

Phylogenetics for Predicting Virus Evolution

Anticipating next seasons influenza strains

Paul Wiesemeyer

Problem



HOUSTON,
we have a — CHOO!

- Estimated 3 – 11% of the population catch symptomatic influenza each season [Tokars et al., 2018]
- Influenza evolves rapidly, evading immune system recognition and vaccines
- Epidemics and pandemics caused by RNA recombination events
- Science is slow responding to health issues

Problem

- $\sim 8 \text{ months}$ from submission to publication of a medical paper [AAMC, 2018]
- $\sim 6 \text{ months}$ from vaccine strain selection to distribution:

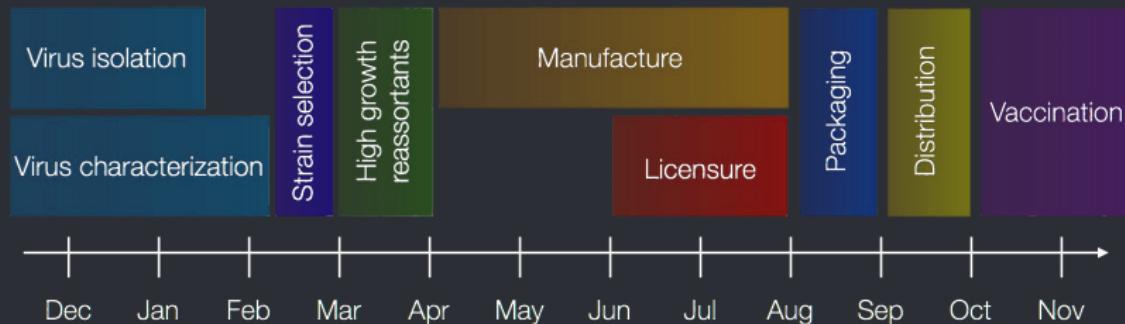


Figure 1: from [Bedford, 2015]

- compare: $\sim 1.6 \text{ days}$ doubling time of H1N1 (2009) [Mostaço-Guidolin et al., 2011]

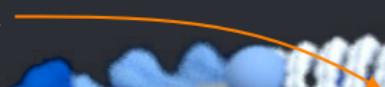
Outline

1. Influenza
2. Phylogenetics
 - 2.1 The Molecular Clock
3. Predicting the next strain of influenza
 - 3.1 Predicting Virus Evolution
 - 3.2 The Hemagglutination Inhibition Assay
 - 3.3 Mapping Antigenicity to the Tree
 - 3.4 Predictions
4. *Nextstrain*
 - 4.1 Narrative
5. Outlook

Influenza

a closer look

8 single stranded RNA



Neuraminidase (NA)



Hemagglutinin (HA)



Image from [CDC, 2020]

Influenza jumps far

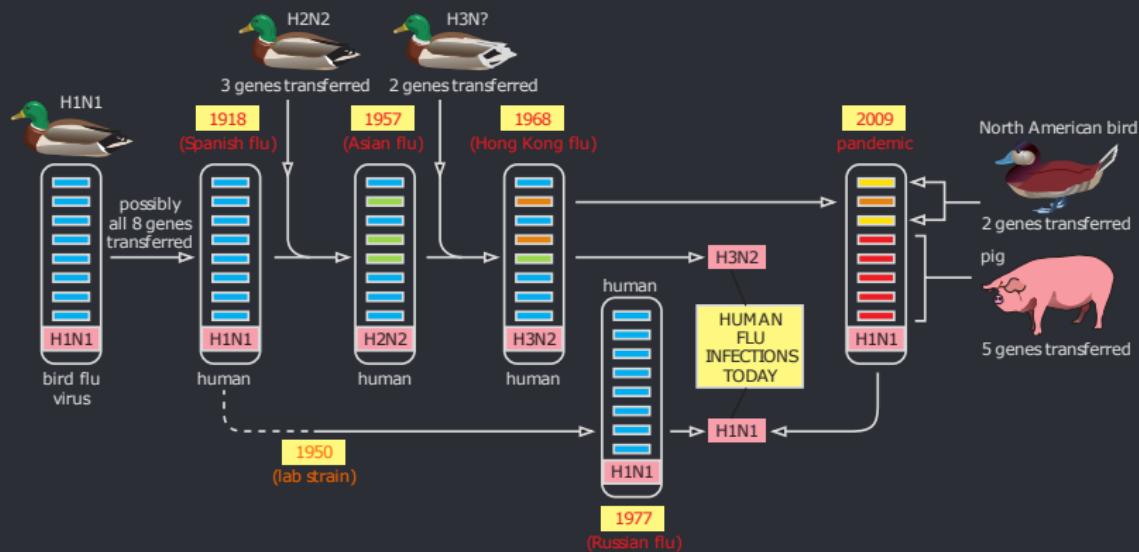


Figure 2: recent influenza A evolutionary shift events [Alberts, 2015]

