












An 800-year longitudinal study of *Yersinia pestis* in Denmark captures the rise and fall of a plague pandemic.

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Introduction

Results

Discussion

Materials and Methods

We sampled 300 individuals across 13 archaeological sites in Denmark (Figure ??, SI Table 1). Site occupation dates spanned from the 11th to the 19th century CE. We estimated individual date ranges (+/- 100 years) based on burial position, which can be categorized according to cultural shifts that occurred in Denmark throughout the medieval and early modern period [1,2]. When the original stratigraphic context was preserved, we were able to refine these individual estimates further (+/- 50 years).

DNA was extracted from teeth and dental pulp according to a specialized protocol for ancient DNA [3]. 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*. Extracts showing amplification in at least 4/6 replicates were converted into paired-end sequencing libraries [4,5]. Targeted capture of the *Y. pestis* genome was performed using previously designed probes [6] and sequenced on an Illumina platform.

Sequenced molecules were aligned to the reference genome (CO92) using the *nf-core/eager* pipeline [7]. To phylogenetically place these new samples, we downloaded a comparative dataset of 39 publicly available *Y. pestis* genomes dated to the Second Pandemic. We selected an additional 8 *Y. pestis* genomes that belong to the basal phylogroup (0.ANT3) to serve as an outgroup. A maximum-likelihood phylogeny was estimated across 10 independent runs of IQTREE [8]. Branch support was evaluated using 1000 iterations of the ultrafast bootstrap approximation [9], with a threshold of 95% required for strong support. The outgroup clade (0.ANT3) was used to estimate the root position and was subsequently pruned from the phylogeny for downstream analysis and visualization.

To tip-date each sample using the associated *Y. pestis* DNA, we first evaluated the degree of temporal signal in the data. We performed a Bayesian Evaluation of Temporal Signal (BETS [10]) using a strict clock and an uncorrelated lognormal (UCLN) relaxed clock. 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 150,000,000 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` [11] and Auspice [12], a component of the Nextstrain visualization suite.

Data Availability

Acknowledgements

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