Plague Denmark Paper

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Abstract

Background

The sequencing of ancient pathogen genomes has resulted in vast advancements to our understanding of the second plague pandemic. However, due to limited sample availability, debate remains about the Plague's origins, routes of dissemination, genomic diversity, and persistence. Specifically, Scandinavia has a unique history with regards to plague persistence as it is home to the oldest known strain of plague to date and has been ravaged by historic epidemics, only for the plague to have disappeared from this region in the modern era.

Problem

It is unknown to what extent local plague reservoirs fed the recurring epidemics in Scandinavia as compared to the continual introduction of globally circulated strains. Previously studied historical records primarily derive from large commercial centres such as London, which are contrasted by countries such as Denmark where the archives have retained limited information about the spread of the plague and its impact on society.

Objectives

To confidently identify and sequence ancient *Yersinia pestis* from Danish archaeological sites across a wide geographic and temporal range. With the aim of performing genomic analyses to estimate the timing, spread, and evolutionary changes occurring within Danish plague strains as compared to neighboring regions.

Significance

First, there have been relatively few studies that explore the genetics of plague in Scandinavia across time and geography, particularly in Denmark. Second, this paper contributes to a larger body of epidemiological literature that considers the patterns and mechanisms by which diseases emerge, propagate, and go extinct.

Sites and Samples

326 individuals were sampled across 6 regions from 14 archaeological sites (Table 1). The site occupation dates span from the 10th to 18th centuries which encompasses the Viking Age (8th - 10th century), the Medieval Period (11th - 16th century) and the Early Modern Period (16th - 19th century) in Denmark.

Table 1: Summary of archaeological sites sampled in this study.

Region	Site Name	Site Code	Site Occupation	Samples
Ribe	Ribe Gräbrødre	ASR 1015	1200 - 1560	53
	Ribe Lindegärden	ASR 2391	900 - 1000	5
		ASR 13/13II	900 - 1000	15
		ASR 13II	1200 - 1560	28
Viby	Nordby	FHM 3970	1050 - 1250	36
Horsens	Monastery Church	HOM 1272	1600 - 1800	50
	Ole Wormsgade	HOM 1649	1100 - 1500	17
	Sejet	HOM 1046	1150 - 1574	25
	Tirup	VKH 1201	1150 - 1350	12
Hågerup	Hågerup	ØHM 1247	1100 - 1555	7
Refshale	Refshale	Refshale	1100 - 1350	19
Viborg	Sct. Mikkel	JAH 1-77	1000 - 1529	4
	The Catholic Church	VSM 09264	1100 - 1529	6
	Sct. Mathias	VSM 855F/906F	1100 - 1529	23
	Sct. Drotten	VSM 902F	1100 - 1529	8
	Faldborg	VSM 29F	1100 - 1600	17
Total				326

