Using genetic data at multiple scales to understand constraints on viral adaptation

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CIRM - Luminy
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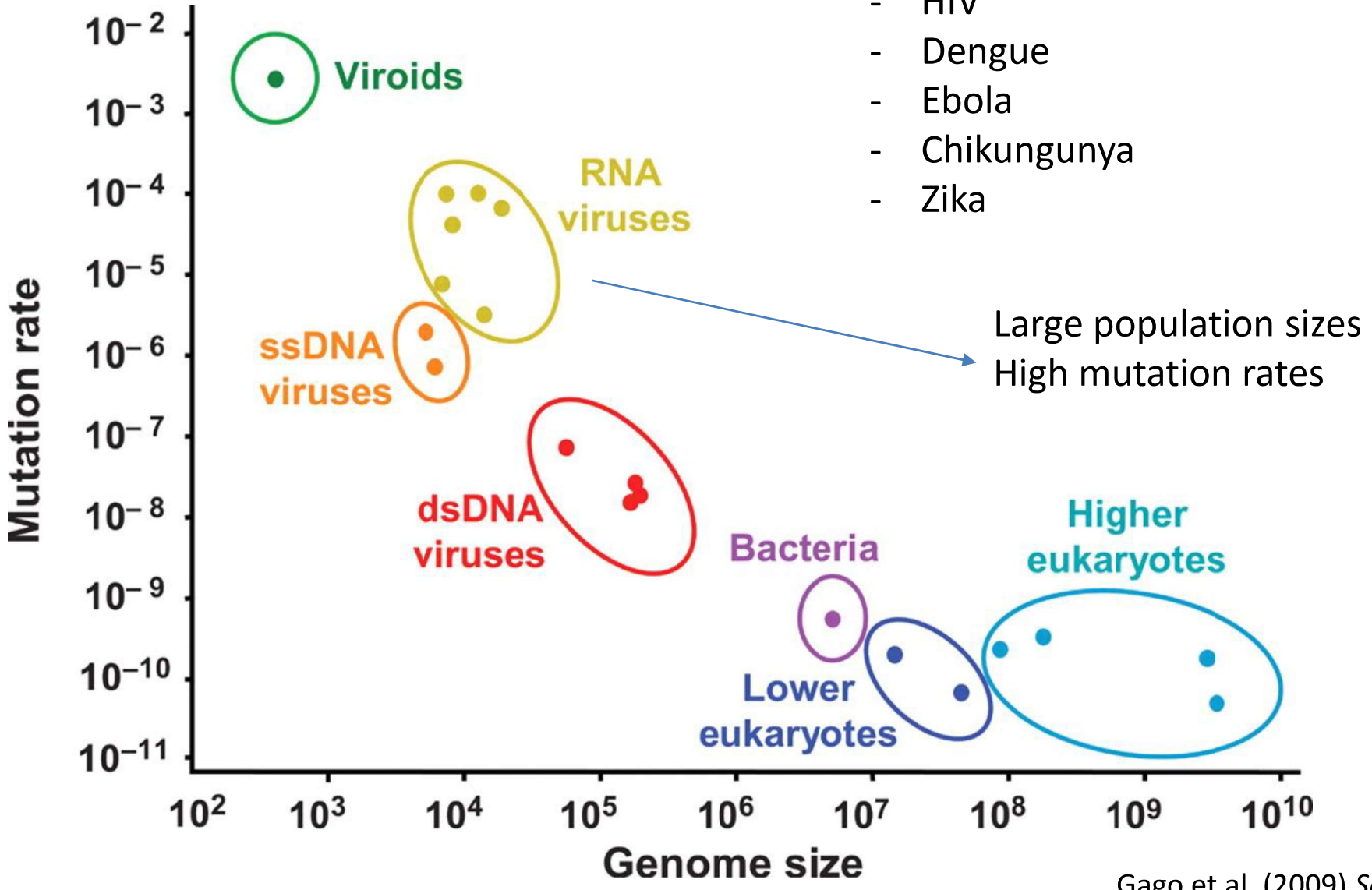
RNA viruses

- Influenza
- HIV
- Dengue
- Ebola
- Chikungunya
- Zika

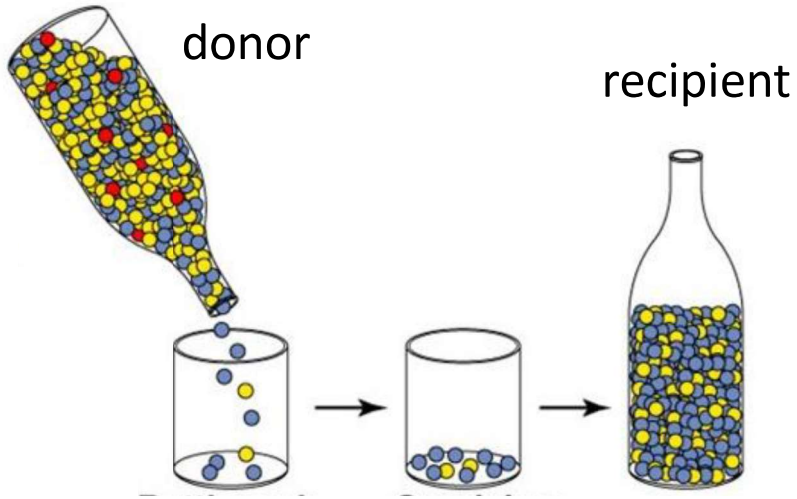


Large population sizes
High mutation rates

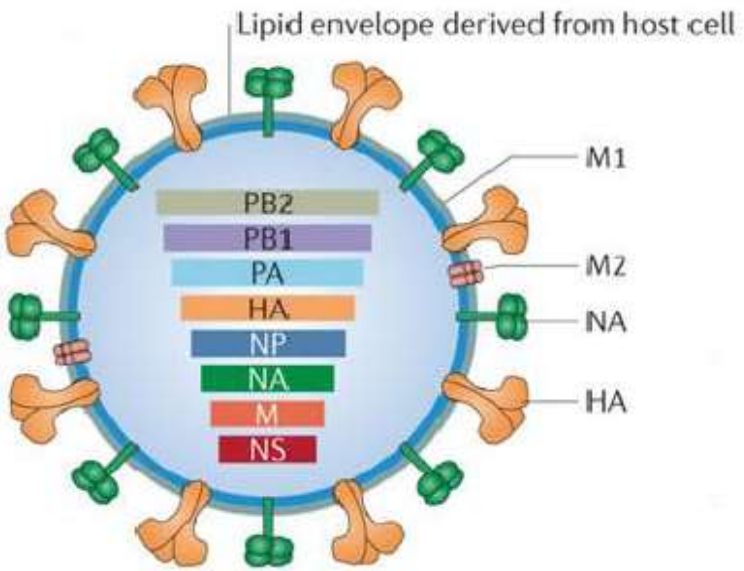
Gago et al. (2009) *Science*

What are some evolutionary constraints to viral adaptation?

