Clinical and Epidemiological Virology,
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Introduction to molecular epidemiology and infectious disease phylodynamics

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SISMID, July 20-22, 2016

This course (SISMID module 13)

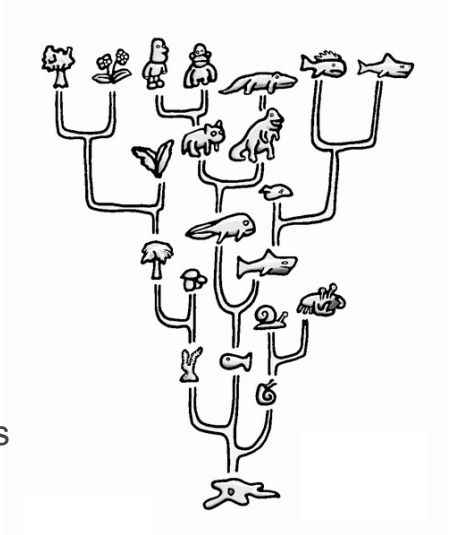
- Wednesday, July 20
  - ➡ Introduction
  - ➡ Alignment, substitution models and phylogenetic inference
- Thursday, July 21
  - ➡ Phylogenetic inference practical
  - ➡ Bayesian phylogenetics
  - ➡ Molecular clocks and model testing
  - ➡ BEAST practical
- Friday, July 22
  - ➡ Viral epidemiology and the coalescent
  - ➡ BEAST practical
  - ➡ Phylogeography
  - ➡ BEAST practical
- Bonus
  - ➡ Phylo-Alignment
  - ➡ Recombination
  - ➡ Robust Counting

http://rega.kuleuven.be/cev/ecv/

(We are here to cater for your needs!)

Molecular evolution and phylogenetics

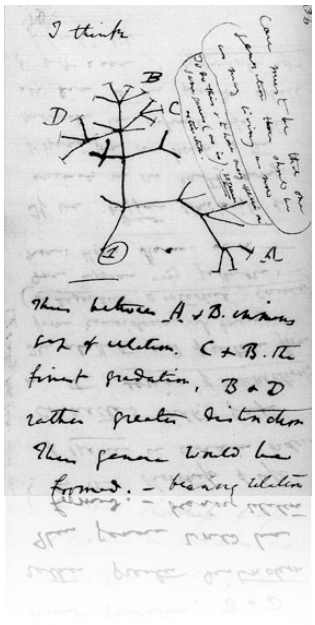
- biological **sequences** (DNA, RNA, protein) contain information about the processes and events that formed them
- this information is often **scrambled**, **fragmentary**, **hidden**, or **lost** completely
- our aim is to use **mathematical models** to recover and decipher this information
- The central concept is a **phylogeny**: a diagram depicting the ancestral relationships among characters or genetic sequences



```
HIV-1 (UK)   ATC---TGCTAAAGCATATGACACAGAGGTACATAATGTTT
HIV-1 (USA)  ATCGGATGCTAGAGCTTATGATACAGAGGTACA---TGTTT
```

Phylogenetics

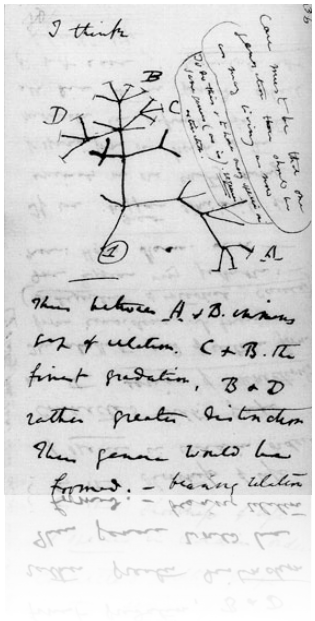
- Darwin, 1837

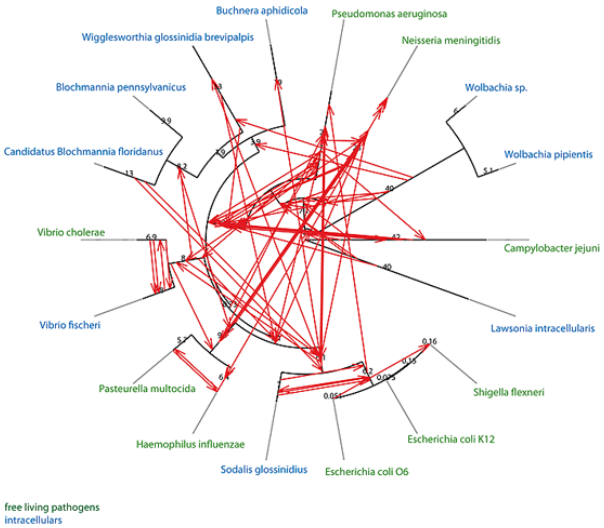


- Haeckel, 1866

Phylogenetics

- Darwin, 1837





Genome distance tree with lateral gene transfers

Information in (viral) molecular sequences

- Genetic distances among strains
- Phylogeny
  - ➡ subtyping/classification
  - ➡ identification of transmission clusters
  - ➡ association with risk factors / traits
  - ➡ forensics
- Dates of historical events
- Evolutionary processes
  - ➡ recombination
  - ➡ natural selection
- Epidemiological processes
  - ➡ transmission rates
  - ➡ movement among locations
- Phenotypic trait evolution?

```
HIV-1 (UK)   ATC---TGCTAAAGCATATGACACAGAGGTACATAATGTTT
HIV-1 (USA)  ATCGGATGCTAGAGCTTATGATACAGAGGTACA---TGTTT
```

Our goal

MOLECULAR SEQUENCES

Alignment Methods — BIOINFORMATICS

ALIGNMENT

Sequence Evolution Models
Phylogenetic Methods — PHYLOGENETICS

EVOLUTIONARY TREE

(time scale = genetic distance)

Molecular Clock Models — PHYLOGENETICS

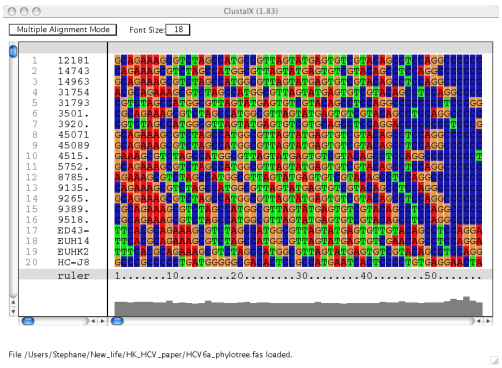
EVOLUTIONARY TREE

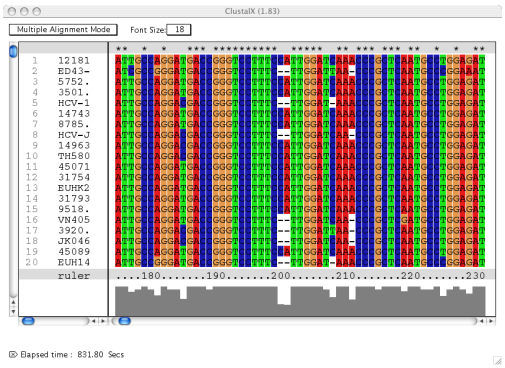
(time scale = years)

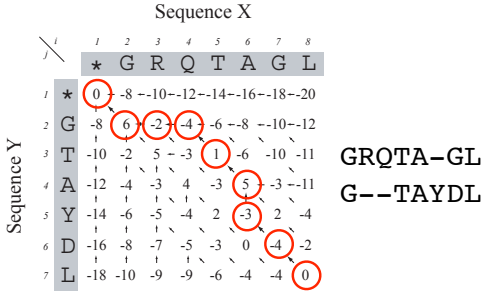
Phylodynamic Models — POPULATION GENETICS

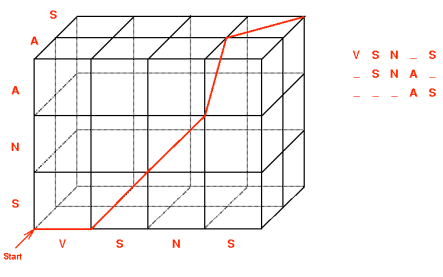
EPIDEMIOLOGY

Sequence alignment

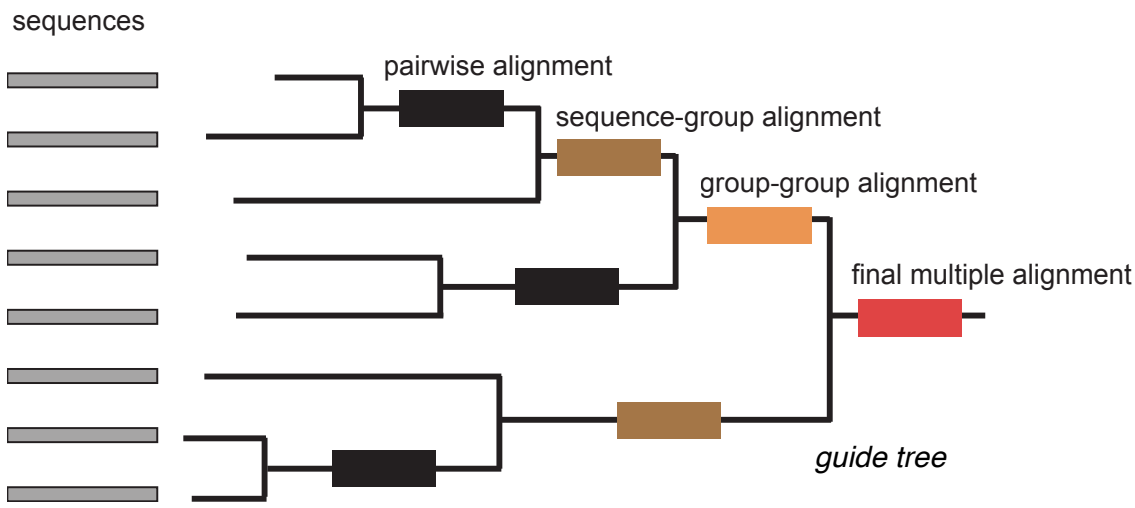








Progressive alignment



http://www.kuleuven.be/aidslab/phylogenybook/Table3.1.html

Genetic distances

```
SIVcpz    ATGGGTGCGA GAGCGTCAGT TCTAACAGGG GGAAAATTAG ATCGCTGGGA
HIV-1     ATGGGTGCGA GAGCGTCAGT ATTAAGCGGG GGAGAATTAG ATCGATGGGA

SIVcpz    AAAAGTTCGG CTTAGGCCCG GGGGAAGAAA AAGATATATG ATGAAACATT
HIV-1     AAAAATTCGG TTAAGGCCAG GGGGAAAGAA AAAATATAAA TTAAAACATA

SIVcpz    TAGTATGGGC AAGCAGGGAG CTGGAAAGAT TCGCATGTGA CCCCGGGCTA
HIV-1     TAGTATGGGC AAGCAGGGAG CTAGAACGAT TCGCAGTTAA TCCTGGCCTG

SIVcpz    ATGGAAAGTA AGGAAGGATG TACTAAATTG TTACAACAAT TAGAGCCAGC
HIV-1     TTAGAAACAT CAGAAGGCTG TAGACAAATA CTGGGACAGC TACAACCATC

SIVcpz    TCTCAAAACA GGCTCAGAAG GACTGCGGTC CTTGTTTAAC ACTCTGGCAG
HIV-1     CCTTCAGACA GGATCAGAAG AACTTAGATC ATTATATAAT ACAGTAGCAA

SIVcpz    TACTGTGGTG CATACATAGT GACATCACTG TAGAAGACAC ACAGAAAGCT
HIV-1     CCCTCTATTG TGTGCATCAA AGGATAGAGA TAAAAGACAC CAAGGAAGCT

SIVcpz    CTAGAACAGC TAAAGCGGCA TCATGGAGAA CAACAGAGCA AAACTGAAAG
HIV-1     TTAGACAAGA TAGAG--GAA -----GAGCA AAACAAAAGT AA---GAAAA

SIVcpz    TAACTCAGGA AGCCGTGAAG GGGGAGCCAG TCAAGGCGCT AGTGCCTCTG
HIV-1     AAGCACAGCA AGC-----AG CAGCTGACA- -CAGGACAC- AG--CAGC--

SIVcpz    CTGGCATTAG TGGAAATTAC
HIV-1     CAGG--TCAG CCAAAATTAC
```

