Imperial College London

MRC Medical Research Council

How will the control of infectious disease be improved by genomic analysis?

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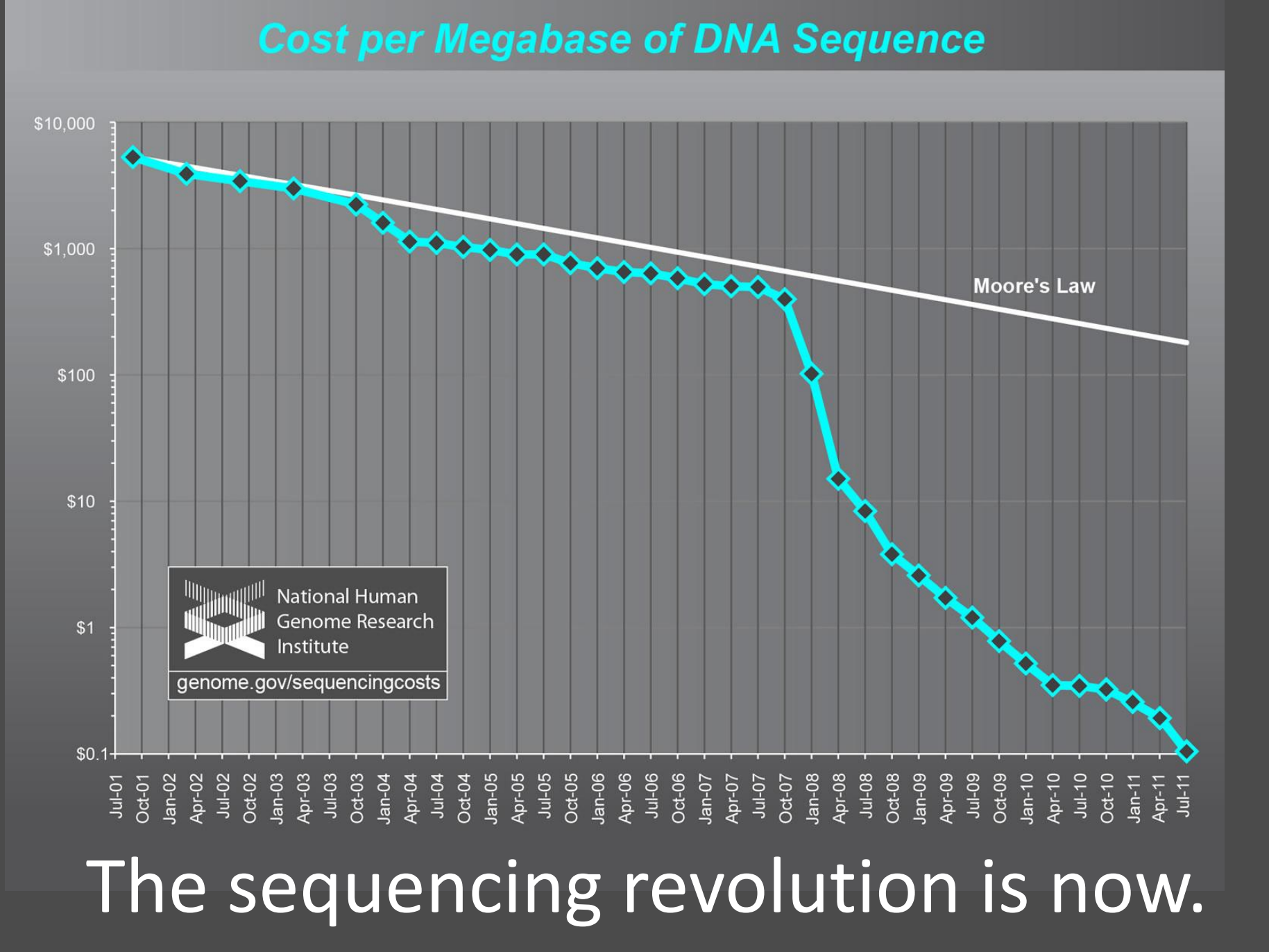
Dept of Infectious Disease Epidemiology



The sequencing revolution is now.

Desktop sequencing in every lab and in every hospital ward

Sequencers are the new microscopes

Why sequence?

- To track pathogens and outbreaks
- To identify factors important for disease
- To tailor treatment
- To understand evolution

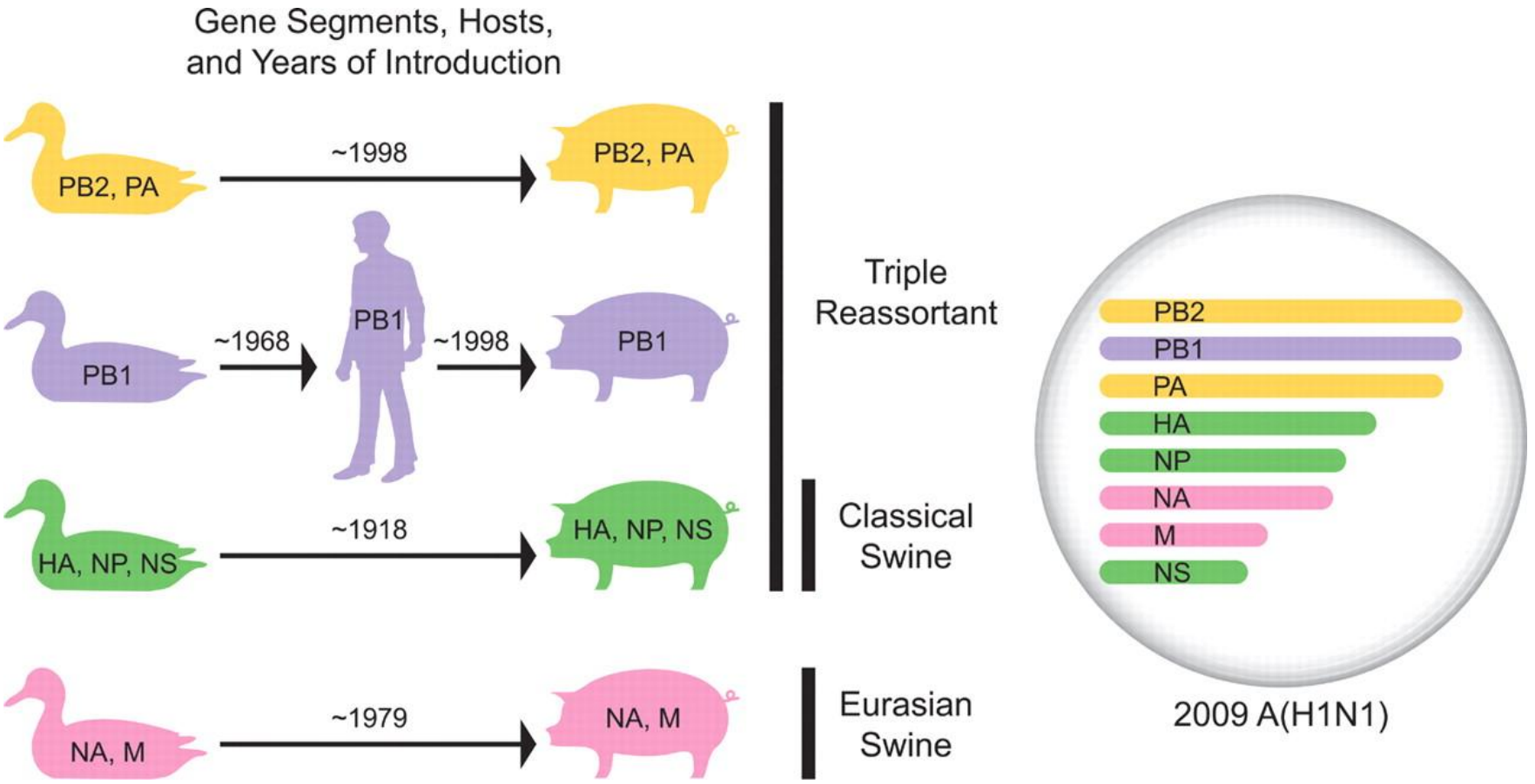
Two Examples:

- The 2009 H1N1 influenza pandemic
- The PMEN-1 lineage of *Streptococcus pneumoniae*

- **The 2009 H1N1 influenza pandemic**

- Tracking bacterial spread and evolution

In April 2009, virological surveillance triggered a pandemic



Garten et al Science 2009, Smith et al Nature 2009

Genetic data was shared in days

NCBI

Influenza Virus Resource
Information, Search and Analysis

HOME | SEARCH | SITE MAP | Flu home | Database | Genome Set | Alignment | Tree | BLAST | Annotation | FTP | Help | Contact us

Influenza Virus Resource presents data obtained from the NIAID Influenza Genome Sequencing Project as well as from GenBank, combined with tools for flu sequence analysis and annotation. In addition, it provides links to other resources that contain flu sequences, publications and general information about flu viruses.

