to an alignment of *Y. pestis* genomes which included 40 other isolates (Figure 1B). Interestingly, Danish strains did not cluster togther, but rather within existing diversity of medieval and early modern *Y. pestis* in Europe with strong support (posterior: 1.0) (Figure 3). We found no evidence to suggest that these strains were the descendant of the Neolithic lineages of *Y. pestis* in Scandinavia (5000 YBP) [[9](#ref-AQa9Tn4j)].

We found no evidence of *Y. pestis* in Denmark between 1000 and 1300 CE. The factors influencing the preservation of ancient DNA are wide-ranging and complex, thus the absence of evidence cannot prove evidence of absence. That being said, we sampled a minimum of 85 individuals and a maximum of 165 individuals that pre-date the 14th century (Figure 2A). With a mean positivity rate observed in this study (8.3%), we expected to detect *Y. pestis* in 7 to 13 individuals from this time frame if it were present. We therefore interpret our negative results from this period as tentative evidence that *Y. pestis* was a relatively new pathogen in medieval Denmark, that did not become abundant and/or widespread until at least the 14th century.

The earliest evidence of *Y. pestis* in Denmark was found in the town of Ribe from two individuals associated from the first half of the 14th century, dated to 1333 (1301-1366) and 1350 (1319-1383). These estimates are highly congruent with the historical record, which first documented the appearance of plague in Denmark at Ribe in 1349 [[13](#ref-1G9pdnarW)]. Furthermore, these strains fell within an unresolved cluster (posterior: 0.15) of samples from Northern and Western Europe (Figure 3) which has previously been linked to the clonal spread of the Black Death (1343-1356) [[1](#ref-1CPIgshmC)]. Our molecular dates support this historical association, albeit only weakly, as the precise epidemic period cannot be resolved due to the large confidence intervals of our estimates [input range here] (>50 years).

The next period in which we identified *Y. pestis* was in the latter half of the 14th century from a third individual from Ribe, dated to 1370 (1336-1408) strongly clustered (posterior: 0.99) with post-Black Death samples from The Netherlands and Russia. These samples have previously been attributed to the *pestis secunda* (1357-1366) [[14](#ref-1BWm60ySL)], although we find the *pestis tertia* (1364-76) [[12](#ref-13NOJLbvF)] to be an equally likely candidate. This clade also has broader epidemiological significance, as it is directly ancestral to the Third Pandemic of plague (19th-20th century) [[1](#ref-1CPIgshmC)]. Our results therefore reveal new global connections, as the same lineage that afflicted medieval Danish populations would later re-emerge to cause modern epidemics of plague, including recent outbreaks in Madagascar [[15](#ref-Oxqt5mfU)].