chimpanzee SIV vs HIV-1 envelope gene

Not all mutations are equally likely

- some point substitutions are more likely to occur than others:

  transitions are more likely than transversions

  - *transitions*:

    purine↔purine or pyrimidine↔pyrimidine

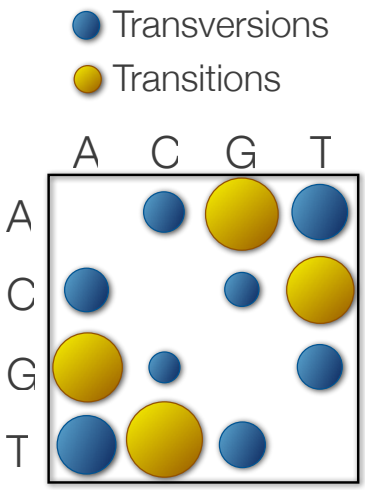
    A↔G C↔T

  - *transversions*:

    purine↔pyrimidine

    A↔C A↔T

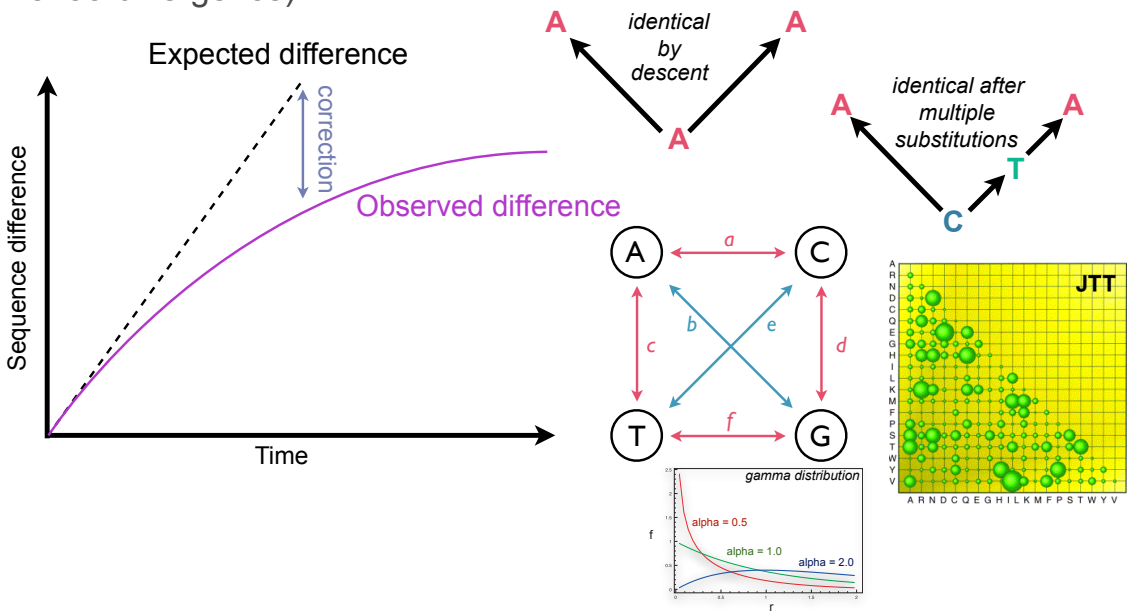
    G↔C G↔T



Unambiguous changes on most parsimonious tree of Ciliate SSUrDNA

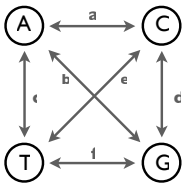
Substitution models

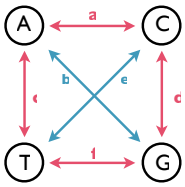
- During evolution, 'multiple hits' can occur at a single position: the evolutionary distance is almost always larger than the dissimilarity (% nt or aa divergence)

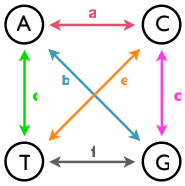


Nucleotide substitution models

Simplest (few parameters)







Most complex (many parameters)

1. Base frequencies are equal and all substitutions are equally likely (Jukes-Cantor) *(a=b=c=d=e=f)*

↓

2. Base frequencies are equal but transitions and transversions occur at different rates (Kimura 2-parameter) *(a=c=d=f, b=e)*

↓

3. Unequal base frequencies and transitions and transversions occur at different rates (Hasegawa-Kishino-Yano) *(a=c=d=f, b=e)*

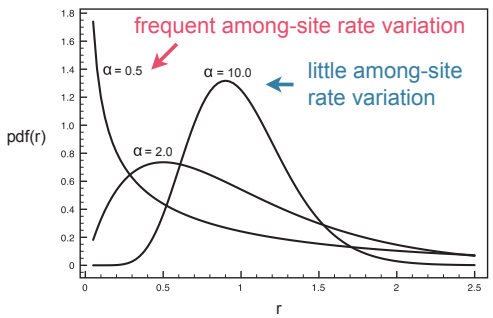
↓

4. Unequal base frequencies and all substitution types occur at different rates (General Reversible Model) *(a, b, c, d, e, f)*

Does this matter?

Estimated genetic distances between SIVcpz and HIVlai, under different substitution models:

Observed % mismatches	= 0.406
JC (Jukes-Cantor)	= 0.586
HKY (Hasegawa-Kishino-Yano)	= 0.611
GTR (General Time Reversible)	= 0.620
GTR + gamma	= 1.017



Gene	α
Prolactin	1.37
Albumin	1.05
C-myc	0.47
Ctyochrome β (mtDNA)	0.44
Insulin	0.40
D-loop (mtDNA)	0.17
12S rRNA (mtDNA)	0.16

Phylogenetic reconstruction

MOLECULAR SEQUENCES

Alignment Methods — BIOINFORMATICS

ALIGNMENT

Sequence Evolution Models
Phylogenetic Methods — PHYLOGENETICS

EVOLUTIONARY TREE

(time scale = genetic distance)

Molecular Clock Models — PHYLOGENETICS

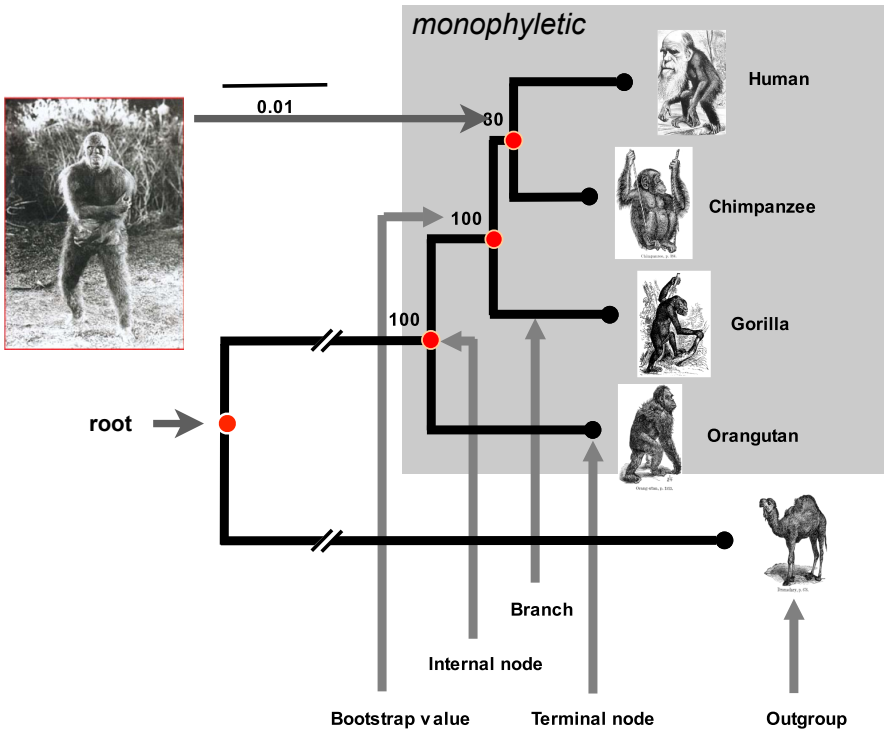
EVOLUTIONARY TREE

(time scale = years)

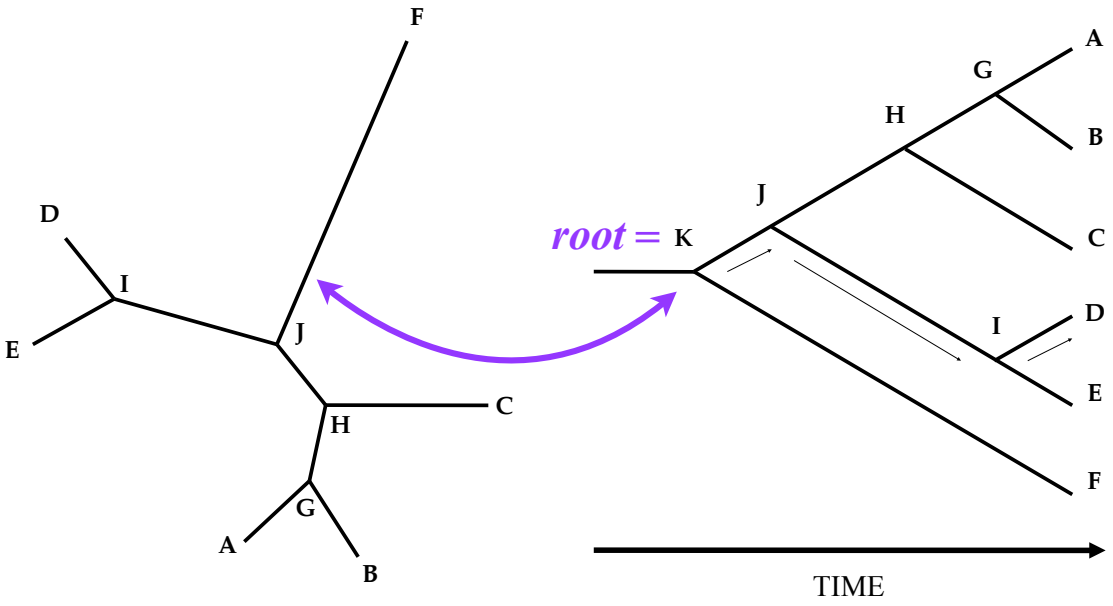
Phylodynamic Models — POPULATION GENETICS

EPIDEMIOLOGY

What is a tree?