1. **Transmission bottlenecks between donors and recipients**



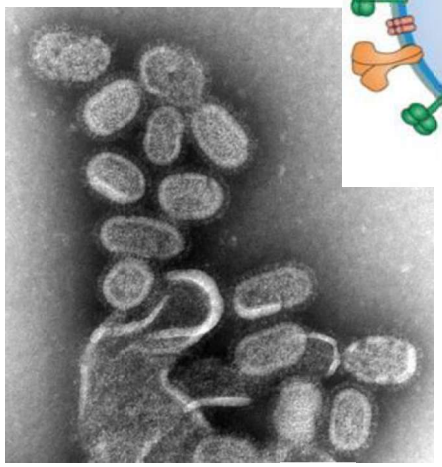
2. **Genetic linkage**

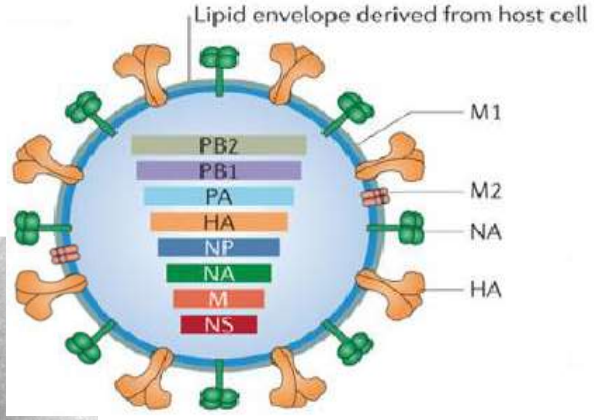


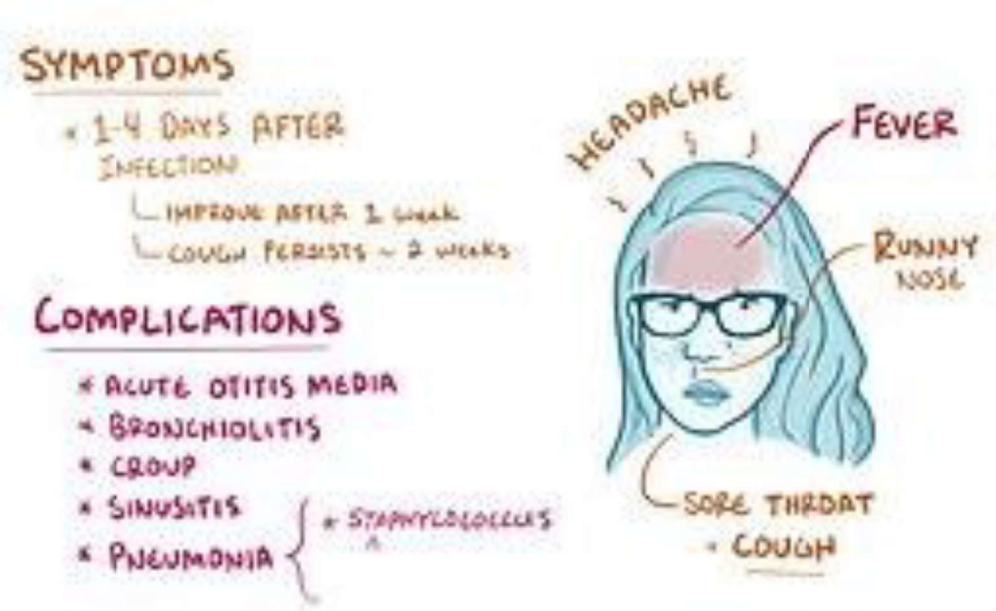
What are some evolutionary constraints to influenza adaptation?

1. **Transmission bottlenecks between donors and recipients**
   - Influenza transmission bottleneck size

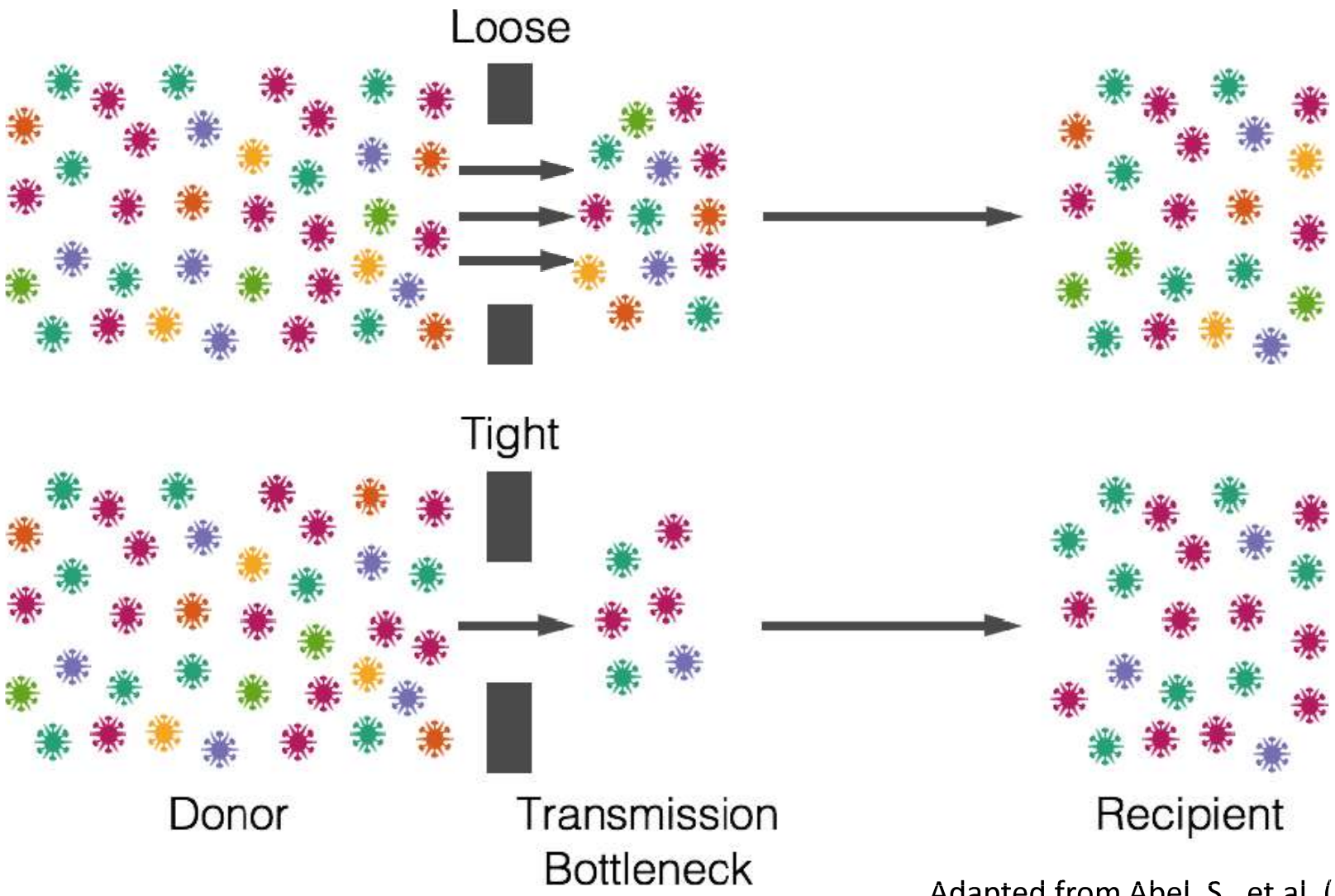
2. **Genetic linkage**
   - Deleterious mutations shaping influenza's antigenic evolution