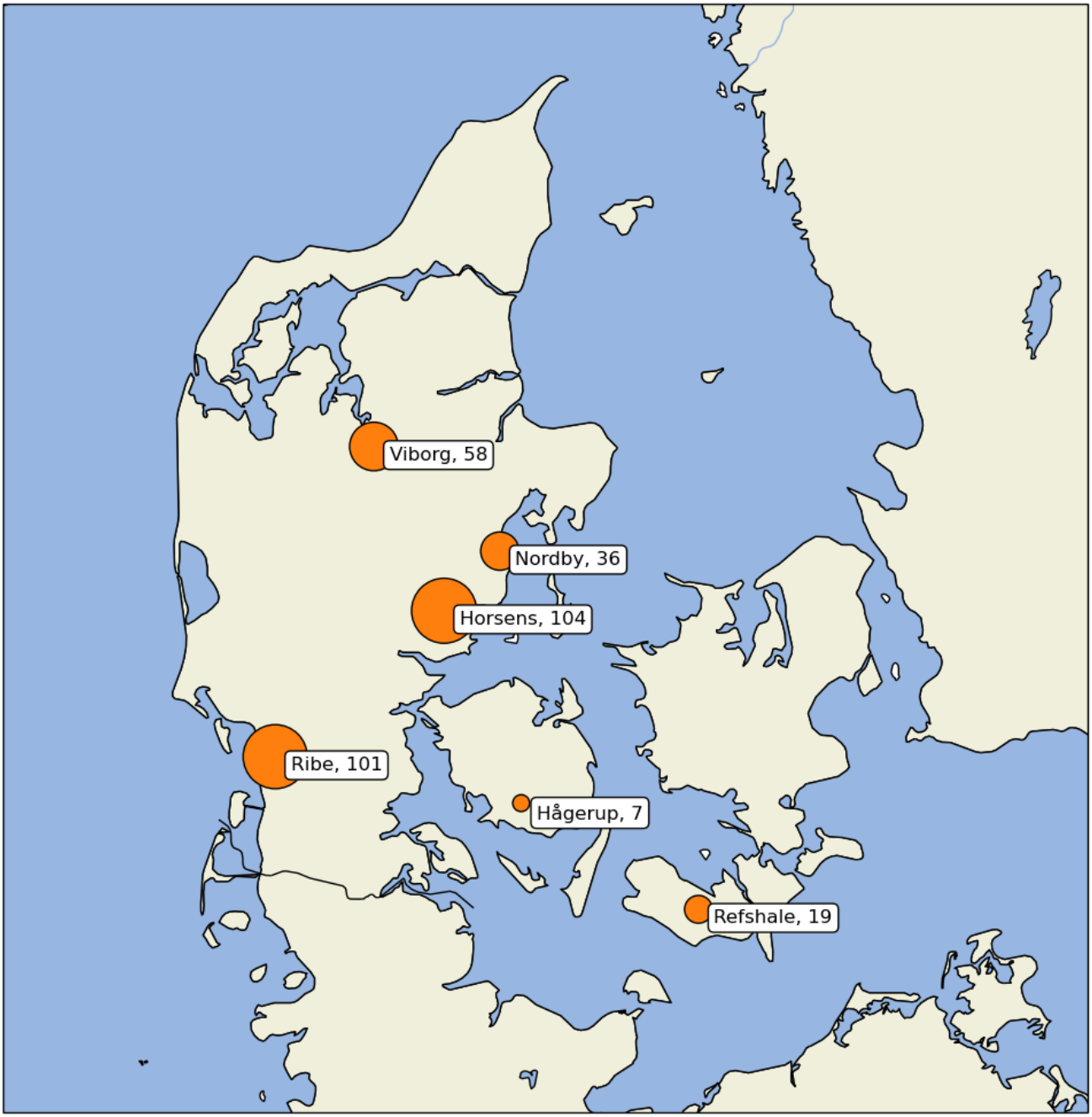

Figure 1: Geographic map of archaeological sites by region.

Plague Detection

13 individuals were identified as plague-positive based on a combination of PCR assays, shotgun sequencing, and targeted enrichment for the *Y. pestis* whole genome (Tables 2 and 3). Of the 13 individuals, 9 had chromosomal coverage sufficient for phylogenetic analyses.

Overall, plague was detected in 4% of all individuals in this study. When excluding plague-negative archaeological sites, this estimate rises to 8.2% of individuals. *Y. pestis* was observed exclusively in the Medieval Period, with no evidence of plague in the Viking Age settlements at Ribe Lindegärden or the Early Modern cemetery at Horsens.

> - Absence of plague in Viking/Early Modern could easily be a false negative.
> - These periods are sparsely sampled, with fewer sites and individuals sampled.
> - Are G25A and G25B two individuals from the same grave?

Table 2: Plague positive summary of high coverage genomes.

Arch ID	Project ID	Site	PCR	Human (%)	Plague (%)	CHROM	pCD1	pMT1	pPCP1
G16	D71	Ribe Gräbrødre	6/6	5.95	0.18	22.6	39.4	14.7	4.6
G861 x1035	D75	Ribe Gräbrødre	6/6	4.42	0.23	17.4	40.2	16.5	3.4
G25B x98	R36	Ribe Gräbrødre	6/6	8.41	0.25	24.0	51.8	14.9	5.8
G25A	D62	Ribe Gräbrødre	6/6	1.12	0.10	3.8	10.5	2.5	0.9
G207	D72	Ribe Gräbrødre	6/6	12.94	0.04	6.0	13.5	5.8	2.2
A146 x3011	P187	Sejet	6/6	0.68	0.01	4.9	18.4	6.6	52.2
G371	P212	Tirup	6/6	0.61	0.04	6.7	26.3	8.5	56.6
Gr GC 15	D51	Faldborg	6/6	0.67	0.05	9.0	25.4	8.1	2.0
A1480 x1480	P387	Ole Wormsgade	6/6	0.04	0.01	6.5	21.7	5.0	75.0

Table 3: Plague positive summary of low coverage genomes.