Influenza

runs fast

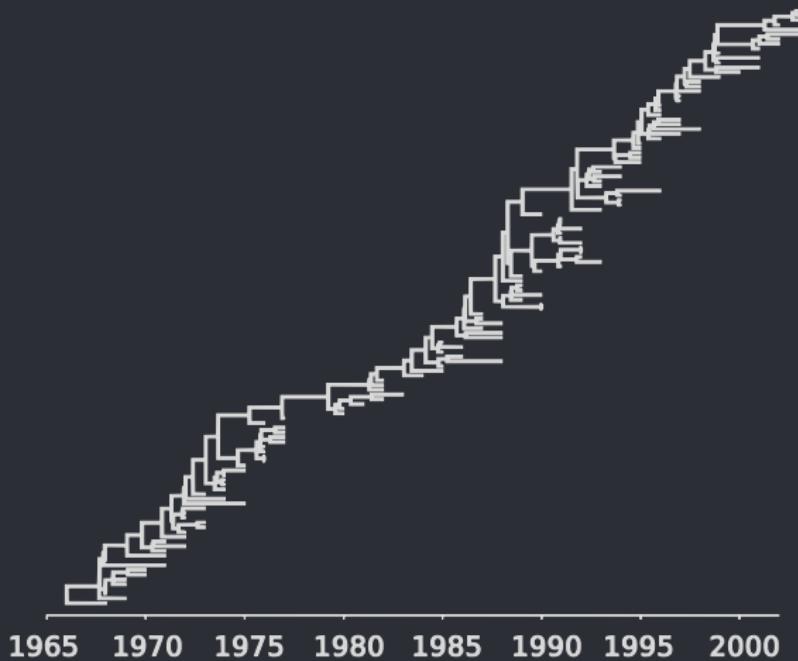


Figure 3: Phylogeny of Influenza A/H3N2 Hemagglutinin [Volz et al., 2013]

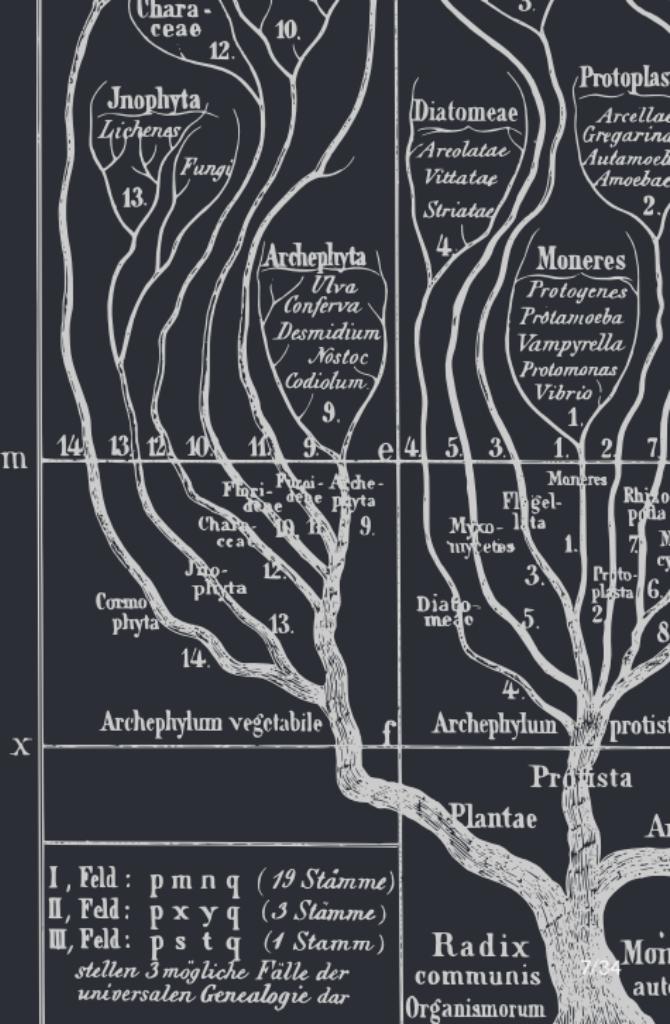
Phylogenetics

Terminology

Tree—a simply connected graph

- branches
- nodes (vertices)
- leaves (endpoints)
- root \Rightarrow parents, children
 \Rightarrow clades:
a branch with its children

Image from [Haeckel, 1866]



I, Feld : p m n q (19 Stämme)
II, Feld: p x y q (3 Stämme)
III, Feld: p s t q (1 Stamm)
stellen 3 mögliche Fälle der
universalen Genealogie dar

Radix
communis
Organismorum

Mon
auto

Phylogenetics

Concept: Parsimony

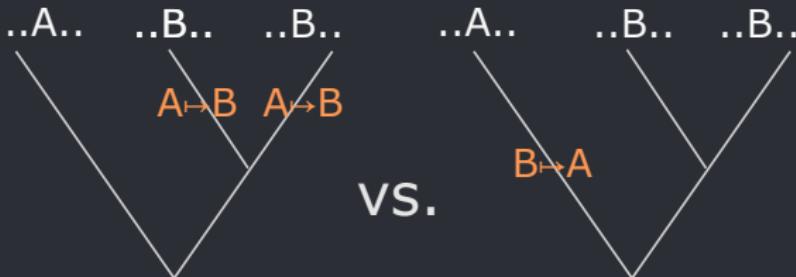


Figure 4: Reconstructing a tree requires likelihood considerations

Minimize the number of mutations to get the most likely tree(s)

Problem: for N leaves: # possible trees $\propto 2^N (N - 1)!$

⇒ We can usually just compute a minute fraction of these.