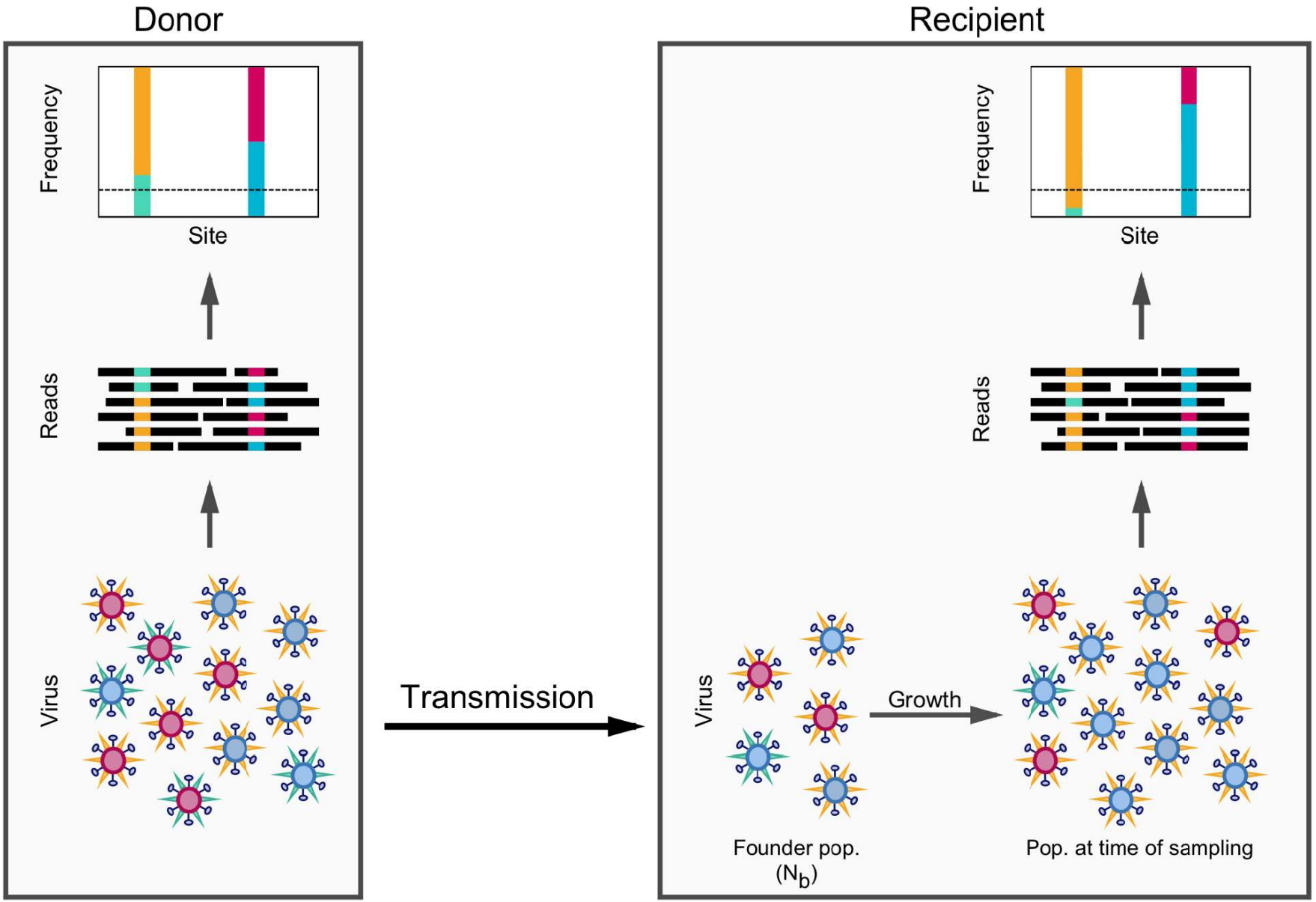
Transmission bottleneck sizes



Adapted from Abel, S., et al. (2015) *PLoS Path.*

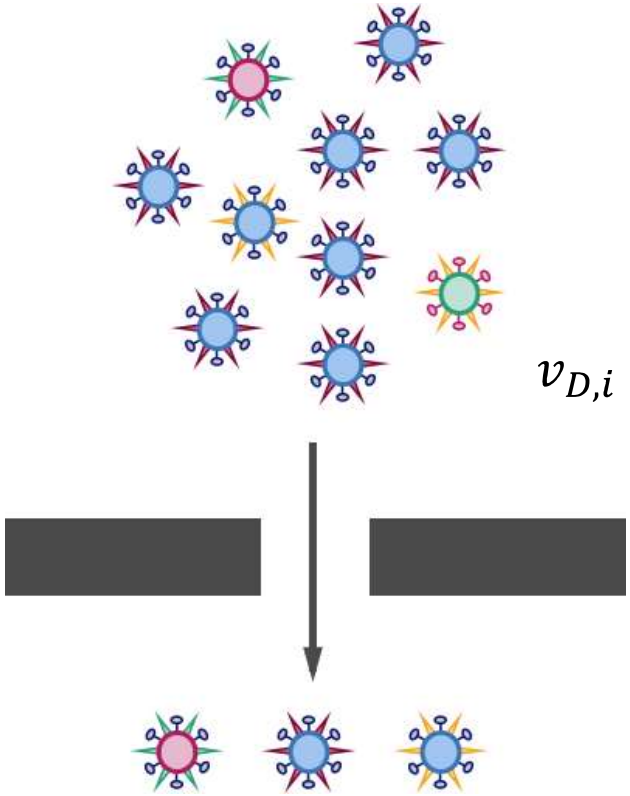
Looser bottlenecks enable more rapid viral adaptation

Estimating transmission bottleneck sizes using NGS data



Existing methods to estimate N_b using NGS data

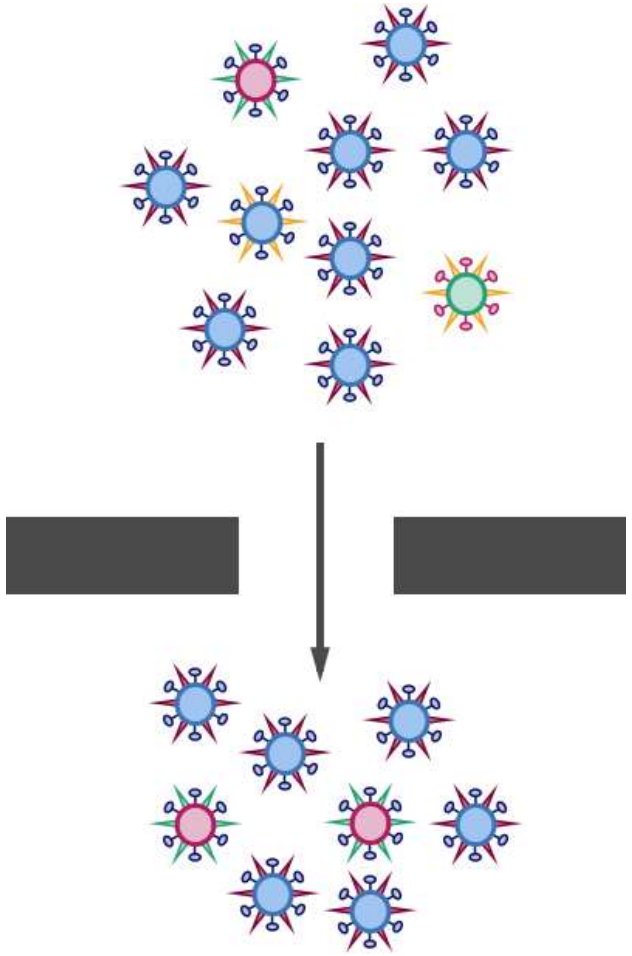
Presence/absence method



Probability not transmitted: $\left(1 - v_{D,i}\right)^{N_b}$

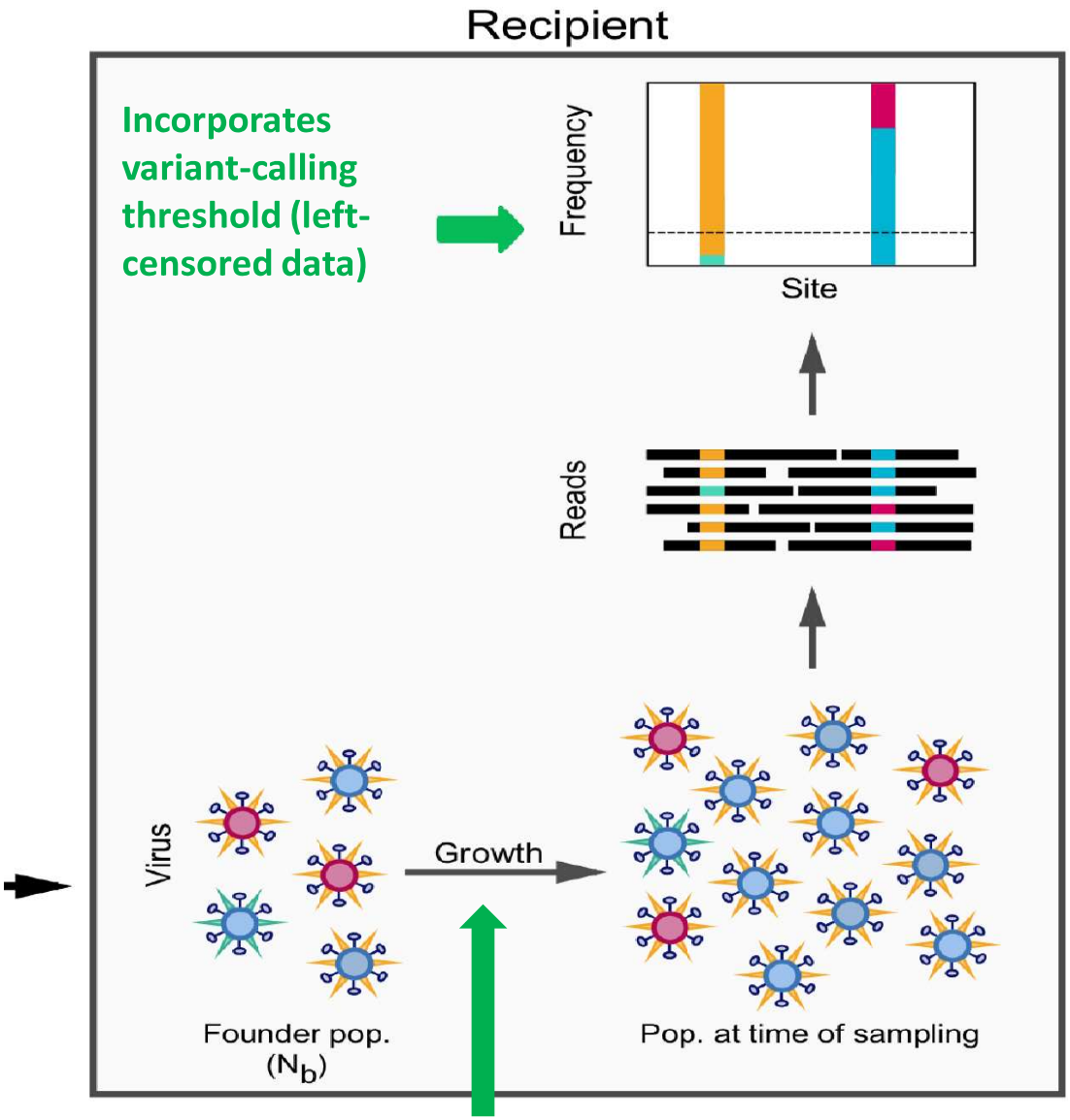
Probability transmitted: $1 - \left(1 - v_{D,i}\right)^{N_b}$