Arch ID	Project ID	Site	PCR	Human (%)	Plague (%)	CHROM	pCD1	pMT1	pPCP1
A1155 x1155	P384	Ole Wormsgade	4/6	0.11	0.01	1.1	4.8	1.4	19.6
Gr ID 319	R21	Faldborg	6/6	0.85	0.01	2.6	3.8	2.3	0.4
A19 X21	D24	Hågerup	6/6	0.55	0.01	2.6	6.1	1.9	0.7
X1265	P246	Ribe Lindegärden	6/6	0.03	0.01	0.1	0.1	0.1	3.2

Skeletal and Molecular Dating

I'm relying heavily on the discussion in Boldsen (2009) [1], as quoted here:

"The dating of individual skeletons is a fundamental problem in historical studies like this, and even the period of usage of each cemetery raises some serious problems. However, most cemeteries have at least some documentary sources broadly framing them in time. The most intensely studied skeletal samples, Tirup and Westerhus, are really the only exceptions in being dated solely on archaeological evidence (Kieffer-Olsen et al. 1986, Sivěn 2005)."

"In medieval graves the position of the arms in relation to the rest of the skeleton in the grave is the only feature that systematically indicates dating of the burial within the temporal frame provided by the period of usage of the cemetery. Arm position dating is primarily based on work by Redin (1976) and Kieffer-Olsen (1993). The successive stages of arm position from A (the arms besides the body) over B (hand joint over the lower part of the abdomen and usually found in the pelvis) and C (the forearms over the upper part of the abdomen and the elbows flexed in an approximately right angle) to D (the hands placed on the shoulders, forearms often crossed over the chest) have primarily been described by Kieffer-Olsen (1993) but Jantzen et al. (1994) have slightly modified the transition dates between the various stages.".

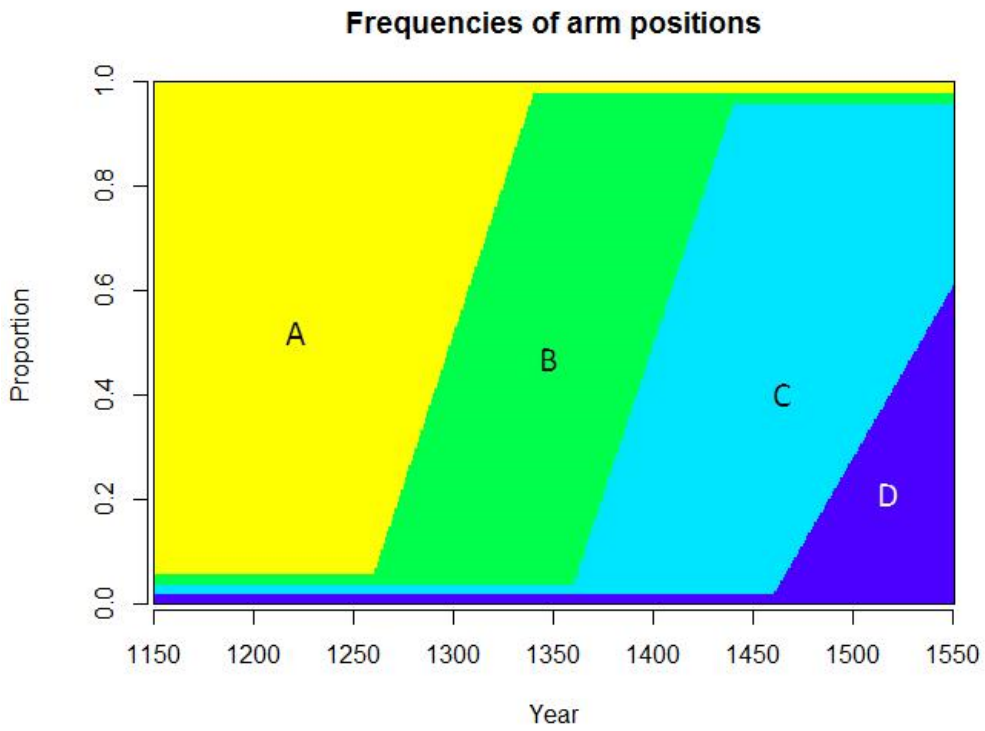

Figure 2: Arm position dating.