Phylogenetics

Example algorithm

	A	B	C	D	E	F
A		9	2	4	9	10
B		9	6	2	10	
C			5	9	10	
D				6	10	
E					10	
F						

N sequences of lengths $\ell_1 \dots \ell_N$ (not identical due to indels, seq. errors)

- Compare pairwise, assign a distance, make $N \times N$ table
- Lowest distances group together, form new subunit.
- Iterate. When comparing to sets of sequences, use arithmetic mean.

Result: small number of locally most likely trees,
not necessarily global maximum.

Computing scales with N^2

Better: Markov Chain Monte Carlo

Phylogenetics

Concept: *The Molecular Clock*

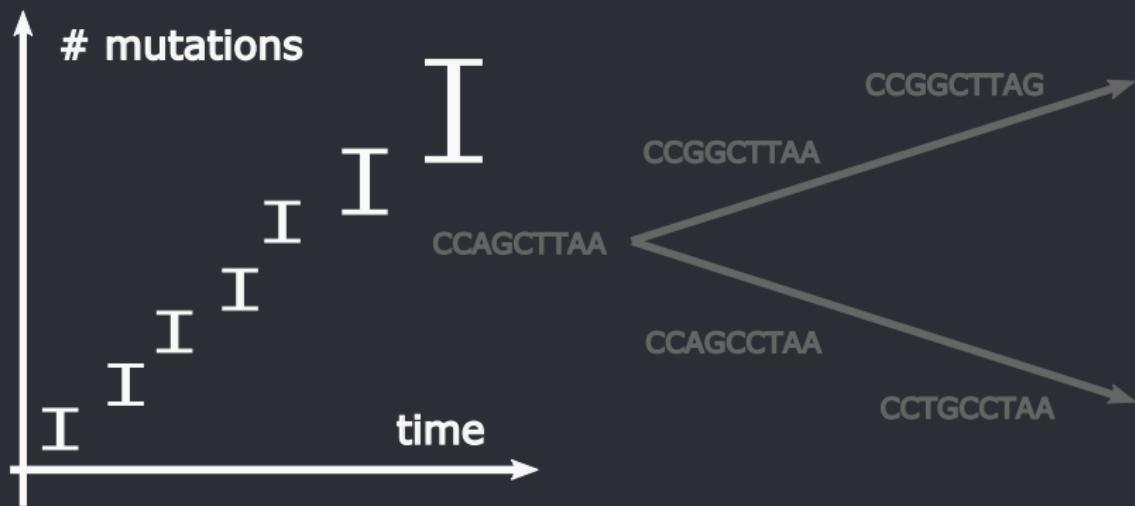


Figure 5: Linear time-mutation relationship

Phylogenetics

Concept: The Molecular Clock

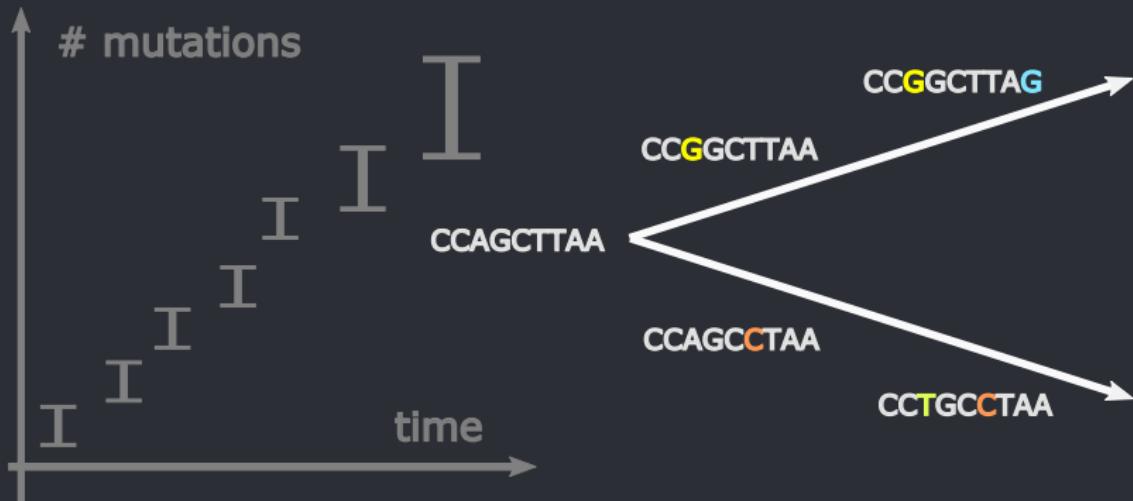


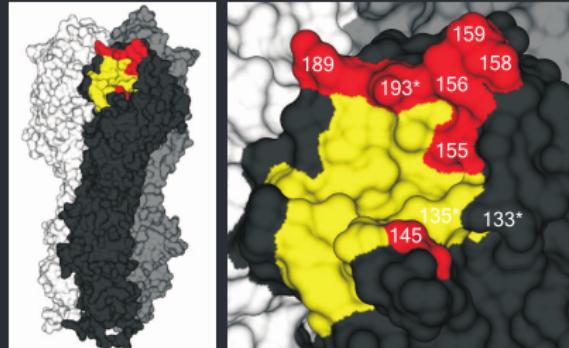
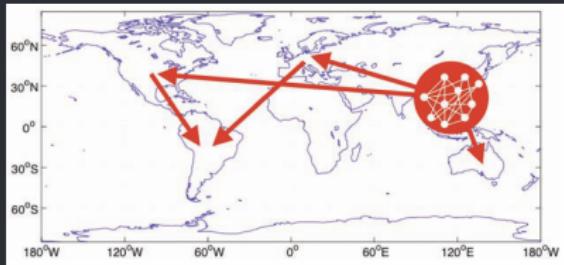
Figure 5: Linear time-mutation relationship

Probability of s.c. *silent* mutations can be taken constant.