Frequency method



Single generation WF model (binomial sampling)

Additional factors incorporated into our frequency method

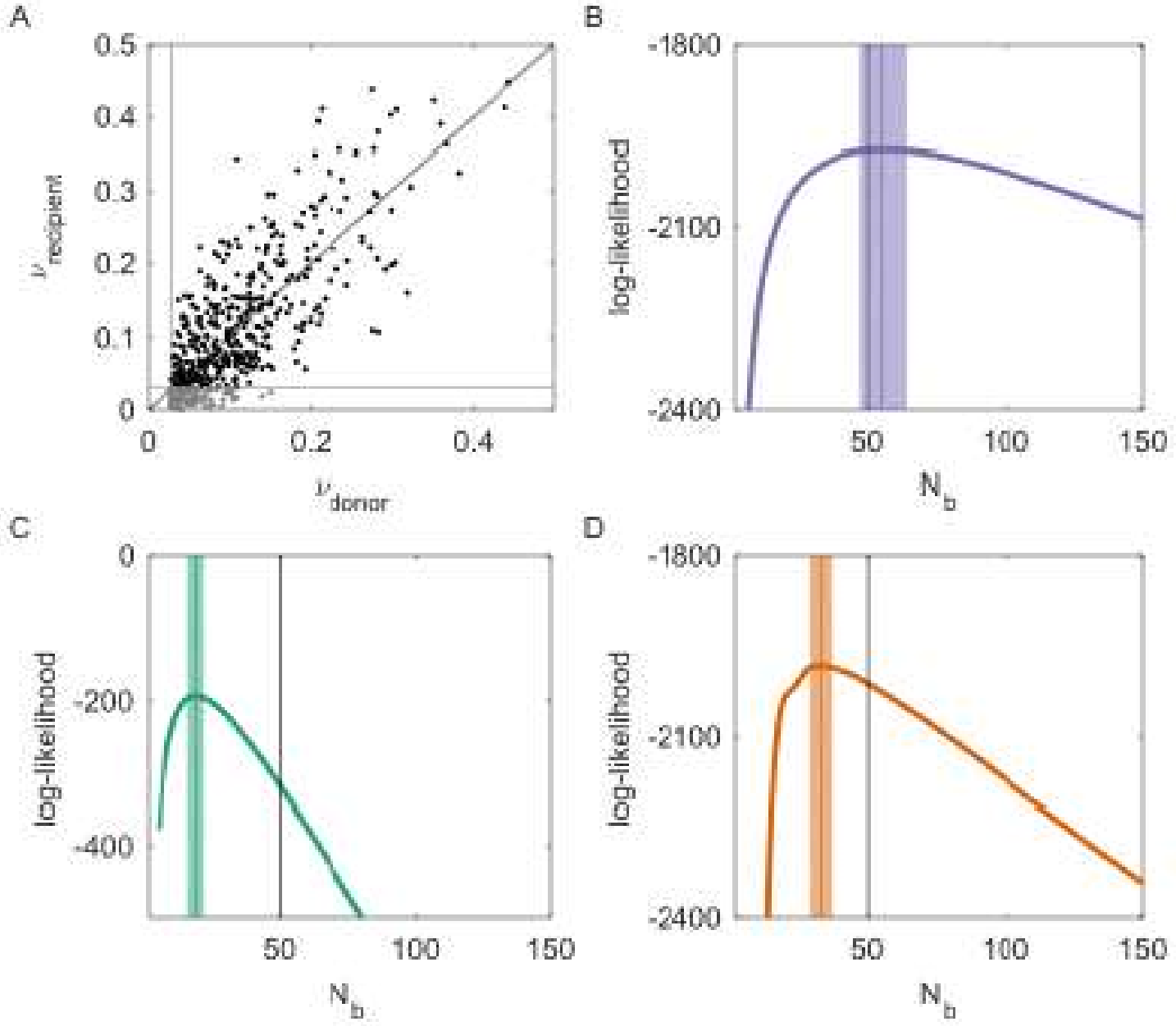


Leads to likelihood calculations based on a beta or beta-binomial distribution

Sobel Leonard, A., Weissman, D., Greenbaum, B., Ghedin, E., Koelle, K. (2017). *Journal of Virology*

Incorporates demographic noise in viral replication dynamics (stochastic growth)

Results on simulated NGS dataset (with N_b = 50)



Presence/ absence

Betabinomial sampling

WF model (binomial sampling)

Influenza A cohort study

Leo Poon

Ben Cowling

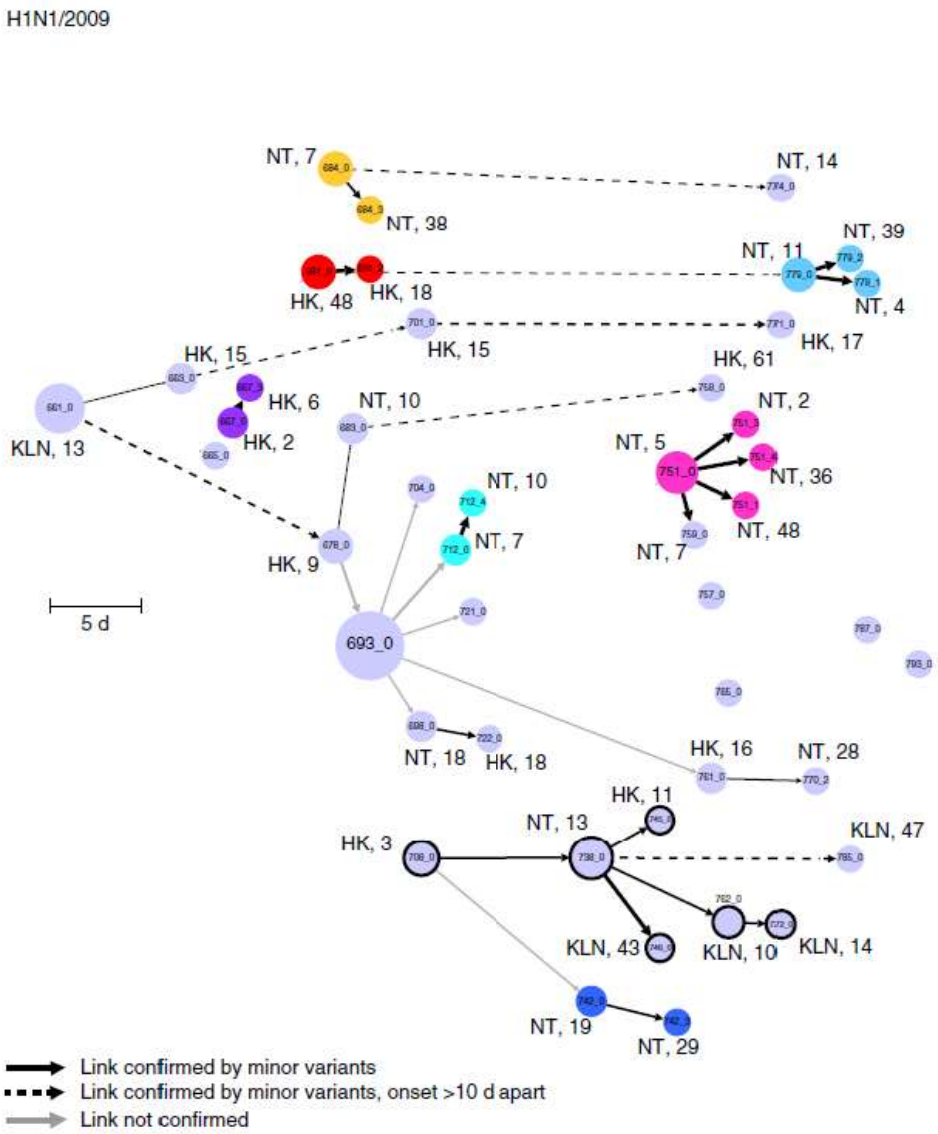
Elodie Ghedin

- Hong Kong study
- July-August 2009
- 84 individuals (67 index + 17 household members)
- H3N2 and H1N1p virus samples
- Metadata on individuals