The skeletal and molecular dates of the 9 high coverage *Y. pestis* genomes are presented in Table 4 and Figure 4. Almost all molecular dates had overlap with the archaeological dates as determined by site occupation period and burial patterns. The exception to this pattern was individual G371 from the site of Tirup. To investigate this disparity, we performed 14C radiocarbon dating on this sample (Figure 3).

The radiocarbon estimate largely agrees with archaeological dates, with an estimated mean date of 1260 CE (+/- 75 yrs with 1 sigma). Thus there is robust evidence that the individual lived and died sometime between the late 12th and early 14th century

However, the associated *Y. pestis* genome is dated to the 15th century. While the 2 sigma distribution of the radiocarbon date does extends into the 15th century, there remains the substantial conflict with the known site occupation dates.

> - How can a pathogen appear to have lived 100 years after its associated host?

Table 4: Summary of the *Y. pestis* molecular dates. The estimated tip date reflects the 95% highest posterior density.

ID	Region	Site	Site Occupation	Arm Position	Skeletal Date	Tip Date
G16	Ribe	Ribe Gräbrødre	1200 - 1560	C	1350 - 1550	1310 - 1388
G861 x1035	Ribe	Ribe Gräbrødre	1200 - 1560	C	1350 - 1550	1489 - 1567
G25B x98	Ribe	Ribe Gräbrødre	1200 - 1560	C	1350 - 1550	1327 - 1414
G25A	Ribe	Ribe Gräbrødre	1200 - 1560	C	1350 - 1550	1295 - 1375
G207	Ribe	Ribe Gräbrødre	1200 - 1560	C	1350 - 1550	1477 - 1551
A146 x3011	Horsens	Sejet	1150 - 1574	B	1250 - 1425	1397 - 1470
A1480 x1480	Horsens	Ole Wormsgade	1100 - 1500	?	?	1384 - 1473
G371	Horsens	Tirup	1150 - 1350	B	1250 - 1425	1419 - 1490
Gr GC 15	Viborg	Faldborg	1100 - 1600	C	1350 - 1550	1539 - 1655

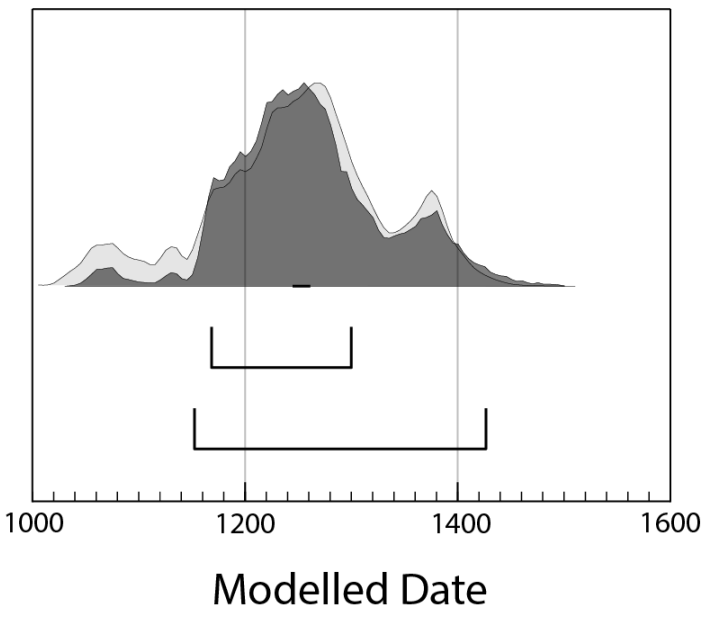

Figure 3: Radiocarbon dating of G371.