Tree terminology: unrooted and rooted



Tree Terminology

Phylogeny

Genealogy

Phylogenetic reconstruction

- **CLUSTERING APPROACHES:** These begin with a genetic distance between each pair of sequences. A 'clustering algorithm' then transforms the genetic distances into a tree.
  - e.g. UPGMA, Neighbour-Joining
  - Simple, faster.
  - No measure of how good the estimated tree is (non-statistical)

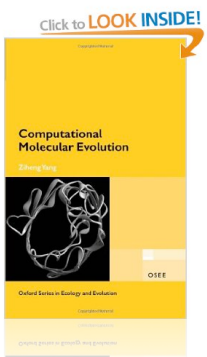
- **OPTIMALITY METHODS**: These define a score for each possible tree. 'Search algorithms' are then used to find the tree with the highest score.
  - e.g. Parsimony, Maximum Likelihood (& Bayesian Inference)
  - More complex, slower. Search may not locate the 'best' tree.
  - Quality of each tree can be directly compared (statistical)

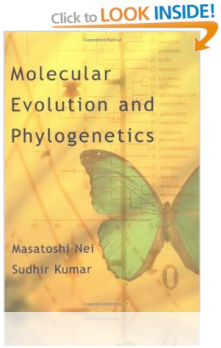
Phylogenetic reconstruction

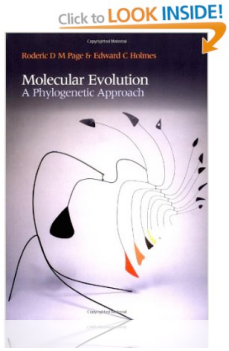
- For n taxa, there are:
  $(2n-3)!/[(2^{n-2})*(n-2)!]$
  rooted, binary trees

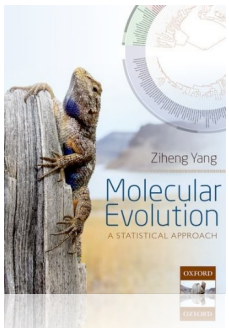
# taxa	# trees	
4	15	enumerable by hand
5	105	enumerable by hand on a rainy day
6	945	enumerable by computer
7	10395	still searchable very quickly on computer
8	135135	a bit more than the number of hairs on your head
9	2027025	population of Glasgow
10	34459425	≈ upper limit for exhaustive searching; about the number of possible combinations of numbers in the National Lottery
20	8.20×10^{21}	≈ upper limit for branch-and-bound searching
48	3.21×10^{70}	≈ the number of particles in the universe
136	2.11×10^{267}	=number of trees to choose from in the "Out of Africa" data (Vigilant et al., 1991)

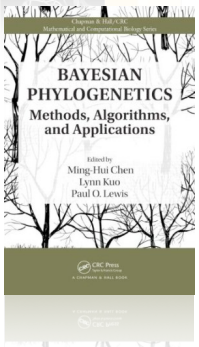
Phylogenetic inference: books

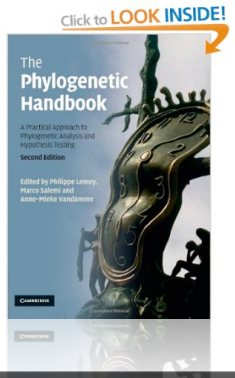


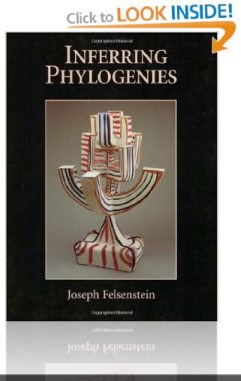












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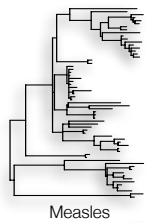
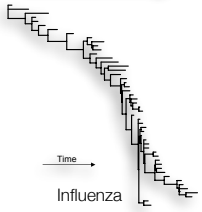
Computer Software: http://evolution.genetics.washington.edu/phylip/software.html

Phylodynamics™

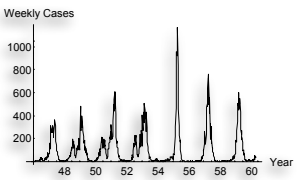
GENETIC DIVERSITY
(phylogenetics & molecular evolution)

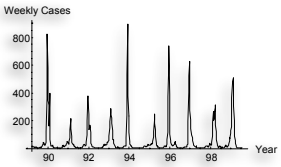


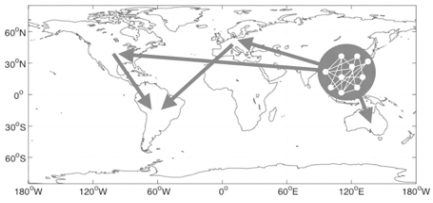


NATURAL SELECTION
(population genetics & immunology)

EPIDEMIC DYNAMICS
(mathematical epidemiology)







Unifying principle

> “Rapidly evolving pathogens are unique in that their ecological and evolutionary dynamics occur on the same timescale and can therefore potentially interact.”

Pybus & Rambaut (2009) Nat. Rev. Genetics 10:540-50

Fundamental Phylodynamic Questions

- How genetically diverse is a pathogen population?
- How do pathogen genomes change through time?
- How does pathogen genetic diversity vary through time and space?
- What are the effects of pathogen genetic diversity on virulence, transmissibility, resistance to treatment, etc.

Specific questions

- When did a epidemic start?
- Where did it come from?
- How fast is it transmitting?
- In what direction is it spreading?
- Are hosts X, Y & Z epidemiologically linked?
- Of how many strains is the epidemic composed?
- Are strains associated with particular transmission routes?
- What adaptations has it accrued?

Fundamental Phylodynamic Questions

- How genetically diverse is a pathogen population?
- How do pathogen genomes change through time?
- How does pathogen genetic diversity vary through time and space?
- What processes and/or events determine these changes?
- What are the effects of pathogen genetic diversity on virulence, transmissibility, resistance to treatment, etc.

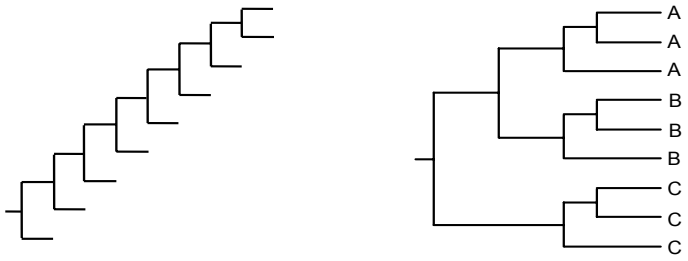
Measuring sequence diversity

- Not as straightforward as you might think...
- Are your pathogen sequences all sampled at the same time?

  If sequences not sampled over time it's difficult to separate the effects of diversity and divergence on genetic diversity.

- Are you measuring sample diversity or population diversity?

  The former is simply a summary of your data, the latter is an inference about the population you have sampled. Sequences should be sampled randomly to estimate the latter.



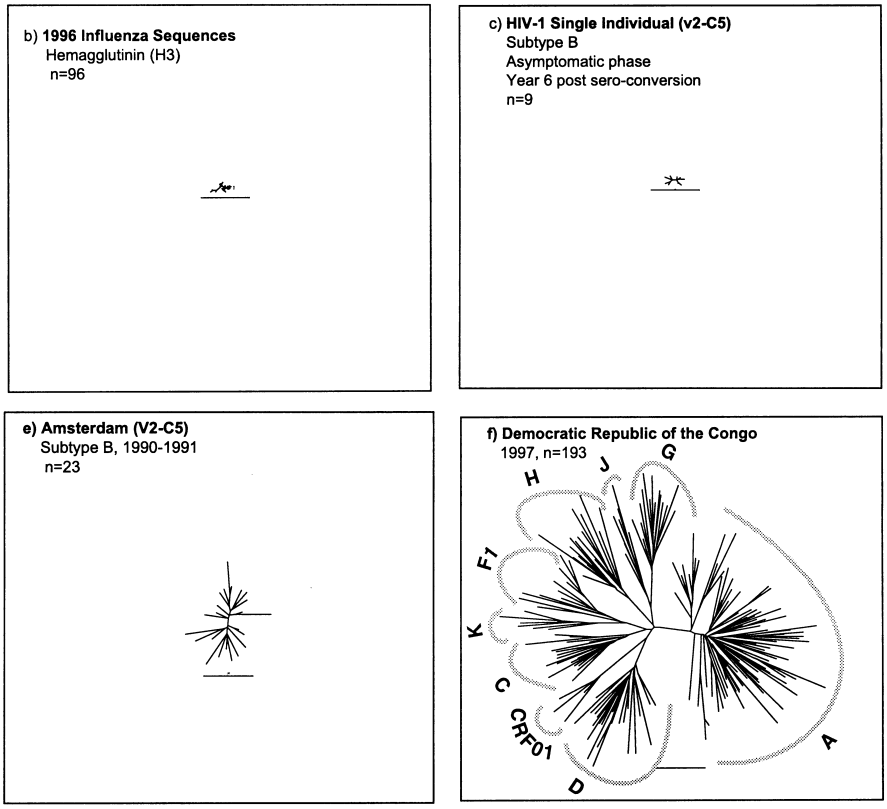
Measuring sequence diversity

- Are you studying an inter-host or intra-host population?

  For the former, each sequence represents a different infection. For the latter, each sequence represents a different virion within an infected individual. The measure of diversity must be interpreted accordingly.

- How do we deal with intra-host diversity when studying the inter-host level?
- Intra-host diversity is low for most acute infections (e.g. influenza) but can be high for chronic infections (e.g. HIV).

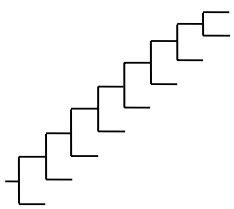
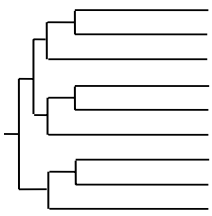
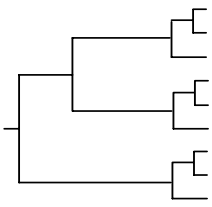
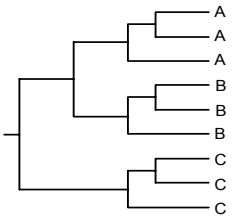
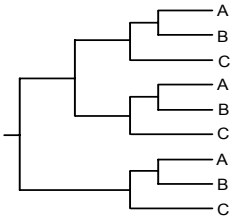
Example: diversity of HIV-1 versus influenza



Scale bar represents a genetic distance of 0.1 substitutions per site.

Korber et al. 2001. British Medical Bulletin 58:19-42

Phylodynamic Patterns

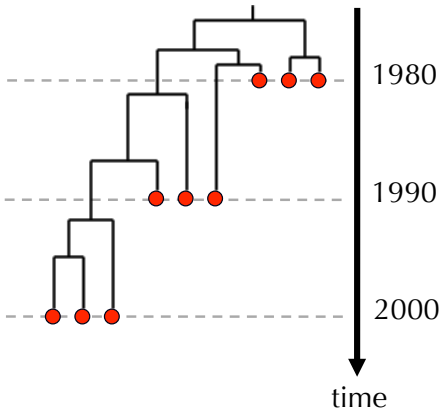
	Continual Immune Selection	Weak/No Immune Selection	
		Population dynamics	Spatial dynamics
Idealised Phylogeny Shapes		*Population growth*  *Population decline* 	*Strong spatial structure*  *Weak spatial structure* 
Examples	Human influenza A within-host HIV	among-host HIV among-host HCV	Measles Rabies, Dengue

Fundamental Phylodynamic Questions

- How genetically diverse is a pathogen population?
- How do pathogen genomes change through time?
- How does pathogen genetic diversity vary through time and space?
- What processes and/or events determine these changes?
- What are the effects of pathogen genetic diversity on virulence, transmissibility, resistance to treatment, etc.

‘Phylodynamic’ Data

- Pathogen genomes are sampled at different points in time and from different locations.
- Hence transmission history is estimated on a real time-scale (e.g. years).