Read more about: This resource | Flu database | NIAID Influenza Sequencing Project | Influenza virus biology

- NCBI
  - Growth of flu sequences
  - GenBank sequences from the NIAID Project
  - Assembly Archive
  - Trace Archive
  - NIAID data releasing status
  - RefSeq genomes
  - RefSeq proteins
  - Protein Structures
- Flu resources
  - NIAID Project
  - JCVI Flu
  - CDC Flu
  - Vaccine Selection
  - WHO Flu
- NCBI Viruses

GenBank sequences from 2009 H1N1 influenza outbreak

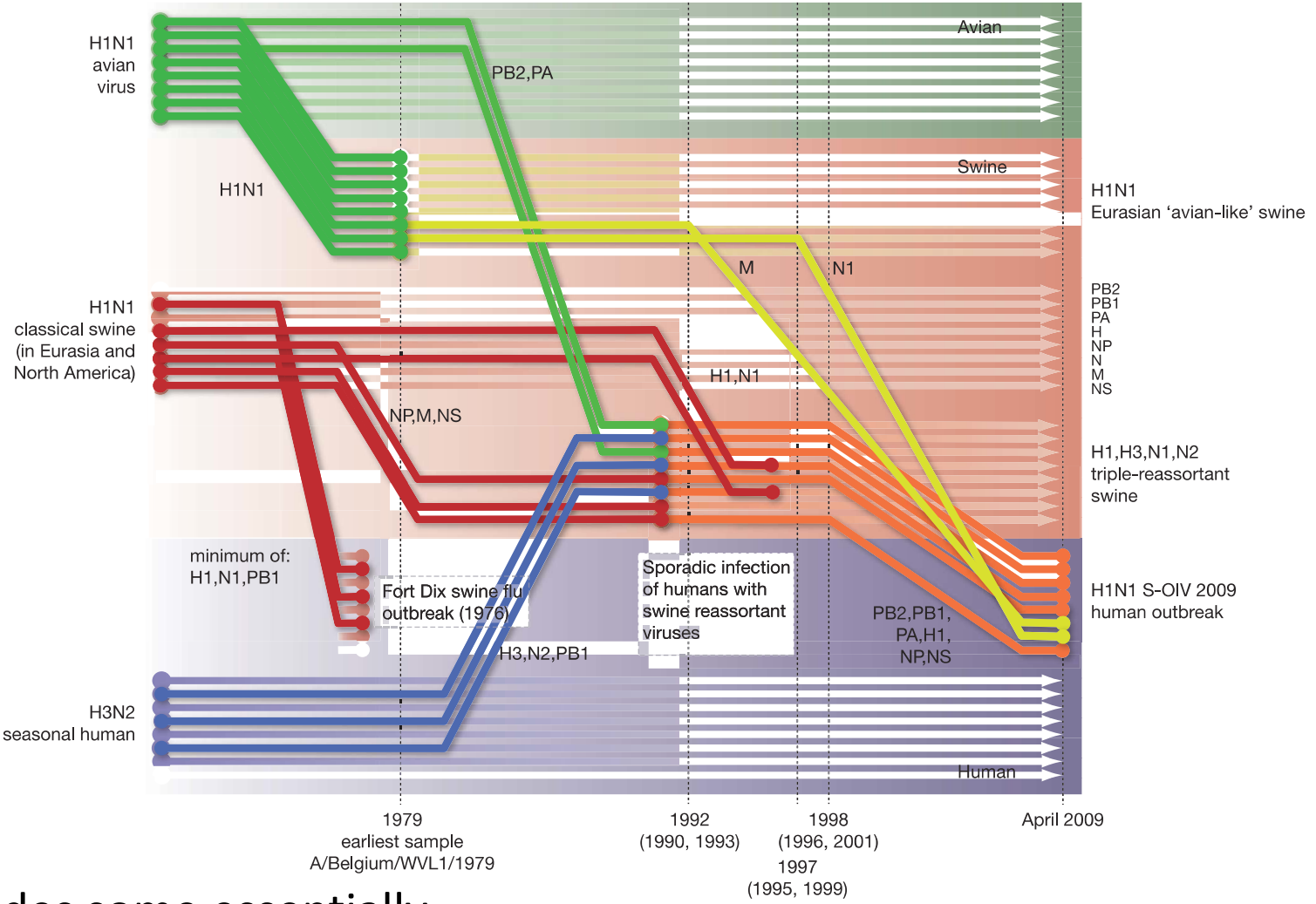
All submitted influenza sequences are available in GenBank as soon as they are processed. The 2009 H1N1 influenza virus sequences are listed on this page and are available for BLAST searching here, and are also available in the **NCBI Influenza Virus Sequence Database**, and can be retrieved with sequences from other influenza viruses for further analyses using tools integrated to the database. **Go to a tutorial for instructions downloading these sequences.** A complete list of GenBank sequences for these viruses can also be obtained through **a special genome project page** or directly from **here**. The result of RPS-BLAST against PDB database, and a summary of amino acid differences in proteins of these viruses are available at Riken National Institute of Japan.

The following 2009 H1N1 influenza virus sequences were submitted to NCBI and are available in GenBank:

June 19, 2009, 8 submitted by University of Padova, Italy; 9 by National Institute for Health and Welfare, Finland; 1 by Laboratory, Biogentec, Sector La Vara S/N, Chile; 10 by National Institute of Infectious Diseases, Japan:

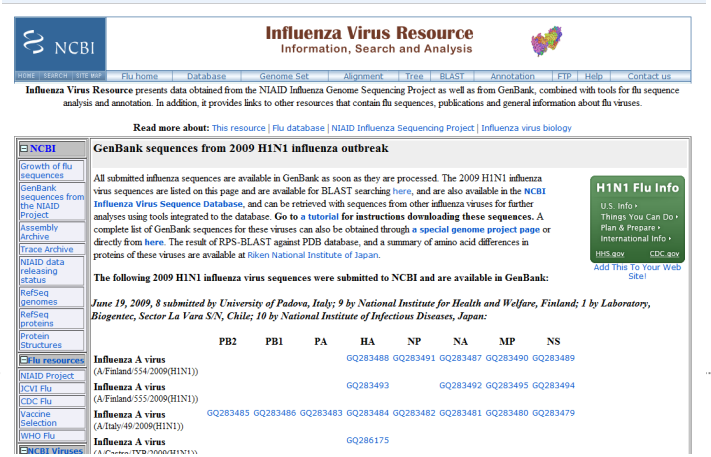
	PB2	PB1	PA	HA	NP	NA	MP	NS
Influenza A virus (A/Finland/554/2009(H1N1))				GQ283488	GQ283491	GQ283487	GQ283490	GQ283489
Influenza A virus (A/Finland/555/2009(H1N1))				GQ283493		GQ283492	GQ283495	GQ283494
Influenza A virus (A/Italy/49/2009(H1N1))	GQ283485	GQ283486	GQ283483	GQ283484	GQ283482	GQ283481	GQ283480	GQ283479
Influenza A virus				GQ286175				