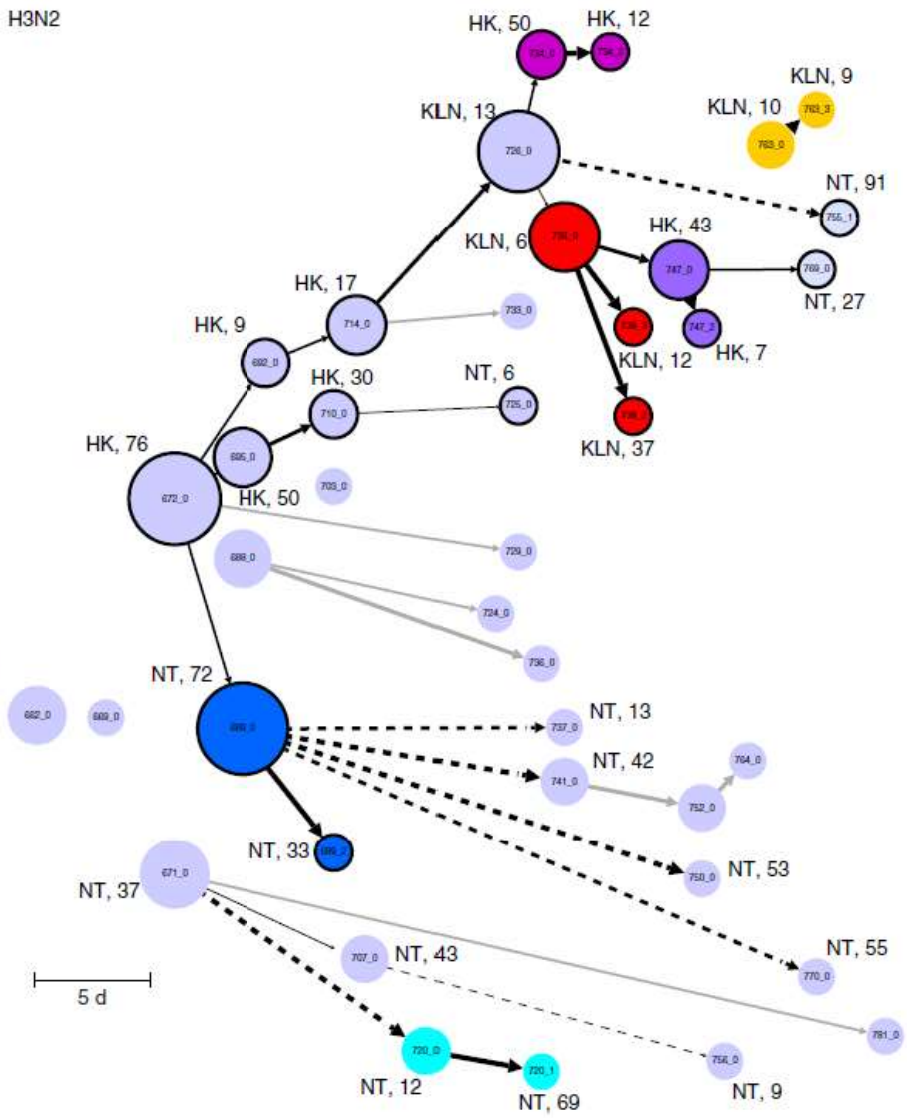
Poon et al. (2016). *Nature Genetics*

Cohort study – identification of transmission pairs

H1N1p - 9 transmission pairs



H3N2 - 7 transmission pairs

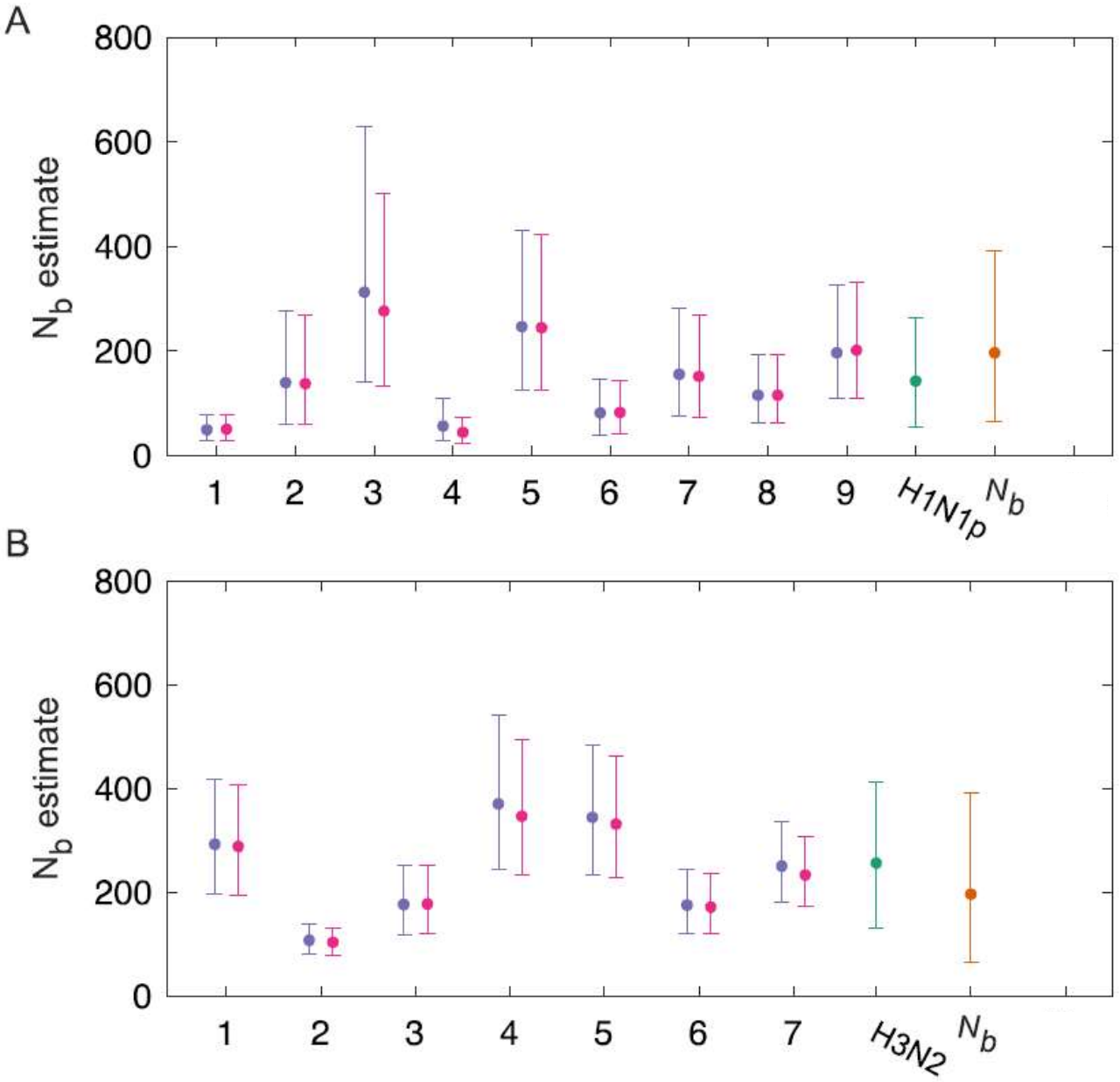


Poon et al. (2016) *Nature Genetics*

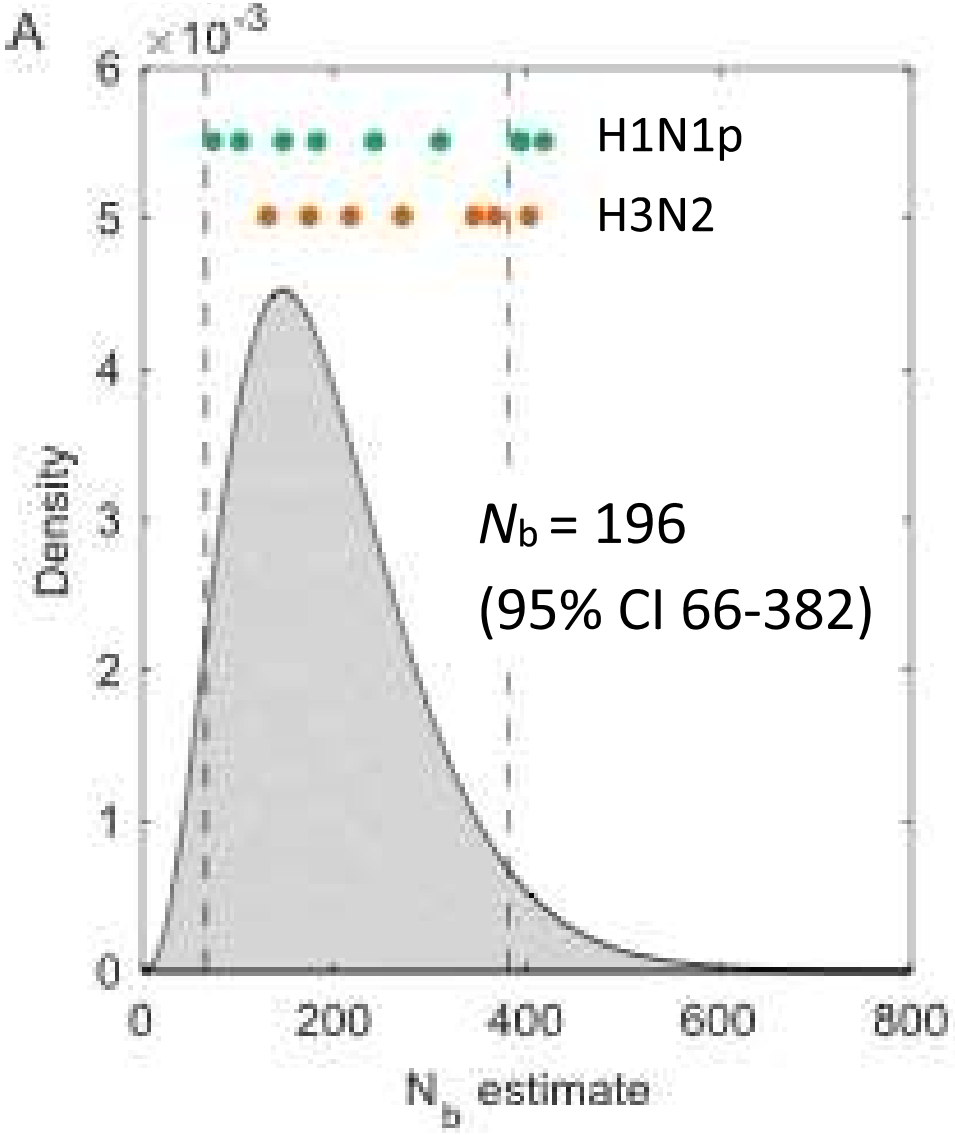
Bottleneck size estimates by transmission pair