MOLECULAR SEQUENCES

Alignment Methods — BIOINFORMATICS

ALIGNMENT

Sequence Evolution Models
Phylogenetic Methods — PHYLOGENETICS

EVOLUTIONARY TREE

(time scale = genetic distance)

Molecular Clock Models — PHYLOGENETICS

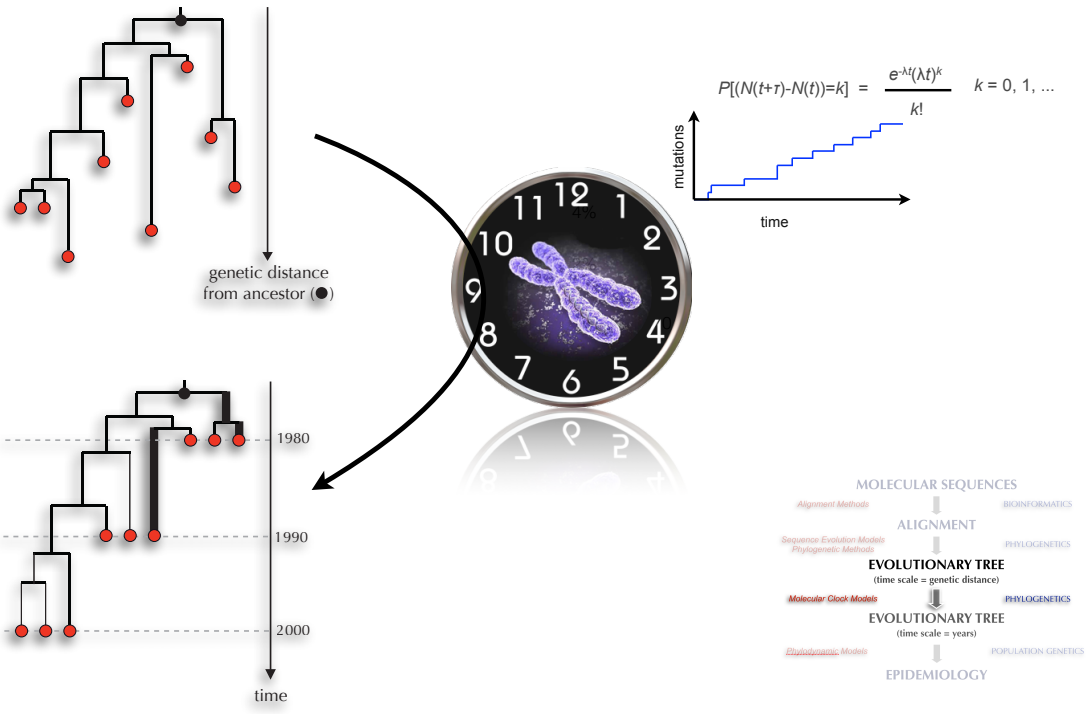
EVOLUTIONARY TREE

(time scale = years)

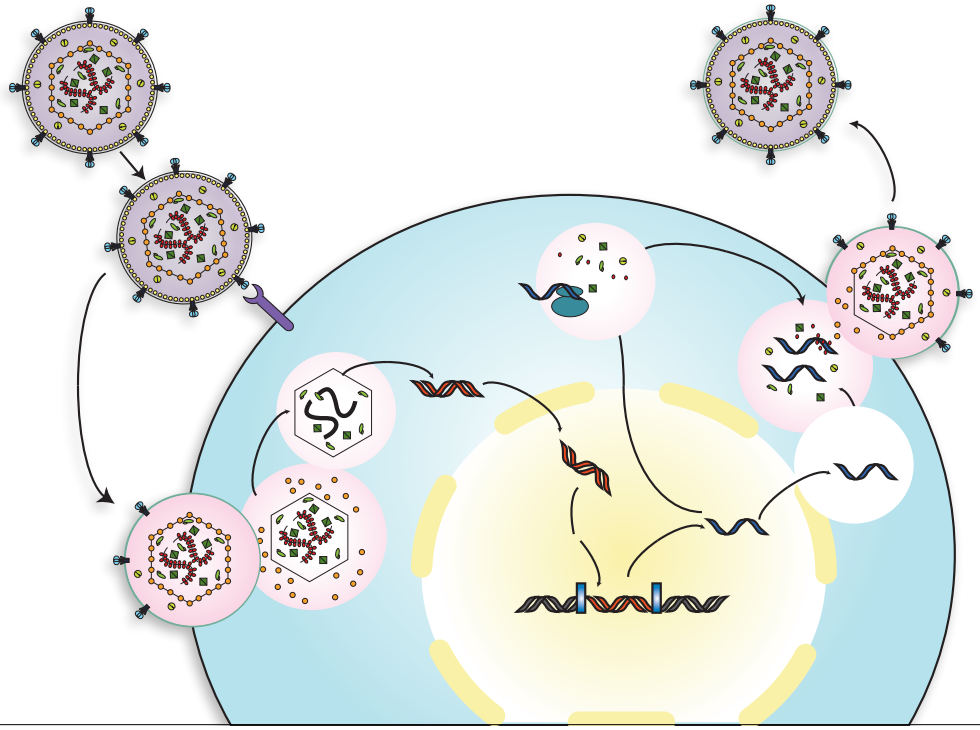
Phylodynamic Models — POPULATION GENETICS