Non-silent—amino acid changing—mutations are under evolutionary pressure.

Predicting the next strain of influenza

Approaches



Epidemiology: Geographical predictor?

Molecular Biology: Is there a telltale mutation site?

Immunology: Hemagglutinin inhibition cartography

Vision: Bring these levels together

The Hemagglutination Inhibition Assay

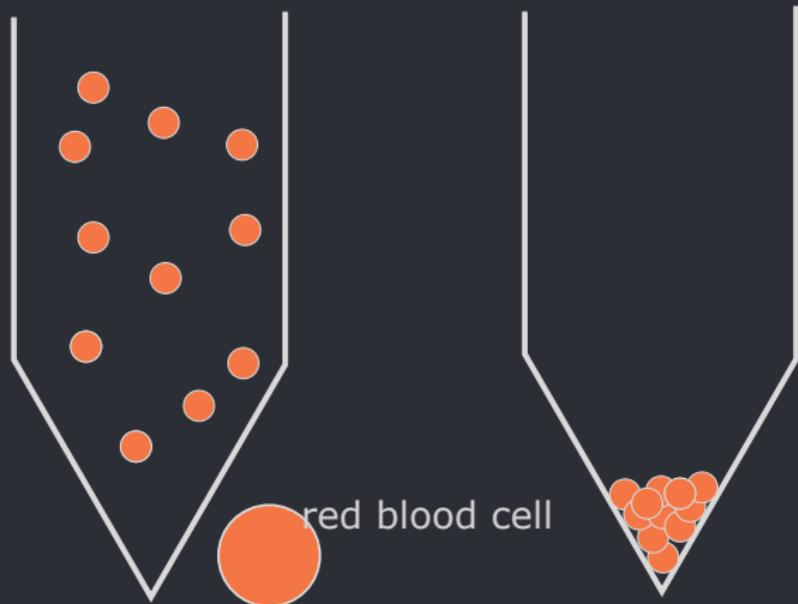


Figure 6: Red blood cells (RBC) precipitate.

The Hemagglutination Inhibition Assay

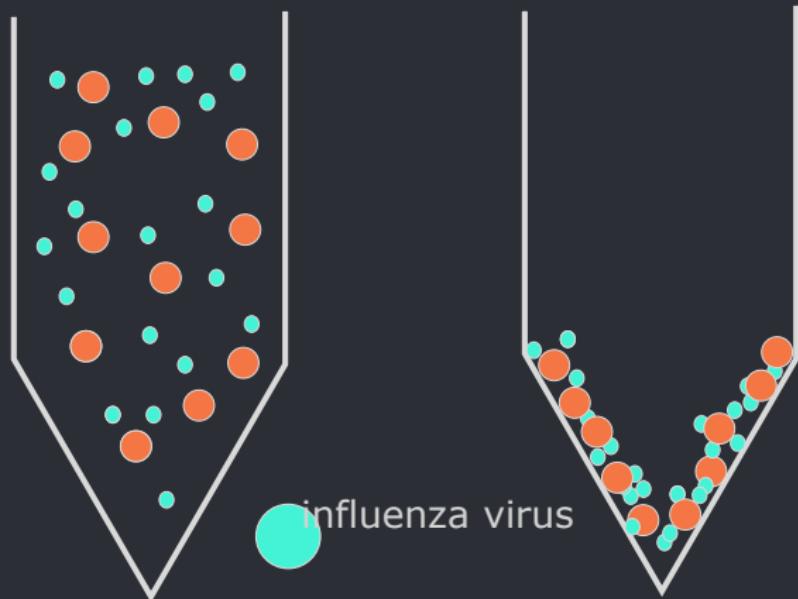


Figure 7: Influenza Hemagglutinin (HA) coagulates the RBC, forming a mat.

