Plague Denmark Paper

Authors

- **Katherine Eaton**
  ⓘD [0000-0001-6862-7756](#) · ⌂ [ktmeaton](#)
  McMaster Ancient DNA Center; Department of Anthropology, McMaster University

- **Ravneet Sidhu**

  McMaster Ancient DNA Center; Department of Biology, McMaster University

- **Hendrik Poinar**
  ⓘD [0000-0002-0314-4160](#)
  McMaster Ancient DNA Center; Department of Anthropology, McMaster University

Introduction

1. When and where does plague (*Y. pestis*) appear in Denmark?
2. How are Danish outbreaks of plague connected to the global Second Pandemic?

Data Collection

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).

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
Nordby	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
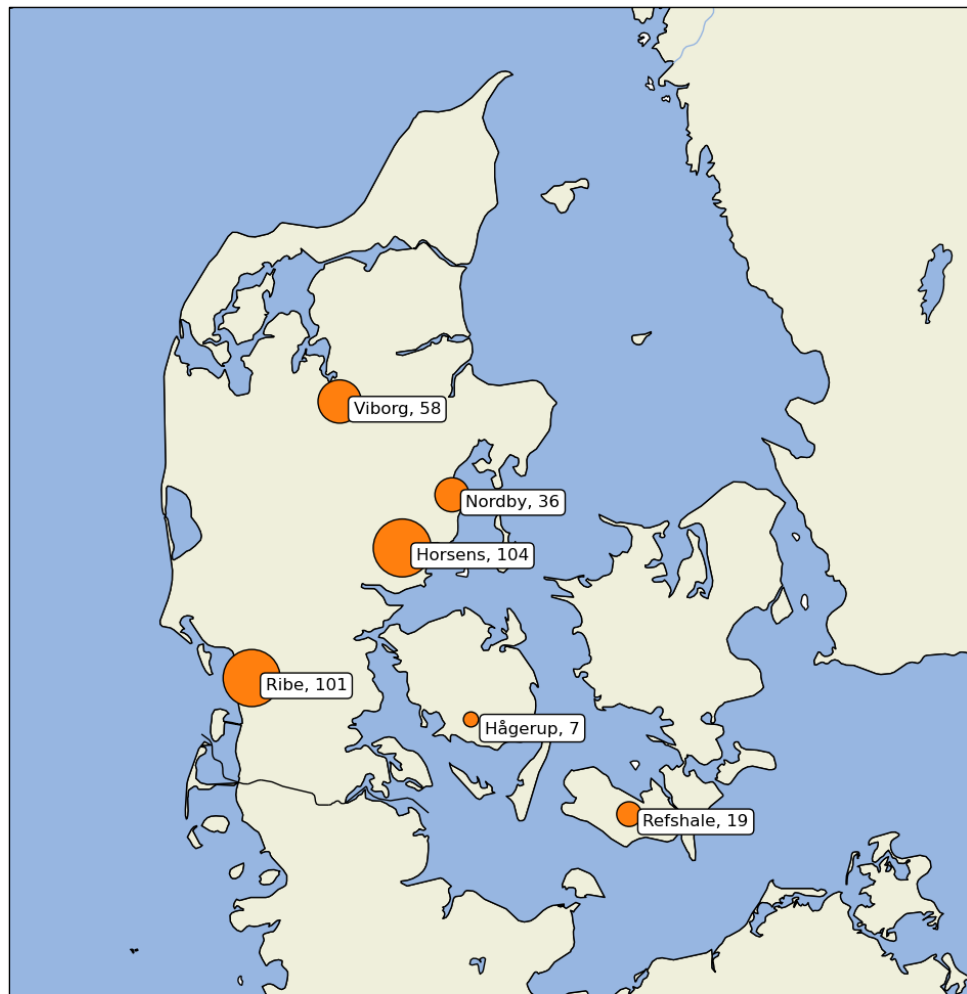
Figure 1: Geographic map of archaeological sites by region.

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 (3X mean coverage).

