












The Plague in Denmark (1000-1800)

Yersinia pestis something

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Introduction

Second Pandemic

- The Second Pandemic of plague devastated Europe (14th - 18th centuries)

Denmark

- The etiology and epidemiology of plague in Scandinavia is contentious.
- Historical sources were sparse and unreliable [1].
- Outbreaks the 14th and 15th centuries were poorly documented.
- "disease terminology is certainly vague and ambiguous... pestis, pestilence and derivatives... were also used to characterize a number of diseases"
- Textual evidence became much more plentiful and precise in the 16th century. At this point, the origins and frequency of epidemics could be more clearly identified.
- *"The outbreaks clearly coincided with larger European incidences of the disease and often the sources expressly state that the infection had come to Denmark from the Baltic region or northern Germany."* [2]
- Plague outbreaks re-occurred approximately every 10 years, lasting 1-5 years [1,3]
- "The plague history of Sweden and Denmark is much less studied than Norway's, but the incidence of importation, seasonality, and temporal rhythms of plague epidemics were clearly different, especially after 1400.47 The reason was evidently that these countries did not have such strong commercial connections with England and mostly imported plague from the Baltic region and the Hanseatic cities, and, in the later part of the plague pandemic, also from the Netherlands." [4]
- One of the most famous digitized databases has no data from Denmark [5; roosen2018DangersNoncriticalUse].

Objectives

- We tested for the presence of *Y. pestis* in medieval and early modern Denmark.
- We used molecular assays to screen the skeletal remains of 298 individuals across 13 archaeological sites, dated to between 1000 and 1800 century CE.
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Results and Discussion

We detected *Y. pestis* in 7 archaeological sites using PCR assays and targeted sequencing (Figure 1A). Across the 7 sites, 8.3% of individuals (13/157) tested positive for *Y. pestis*, ranging from 3.3% at Ribe Lindegården to 14.3% at Hågerup. On the one hand, this positivity rate could be considered 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 osteological paradox [6], in which mortality is selective and the deceased are not representative of the living population. While the exact extrapolation is unclear, our *Y. pestis* positivity rate (3.3 - 14.3%) does align with mortality estimates (5 - 15%) during the later epidemics of the Second Pandemic [2,7].

Of the 13 plague-positive individuals, 9 had sufficient sequencing depth (>3X) of the *Y. pestis* chromosome for phylogenetic analysis (Figure 2B). To estimate a time-scaled phylogeny and dates for each sample, we fit a relaxed molecular clock to an alignment of 49 *Y. pestis* genomes dated to the Second Pandemic (Figure 1B).

We found no evidence of *Y. pestis* in Denmark between 1000 and 1300 CE. However, the factors influencing the preservation of ancient DNA are wide-ranging and complex [8] and 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). Taking the mean positivity rate observed in this study (8.3%), we would expect to detect *Y. pestis* in 7 to 13 individuals from this time frame if it were present. We therefore interpret this 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. Two individuals were associated with *Y. pestis* 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, as the first documented appearance of plague in Denmark was at Ribe in 1349 [3]. 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 Black Death (1343-1356) [9]. 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 (>50 years).

The next period in which we identified *Y. pestis* was in the latter half of the 14th century. A third isolate from Ribe was dated to 1370 (1336-1408) and 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) [10], although we find the *pestis tertia* (1364-76) [2] 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) [9]. 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 the recent outbreaks in Madagascar [11].

We observed a gap in the continuity of plague at Ribe, as no *Y. pestis* was detected there 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* appeared to shift during this period from the eastern coast of Jutland to the western coast. We recovered 3 distinct, and possibly contemporaneous, isolates of *Y. pestis* from 3 sites near Horsens dated to 1429 (1392-1467), 1433 (1403-1464) and 1457 (1427-1487). These genomes were most closely related to 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 [2]. However, recent studies have demonstrated that the directionality and spread of zoonotic diseases cannot be robustly inferred from genomic data alone [???,12]. 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 [13] in future work.

In the 16th century, we once again observed *Y. pestis* at Ribe. We dated two *Y. pestis* isolates from this region to 1513 (1484-1546) and 1525 (1494-1560). Furthermore, we also found evidence of *Y. pestis* in the northern site of Faldborg dated to 1594 (1550-1649). As an estimate of plague's disappearance (1649), this is congruent with the historical record which documents the last recorded outbreak of plague in Jutland to last from 1654-1657. We found no evidence of *Y. pestis* in Denmark after this point, specifically between 1649 and 1800 CE. However, no individuals definitively post-date 1649 CE, although this period could include a maximum of 70 individuals (Figure 2A). We would therefore expect to detect *Y. pestis* in 0 to 2 individuals (3.3%) from this time frame if it were present. 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*, where we performed in-depth (N=298), longitudinal sampling (800 years) within a single country (Denmark). We describe the earliest known appearance of *Y. pestis* in Denmark (14th century), and document the continuity of this pathogen 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 8.3% of Danish individuals. Our phylogenetic analysis of 9 *Y. pestis* genomes 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 disease exposure among Danish populations, such as the site of Tirup, where there is little to no surviving historical evidence. These results are of importance for researchers of both plague and other infectious diseases, as they (1) document unseen 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.