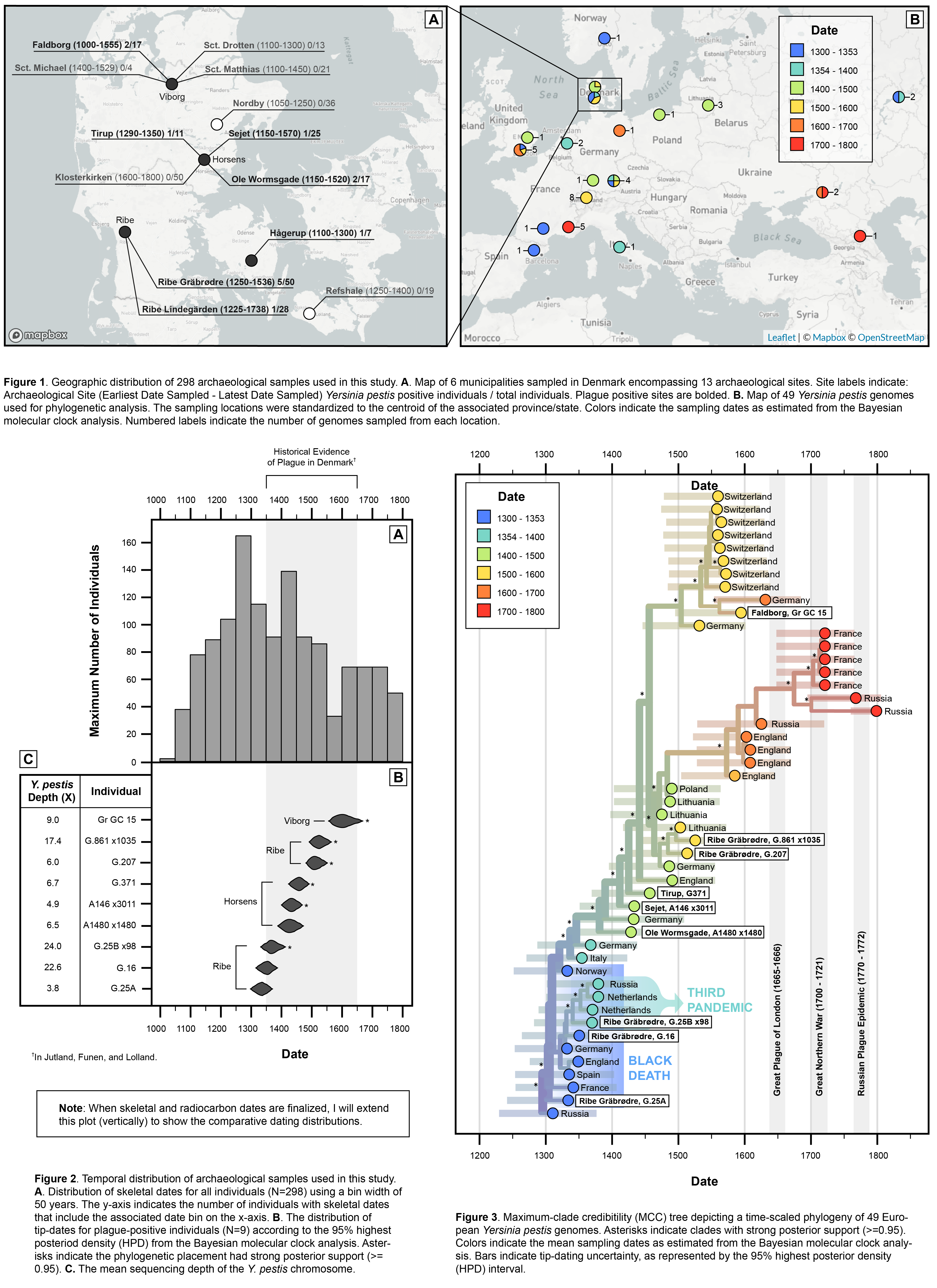
We found no plague at Ribe between 1408 and 1484. This was surprising, as 86% of individuals (43/50) from this site were archaeologically dated to between 1400 and 1536. Instead, the distribution of *Y. pestis* shifts during this period from the eastern coast of Jutland to the western coast of XX. We recovered three distinct, and possibly contemporaneous isolates of *Y. pestis* from three sites near Horsens dated to 1429 (1392-1467), 1433 (1403-1464) and 1457 (1427-1487). Interestingly, these genomes were most closely related to *Y. pestis* strains isolated from individuals sampled in Germany, Lithuania, Poland, and England (Figure 3). This geographic association parallels the historical record, in which outbreaks in Denmark coincided with those in the Baltic region [[12](#ref-13NOJLbvF)]. Recent studies have demonstrated that the directionality and spread of zoonotic diseases cannot be robustly inferred from genomic data alone [**???**,[16](#ref-iPczwfq8)]. Instead, our results establish an epidemiological link between *Y. pestis* and historical case records in Denmark, which could be jointly modeled with greater resolving power [[17](#ref-eoKChWDW)] in future work.