The Hemagglutination Inhibition Assay

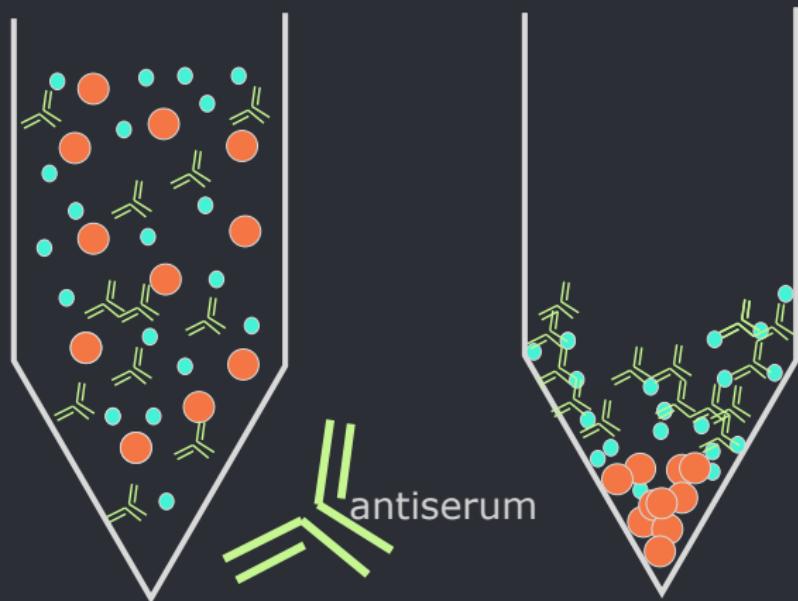


Figure 8: Antisera that fit the HA's epitope site bind to it, letting the RBC sink to the bottom. Effect works up to a certain antigenic distance and antisera concentration.

The Hemagglutination Inhibition Assay

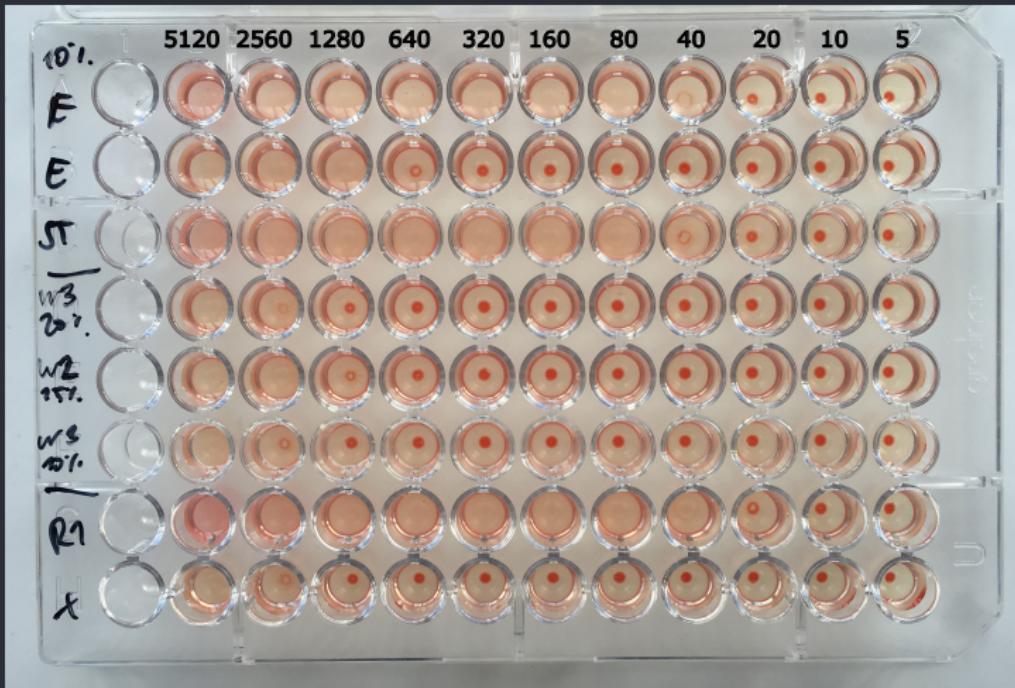


Figure 9: Test 12 different dilutions of one antiserum against 8 different virus strains. The highest dilution that prevents agglutination is called the titer.

The Hemagglutination Inhibition Assay

How this used to be looked at

Post infection ferret sera				
A/Bris	A/Uru	A/HK	A/Perth	
10/07	716/07	1985/09	16/09	
F29/08	F26/08	F21/09	F25/09	

REFERENCE VIRUSES

A/Brasilia/10/2007	2560	2560	80	<
A/Uruguay/716/2007	1280	2560	<	<
A/Hong Kong/1985/2009	80	160	1280	640
A/Perth/16/2009	<	40	640	640

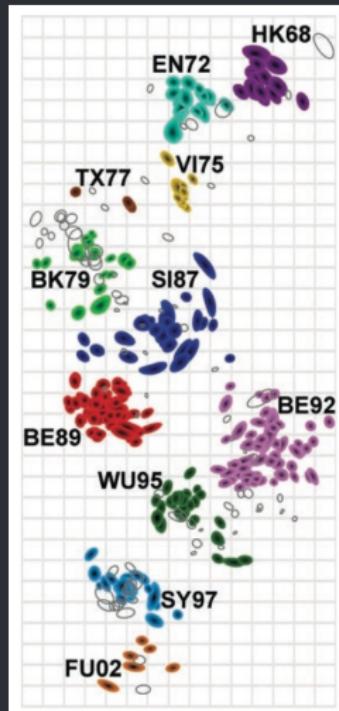
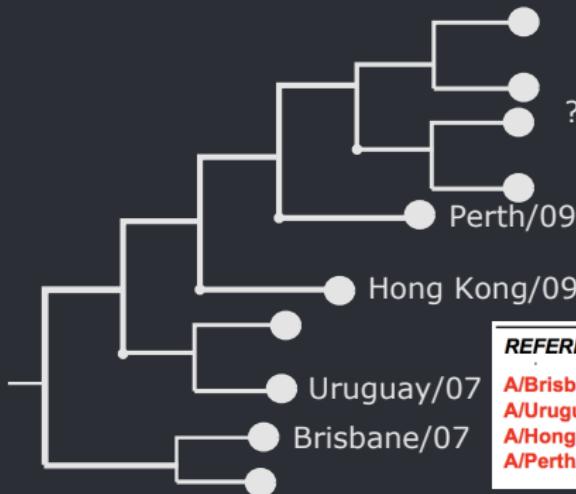