Genomics revealed a complex evolutionary history

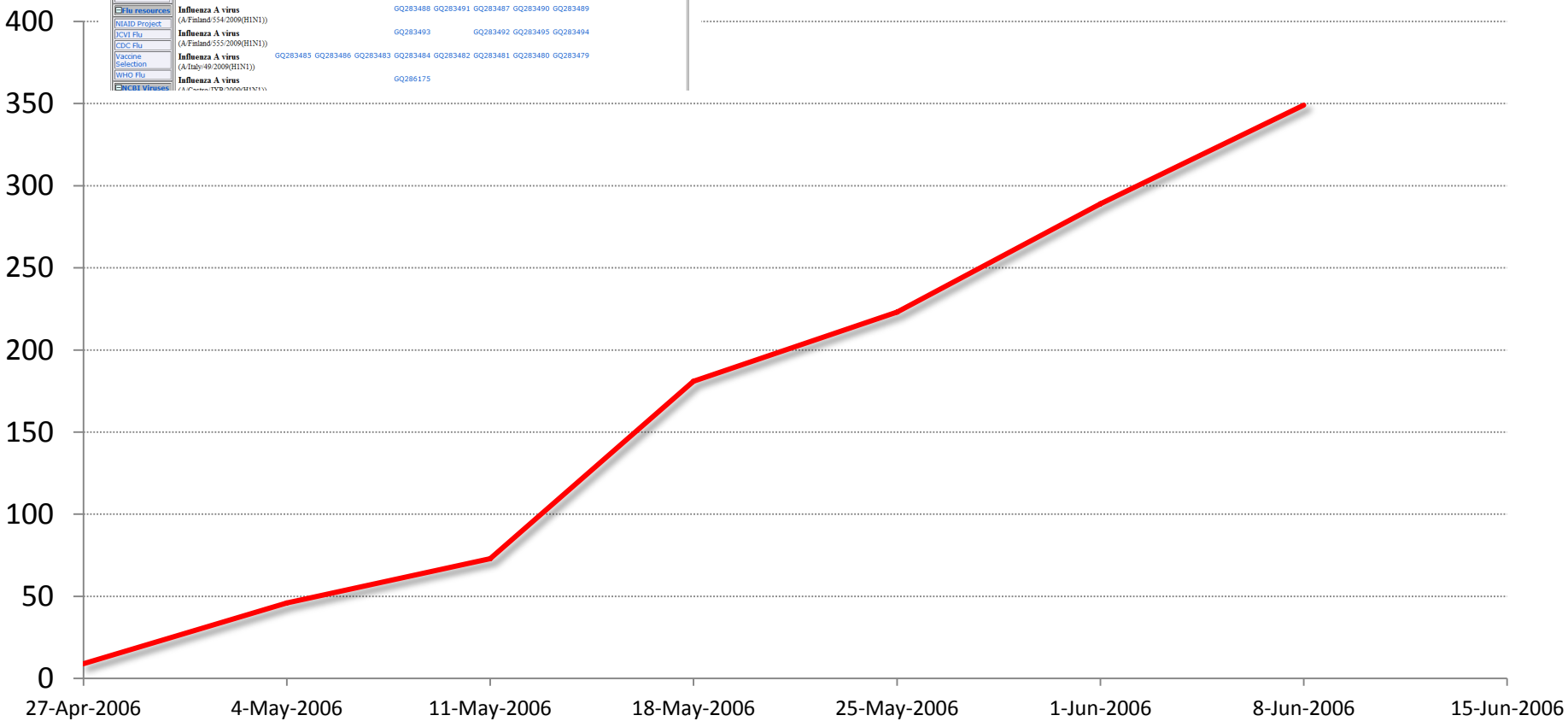


Still includes some essentially human-adapted core genes

Gavin Smith et al, Nature 2009 & 2010

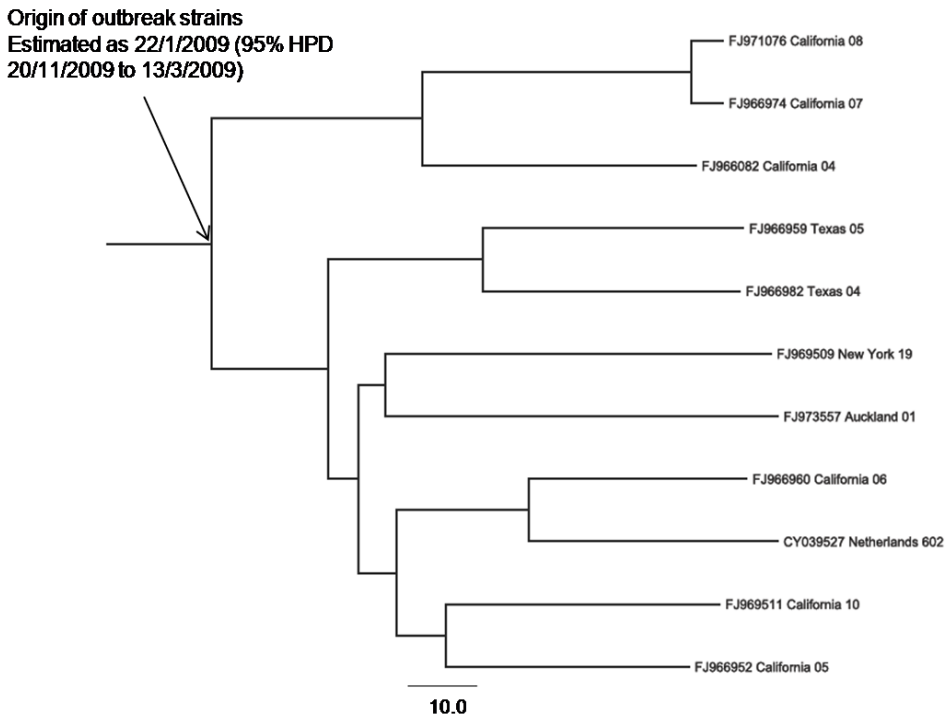


Number of full length HA sequences:



Currently over 2,600 full length HA sequences (for H1N1pdm virus)

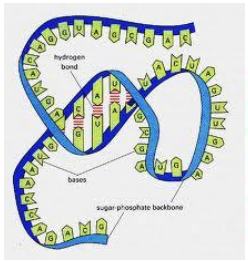
Earliest H1N1pdm trees (4 May)

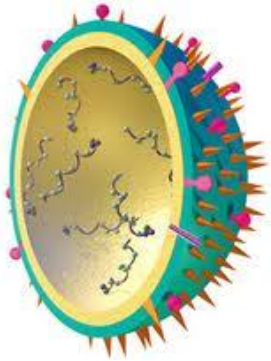


We can already tell from tree structure that the epidemic wasn't growing very fast…

Molecular epidemiology to detect local transmission (EpiInfo)

PHYLODYNAMICS:

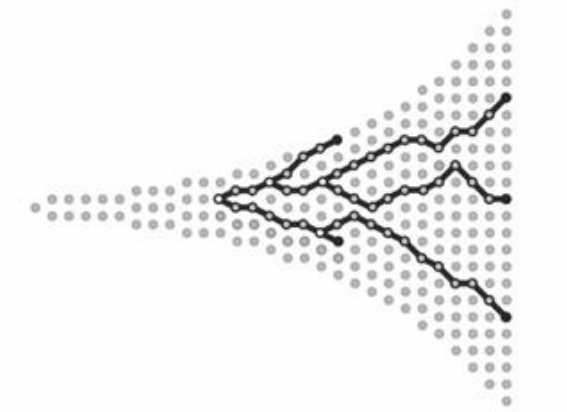


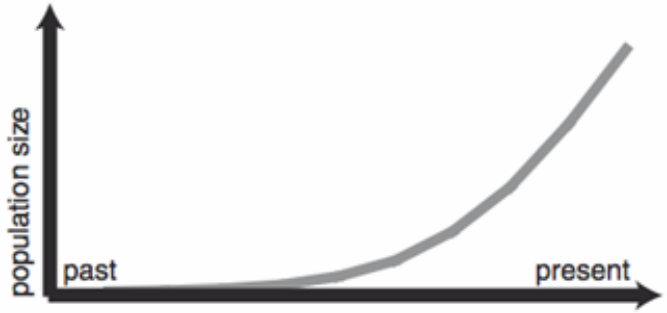


BEAST (Bayesian Evolutionary Analysis Sampling Trees)

aaaagcaaca aaaatgaagg caatactagt agttctgcta
tgcagacaca ttatgtatag gttatcatgc gaacaattcat
actagaaaag aatgtaacag taacacactc tgttaacctt
gaaactatgc aaactaagag gggtagcccc attgcatttg
ctggatcctg ggaaatccag agtgtgaatc actctccaca
tgtggaaaca tctagttcag acaatggaac gtgttaccca