The positivity rate for plague was 4% of all individuals, or 8.2% when excluding plague-negative archaeological sites.

Y. pestis was observed exclusively in the Medieval Period, with no evidence of plague in the Viking Age settlements at Ribe Lindegärden (N=20) or the Early Modern cemetery at Horsens (N=50). However, the absence of evidence for plague in these periods may be a false-negative, given that these time periods are sparsely sampled.

> **Kat's Notes**:
> - 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 Dating

"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, Sivĕn 2005)." [1]. Aspects of dating of cemetery samples has been discussed for Nordby (Skov 2002), Refshale (Snedker 1973), St. Mikkel (Vellev 1979).

"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." [1].

Molecular Dating

The molecular dates of the 9 high coverage *Y. pestis* genomes are presented in Table [4](#). All molecular dates had overlap with the expected range of site occupation dates, with the exception of individual G371 from the site of Tirup. To investigate this disparity, we performed 14C radiocarbon dating on this sample (Figure [2](#)). The radiocarbon estimate largely agrees with the site occupation date, with an estimated mean date of 1260 CE (+/- 75 yrs with 1 sigma). The 2 sigma distribution of the radiocarbon date extends to 1430 CE which does overlap with the molecular date.

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

| ID	Site	Site Occupation	Tip Date
G16	Ribe Gräbrødre	1200 - 1560	1310 - 1388
G861 x1035	Ribe Gräbrødre	1200 - 1560	1489 - 1567
G25B x98	Ribe Gräbrødre	1200 - 1560	1327 - 1414
G25A	Ribe Gräbrødre	1200 - 1560	1295 - 1375
G207	Ribe Gräbrødre	1200 - 1560	1477 - 1551
A146 x3011	Sejet	1150 - 1574	1397 - 1470
A1480 x1480	Ole Wormsgade	1100 - 1500	1384 - 1473
G371	Tirup	1150 - 1350	1419 - 1490
Gr GC 15	Faldborg	1100 - 1600	1539 - 1655
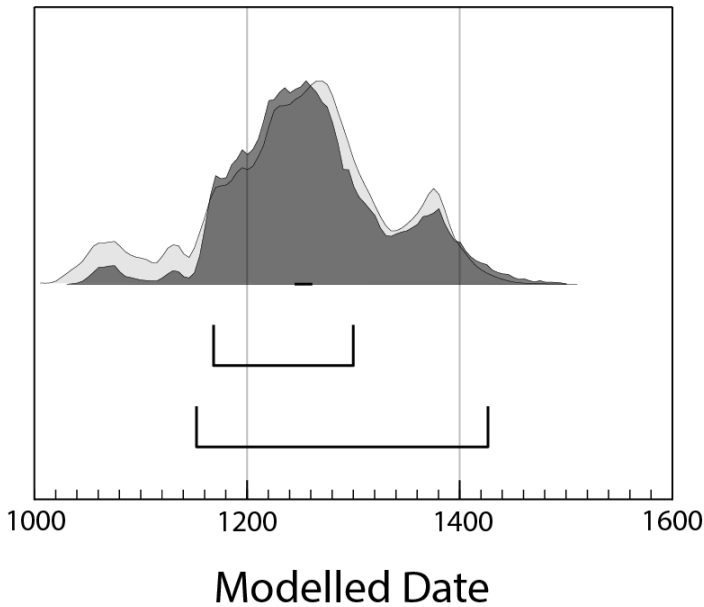
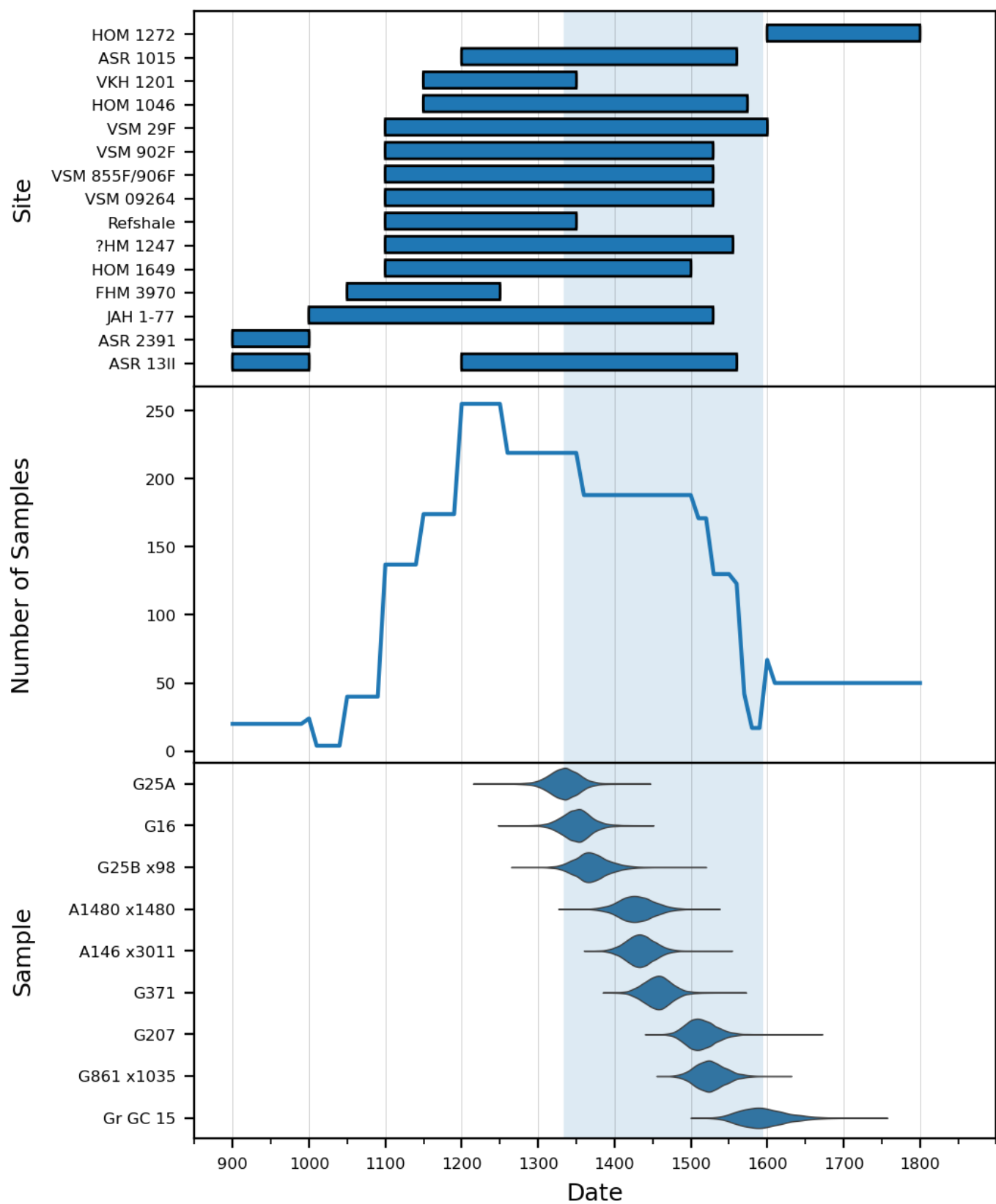
Figure 2: Radiocarbon dating of G371.

Figure 3: Timeline of archaeological sites and plague-positive individuals. The shaded range spans the highest probability period from the oldest to the youngest sample.

