The Genome of the Black Death:
Full Genome analysis of ancient Pathogens

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History of infectious Disease:
Most infectious diseases occurred during the Neolithic due to **Zoonosis**, e.g. measles, smallpox, flu, tuberculosis, plague, leprosy, or pertussis.
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Major pandemics in history
Plague of Athens 4th century BC (measles ?)
Antonine plague 2nd century AD (smallpox?)
Justinian plague 6th century AD (bubonic plague?)
Black Death 14th century AD (bubonic plague)
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Since the 1980s we see a new wave of re-emerging infectious diseases, e.g. HIV, SARS, Hanta, Ebola, Lyme disease, and antibiotic resistance e.g. MDR-Tb
The Black Death

• The Black Death was one of the most devastating epidemics in human history

• It lasted only 5 years (1347-1351) and 25-50 million Europeans died of the epidemic
Yersinia pestis

Bubonic plague Infection cycle:

1. Squirrel
2. Flea
3. Rat

- Sylvatic plague
- Urban plague

- Bubonic plague
- Pneumonic plague

Bubo

infected flea

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- 2000 cases each year
- 1-5% mortality
Genetic components of *Y. pestis*

Shared with non-pestis ancestors

- pCD1 70kb
- chromosome 4.6Mb

Y. pestis-specific plasmids

- PCP1 9.6kb
- pMT 100kb

High-copy (ca. 100 per cell)
Plasminogen activator gene
• First genome of *Y.pestis* sequenced in 2001 (Parkhill et al.)

• 20 genomes of modern *Y.pestis* strains now sequenced (Morelli et al., 2010)

• Highly similar: 2700 positions differences in total (Morelli et al., 2010)
# Modern Plague vs. Ancient Plague

<table>
<thead>
<tr>
<th>Differences</th>
<th>Modern Plague</th>
<th>Black Death</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Symptoms</strong></td>
<td>• A single bubo</td>
<td>• Multiple buboes and black pustules</td>
</tr>
<tr>
<td></td>
<td>• Apathy, depression</td>
<td>• Hysteria, extreme thirst, pain, headache, vomiting</td>
</tr>
<tr>
<td></td>
<td><strong>Transmissic trends</strong></td>
<td><strong>Transmissic trends</strong></td>
</tr>
<tr>
<td></td>
<td>• Slow rate of transmission</td>
<td>• Fast rate of transmission</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Person to person transmission (?)?</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Always present</td>
</tr>
<tr>
<td><strong>Climate</strong></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>• Outbreaks occurred at any time of the year, regardless of climatic conditions (heat or humidity)</td>
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<td><strong>Other probable suspects:</strong></td>
<td>• Anthrax (Twigg, 1984)</td>
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<td></td>
<td>• Filovirus, e.g. Ebola (Scott and Duncan, 2001)</td>
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<tr>
<td></td>
<td>• An as yet unidentified virus (Cohn, 2003)</td>
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</table>
Objectives:

- Identify the causative agent of the Black Death
- Study the Evolution of the ancient pathogen
- Find explanation for higher mortality of medieval plague
The Black Death Pathogen

Pathogen DNA exists in low quantity

Polymerase Chain Reaction (PCR)
Ancient Data (with PCR):

- Drancourt et al. 1998: 133 bp to 300 bp  Yes
- Raoult et al. 2000: 147 bp  Yes
- Gilbert et al. 2004: 134-300 bp  No
- Drancourt et al. 2004: 292-387 bp  Yes
- Wiechmann et al. 2005: 148 bp  Yes
- Haensch et al. 2010: 80-170 bp  Yes
- PCR can not access the vast majority of the ancient DNA
Next Generation Sequencing

ABI Sanger Sequencing
~$10^2$ fragments per run

2004

454 GS20
~$10^5$ fragments per run

2005

Illumina GA2
~$10^8$ fragments per run

Illumina HiSeq
~$10^9$ fragments per run

2010

2012

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Next Generation Sequencing

Sequencing Library

A

ancient DNA

artificial “adaptors”

B
Next Generation Sequencing

**PCR**

- 20nt → 20nt → 60nt

**Direct sequencing**

- A ← B

- 20nt → 60nt

- All size fragments available
- Library can be “immortalized”
- damage patterns can be preserved

- Short molecules not available
- Extremely wasteful of extract
- damage patterns are not preserved
Shot Gun Sequencing
4% Human DNA
0.005% Human mtDNA
0.0005% Human Pathogen DNA