* **The missing citation is our other manuscript (in prep) on global plague phylogenetics.**

In the 16th century, we once again observed *Y. pestis* at Ribe from two individuals dated to 1513 (1484-1546) and 1525 (1494-1560). Furthermore, we also found evidence of *Y. pestis* in the northern site of Faldborg, some XX km away, dated to 1594 (1550-1649). Our estimate of plague’s disappearance (1649), is congruent with the historical record which documents the last recorded outbreak of plague in Jutland in the years 1654-1657 [[3](#ref-pePeAsdw)]. We found no evidence of *Y. pestis* in Denmark after this point, between 1649 and 1800 CE. While, no individuals definitively post-date 1649 CE, the period could potentially include a maximum of 70 individuals (Figure 2A) for which would expect to detect *Y. pestis* in 0 to 2 individuals (3.3%). Our results do not differ from this expectation, and are therefore not informative with regards to the disappearance of *Y. pestis* in Denmark. To address this question, additional samples would be required from the 17th and 18th centuries..

## Conclusion

This study marks the first population-level analysis of ancient *Y. pestis*, from an in-depth (N=298), longitudinal sampling (800 years) within a single country (Denmark). We have described the earliest known appearance of *Y. pestis* in Denmark (14th century), and document its continuity in Scandinavia over a period of 400 years (17th century). Furthermore, we provide the first positivity rates of historical plague from molecular evidence, as we detected *Y. pestis* in an average of 8.3% of Danish individuals. Our phylogenetic analysis was highly congruent with the sparse textual evidence of *pestilence* in Denmark, with regards to the timing of outbreaks and geographic ties to the Baltic region. We also provide novel evidence of plague exposure among Danish populations, such as the site of Tirup, where there is no surviving historical evidence. These results are of importance for both researchers of plague and other infectious diseases, as they (1) illuminate undocumented pathogens in the historical record, (2) reveal new connections between our past and present experience of plague, (3) broaden our understanding of the epidemiology of re-emerging diseases.



## Materials and Methods

We sampled 298 individuals across 13 archaeological sites in Denmark (Figure 1A, Dataset S1). Site occupation dates spanned from the 11th to the 19th century CE. We estimated individual date ranges based on burial position, which was categorized according to cultural shifts that occurred in Denmark throughout the medieval and early modern period [[18](#ref-eD3kpkYB)]. When the original stratigraphic context was preserved, we refined these individual estimates further. For individuals with ambiguous or conflicting archaeological estimates, we performed radiocarbon dating when additional destructive sampling was permitted.

DNA was extracted from teeth (root or dental pulp) according to a specialized protocol for ancient DNA [[19](#ref-9kFCN7oR)]. Reagent blanks were introduced as negative controls to monitor DNA contamination in subsequent steps. We screened for plague using a PCR assay that targets the *pla* virulence gene in *Yersinia pestis* [[20](#ref-ACt53Sow)]. Extracts showing amplification in at least 4/6 replicates were converted into paired-end sequencing libraries [[21](#ref-sVvw7Kko)]. Targeted capture of the *Y. pestis* genome was performed using previously designed probes [[20](#ref-ACt53Sow)] and sequenced on an Illumina platform.

Sequenced molecules were aligned to a reference genome using the *nf-core/eager* pipeline [[22](#ref-17yD9OrGW)]. To phylogenetically place these new samples, we downloaded a comparative dataset of 40 high-coverage *Y. pestis* genomes (>3X) dated between the 14th and 18th centuries. We then constructed a multiple alignment with the [snippy](https://github.com/tseemann/snippy) pipeline, which included 356 variation positions and 4,289,810 constant sites.

To tip-date each genome, we performed a Bayesian Evaluation of Temporal Signal (BETS) [[23](#ref-zikRADit)] with BEAST2 [[24](#ref-U9NYNgQR)]. We assumed a constant population size and compared the use of a strict clock and an uncorrelated lognormal (UCLN) relaxed clock. Diffuse normal priors were constructed for all tip-dates, using the mean radiocarbon/mortuary date and half the uncertainty as the standard deviation. All samples were assigned equivalent priors with a mean date of 1330 CE and a standard deviation of 115 years. Bayes factors were calculated by comparing the marginal likelihoods of each candidate model, as estimated with a generalized stepping stone (GSS) computation. The model with the highest marginal likelihood was then run for 100,000,000 generations to ensure the effective sample size (ESS) of all relevant parameters was greater than 200.

Sebastian and Leo had important cautionary notes about my priors being improper. Could we meet to discuss?