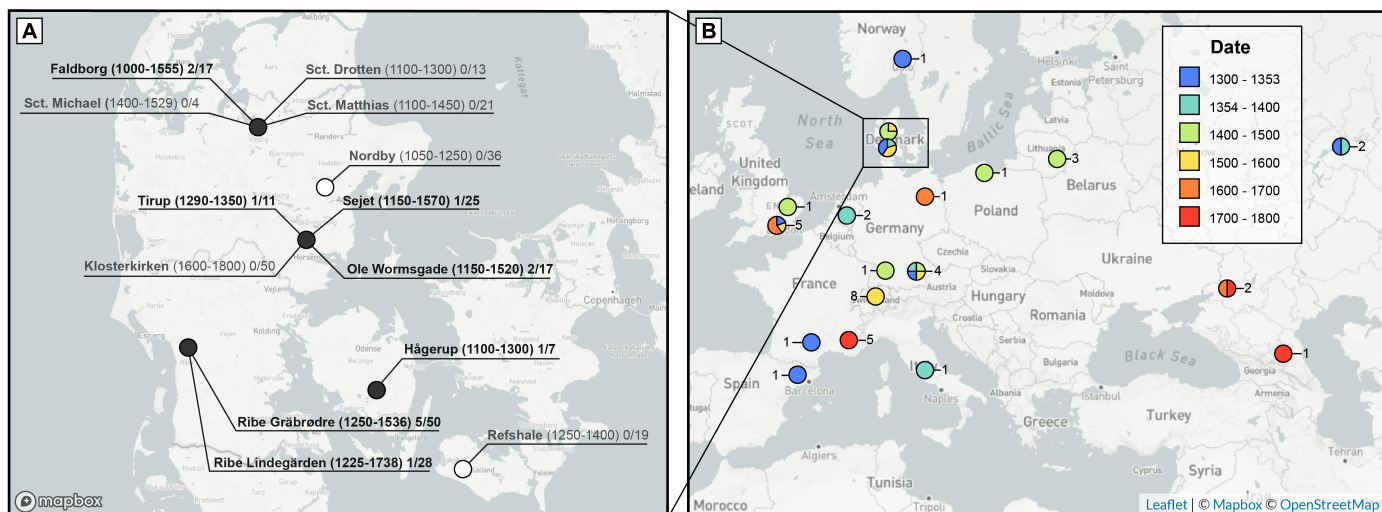
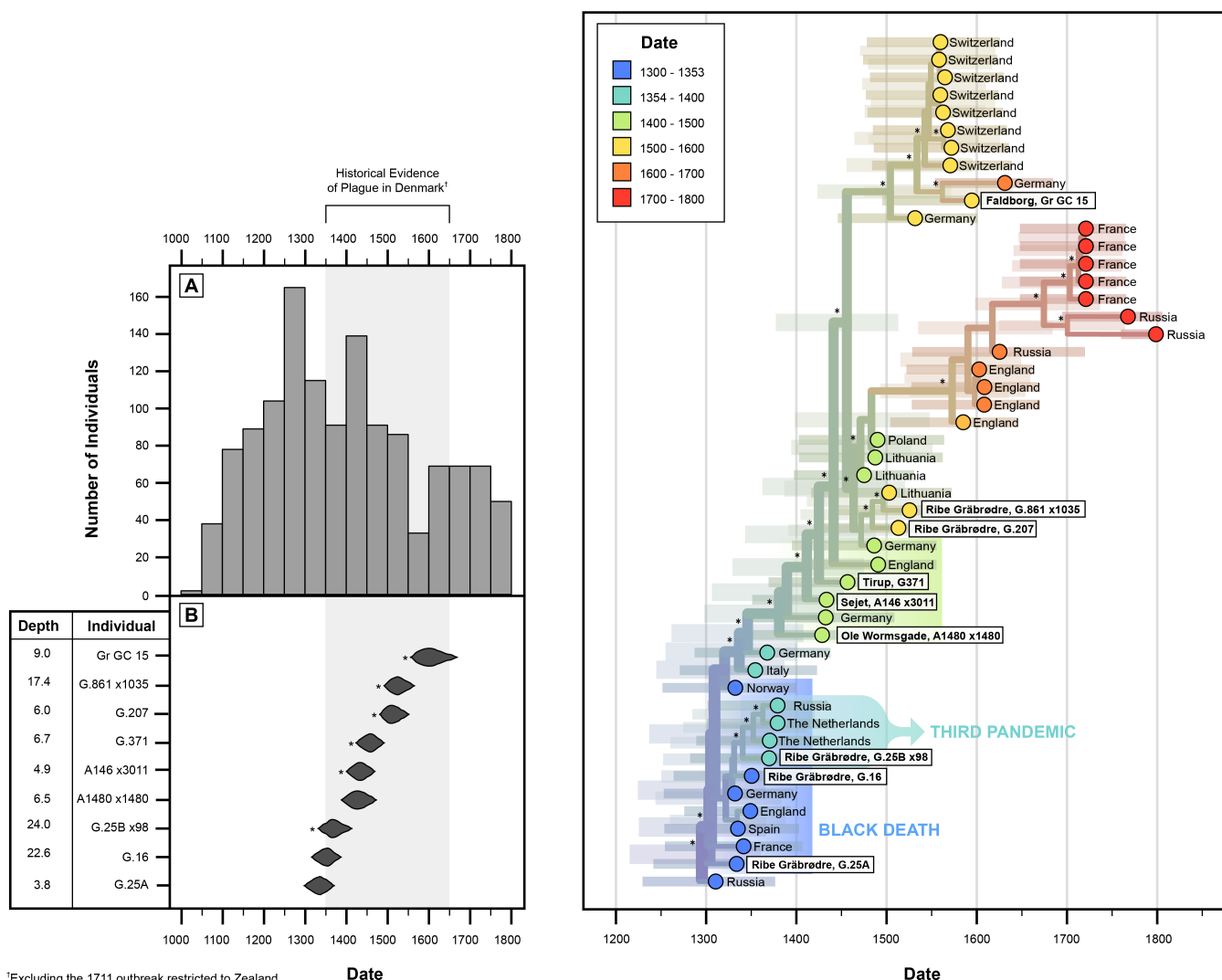