Table from [Bedford, 2015],

cartography from [Smith et al., 2004]

(one gridline is one \log_2 titer i.e. two-fold dilution)

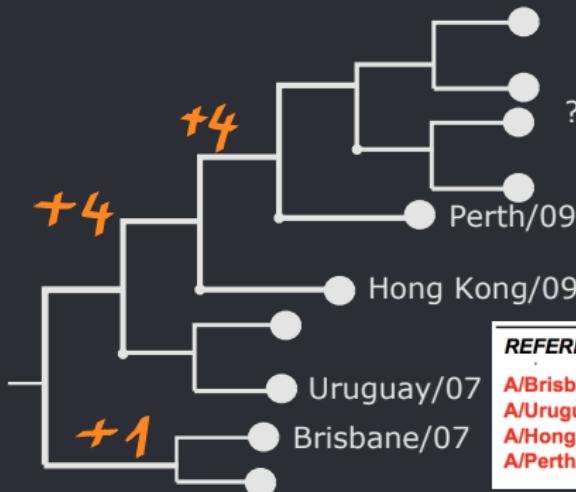
Mapping Antigenicity to the Tree



REFERENCE VIRUSES	A/Bris	A/Uru	A/HK	A/Perth
A/Brisbane/10/2007	2560	2560	80	<
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Figure 10: Mapping the chart to the tree inferred from sequences. [Bedford, 2015]

Mapping Antigenicity to the Tree



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Figure 10: Mapping the chart to the tree inferred from sequences. [Bedford, 2015]

Mapping Antigenicity to the Tree

A literal toy model:



Figure 11: [rosipaw, 2010]

The Model — [Neher et al., 2016] — I

$T_{a\beta}$ HI titer of virus a against antiserum β (virus b)

$H_{a\beta}$ \log_2 relative titer (we'll use this one)

$$H_{a\beta} = \log_2(T_{b\beta}) - \log_2(T_{a\beta}) \quad (1)$$

$\hat{H}_{a\beta}$ predicted \log_2 relative titer

v_a avidity of virus a (=greediness)

p_β potency of antiserum β (=effectiveness)

D_{ab} genetic component of titer drop

$$\hat{H}_{a\beta} = v_a + p_\beta + D_{ab} \quad (2)$$

The Model — [Neher et al., 2016] — II

What remains is split up into a sum over individual branch contributions $d_i \geq 0$:

$$D_{ab} = \sum_{i \in (a \dots b)} d_i \quad (3)$$

Where the sum is over the path connecting virus a and virus b corresponding to antiserum β . Now we want

$$\hat{H}_{a\beta} \stackrel{!}{=} H_{a\beta} \quad (4)$$

to that end we minimize a cost function C of the whole tree:

$$C := \sum_{a,\beta} (\hat{H}_{a\beta} - H_{a\beta})^2 + \lambda \sum_i d_i + \gamma \sum_a v_a^2 + \delta \sum_\alpha p_\alpha^2 \quad (5)$$

Predictions of antigenicity

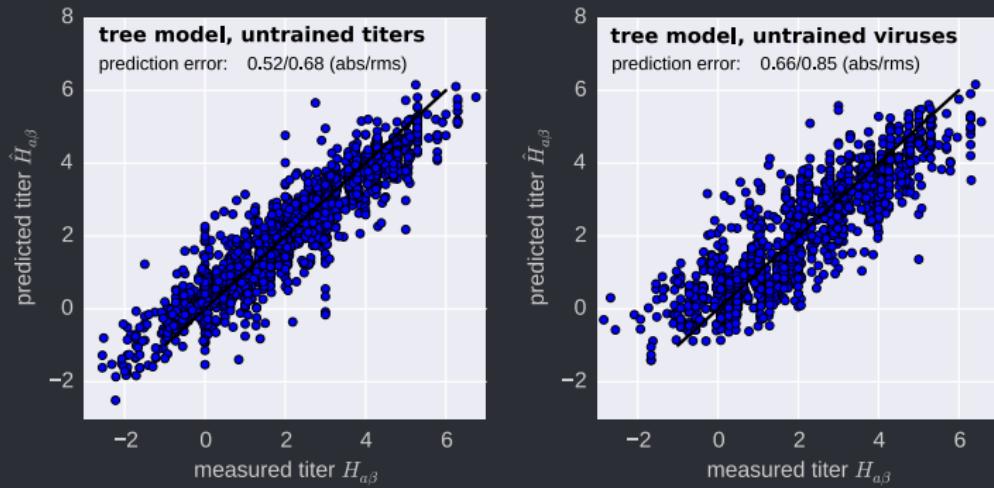


Figure 12: On the left, 10% randomly picked measurements were omitted, on the right, 10 % of entire titer columns were held back, as if it was a new clade. Dataset: Influenza A/H3N2 (12y) [Neher et al., 2016]

Predictions

corroborating "tree-likeness"