+

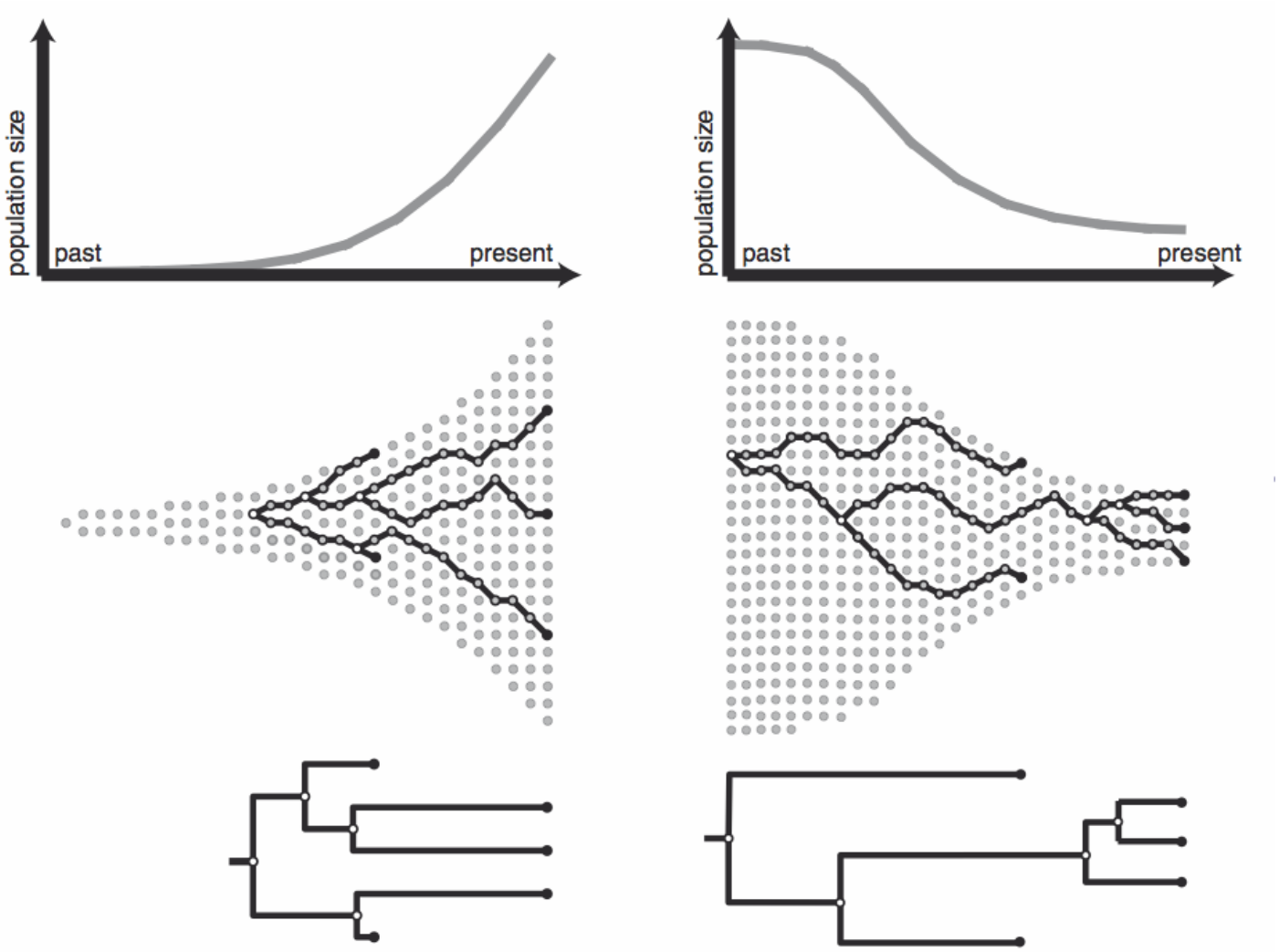




Bayesian Skyline Plot

BSP

The coalescent with variable population size



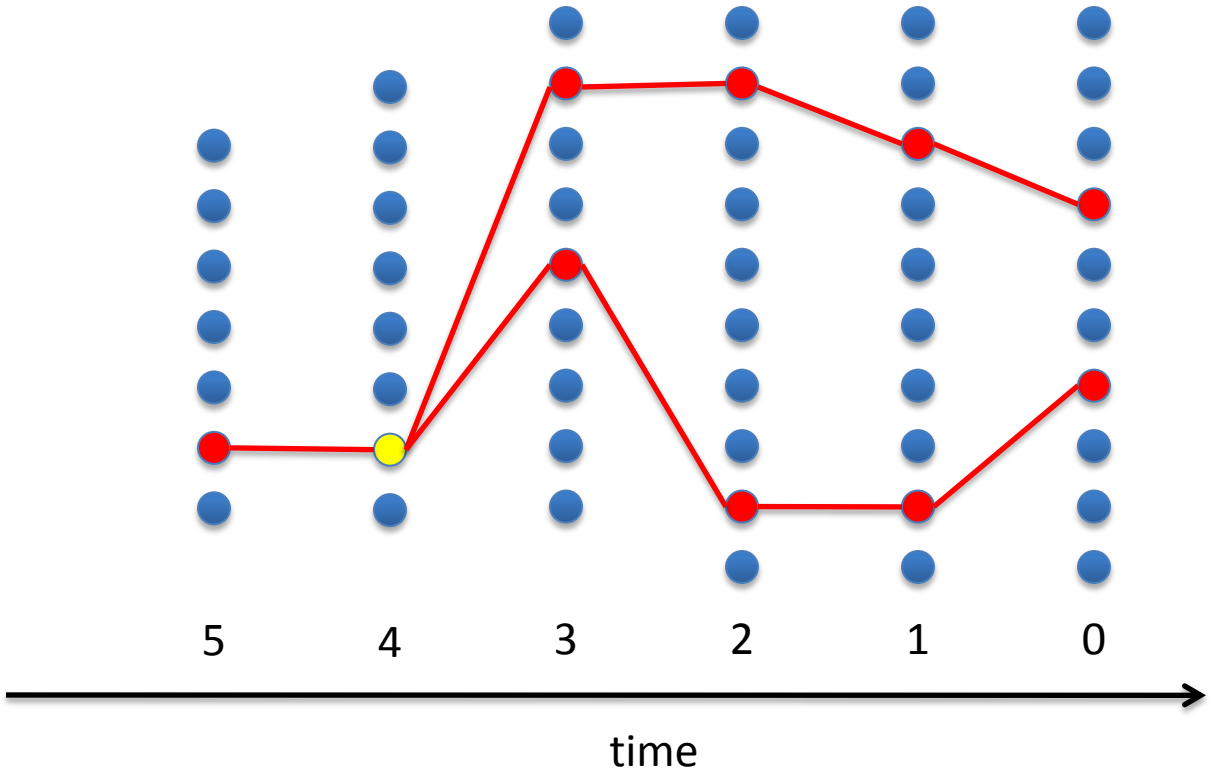
Lemey et al, AIDS Rev 2006

The coalescent

Consider 2 infected people randomly chosen from N_t total infected people

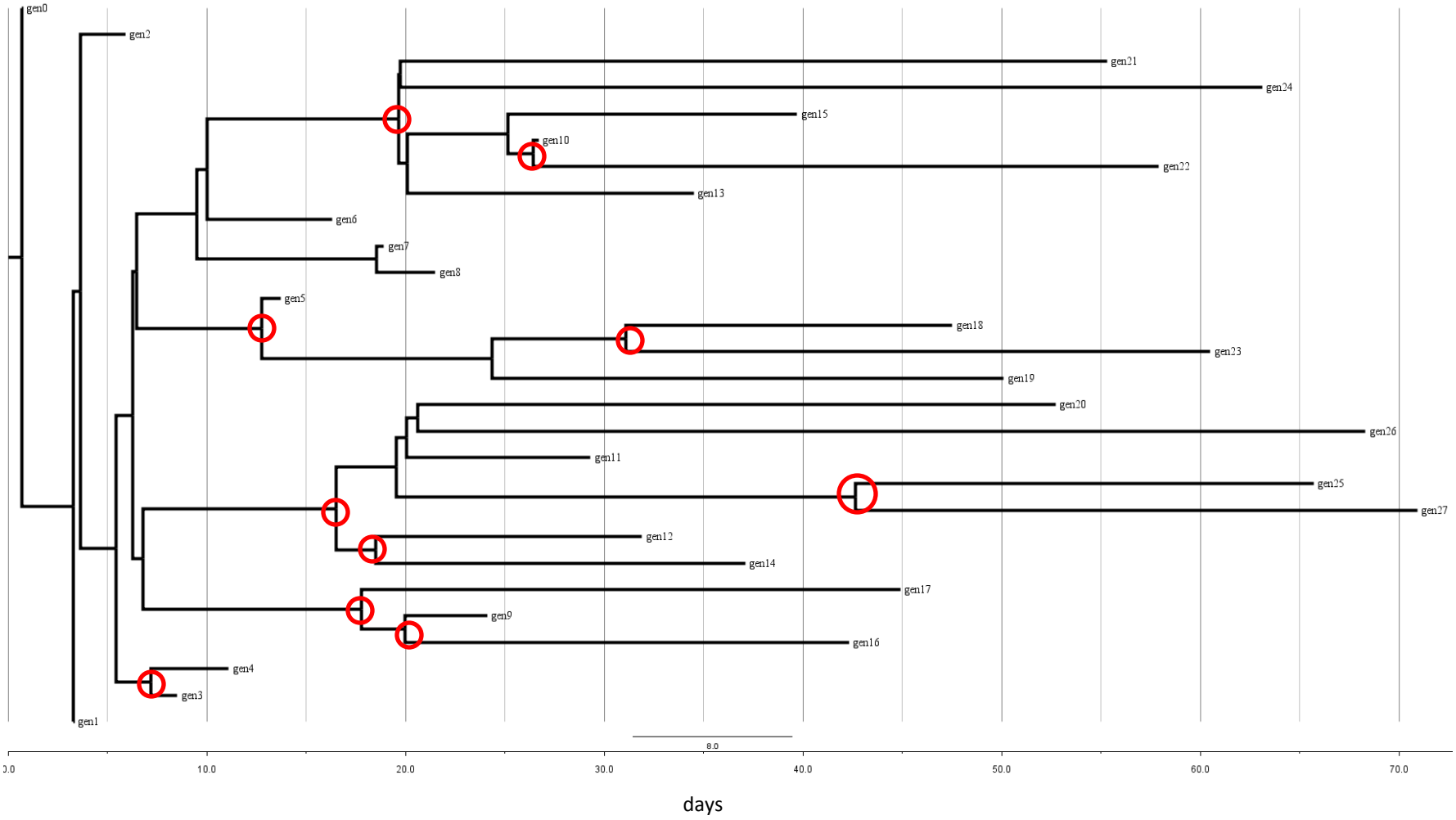
The coalescent

Probability they shared a common ancestor time T ago is $\frac{1}{N_T}\prod_{x=1}^{(T-1)}\left(1-\frac{1}{N_x}\right)$



Time is measured in generations of infection

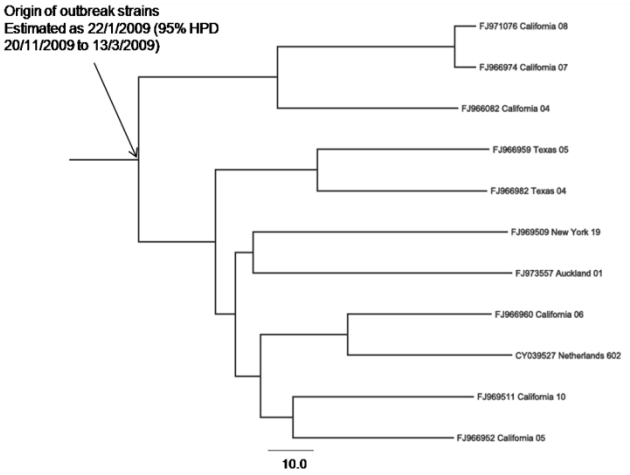
The coalescent can thus be used to reconstruct the number infected from a phylogeny…

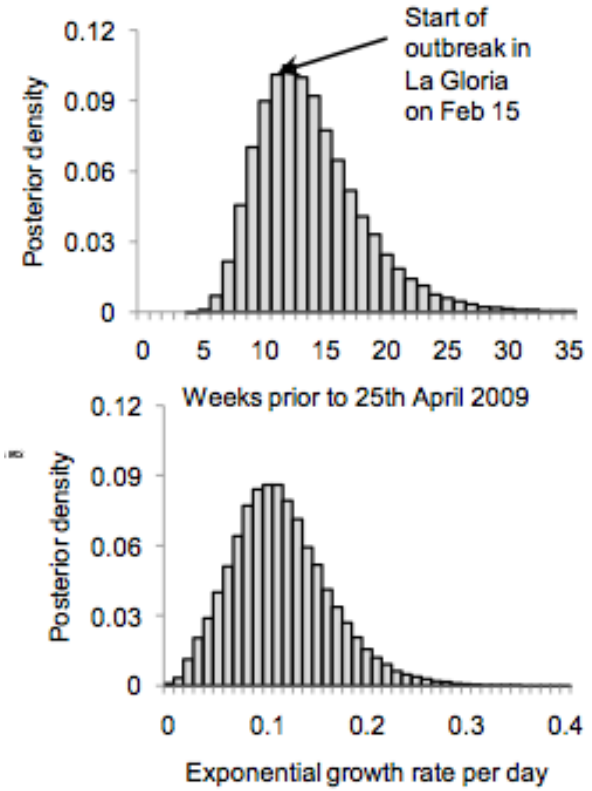


○ coalescent events

…provided a suitable model is used to relate mutations to time (*molecular clock model*)

Earliest H1N1pdm trees (4 May)