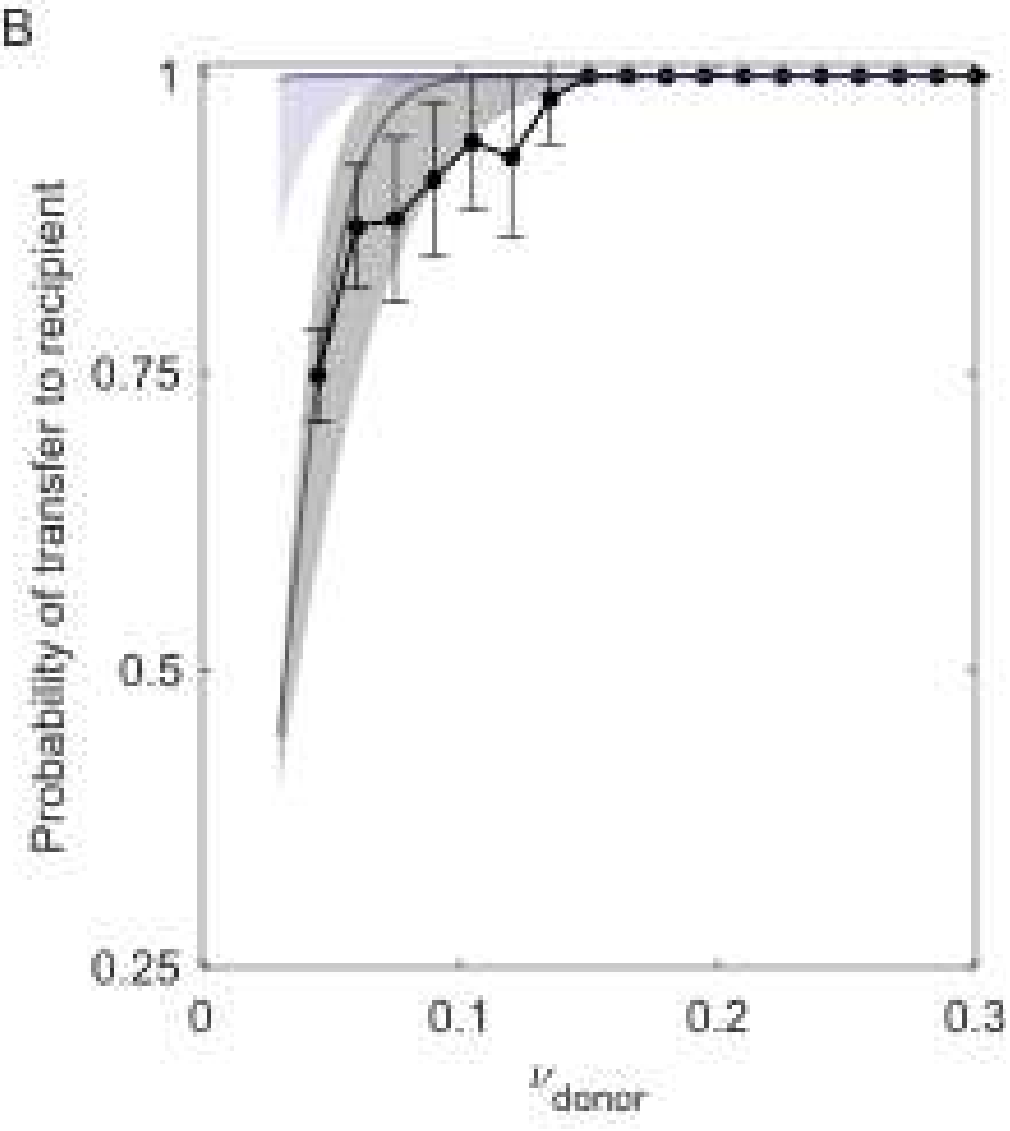
H1N1p

H3N2



Overall bottleneck size estimates

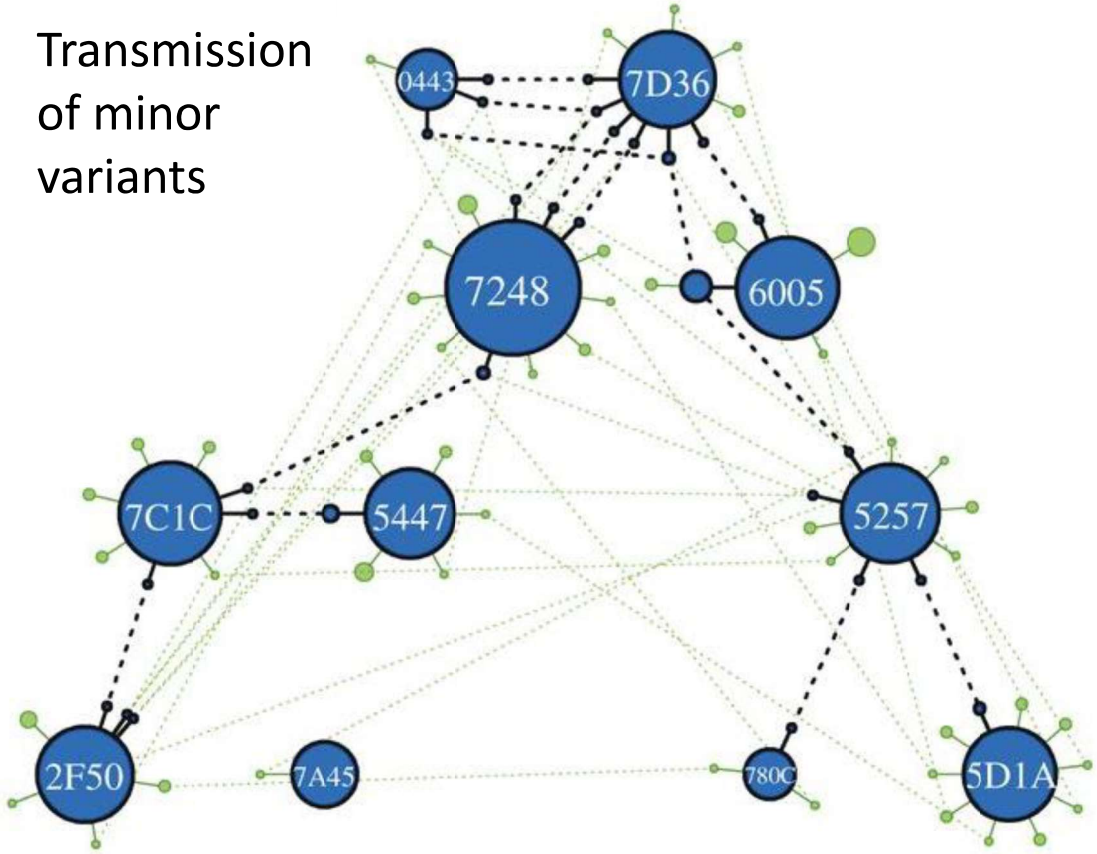




We found that the transmission bottleneck of influenza A is loose and highly variable across transmission pairs. (**Transmission bottlenecks may not play a substantial role in slowing influenza's rate of adaptation?**)