EPIDEMIOLOGY

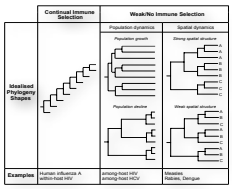
Molecular clocks

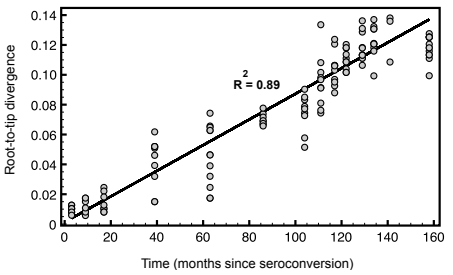


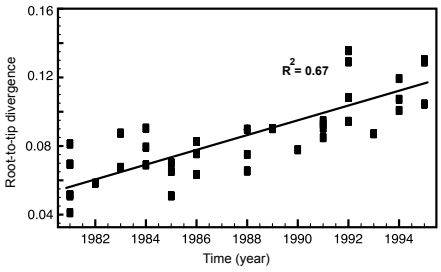
HIV: the ultimate evolver

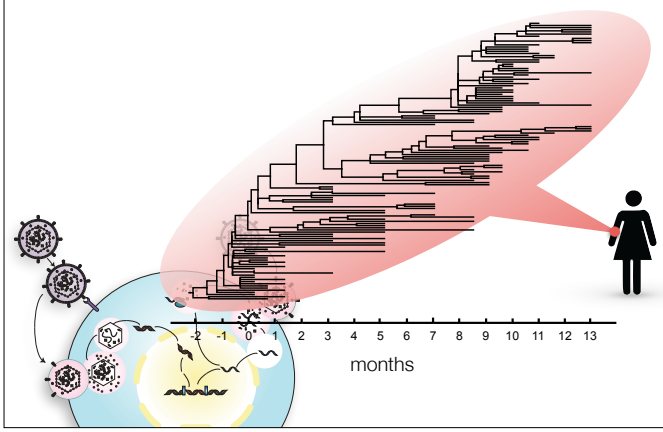


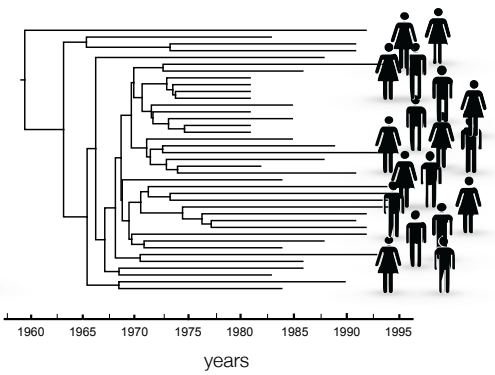
measurable evolution of HIV-1





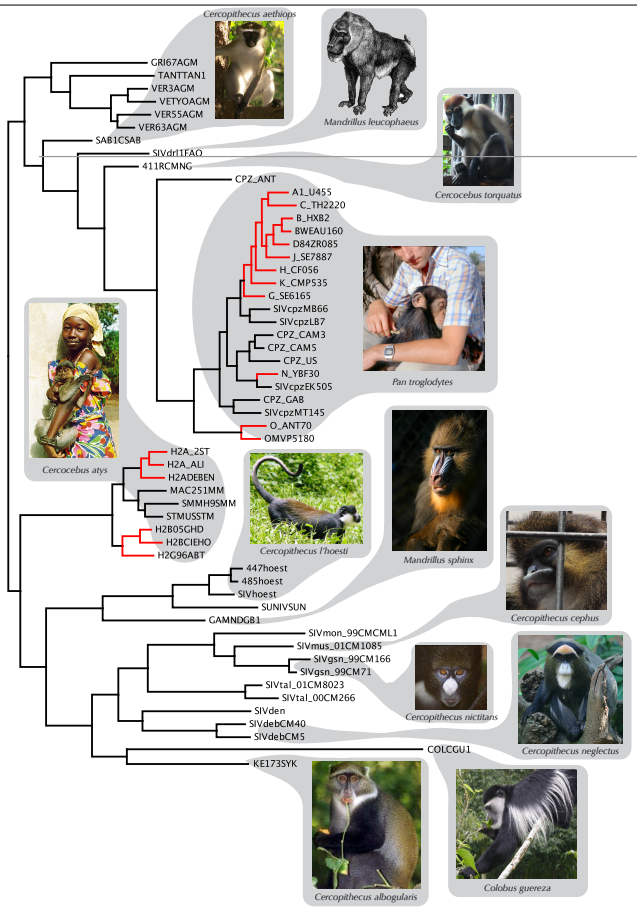


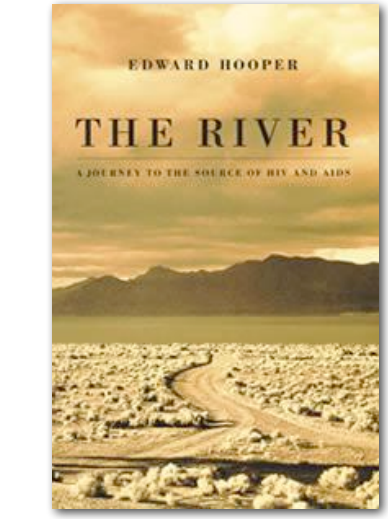


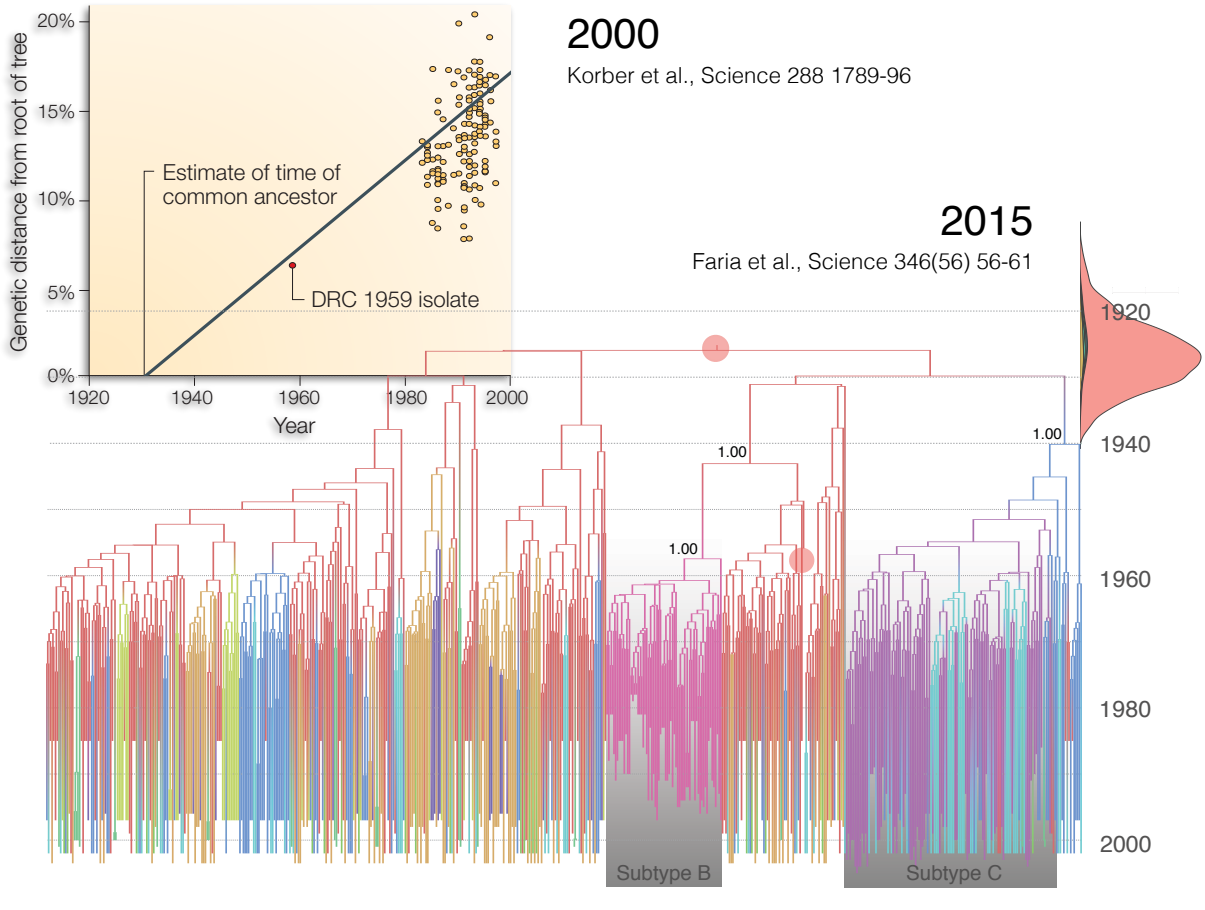


Lemey et al 2006 AIDS Rev

The origin of HIV-1







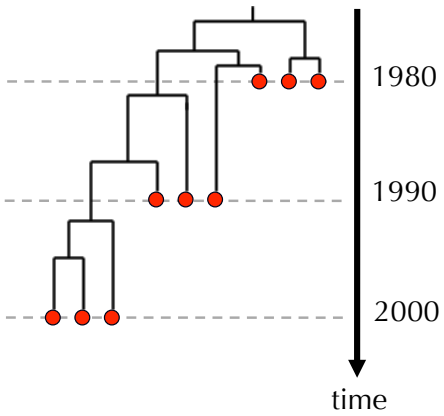
‘Phylodynamic’ Data

- Pathogen genomes are sampled at different points in time and from different locations.
- Hence transmission history is estimated on a real time-scale (e.g. years).
- The ability to genetically distinguish sequences sampled at different times depends on:

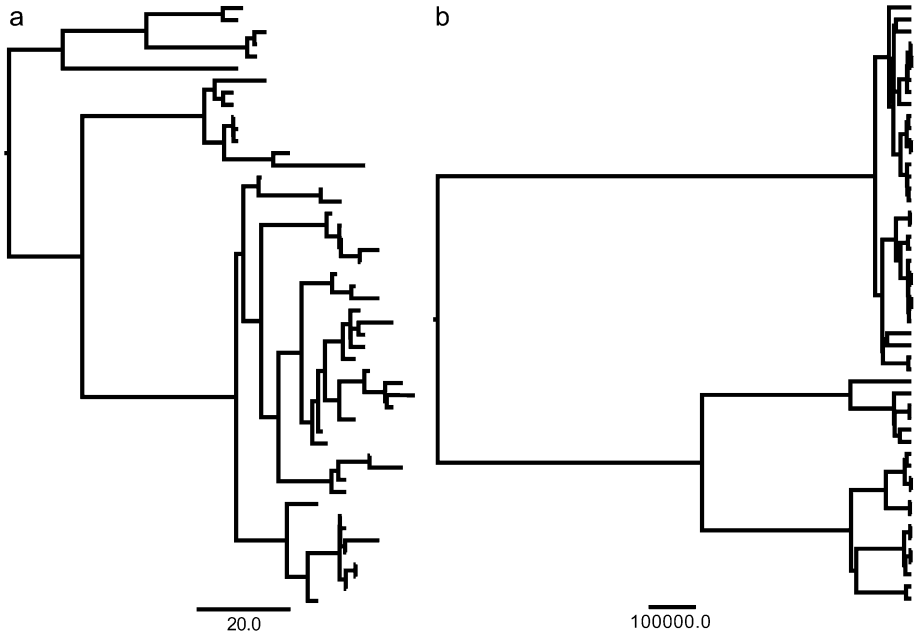
  (i) the rate of evolution of the gene/ genome that is obtained

  (ii) the length of time between samples

  (iii) the sequence length of the gene/ genome that is obtained



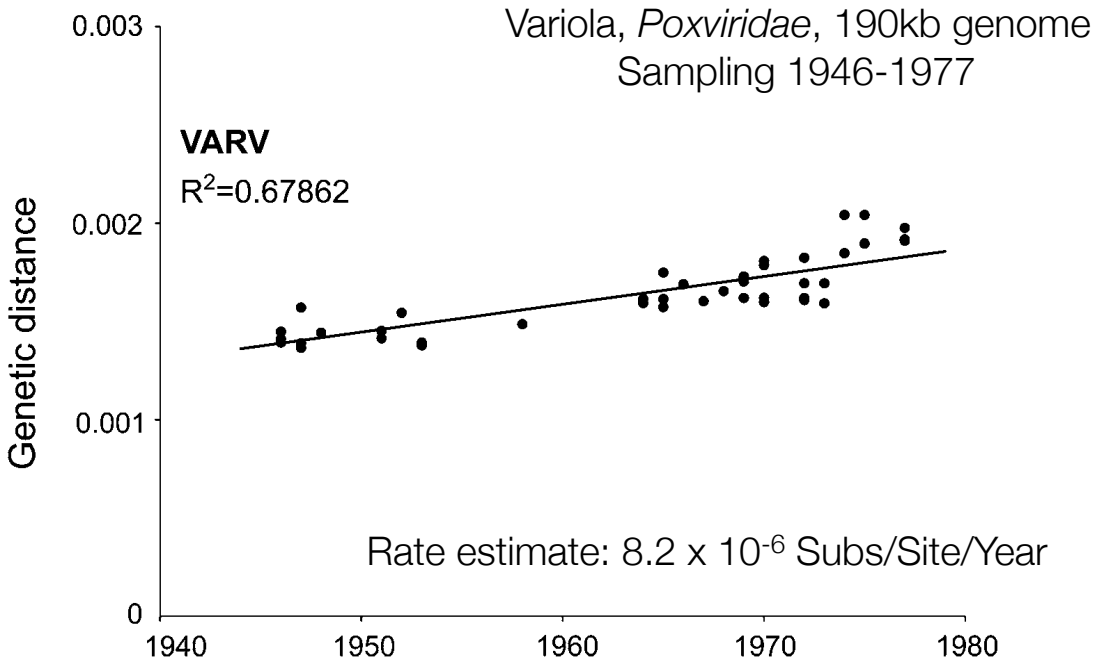
‘Phylodynamic’ Data

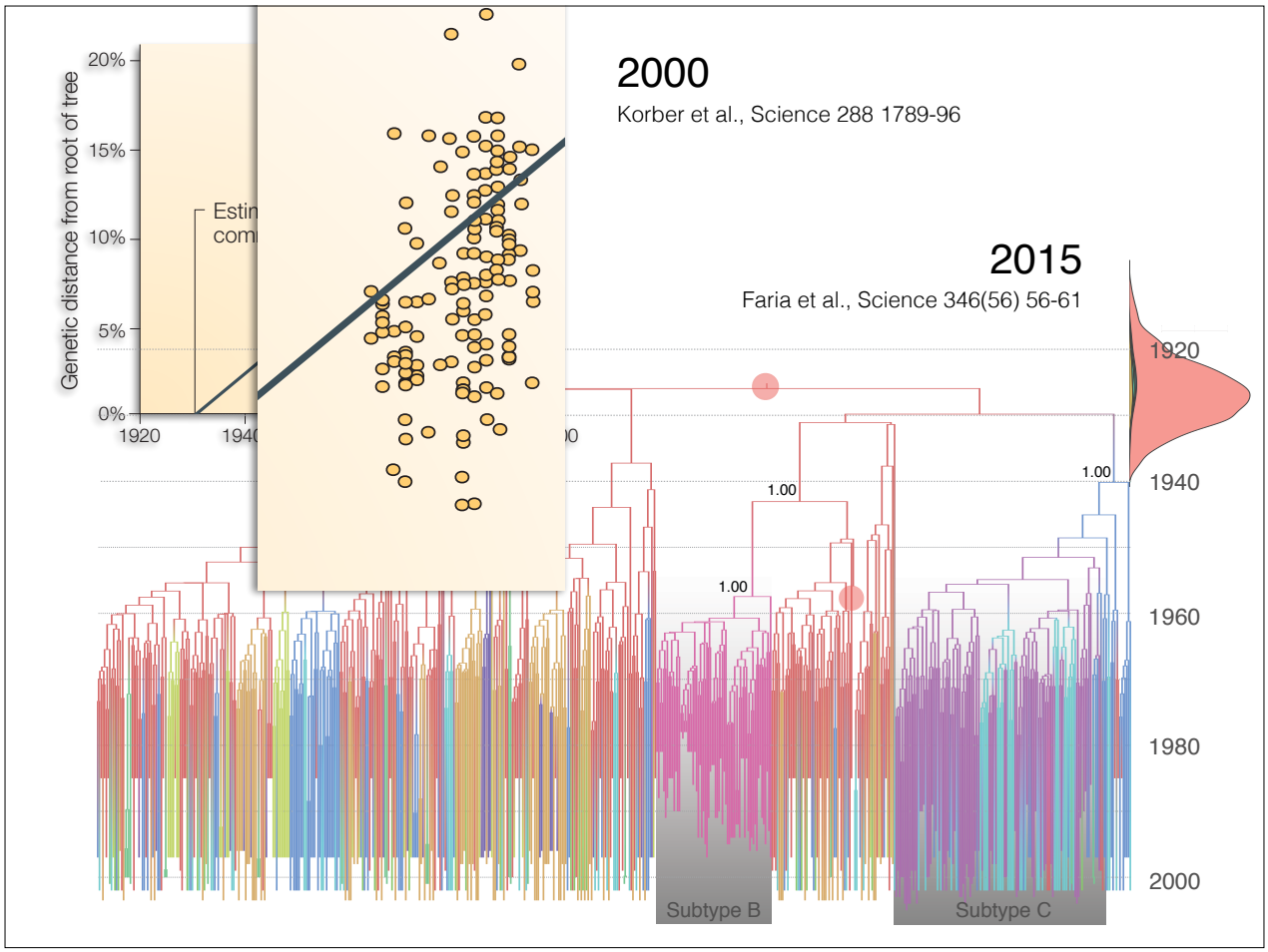


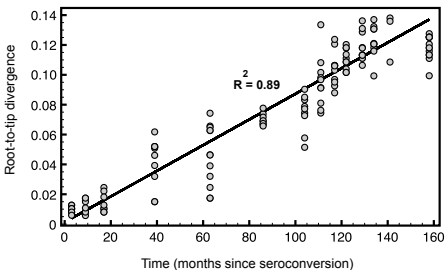
10^{-4} subs/site/year

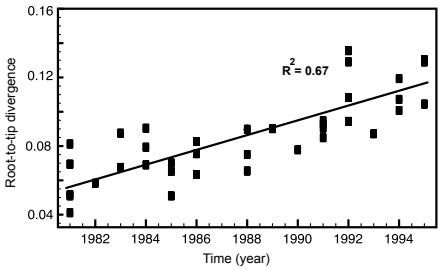
10^{-8} subs/site/year

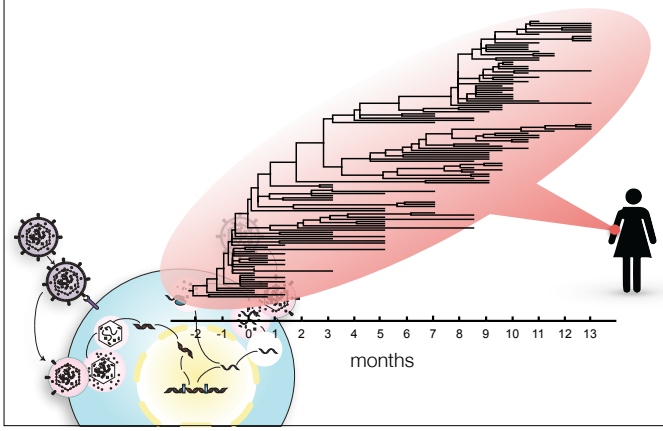
A DNA virus (smallpox)