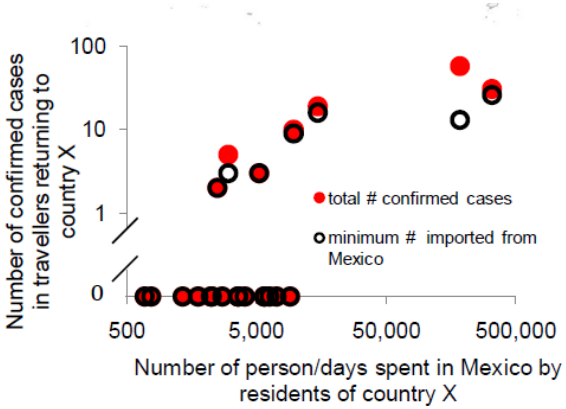


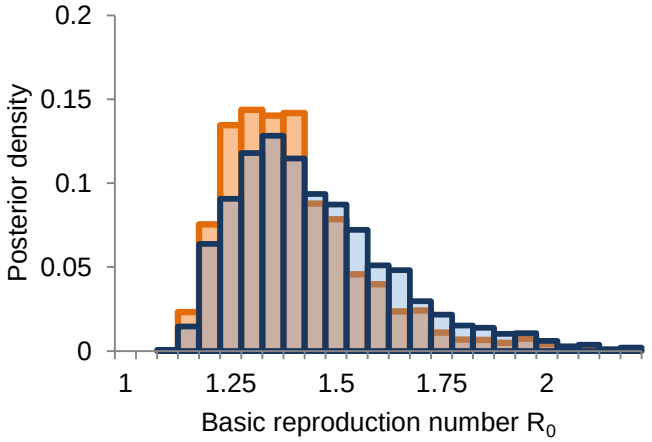
Can already tell that the epidemic wasn't growing very fast

Based on <u>assuming</u> exponential growth: strong assumptions → robust estimates

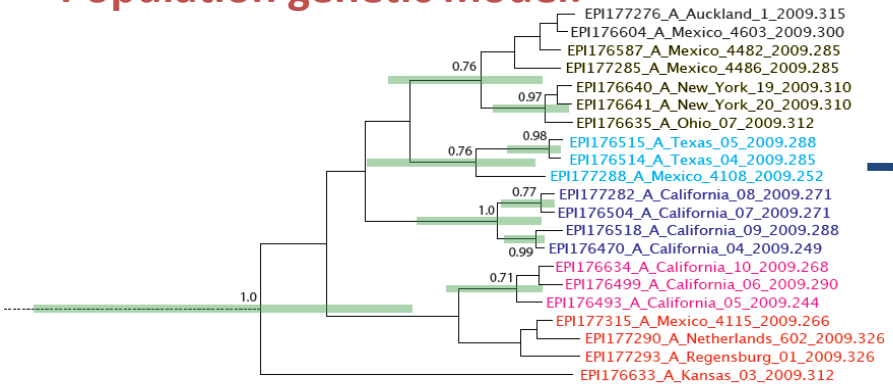
9 May: Updated trees

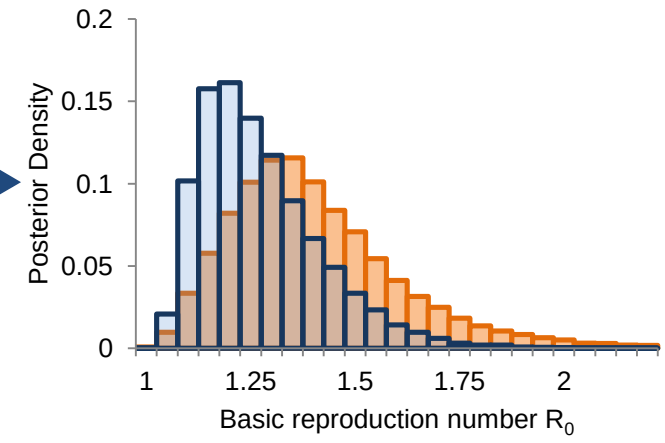
Epidemiological model:





Population genetic model:

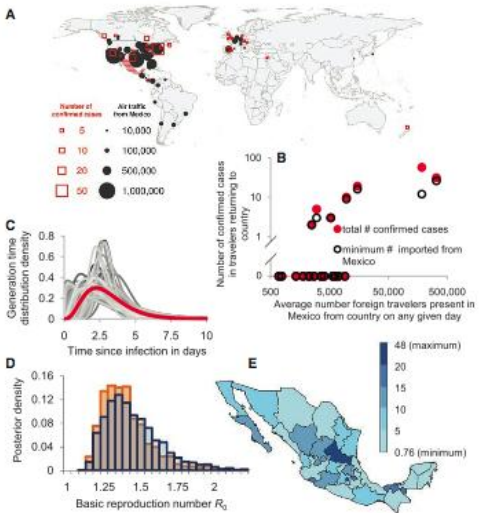




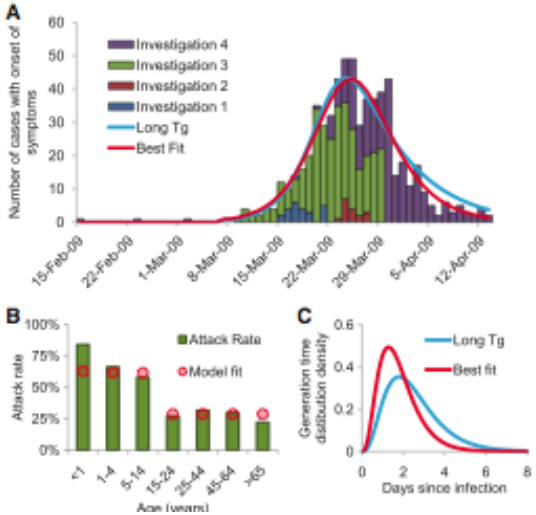
Orange – first iteration

Blue – updated – more effort to obtain ‘random’ unlinked sample

Fraser et al, Science 2009

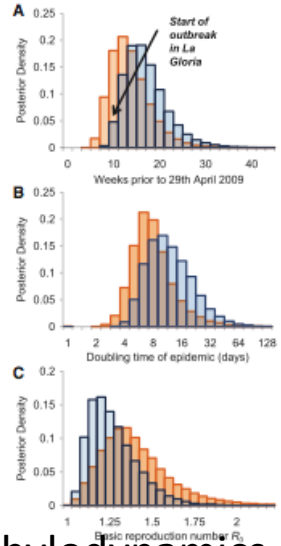


Back-calculation

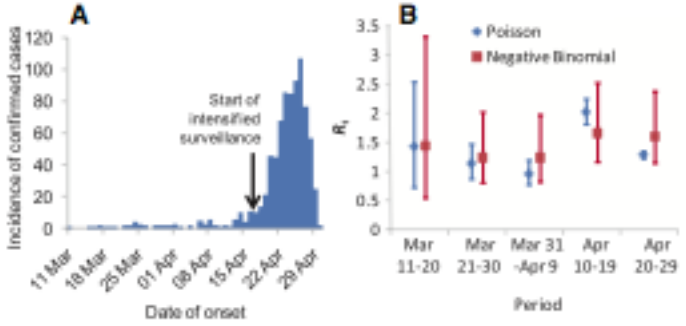


Outbreak investigation

Effective reproduction number



Phylodynamics

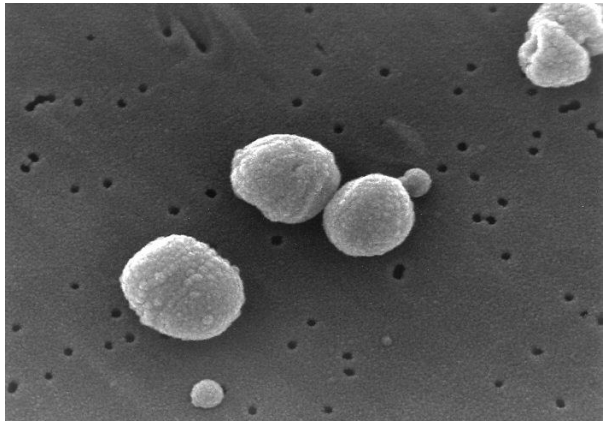


Time-series analysis

- The 2009 H1N1 influenza pandemic

- **Tracking bacterial spread and evolution**

Streptococcus pneumoniae

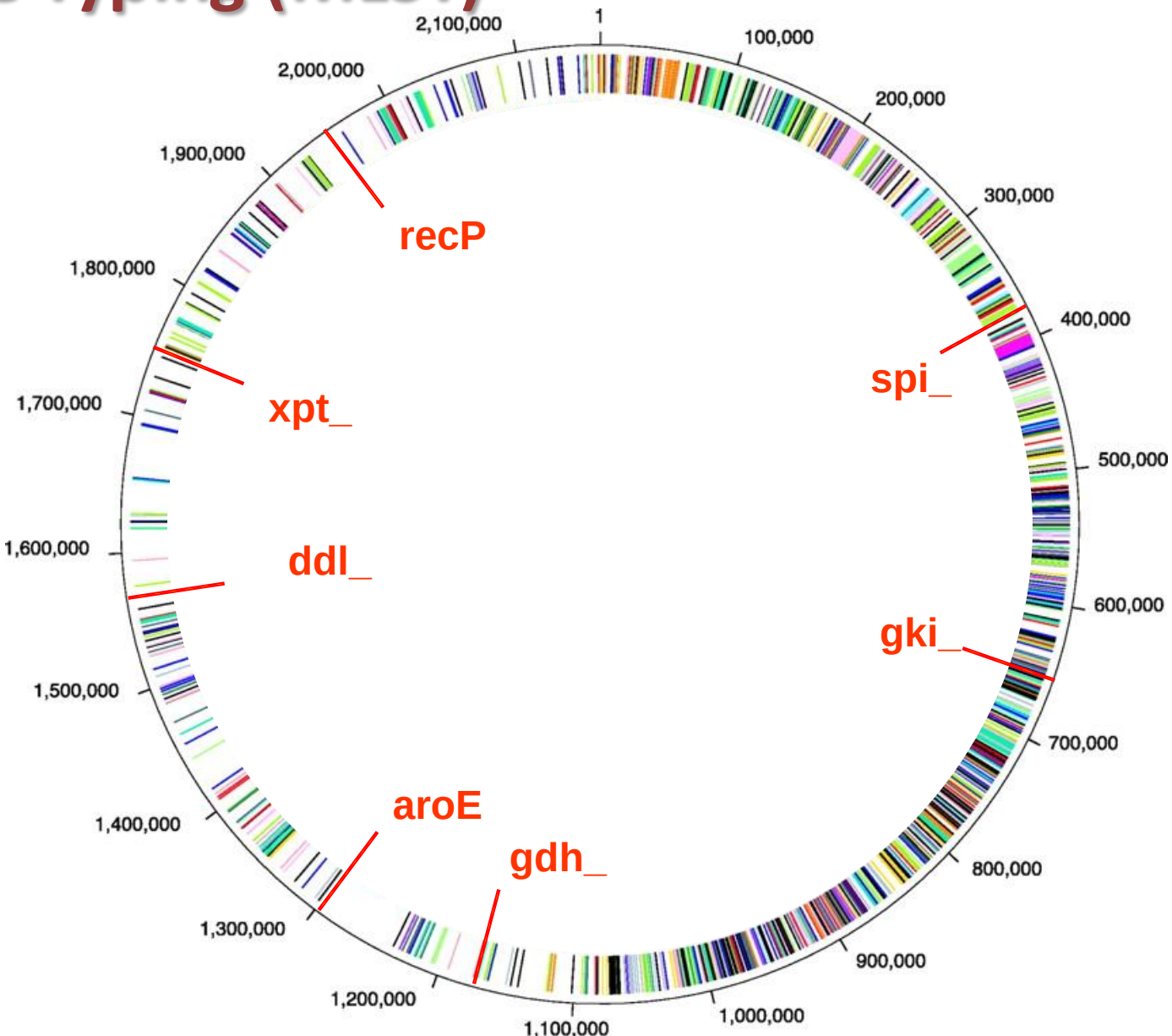


- Gram +ve, commonly carried.
- Near ubiquitous in children.
- Causes otitis, pneumonia, invasive disease, meningitis, ….
- Causes 10% of all paediatric mortality.
- Diverse patterns of virulence and resistance.
- Antigenically diverse (92 serotypes).
- 7- and 13-valent vaccines now available.
- Naturally competent – recombinogenic.

Vaccine-caused Serotype Replacement

- Serotype replacement was complete in US in <10 years (Hanage et al, Epidemics 2009).
- Disease levels decreased approx 2/3 in US, but very little decline in the UK (HPA UK).
- Huge ecological perturbation, with unknown effect on antibiotic resistance and virulence factors
- What are implications for global roll-out?

Probing the core genome with Multi-Locus Sequence Typing (MLST)

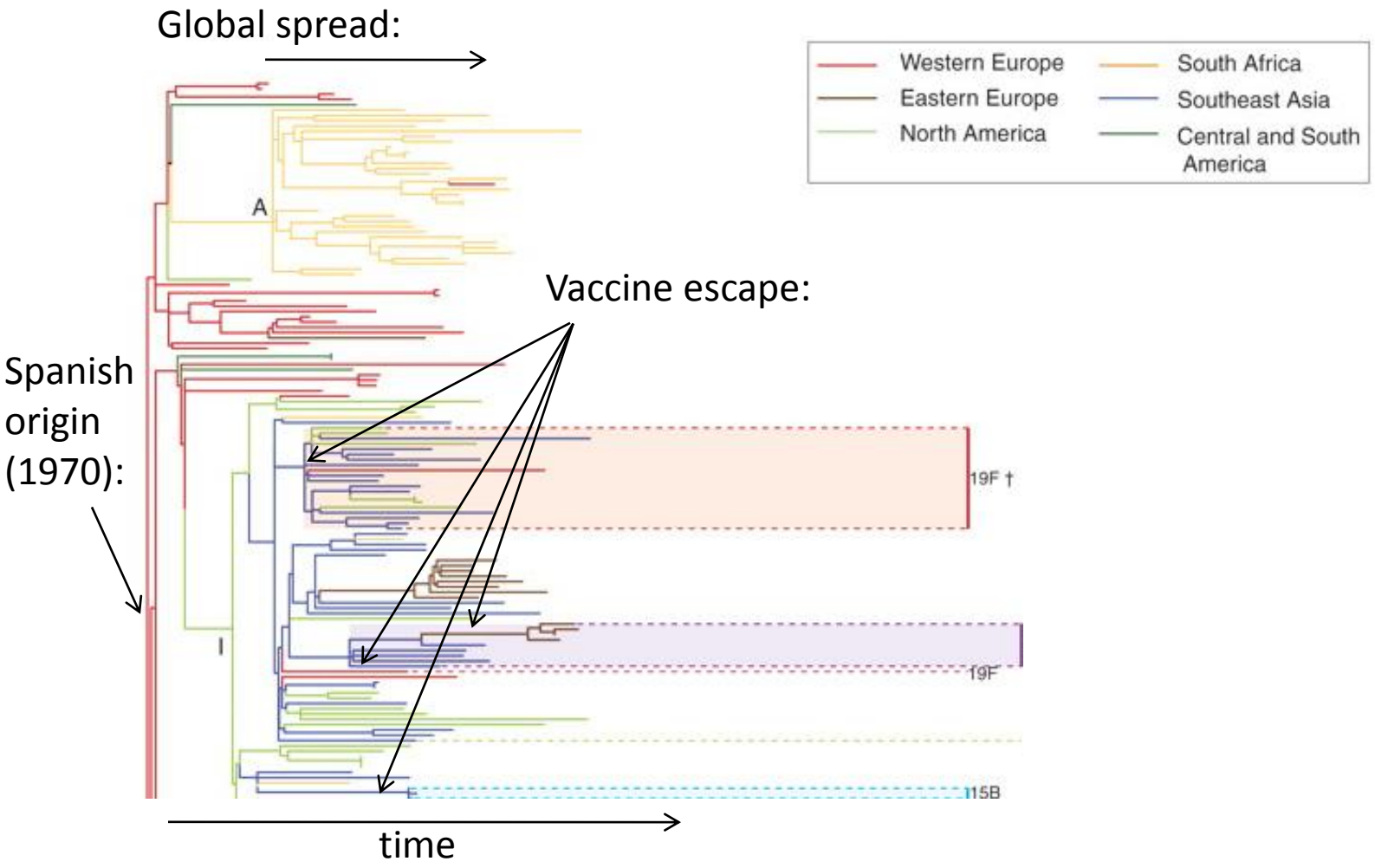


A population genomic analysis

- Focus on PMEN-1 lineage:
  - Earliest recognised multi-drug resistant lineage of *Streptococcus pneumoniae* (penicillin, chloramphenicol, tetracycline, occasionally: fluoroquinolones & rifampicin, …)
  - Predominantly serotype 23F/ST81
  - Caused 40% of invasive disease in USA in 1990s
  - Member of the highly mosaic cluster (*based on BAPS/MLST analysis*)

- Full genomes from 241 isolates

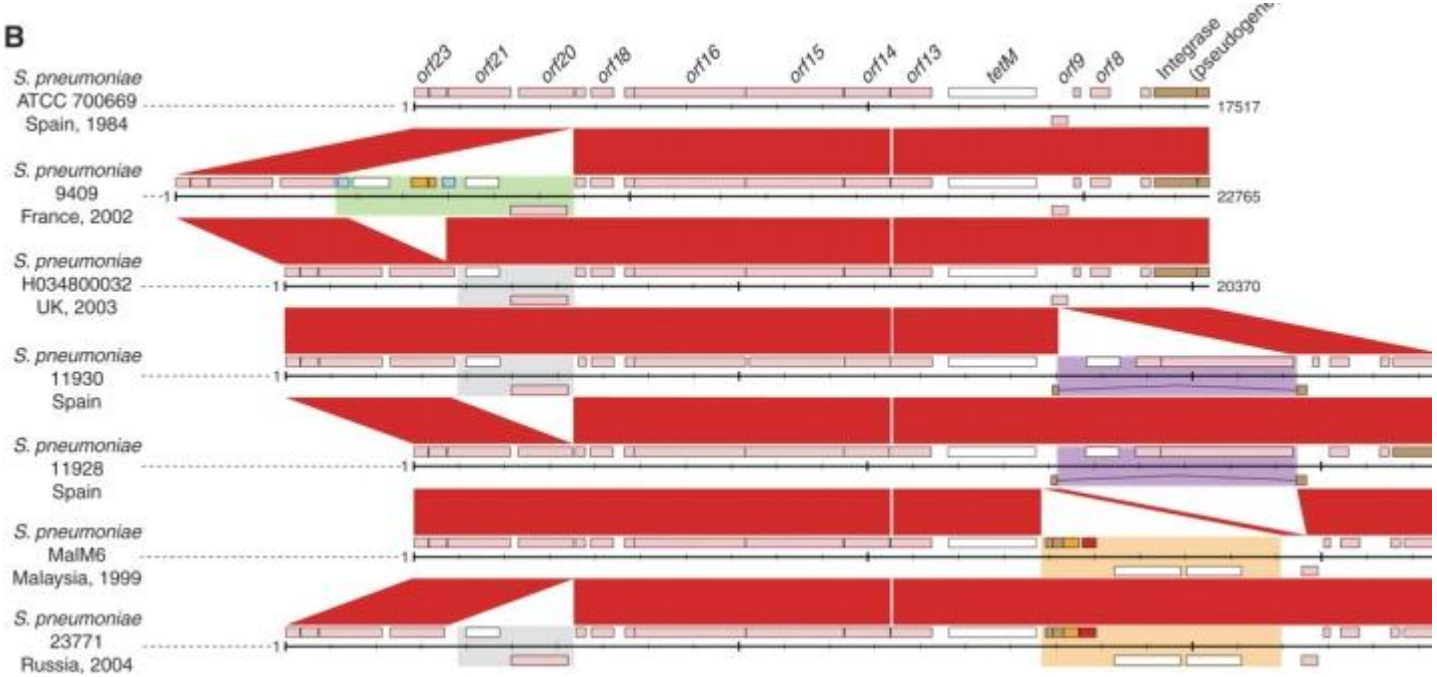
Rapid Pneumococcal Evolution in Response to Clinical Interventions