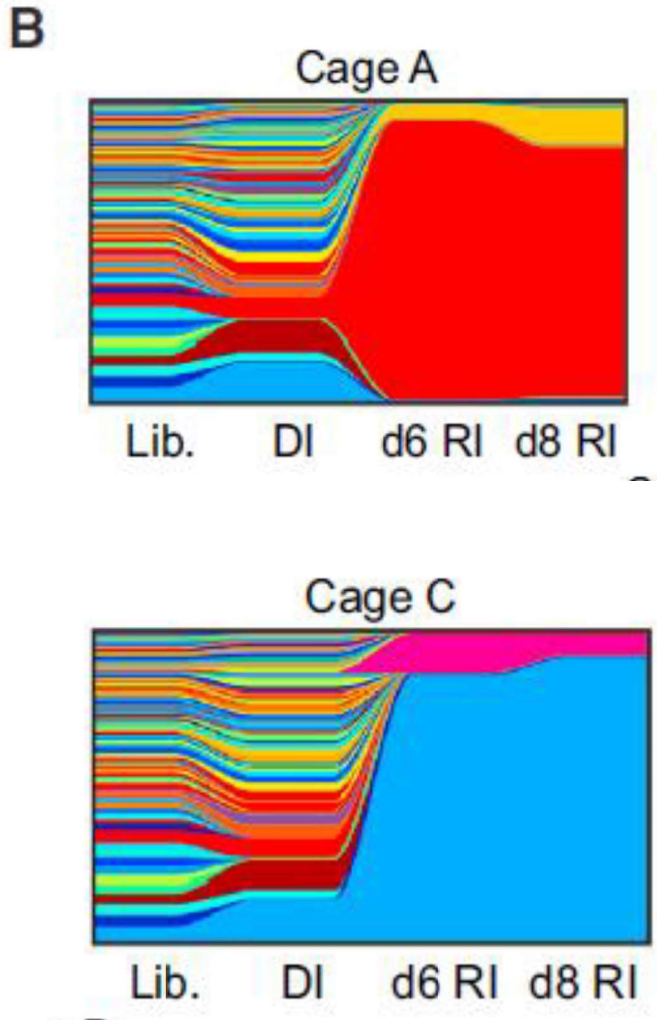
Equine H3N8 (also in Swine H1N1)

Transmission of minor variants



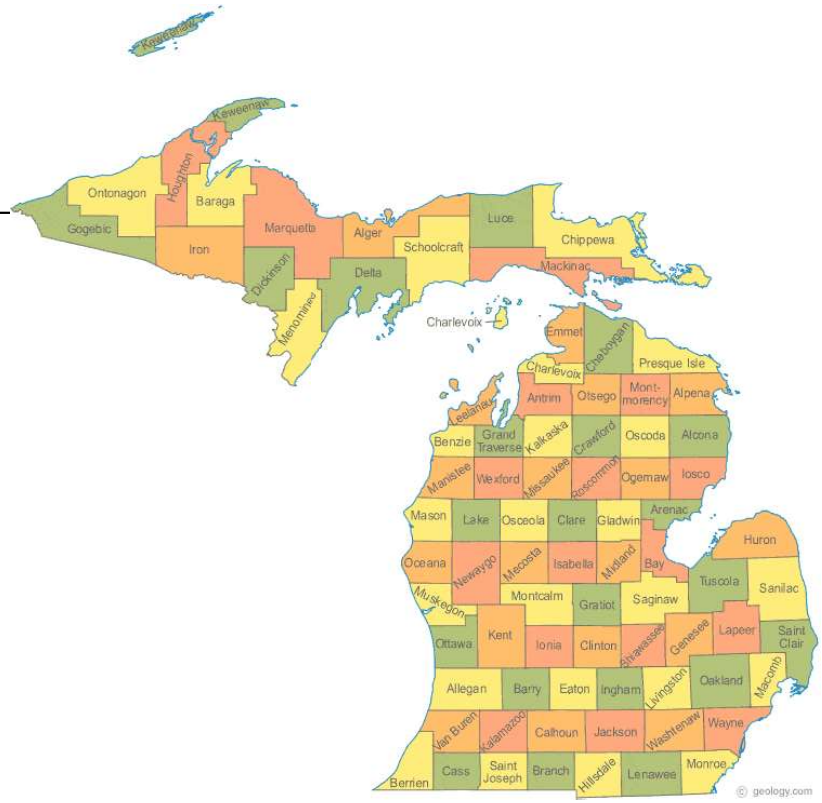
Stack et al. (2013) Proc. Biol. Sci. (2013), among others

but…

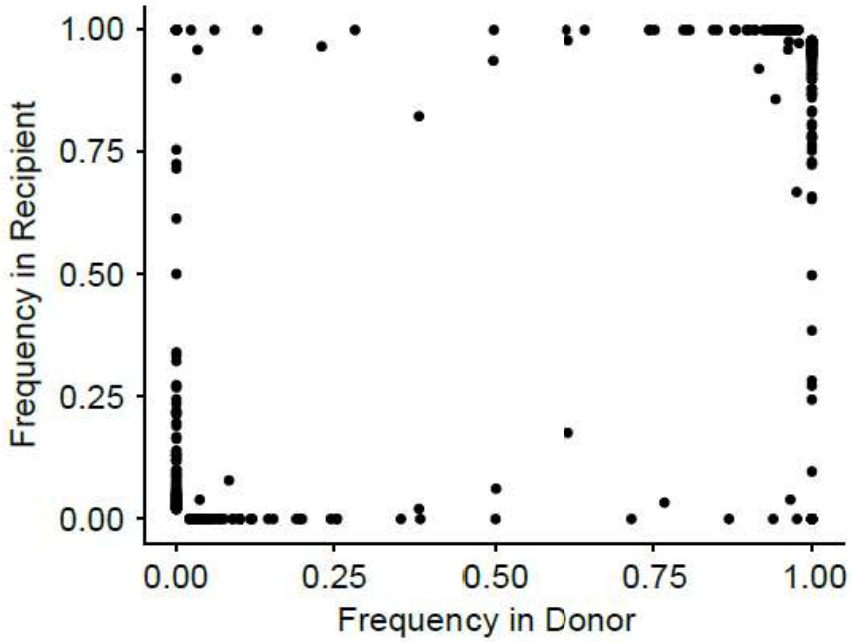


Varble et al. Cell Host & Microbe (2014)

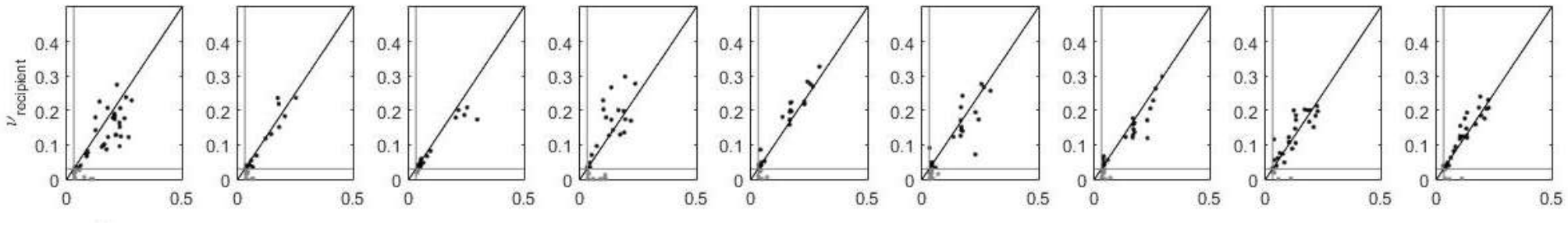
Recent study by McCrone et al. (2018) *eLife*



Transmission bottleneck: 1-2 virions
(via presence/absence and betabinomial methods)