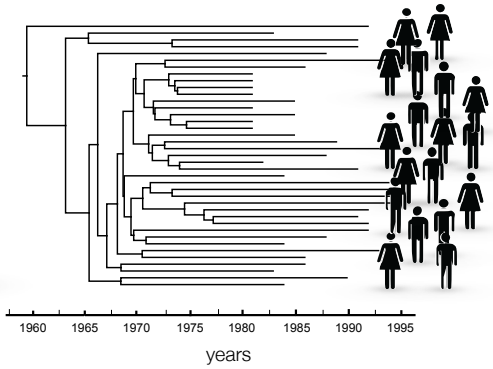




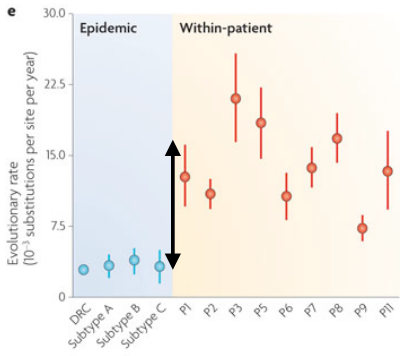








Lemey et al 2006 AIDS Rev

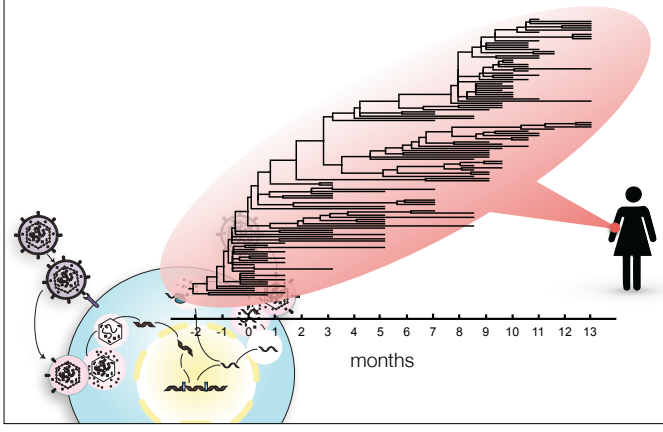


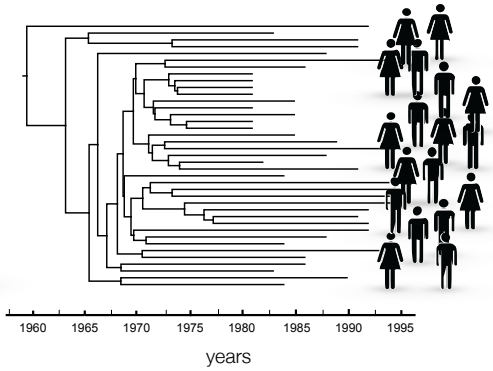
Pybus and Rambaut, NGR, 2009

Vrancken et al., PLoS Comp Bio, 2014

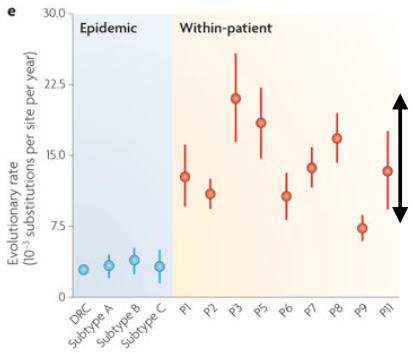
Mixed effects model:

$$\log \mu_i = \theta_i + \beta X_i$$





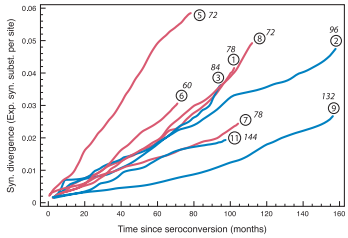
Lemey et al 2006 AIDS Rev



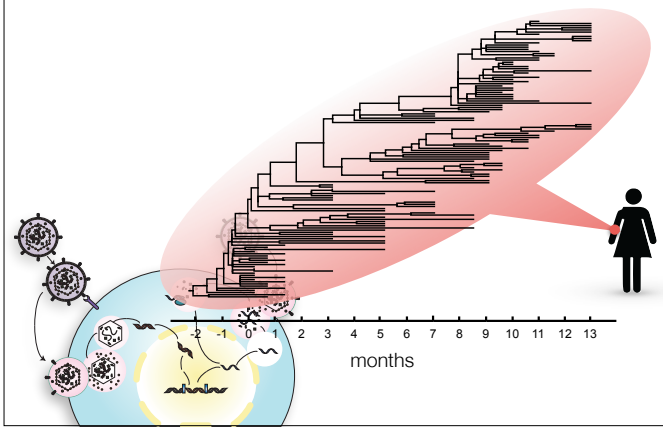
Pybus and Rambaut, NGR, 2009

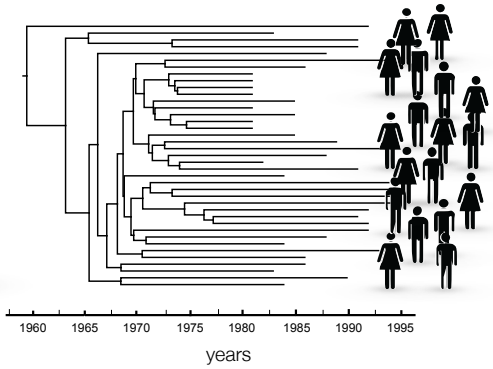
Edo-Matas et al., Mol Biol Evol, 2011

$$\log\theta_i = \beta_0 + \delta_{\text{LTNP}}\beta_{\text{LTNP}}\text{LTNP}_i + \delta_{\Delta 32}\beta_{\Delta 32}\Delta 32_i + \varepsilon_i$$



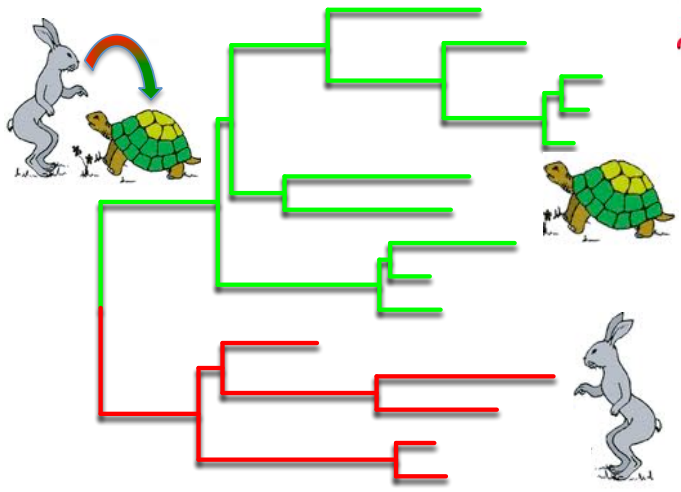
Lemey et al., PLoS Comp Bio, 2007





Lemey et al 2006 AIDS Rev

What drives the tempo of pathogen evolution?

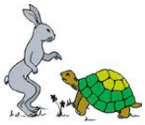


Pathogen factors

Mutation rate

Life cycle/replication dynamics

Host factors



Life history

Seasonality

Metabolic rate etc.

Historical factors

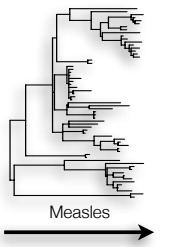
Pathogen phylogeny

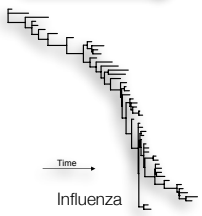
Fundamental Phylodynamic Questions

- How genetically diverse is a pathogen population?
- How do pathogen genomes change through time?
- How does pathogen genetic diversity vary through time and space?
- What processes and/or events determine these changes?
- What are the effects of pathogen genetic diversity on virulence, transmissibility, resistance to treatment, etc.

Phylodynamics™

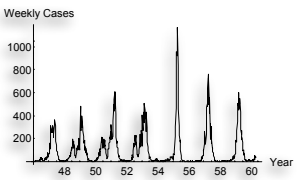
GENETIC DIVERSITY
(phylogenetics & molecular evolution)

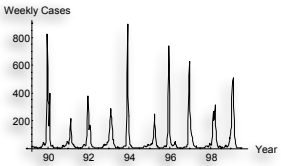


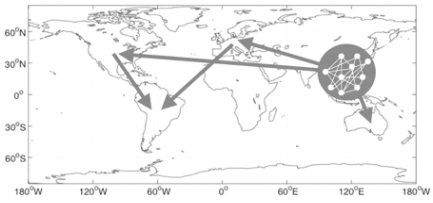


NATURAL SELECTION
(population genetics & immunology)

EPIDEMIC DYNAMICS
(mathematical epidemiology)

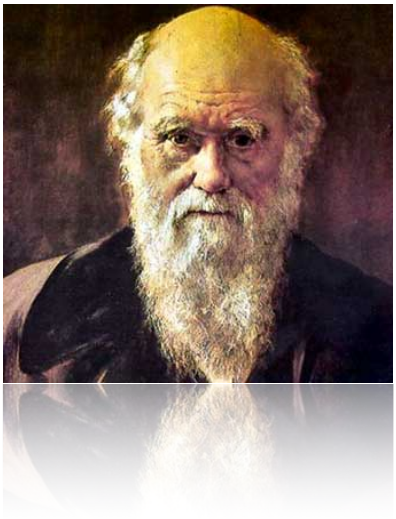






Evolutionary processes: natural selection

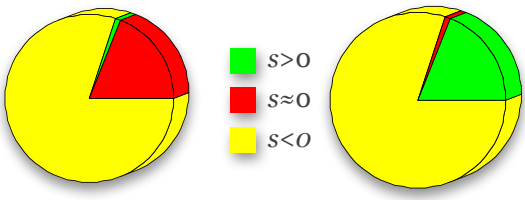
- "the preservation of favourable variations and the rejection of injurious variations, i call natural selection. variations neither useful nor injurious would not be affected by natural selection, and would be left a fluctuating element"
  - darwin, the origin of species