Figure 1. Geographic distribution of 298 archaeological samples used in this study. **A.** Map of 6 municipalities sampled in Denmark encompassing 13 archaeological sites. Site labels indicate: **Archaeological Site** (Earliest Date Sampled - Latest Date Sampled) *Yersinia pestis* positive individuals / total individuals. Plague positive sites are bolded. **B.** Map of 49 *Yersinia pestis* genomes used for phylogenetic analysis. The sampling locations were standardized to the centroid of the associated province/state. Colors indicate the sampling dates as estimated from the Bayesian molecular clock analysis. Numbered labels indicate the number of genomes sampled from each location.



[†]Excluding the 1711 outbreak restricted to Zealand.

Figure 2. Temporal distribution of archaeological samples used in this study. **A.** Distribution of skeletal dates for all individuals (N=298) using a bin width of 50 years. The y-axis indicates the number of individuals with skeletal dates that include the associated date bin on the x-axis. **B.** The distribution of tip-dates for plague-positive individuals (N=9) with sufficient depth (>3X) of the *Y. pestis* chromosome. Asterisk indicate the phylogenetic placement had strong posterior support (≥ 0.95).

Figure 3. Maximum-clade credibility (MCC) tree depicting a time-scaled phylogeny of 49 *Yersinia pestis* genomes dated to the Second Pandemic. Asterisks indicate clades with strong posterior support (≥ 0.95). Colors indicate the mean sampling dates as estimated from the Bayesian molecular clock analysis. Bars indicate tip-dating uncertainty, as represented by the 95% highest posterior density (HPD) interval.

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 [14]. 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 and dental pulp according to a specialized protocol for ancient DNA [15]. 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* [16]. Extracts showing amplification in at least 4/6 replicates were converted into paired-end sequencing libraries [17]. Targeted capture of the *Y. pestis* genome was performed using previously designed probes [16] and sequenced on an Illumina platform.

Sequenced molecules were aligned to a reference genome using the *nf-core/eager* pipeline [18]. To phylogenetically place these new samples, we downloaded a comparative dataset of 40 high-coverage *Y. pestis* genomes (>3X) dated to the Second Pandemic. We then constructed a multiple alignment with the *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) with BEAST2 [19][20]. 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 Danish 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.

Data visualization was performed using the python package *seaborn* and *auspice*, a component of the Nextstrain visualization suite [21].

Data Availability

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

Acknowledgments

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

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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)