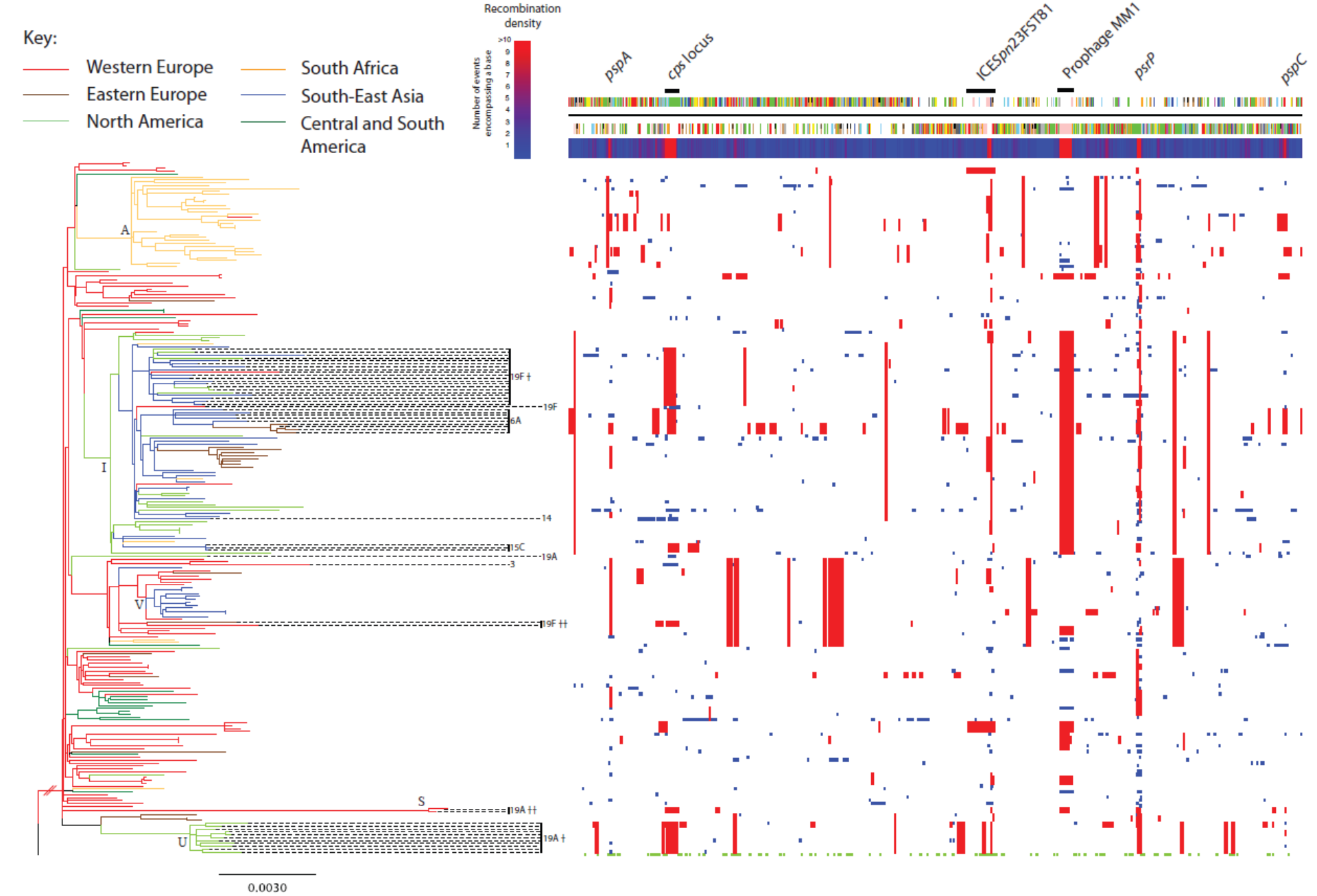


Croucher et al, Science 2011

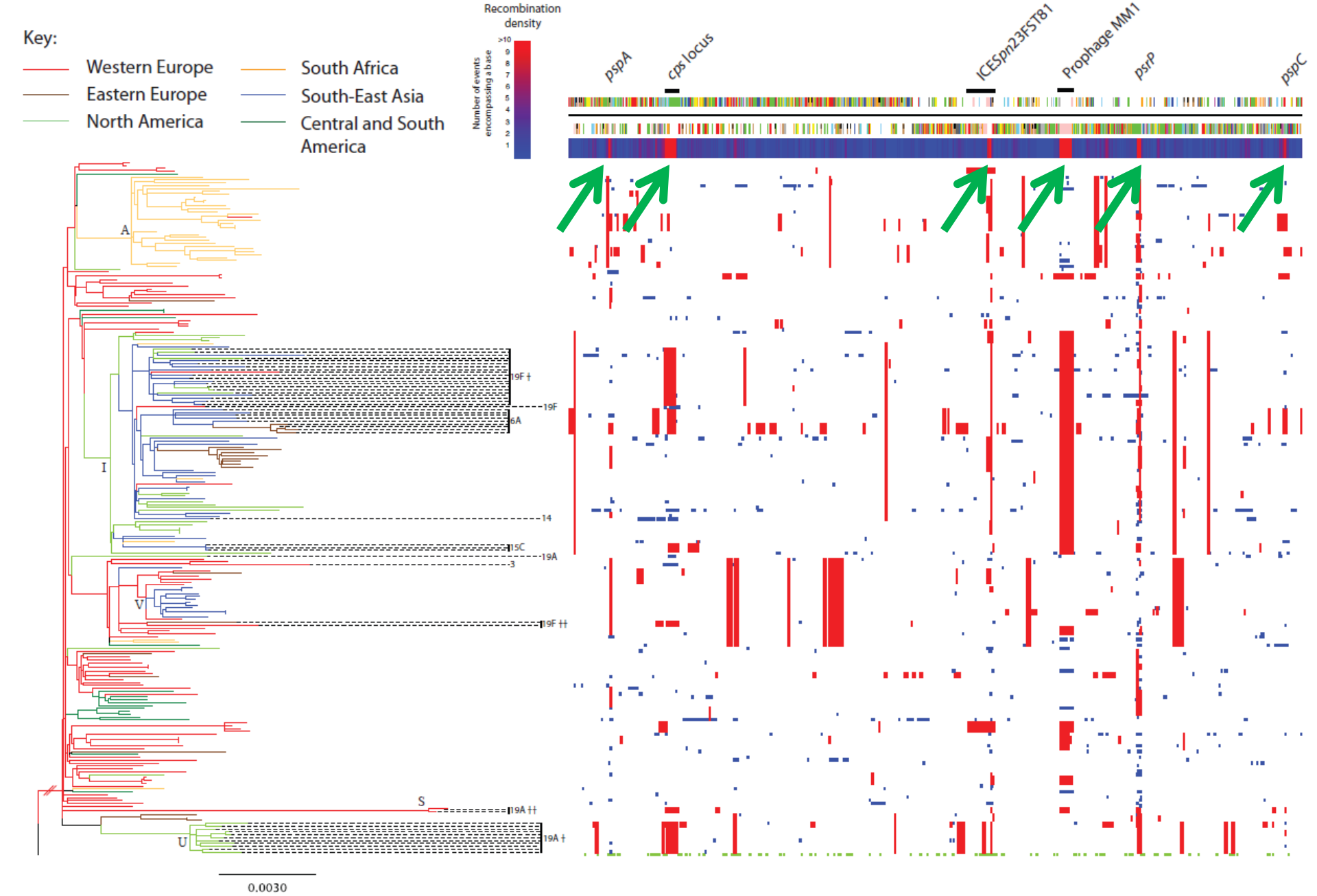
Multiple acquisition & loss of antibiotic resistance

- Whole lineage is resistant to penicillin, chloramphenicol & tetracycline.
- Fluoroquinolone resistance mutations acquired & lost, seemingly random.
- Macrolide resistance cassettes acquired repeatedly through horizontal gene transfer