Phylogeny

The time-scaled phylogeny reveals geographic and temporal structure during the Second Pandemic (Figure 4).
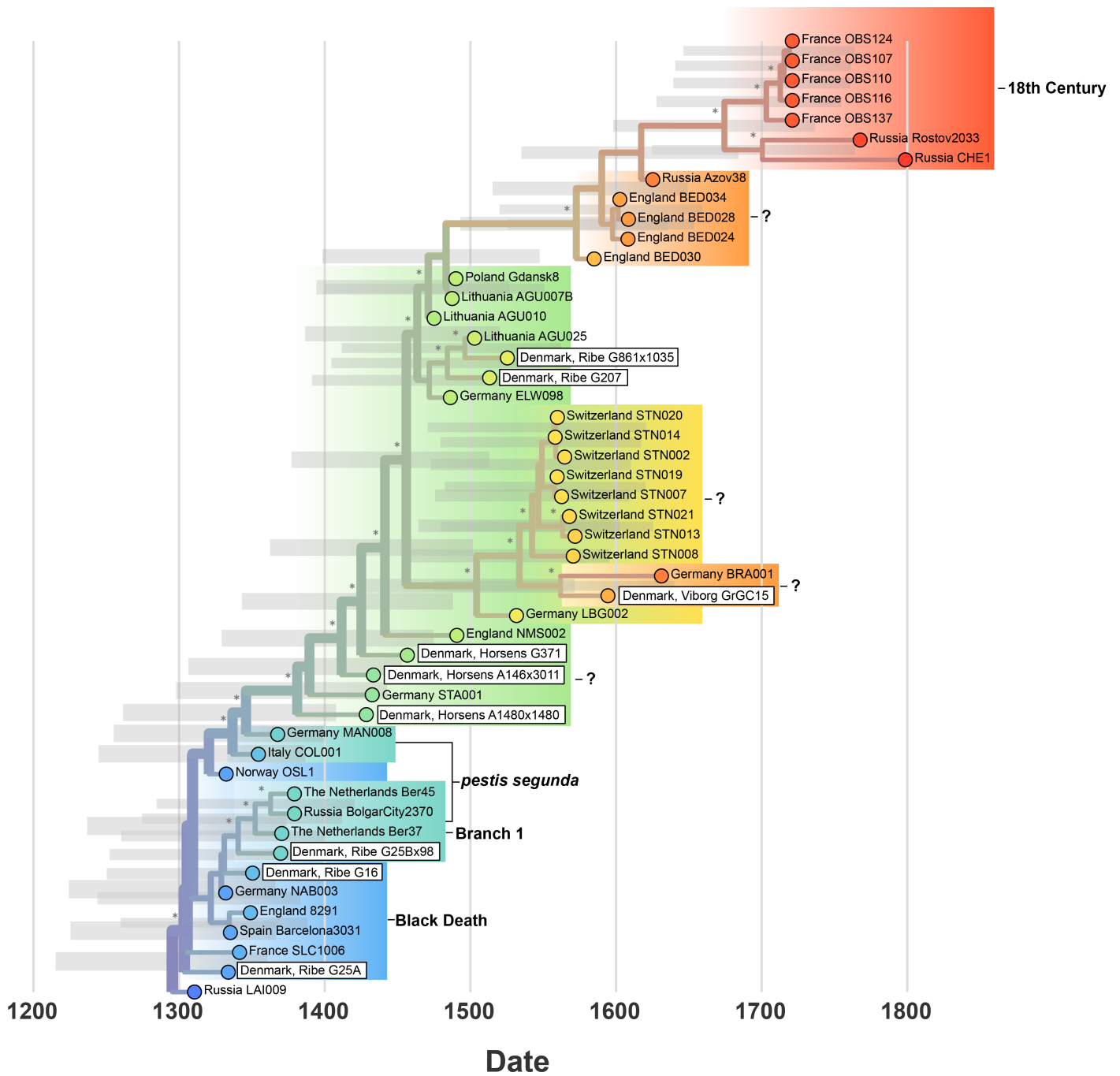

Figure 4: Bayesian time-scaled phylogeny.