Data visualization was performed using the python package *seaborn* and *auspice*, a component of the Nextstrain visualization suite [[25](#ref-S0T839fB)].

## Data Availability

Raw sequence reads have been deposited in NCBI BioProject PRJNAXXXXX. Archaeological metadata is provided in the supplementary information (Dataset SI).

## Acknowledgments

To Be Done, so many people to recognize and thank :)

## References

1. **Phylogeography of the second plague pandemic revealed through analysis of historical Yersinia pestis genomes**   
Maria A. Spyrou, Marcel Keller, Rezeda I. Tukhbatova, Christiana L. Scheib, Elizabeth A. Nelson, Aida Andrades Valtueña, Gunnar U. Neumann, Don Walker, Amelie Alterauge, Niamh Carty, … Johannes Krause  
*Nature Communications* (2019-10-02) <https://www.nature.com/articles/s41467-019-12154-0>   
DOI: [10.1038/s41467-019-12154-0](https://doi.org/10.1038/s41467-019-12154-0)

2. **“In These Perilous Times”: Plague and Plague Policies in Early Modern Denmark**   
Peter Christensen  
*Medical History* (2003-10) <https://www.cambridge.org/core/journals/medical-history/article/in-these-perilous-times-plague-and-plague-policies-in-early-modern-denmark/EFF71835DE9EBFB610E35451FD7A0A86>   
DOI: [10.1017/s0025727300057331](https://doi.org/10.1017/S0025727300057331)

3. **The Black Death and Later Plague Epidemics in the Scandinavian Countries: Perspectives and Controversies**   
Ole Jørgen Benedictow  
*De Gruyter Open Poland* (2016-12-19) <https://www.degruyter.com/document/doi/10.1515/9788376560472/html>   
ISBN: [978-83-7656-047-2](https://worldcat.org/isbn/978-83-7656-047-2)

4. **Yersinia pestis–etiologic agent of plague**   
R. D. Perry, J. D. Fetherston  
*Clinical Microbiology Reviews* (1997-01)   
PMID: [8993858](https://www.ncbi.nlm.nih.gov/pubmed/8993858) · PMCID: [PMC172914](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC172914)

5. **Assessing the origins of the European Plagues following the Black Death: a synthesis of genomic, historical and ecological information**   
Barbara Bramanti, Yarong Wu, Ruifu Yang, Yujun Cui, Nils Chr Stenseth  
*bioRxiv* (2021-04-20) <https://www.biorxiv.org/content/10.1101/2021.04.20.440561v1>   
DOI: [10.1101/2021.04.20.440561](https://doi.org/10.1101/2021.04.20.440561)

6. **Climate-driven introduction of the Black Death and successive plague reintroductions into Europe**   
Boris V. Schmid, Ulf Büntgen, W. Ryan Easterday, Christian Ginzler, Lars Walløe, Barbara Bramanti, Nils Chr Stenseth  
*Proceedings of the National Academy of Sciences* (2015-03-10) <http://www.pnas.org/content/112/10/3020>   
DOI: [10.1073/pnas.1412887112](https://doi.org/10.1073/pnas.1412887112) · PMID: [25713390](https://www.ncbi.nlm.nih.gov/pubmed/25713390)

7. **Trade routes and plague transmission in pre-industrial Europe**   
Ricci P. H. Yue, Harry F. Lee, Connor Y. H. Wu  
*Scientific Reports* (2017-10-11) <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5636801/>   
DOI: [10.1038/s41598-017-13481-2](https://doi.org/10.1038/s41598-017-13481-2) · PMID: [29021541](https://www.ncbi.nlm.nih.gov/pubmed/29021541) · PMCID: [PMC5636801](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5636801)

8. **Dangers of Noncritical Use of Historical Plague Data**   
Joris Roosen, Daniel R. Curtis  
*Emerging Infectious Diseases* (2018-01) <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5749453/>   
DOI: [10.3201/eid2401.170477](https://doi.org/10.3201/eid2401.170477) · PMID: [null](https://www.ncbi.nlm.nih.gov/pubmed/null) · PMCID: [PMC5749453](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5749453)