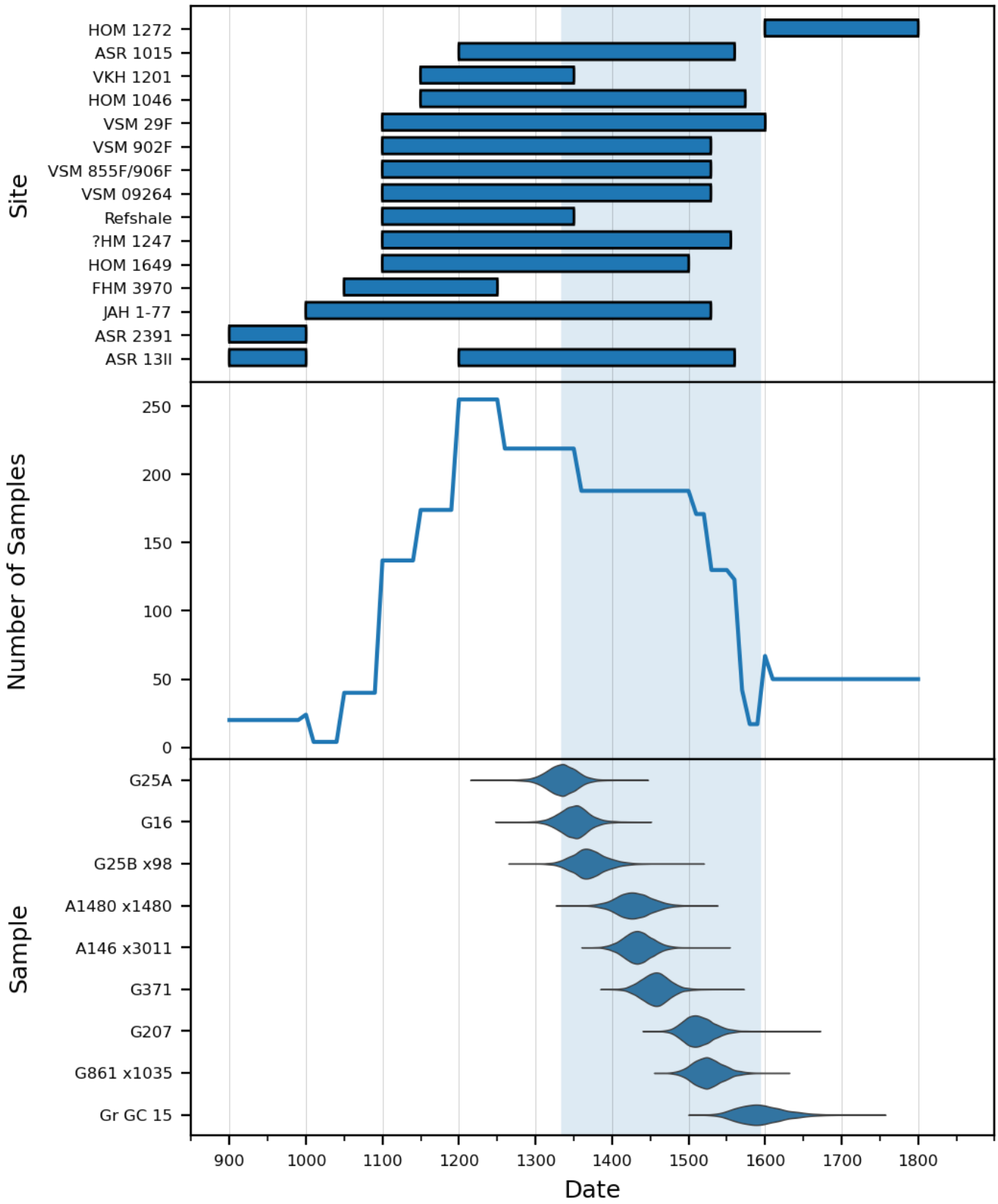

Figure 4: Timeline of archaeological sites and plague-positive individuals. The shaded range spans the highest probability period from the oldest to the youngest sample.

Phylogeny

An examination the time-scaled phylogeny of the Second Pandemic (Figure 5) adds greater nuance to the molecular dating.