Evolutionary processes: natural selection

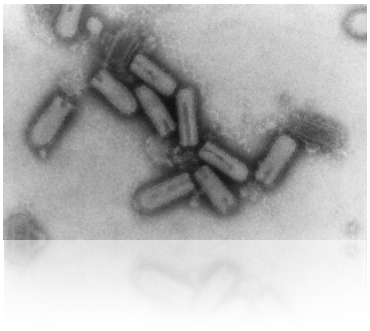
most fixed mutations are neutral

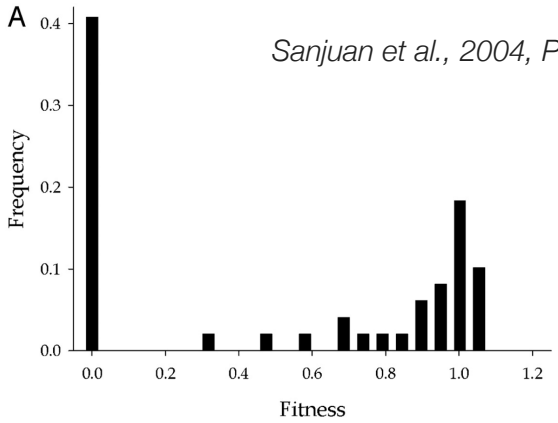
neutralist model
motoo kimura



most fixed mutations are advantageous

selectionist model
john gillespie

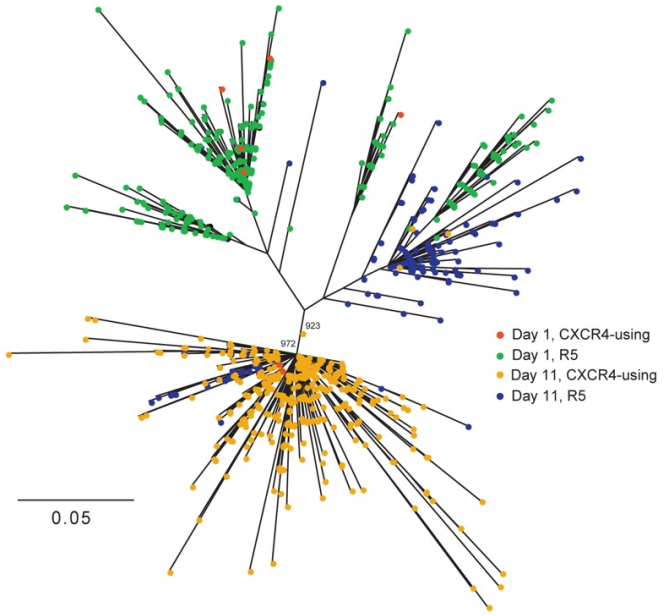




Sanjuan et al., 2004, PNAS

Evolutionary processes: natural selection

- Immune escape (antibodies*, T-cells*, innate immune responses)
- Antiviral drug resistance
- Vaccine escape mutations
- Cell & tissue tropism
- Inter-host viral transmission (i.e. for viral emergence)



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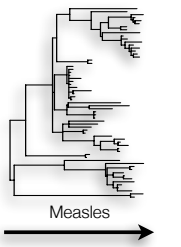

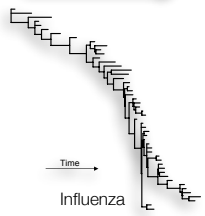

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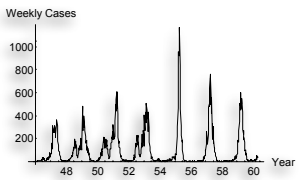

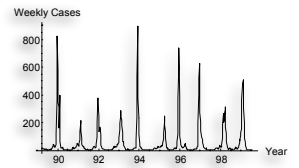

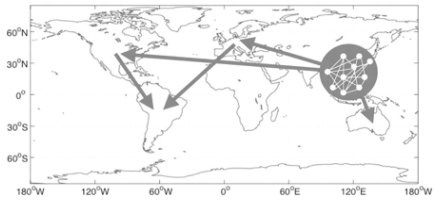

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(time scale = genetic distance)

Molecular Clock Models

PHYLOGENETICS

EVOLUTIONARY TREE

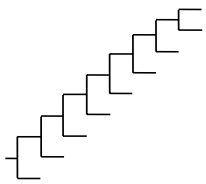
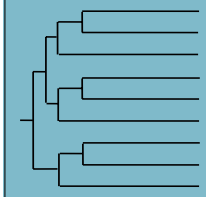
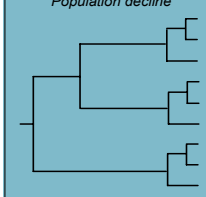
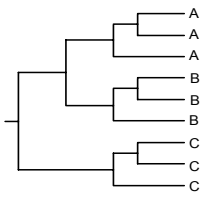
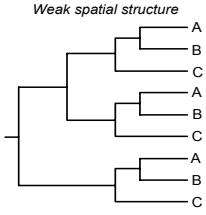
(time scale = years)

Phylodynamic Models

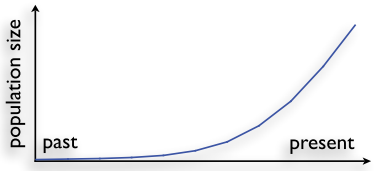
POPULATION GENETICS

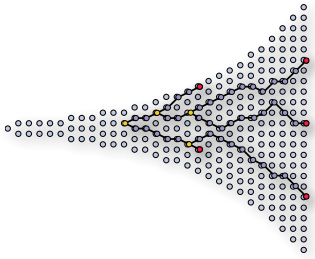
EPIDEMIOLOGY

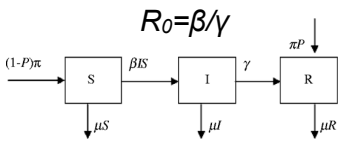
Phylodynamic Patterns

	Continual Immune Selection	Weak/No Immune Selection	
		Population dynamics	Spatial dynamics
Idealised Phylogeny Shapes		*Population growth*  *Population decline* 	*Strong spatial structure*  *Weak spatial structure* 
Examples	Human influenza A within-host HIV	among-host HIV among-host HCV	Measles Rabies, Dengue

Demography and coalescent theory







- The rate at which lineages 'coalesce' depends on population size and population structure.

Kingman JFC (1982) Journal of Applied Probability 19A:27–43

- Population dynamics can be reconstructed using parametric or flexible nonparametric models (the 'skyline or skyride plot' method)

Pybus et al. (2000) Genetics 155:1429-37

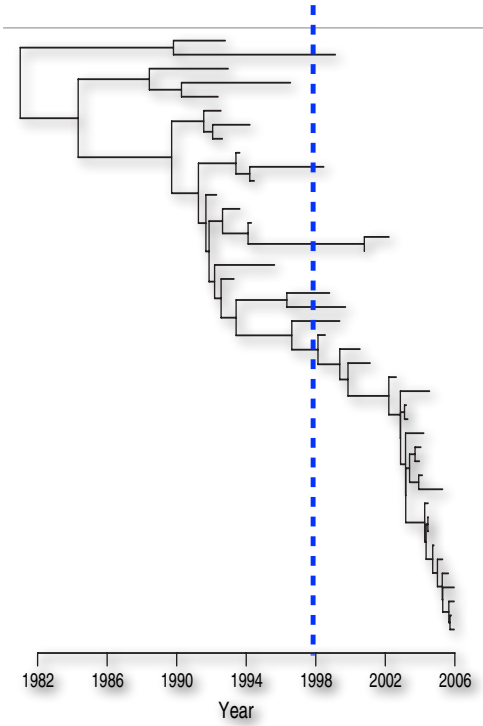
Drummond, Rambaut, Shapiro & Pybus (2005) Mol Biol Evol 22:1185-92

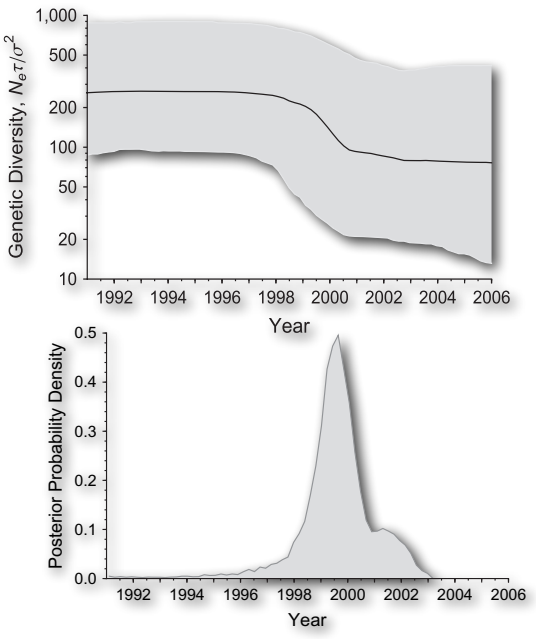
Minin, Bloomquist and Suchard (2008) Mol Biol Evol 25:1459-71

- Birth-death models can also be used as the tree-generative model and just like coalescent models they can be parametrized in terms of compartmental epidemic models.

Stadler et al. (2012) MBE 29:347-357

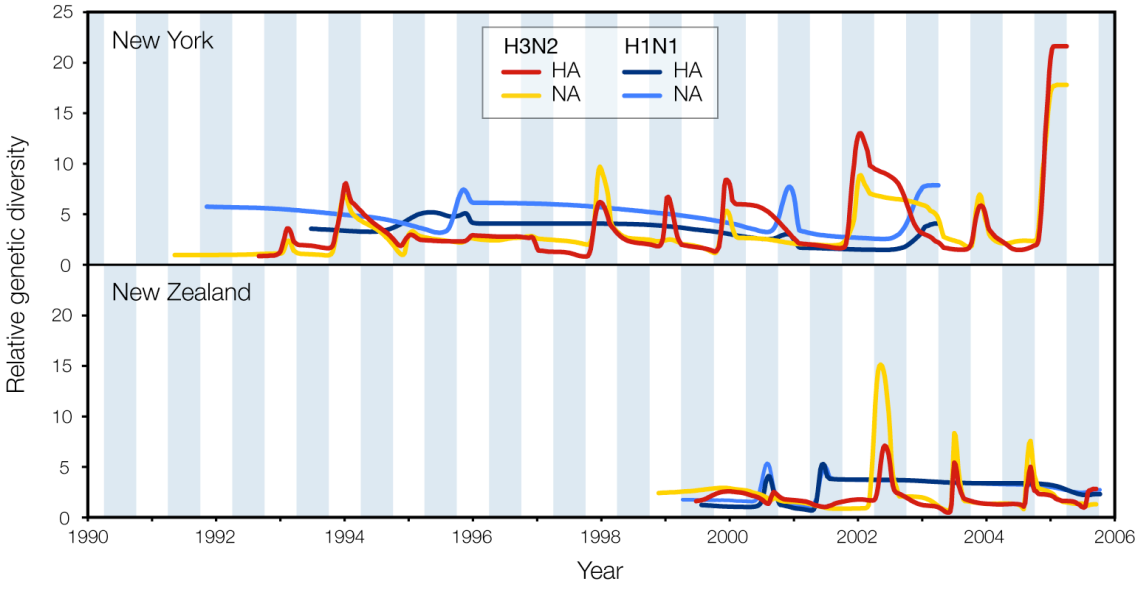
HBV Vaccination in Amsterdam





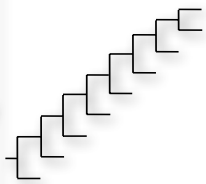
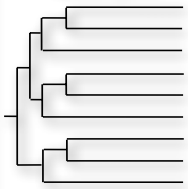
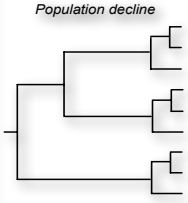
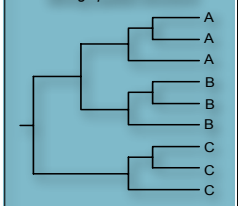
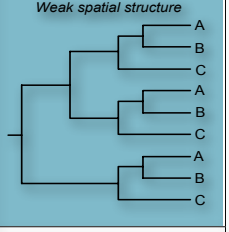
van Ballegooijen et al. 2009. Am. J. Epidemiol. 170:1455-63