9. **Emergence and spread of basal lineages of *Yersinia pestis* during the Neolithic Decline**   
Nicolás Rascovan, Karl-Göran Sjögren, Kristian Kristiansen, Rasmus Nielsen, Eske Willerslev, Christelle Desnues, Simon Rasmussen  
*Cell* (2019-01-10) <https://www.cell.com/cell/abstract/S0092-8674(18)31464-8>   
DOI: [10.1016/j.cell.2018.11.005](https://doi.org/10.1016/j.cell.2018.11.005) · PMID: [30528431](https://www.ncbi.nlm.nih.gov/pubmed/30528431)

10. **The Osteological Paradox: Problems of Inferring Prehistoric Health from Skeletal Samples**   
James W. Wood, George R. Milner, Henry C. Harpending, Kenneth M. Weiss, Mark N. Cohen, Leslie E. Eisenberg, Dale L. Hutchinson, Rimantas Jankauskas, Gintautas Cesnys, Gintautas Česnys, … Richard G. Wilkinson  
*Current Anthropology* (1992) <https://www.jstor.org/stable/2743861>

11. **Black Death Bodies**   
Sharon N. ; Kowaleski Dewitte  
*Fragments: Interdisciplinary Approaches to the Study of Ancient and Medieval Pasts* (2017) <http://hdl.handle.net/2027/spo.9772151.0006.001>

12. **Out of the West: Formation of a Permanent Plague Reservoir in South-Central Germany (1349–1356) and its Implications**   
Philip Slavin  
*Past & Present* (2021-01-25) <https://doi.org/10.1093/pastj/gtaa028>   
DOI: [10.1093/pastj/gtaa028](https://doi.org/10.1093/pastj/gtaa028)

13. **The Black Death**   
Kristina Lenz, Nils Hybel  
*Scandinavian Journal of History* (2016) <https://journals.scholarsportal.info/details/03468755/v41i0001/54_tbd.xml>   
DOI: [10.1080/03468755.2015.1110533](https://doi.org/10.1080/03468755.2015.1110533)

14. **Integrative approach using *Yersinia pestis* genomes to revisit the historical landscape of plague during the Medieval Period**   
Amine Namouchi, Meriam Guellil, Oliver Kersten, Stephanie Hänsch, Claudio Ottoni, Boris V. Schmid, Elsa Pacciani, Luisa Quaglia, Marco Vermunt, Egil L. Bauer, … Barbara Bramanti  
*Proceedings of the National Academy of Sciences* (2018-12-11) <http://www.pnas.org/lookup/doi/10.1073/pnas.1812865115>   
DOI: [10.1073/pnas.1812865115](https://doi.org/10.1073/pnas.1812865115)

15. **The 2017 plague outbreak in Madagascar: Data descriptions and epidemic modelling**   
Van Kinh Nguyen, César Parra-Rojas, Esteban A. Hernandez-Vargas  
*Epidemics* (2018-12)   
DOI: [10.1016/j.epidem.2018.05.001](https://doi.org/10.1016/j.epidem.2018.05.001) · PMID: [29866421](https://www.ncbi.nlm.nih.gov/pubmed/29866421)

16. **Sampling bias and model choice in continuous phylogeography: Getting lost on a random walk**   
Antanas Kalkauskas, Umberto Perron, Yuxuan Sun, Nick Goldman, Guy Baele, Stephane Guindon, Nicola De Maio  
*PLOS Computational Biology* (2021-01-06) <https://journals.plos.org/ploscompbiol/article?id=10.1371/journal.pcbi.1008561>   
DOI: [10.1371/journal.pcbi.1008561](https://doi.org/10.1371/journal.pcbi.1008561)

17. **Inferring infectious disease phylodynamics with notification data**   
Sebastián Duchêne, Francesca Di Giallonardo, Edward C. Holmes, Timothy G. Vaughan  
*bioRxiv* (2019-04-08) <https://www.biorxiv.org/content/10.1101/596700v1>   
DOI: [10.1101/596700](https://doi.org/10.1101/596700)