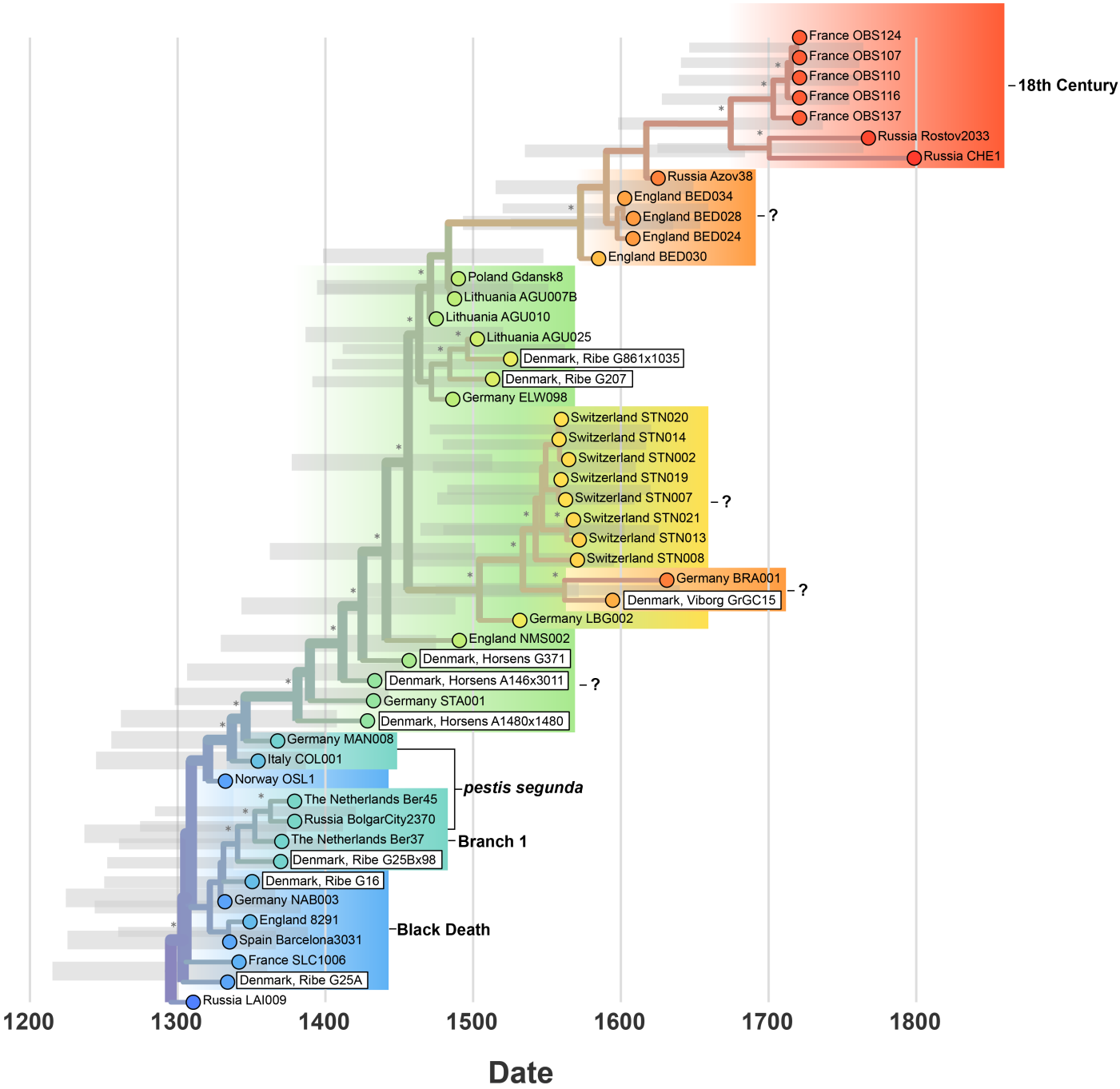

Figure 5: A time-scaled phylogeny of the Second Plague Pandemic. Asterisks indicate branches with strong statistical support. Grey bars indicate the 95% HPD interval on the internal node dates

Denmark and the Black Death

Two individuals from Ribe, G25A and G16, cluster with *Y. pestis* strains dated to the 14th century. This cluster is thought to be associated with the Black Death, as the genomes are nearly identical and have been isolated from all across Europe, thus indicating rapid geographic dispersal. Given the high degree of genetic similarity, the branching order and thus migration patterns, of this epidemic clade cannot be resolved. Our understanding of this event is therefore that of a “burst” radiation, with possibly multiple waves of *Y. pestis* that are migrating faster than the mutation rate.