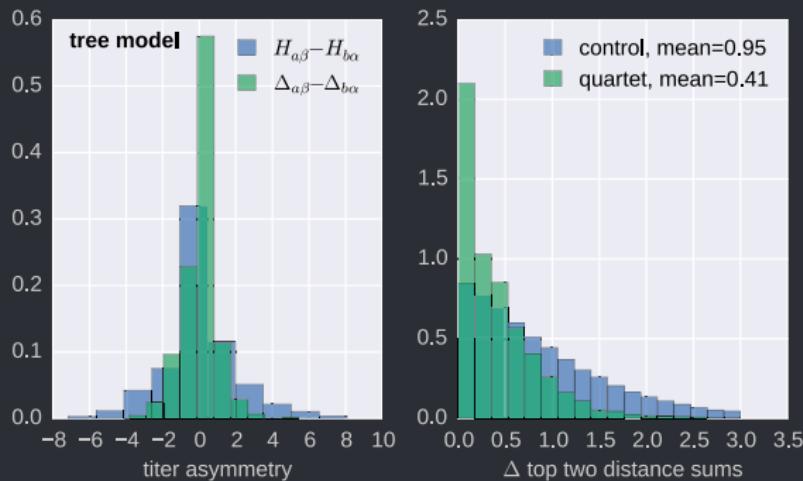


Figure 13: Left: Does subtracting v_a and p_β enforce symmetry? ($\Delta_{a\beta} = D_{ab}$)
Right: showing tree-likeness, employing the quartet rule. [Neher et al., 2016]

Predictions

fixation implications

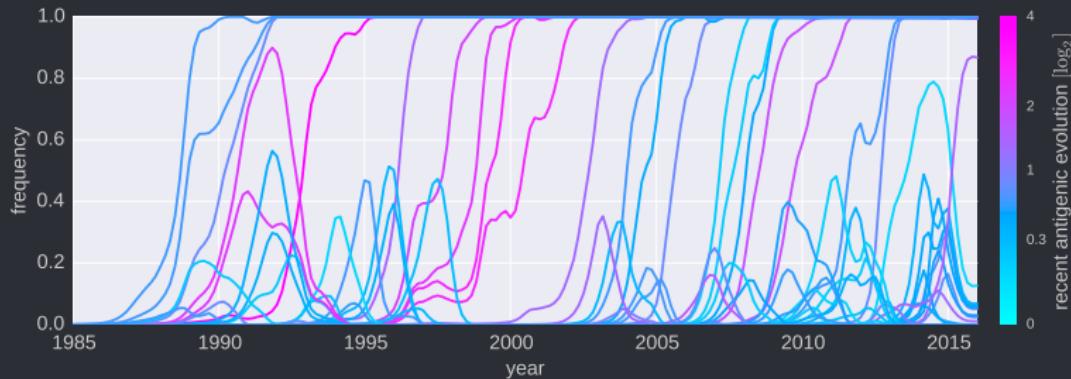


Figure 14: Fraction of samples having a certain mutation plotted over time.
Strains with frequencies smaller than 0.01 were omitted. [Neher et al., 2016]

Predictions

recall:

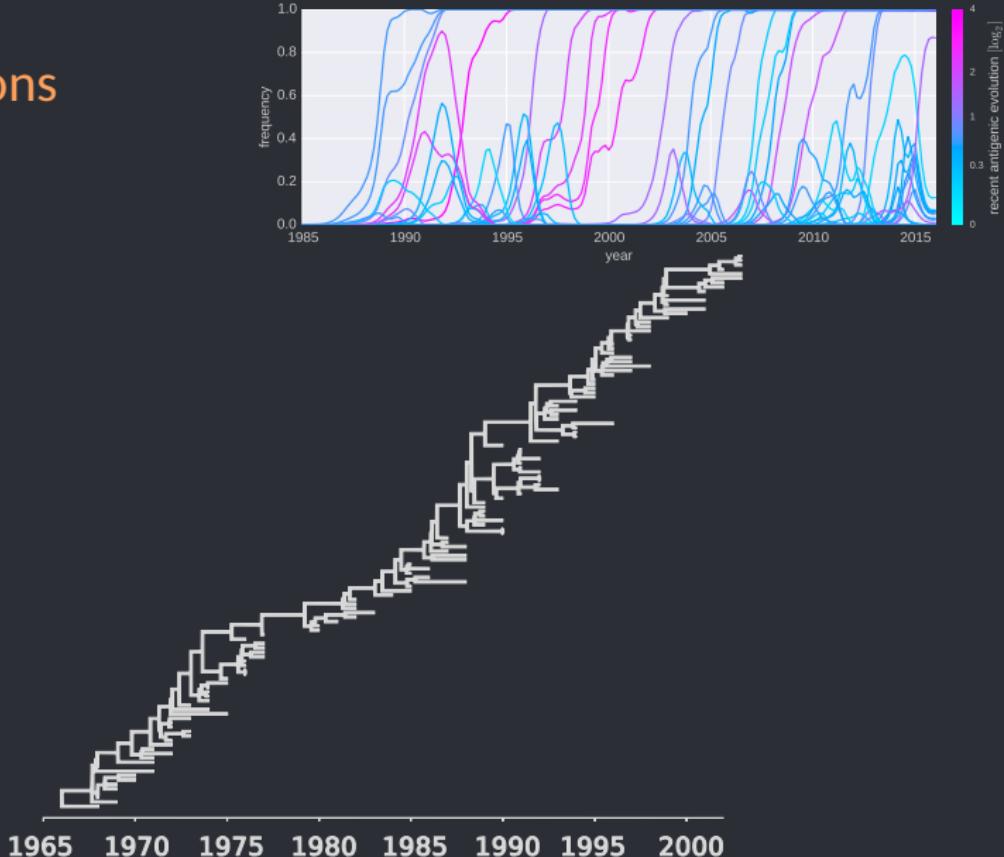


Figure 15: A/H3N2 phylogeny [Volz et al., 2013]