18. **Leprosy in Medieval Denmark — Osteological and epidemiological analyses**   
Jesper L. Boldsen  
*Anthropologischer Anzeiger* (2009) <http://www.jstor.org/stable/29543069>

19. **Complete mitochondrial genome sequence of a Middle Pleistocene cave bear reconstructed from ultrashort DNA fragments**   
J. Dabney, M. Knapp, I. Glocke, M.-T. Gansauge, A. Weihmann, B. Nickel, C. Valdiosera, N. Garcia, S. Paabo, J.-L. Arsuaga, M. Meyer  
*Proceedings of the National Academy of Sciences* (2013-09-24) <http://www.pnas.org/cgi/doi/10.1073/pnas.1314445110>   
DOI: [10.1073/pnas.1314445110](https://doi.org/10.1073/pnas.1314445110)

20. ***Yersinia pestis* and the Plague of Justinian 541–543 AD: a genomic analysis**   
David M Wagner, Jennifer Klunk, Michaela Harbeck, Alison Devault, Nicholas Waglechner, Jason W Sahl, Jacob Enk, Dawn N Birdsell, Melanie Kuch, Candice Lumibao, … Hendrik Poinar  
*The Lancet Infectious Diseases* (2014-04) <https://linkinghub.elsevier.com/retrieve/pii/S1473309913703232>   
DOI: [10.1016/s1473-3099(13)70323-2](https://doi.org/10.1016/S1473-3099(13)70323-2)

21. **Double indexing overcomes inaccuracies in multiplex sequencing on the Illumina platform**   
Martin Kircher, Susanna Sawyer, Matthias Meyer  
*Nucleic Acids Research* (2012-01-01) <https://academic.oup.com/nar/article/40/1/e3/1287690>   
DOI: [10.1093/nar/gkr771](https://doi.org/10.1093/nar/gkr771)

22. **Reproducible, portable, and efficient ancient genome reconstruction with nf-core/eager**   
James A. Fellows Yates, Thiseas C. Lamnidis, Maxime Borry, Aida Andrades Valtueña, Zandra Fagernäs, Stephen Clayton, Maxime U. Garcia, Judith Neukamm, Alexander Peltzer  
*PeerJ* (2021-03-16) <https://peerj.com/articles/10947>   
DOI: [10.7717/peerj.10947](https://doi.org/10.7717/peerj.10947)

23. **Bayesian Evaluation of Temporal Signal in Measurably Evolving Populations**   
Sebastian Duchene, Philippe Lemey, Tanja Stadler, Simon YW Ho, David A Duchene, Vijaykrishna Dhanasekaran, Guy Baele  
*Molecular Biology and Evolution* (2020-11-01) <https://doi.org/10.1093/molbev/msaa163>   
DOI: [10.1093/molbev/msaa163](https://doi.org/10.1093/molbev/msaa163)

24. **BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis**   
Remco Bouckaert, Timothy G. Vaughan, Joëlle Barido-Sottani, Sebastián Duchêne, Mathieu Fourment, Alexandra Gavryushkina, Joseph Heled, Graham Jones, Denise Kühnert, Nicola De Maio, … Alexei J. Drummond  
*PLOS Computational Biology* (2019-04-08) <https://journals.plos.org/ploscompbiol/article?id=10.1371/journal.pcbi.1006650>   
DOI: [10.1371/journal.pcbi.1006650](https://doi.org/10.1371/journal.pcbi.1006650)

25. **Nextstrain: real-time tracking of pathogen evolution**   
James Hadfield, Colin Megill, Sidney M. Bell, John Huddleston, Barney Potter, Charlton Callender, Pavel Sagulenko, Trevor Bedford, Richard A. Neher  
*Bioinformatics* (2018-12-01) <https://academic.oup.com/bioinformatics/article/34/23/4121/5001388>   
DOI: [10.1093/bioinformatics/bty407](https://doi.org/10.1093/bioinformatics/bty407)

## Supplementary Information

These are additional figures/data I anticipate co-authors or reviewers may want in the SI:

* Date distributions by site (1 page, 13 subplots)
* Maximum-likelihood phylogeny (1 page)
* Individual priors (1 page, ~20 subplots)