The Ancestors of the Third Pandemic

Interestingly, the *Y. pestis* genome associated with individual G25Bx98 from Ribe (who was found in the same grave as G25A?) is genetically distinct from the earlier strains from this region. This isolate falls within a clade of high epidemiological significance, which is the ancestral group giving rise to the Third Pandemic of plague. This clade was previously hypothesized to reflect a backward migration of plague from Northern Europe back into Asia. The *Y. pestis* genome retrieved from G25Bx98 tentatively supports hypothesis, as it falls basal to the more derived strains from The Netherlands and Russia.

Regional Variation

In contrast to the genetic homogeneity observed across Europe during the Black Death period, later isolates of *Y. pestis* show strong intra- and inter-regional variation. Plague genomes collected from sites near the city of Horsens are closely related to one another but form independent lineages.

diverges in Ole Wormsgade (A1480x1480), the cemetery of the port city of Horsens. Following this divergence, distinct lineages are observed in the rural settlements of Sejet (A146x3011) and then Tirup (G371).

> **Phase 1: 1300-1450**
> - All *Y. pestis* genomes from the 14th century cluster together.
> - Samples from the early-mid 1300s are widely dispersed across Europe (Figure 6), and have highly similar genetic content resulting poorly resolved branching order. This suggests rapid, epidemic spread, thought to be associated with the Black Death.
> - Samples from the late 1300s also cluster together, and are linked to the *pestis segunda* series of epidemics in Europe.
> - The only Danish samples in Phase I are from Ribe, and fall within both the Black Death and *pestis segunda* groups. - These are primarily coastal sites, Germany and Russia as the exception.

> **Phase 2: 1450-1600**
> - A very curious branching pattern, lots of ‘independent’ emergences rather than monophyletic clades.
> - All Danish samples from the Horsens region fall here, and although they have temporal overlap with each other, the lineages of plague are distinct.
> - ...

> **Phase 3: 1600-1800**
> - ...