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 5), 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.
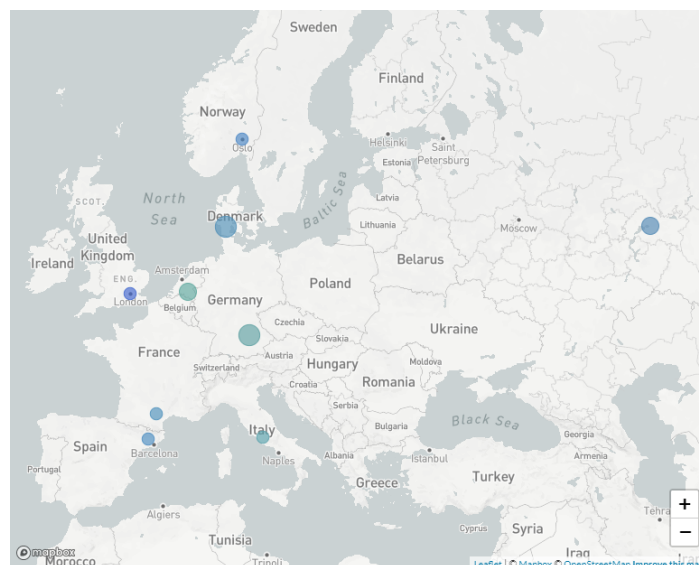- ...

Phase 3: 1600-1800
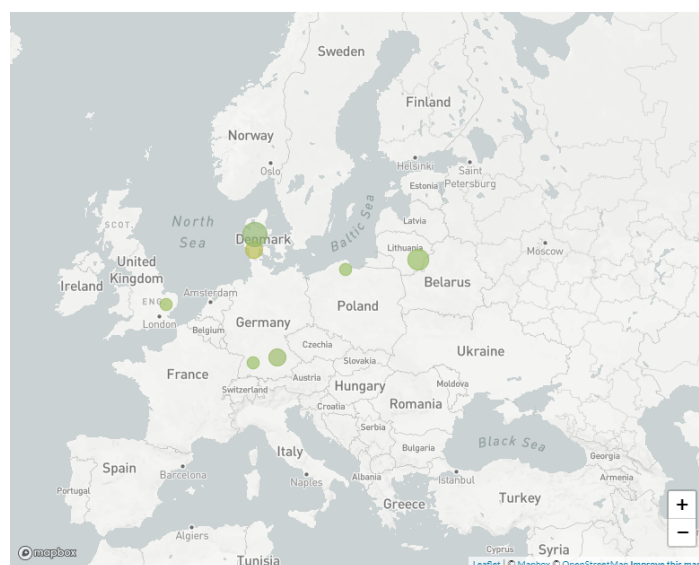- ...

Figure 5: Phase 1: 1300 - 1450

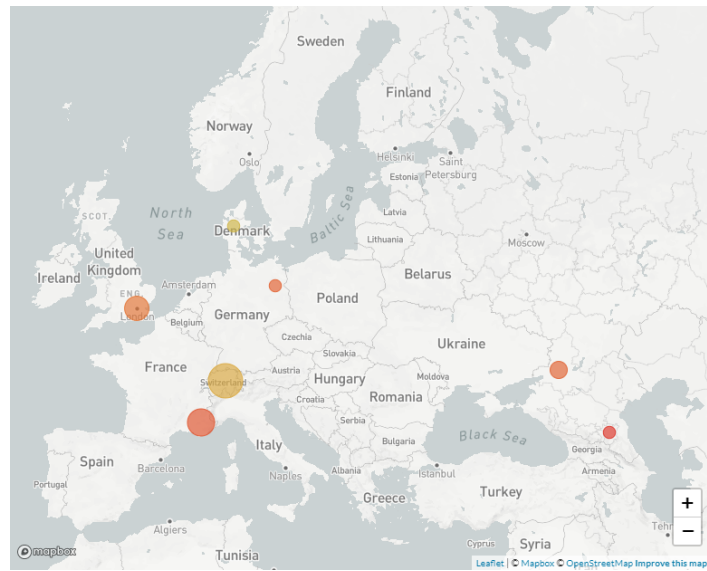**Figure 6:** Phase 2: 1450 - 1600

Figure 7: 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.