1 : 25
1 : 20.000
1 : 200.000

For 1000bp of a human Pathogen one needs 40.000.000 DNA Sequences
For 1000bp concatenated human Pathogen DNA one needs 40.000.000.000

Shot Gun Sequencing
Fishing for Plague DNA

Targeted DNA Enrichment:
Fishing for ancient Pathogen DNA
Fishing for pcp1 Plasmid 10,000 bp

High-copy (ca. 100 per cell)
Virulence genes
East Smithfield Cementary, London

- well reknown Plague Cementary
- used between
- excavated in 1986
- more than 500 Skeletons excavated
- Used 20 skeletons that show Y.pestis DNA with PCR
Extraction and library preparation

DNA extraction

Ancient DNA

immortalization

amplification

Many copies

Used for DNA capture

High throughput sequencing

DNA library
Fishing for pcp1 plasmid

After Maricic et al. 2010, Plos One
### Results – five teeth from two collection

<table>
<thead>
<tr>
<th></th>
<th>Human mitochondrial DNA</th>
<th>Y. <em>pestis</em> PCP1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Saint Nicholas Shambles</strong></td>
<td>110, 500 fragments</td>
<td>0 PCP1 fragments</td>
</tr>
<tr>
<td>pre-Black Death control</td>
<td>3 complete genomes,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 partial genomes</td>
<td></td>
</tr>
<tr>
<td><strong>East Smithfield</strong></td>
<td>202, 450 fragments</td>
<td>23, 112 PCP1 fragments</td>
</tr>
<tr>
<td><strong>Black Death Cemetery</strong></td>
<td>5 complete genomes</td>
<td>98% of PCP at 2 fold coverage</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No differences to modern PCP</td>
</tr>
</tbody>
</table>

Schuenemann et al. 2011, PNAS
Authenticity of Plague DNA

DNA damage profile

Likely sites of DNA damage
Miscoding lesions

C $\rightarrow$ T

Neandertal DNA

fragments: 529
DNA damage over time

Sawyer et al. 2012, PLoS ONE
Authenticity of Plague DNA

DNA damage profile

Likely sites of DNA damage
Miscoding lesions

C $\Rightarrow$ T
Conclusions Study 1:

- Authentic ancient human DNA preservation in Black Death victims
- Authentic Y. pestis DNA preservation in Black Death victims
- Y. pestis was at least one of the causative agents of the Black Death
Fishing for the full medieval Y. pestis genome

- pCD1 Plasmid 70kb
- Chromosome 4.6Mb
- pMT1 Plasmid 100kb
Before Array enrichment

0.0005% Y.pestis

After Array enrichment

60% Y.pestis

Fishing for the full medieval Y.pestis genome

Bos et al. 2011, Nature
Results

99% of the genome
30 fold average coverage

Bos et al. 2011, Nature
Results – Full Genome Analysis

Human pathogenic Y.pestis strains

Black Death
Y.pestis Strain
1283-1347 AD

Justian Plague
6th – 7th century

Antonine Plague
2nd century

Y. pseudo tuberculosis
soil bacteria

Y. pestis
Microtus
rodent pathogen

Black Death
Y. pestis Strain

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After Bos et al., 2011 Nature
Conclusions Study 2:

Medieval *Yersinia pestis* is:

- Not so different from modern strains
- Does not have a single private substitution
- Close to the Most recent common ancestor of human pathogenic *Y.pestis*

The genetic makeup of *Y.pestis* likely not responsible for the increased mortality during the Black Death pandemic.
What contributed to the high mortality in the medieval era?

*Climate?*

*Changes in vector populations?*

*Genetic susceptibility of host populations?*

*Con-current disease?*

*Social conditions?*
Outlook

- samples from earlier Plagues such as Justinian plague

- more Black Death genomes for a better understanding of plague diversity

- study genetic response of human populations to pathogens, e.g. immunity related genes

- more historical pathogens tuberculosis, leprosy, smallpox, syphilis

Ancient Pathogen Genomics

(fossil record for Pathogens)
Dr. Kirsten Bos

Dr. V. Schünemann

Prof. Olaf Riess

Prof. Daniel Huson

Prof. Hendrik Poinar

- Hernan Burbano
- Martin Kircher
- Matthias Meyer
- Svante Pääbo

Carl Zeiss Stiftung
I'M FED UP WITH THIS GUY -
LET'S BECOME PATHOGENIC