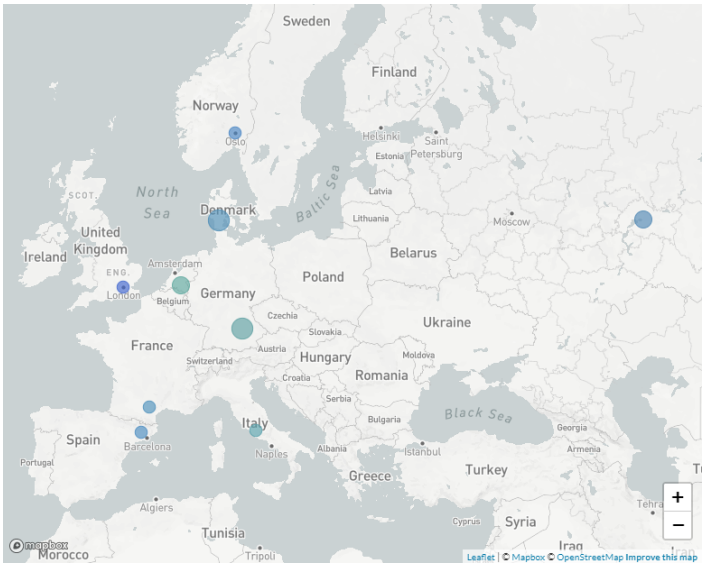

Figure 6: Phase 1: 1300 - 1450

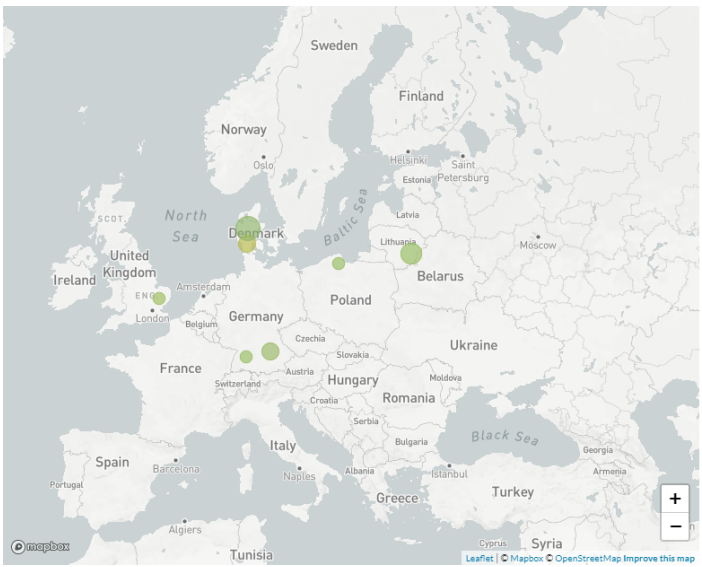

Figure 7: Phase 2: 1450 - 1600

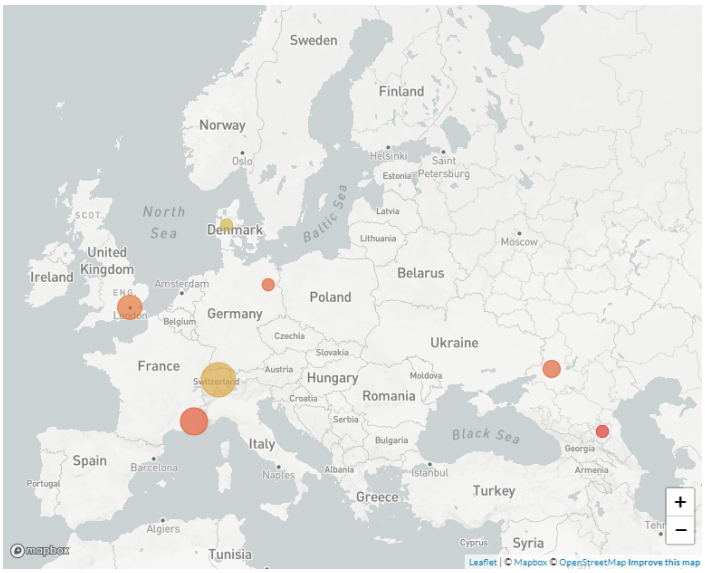

Figure 8: Phase 3: 1600 - 1800

References

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

Appendix

Misc Notes

Table 5: Plague false positive summary.

Arch ID	Project ID	Site Code	PCR	Human (%)	Plague (%)	Chrom	pCD1	pMT1	pPCP1
G70 x212	R44	ASR 1015	3/6	1.48	0.00	0.1	0.1	0.1	0.0
G860	R39	ASR 1015	5/6	0.09	?	?	?	?	?
G364	R43	ASR 1015	4/6	?	?	?	?	?	?
K1167 x1167	P235	ASR 13 II	3/6	?	?	?	?	?	?
A21 x23	D25	ØHM 1247	4/6	0.01	0.00	0.05	0.1	0.0	0.0
G260 K539 x876	R27	VSM 09264	3/6	?	?	?	?	?	?

To estimate dates for the plague-positive individuals, a Bayesian Evaluation of Temporal Signal (BETS) was first performed. Briefly, each candidate model was tested using the correct collection dates of all samples and then compared to the same model with all collection dates assumed to be contemporaneous. Bayes factors (BF) were calculated by comparing the marginal likelihoods of each model, as estimated with a generalized stepping stone (GSS) computation across 100 chains each sampled over 1,000,000 generations.

The BETS analysis revealed decisive support for temporal signal (dates vs. no dates) using both the strict clock (SC) and uncorrelated lognormal relaxed clock (UCLN) (Table 6). A comparison of the strict vs. relaxed clocks using collection date produced decisive support for the relaxed clock.

Table 6: Bayesian Evaluation of Temporal Signal (BETS) summary.

Model	Abbrev.	Dates	Likelihood	Bayes Factor (Dates)	Bayes Factor (Model)
Strict Clock	SC	Yes	-5948088	749	–
		No	-5948837	–	–
Relaxed Clock	UCLN	Yes	**-5947948**	715	140
		No	-5948663	–	–

A time-scaled phylogeny with tip-dating was estimated using a relaxed clock and diffuse normal priors centered around the mean collection date.