Predictions fixation jumps

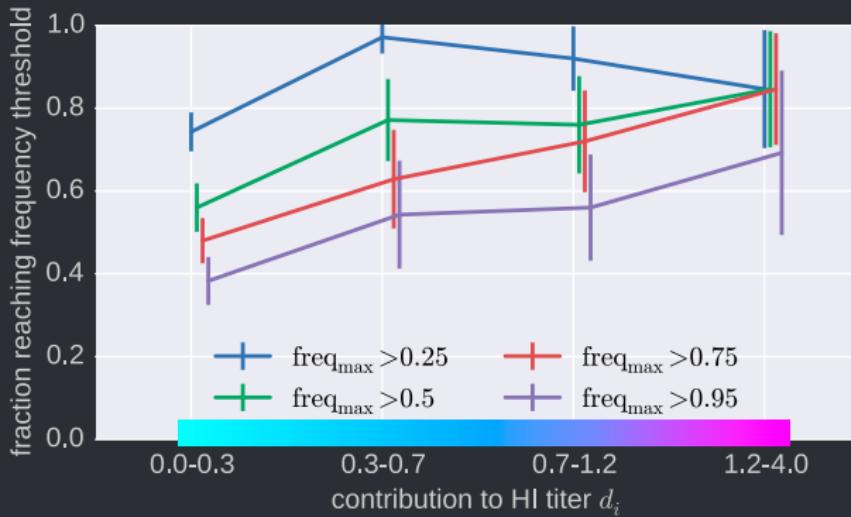
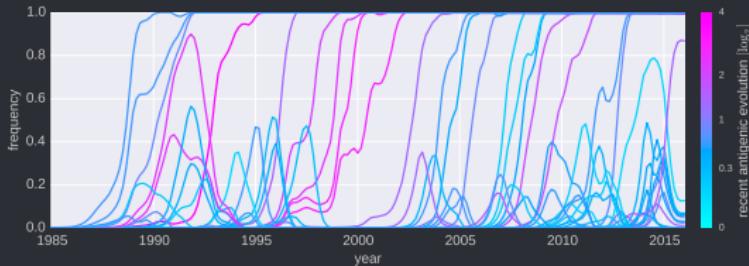
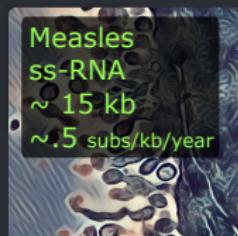
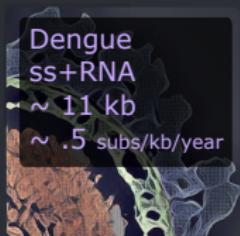
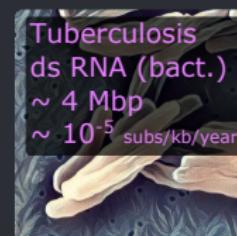
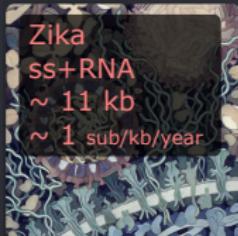
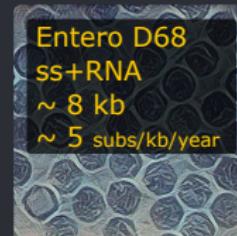
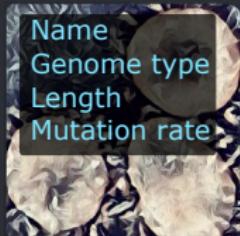


Figure 16: For high recent antigenic evolution traits, 25% prevalence directly entails 75% [Neher et al., 2016]

Nextstrain

Nextstrain— A Growing Platform



Nextstrain— Narrative

<https://nextstrain.org/community/narratives/gitchhiker/virophyle>

Multi Scale Evolution

If a single event mutation occurs, say **D 186 G** in the HA genome, it is subject to multi scale evolutionary selection:

- this RNA instance vs. the other RNA strands in the same cell
- this cell's mutated viruses vs. other viruses inside the host
- this host's viruses vs. viruses in rest of the population
- this population vs. other populations

These scales are difficult to separate. At the population level *epidemiological* processes may dominate.

Outlook

and closing remarks

- Difficult to disentangle levels, therefore integrate visualization
- Include more meta data (symptoms, severity, & c.)
- Interesting: larger evolutionary timescale
- Include other pathogens, like bacteria, CAVEAT: HGT
- My question: what happens if epidemic threshold is crossed even further, and zoonotic forcing is stronger?
- nice to see instance of Red Queen Hypothesis
- Transparency and Community building very important!
- When you find literature on phylodynamics, often animals, but to me this is more difficult.

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