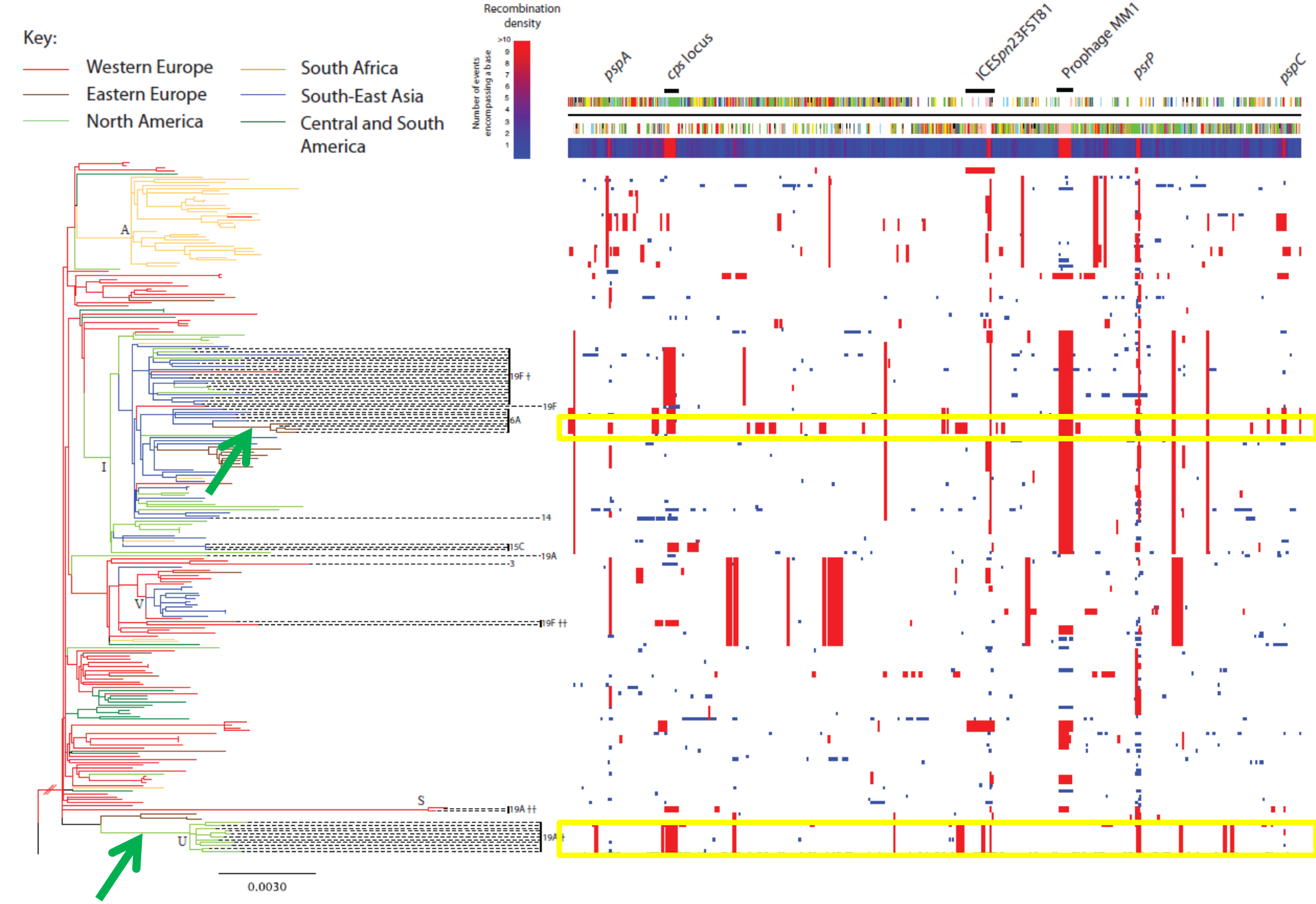




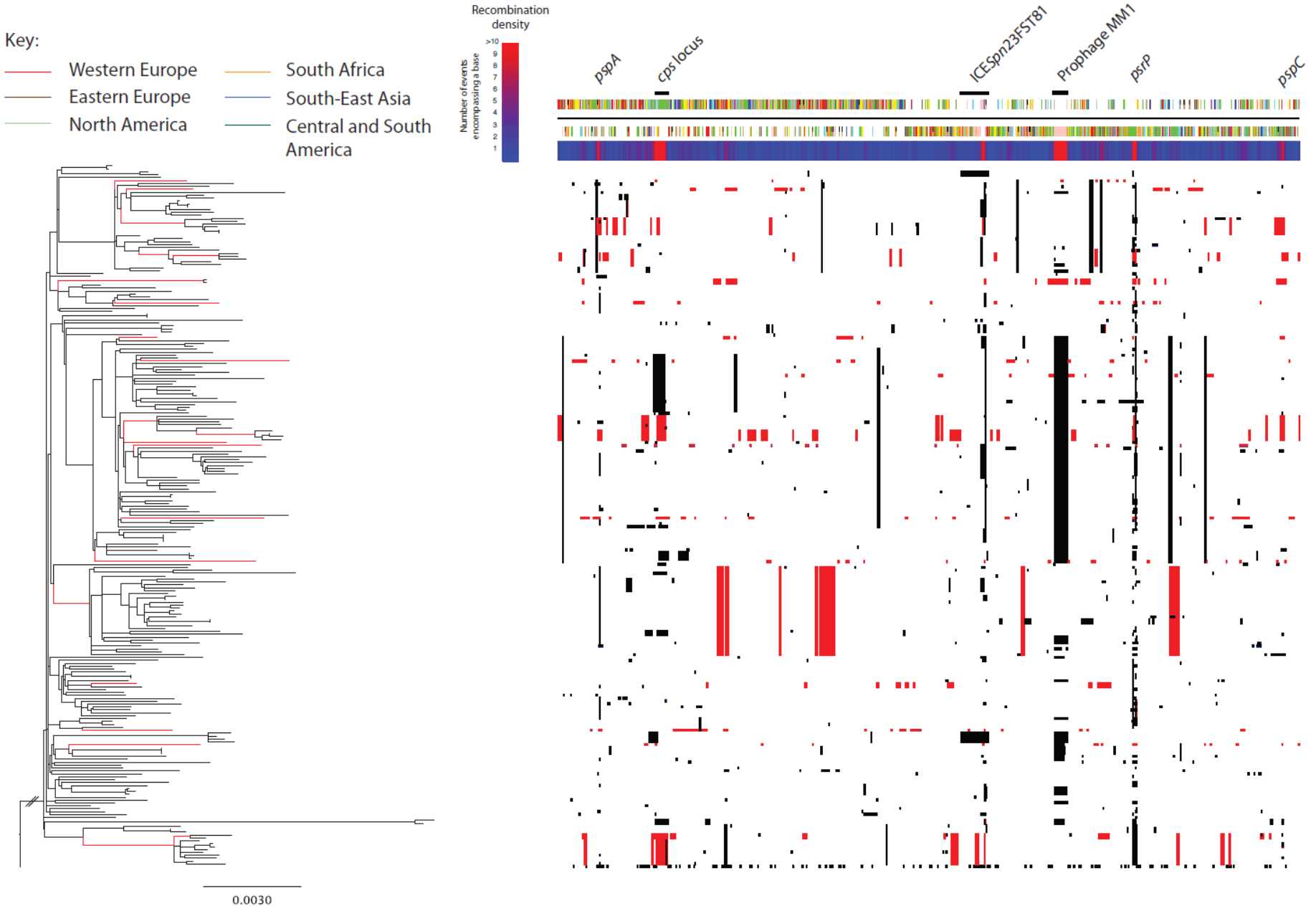
90% of polymorphisms acquired by recombination, covering 75% of genome (identified by SNP density)



Recombination not uniform along genome, but marked by hotspots

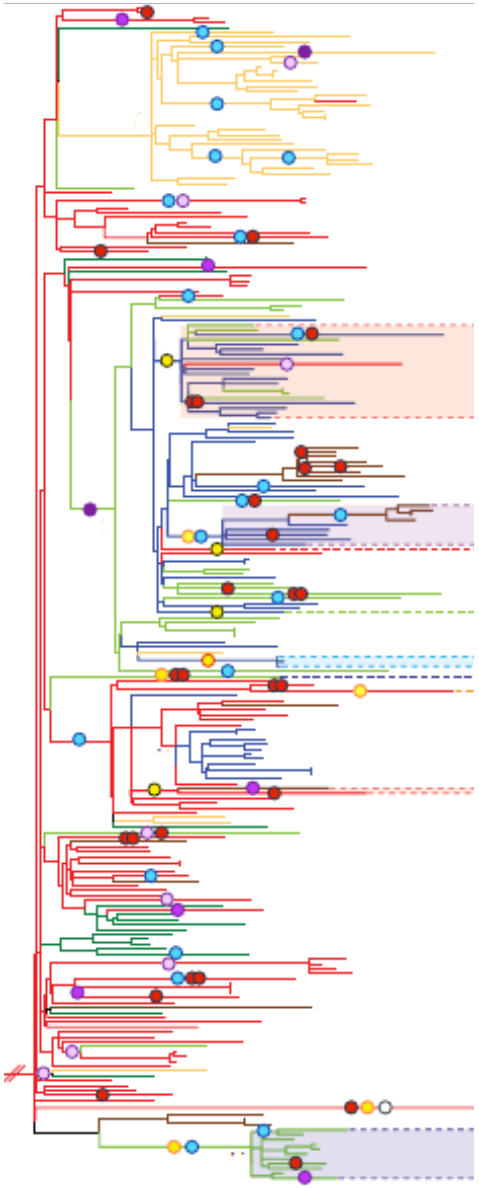


Some suggestion of lineages having experienced hyper-recombination?



Hyper-recombination accounts for over 50% of polymorphisms – role still unclear

Phylogeny reveals a rich selective history



Blue: hyper-recombination events
Yellow: serotype switches
Purple: Acquisition of macrolide resistance cassettes
Red: Fluoroquinolone resistance mutations
White: Abrogation of competence.

With whole genome data, we can appreciate that genetic, genomic and selective events all occur concurrently, not in isolation. This will require new theory.

Summary

- PMEN-1 lineage defined by acquisition of an accessory multidrug-resistant gene (ICE), and rapid spread since 1970
- Further changes in lineage driven by rapid switching in accessory genome (inc. antibiotic resistance and vaccine escape)
- New understanding of mechanisms of recombination

Conclusions

- Sequencing is cheap, and will soon be standard.
- Most infections will be ‘typed’.
- Interpretation & analysis will remain challenging.
- Data storage & sharing, and linkage to meta-data will be extremely challenging, but necessary.