Influenza H3N2 epidemic dynamics



Rambaut et al. 2009. Nature

PhyloGEOdynamic Patterns

	Continual Immune Selection	Weak/No Immune Selection	
		Population dynamics	Spatial dynamics
Idealised Phylogeny Shapes		*Population growth*  *Population decline* 	*Strong spatial structure*  *Weak spatial structure* 
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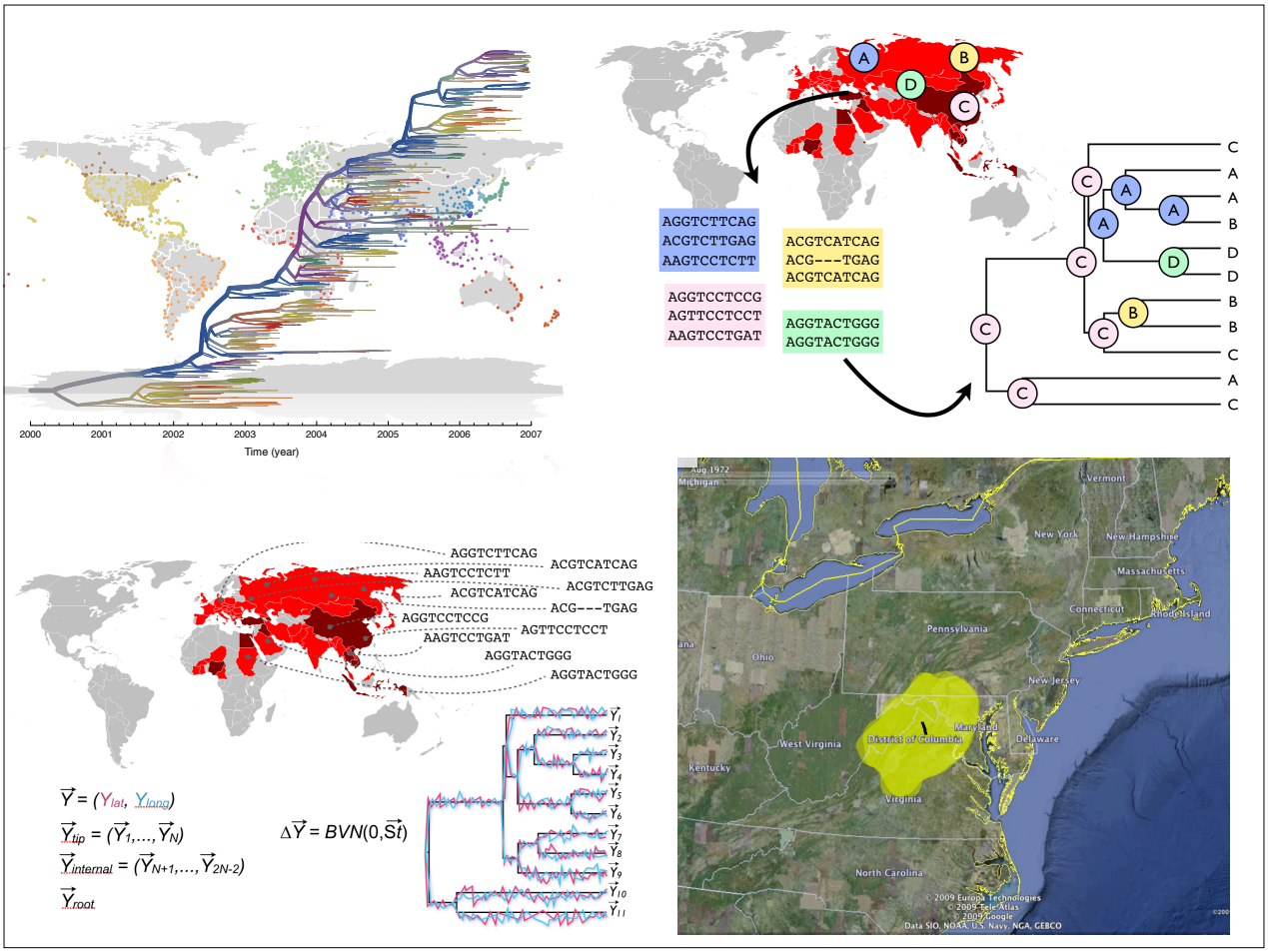
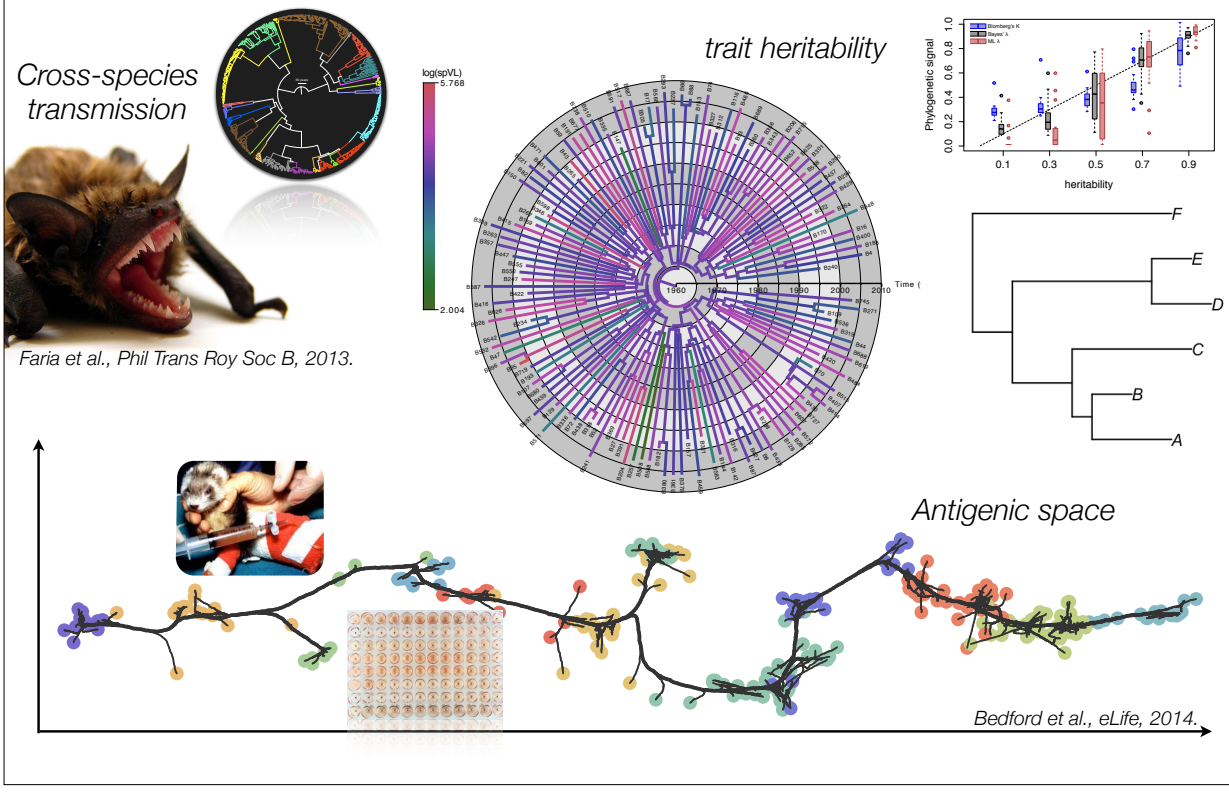
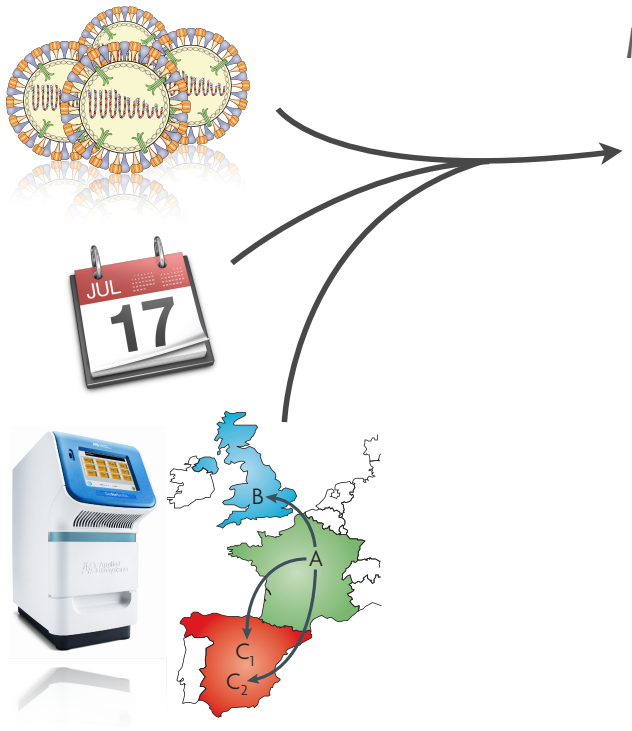


Trait evolution and the comparative approach



Bayesian Evolutionary Analysis Sampling Trees



MOLECULAR SEQUENCES

ALIGNMENT

EVOLUTIONARY TREE

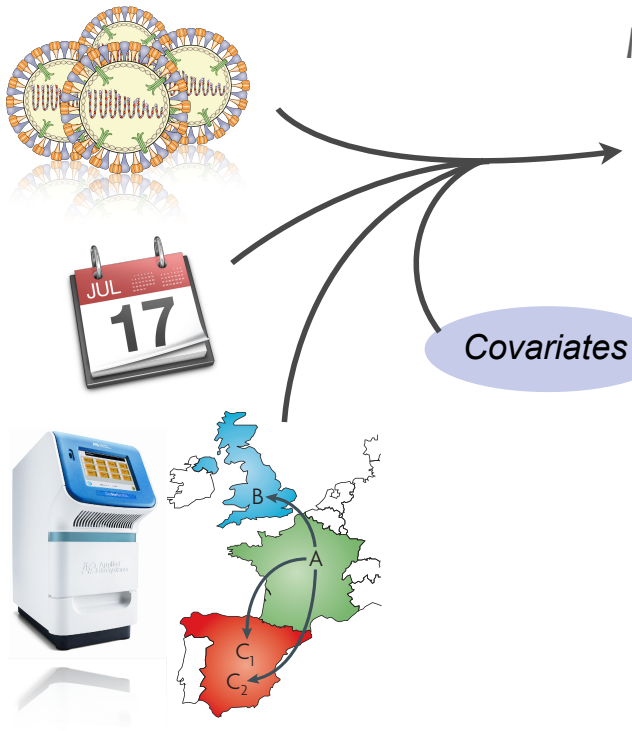
(time scale = genetic distance)

EVOLUTIONARY TREE

(time scale = years)

EPIDEMIOLOGY

Bayesian Evolutionary Analysis Sampling Trees



Covariates

MOLECULAR SEQUENCES

ALIGNMENT

EPIDEMIOLOGY