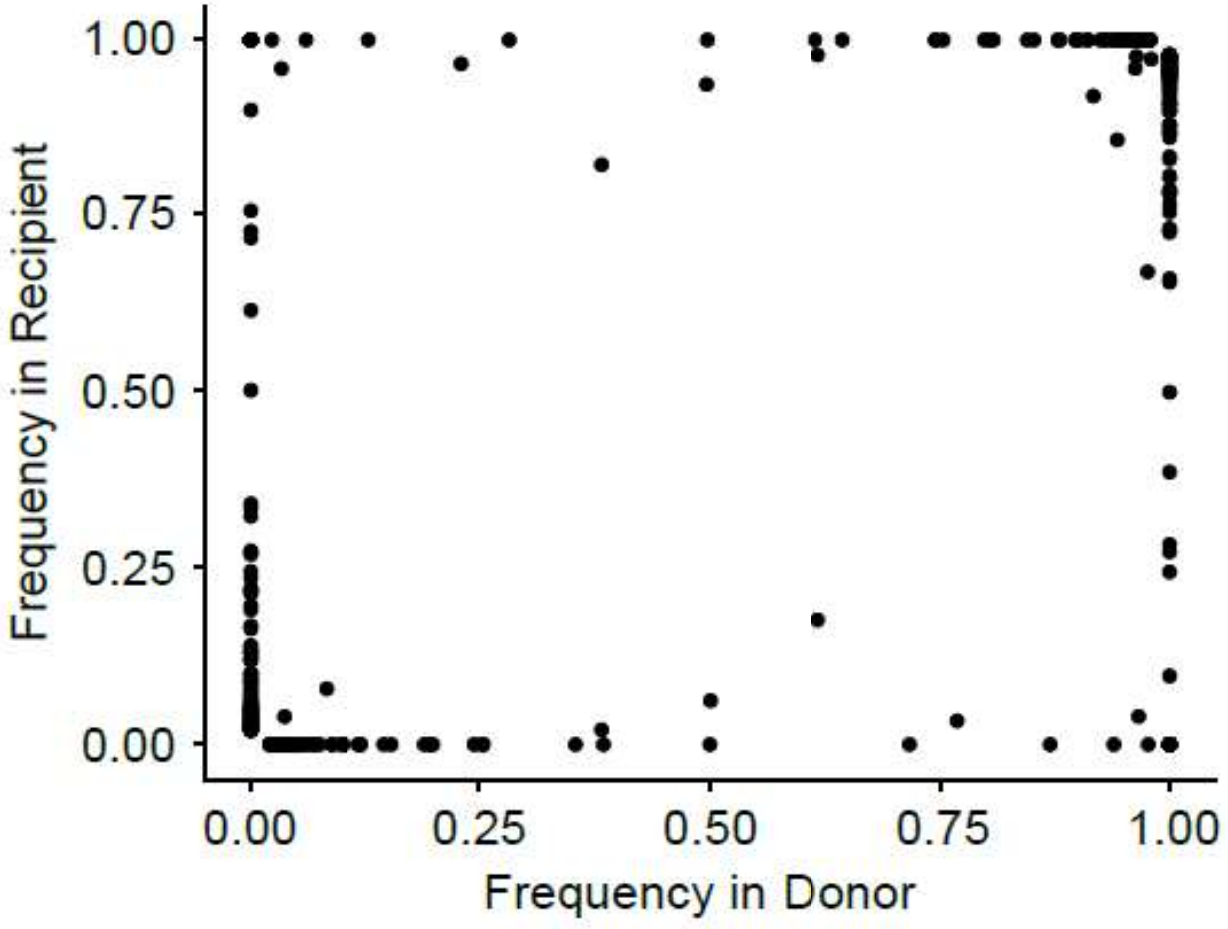


Hong Kong study:



Recent study by McCrone et al. (2018) *eLife*

Transmission bottleneck N_b: 1-2 virions
Calculated over all transmission pairs; the majority of individual transmission pairs had CI of 1-200 virions (few variants in the donor)



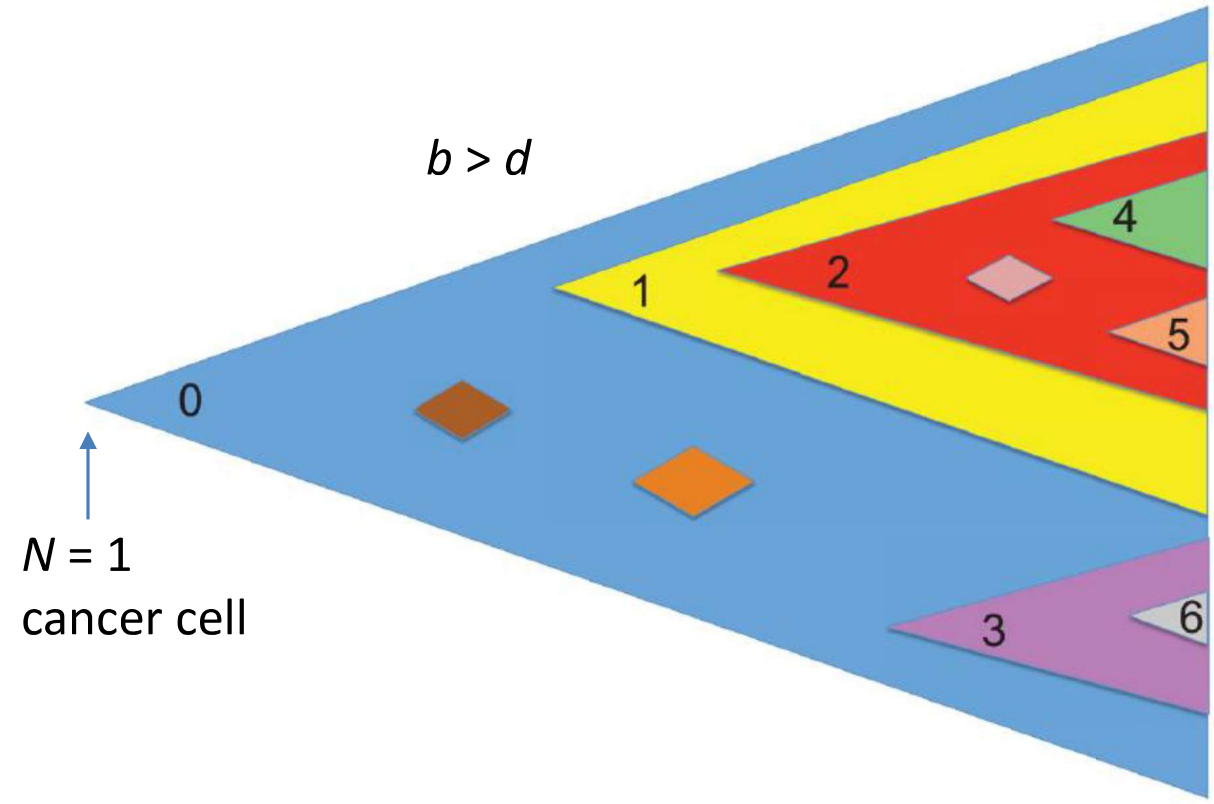
Can we use *de novo* variants in the recipient to estimate N_b more effectively?

Cancer evolution model

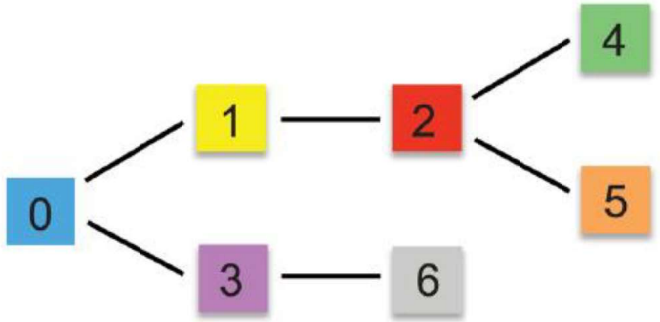
Quantifying Clonal and Subclonal Passenger Mutations in Cancer Evolution

Ivana Bozic[1,2]*, Jeffrey M. Gerold[1], Martin A. Nowak[1,2,3]*

PLOS Computational Biology, 2016



k indexes mutation by arrival order



Parameters: b, d → $\delta = d/b$

u = mutation rate

Derivations in Bozic et al. (2016); multi-type branching process model

Probability of fixation of new mutations: $\rho_k \approx \left(\frac{u}{u - \log\delta}\right)^k$

Increases with u and death-birth ratio δ

Mean number of clonal mutations: $\overline{m}_c = \frac{\delta u}{1-\delta}$

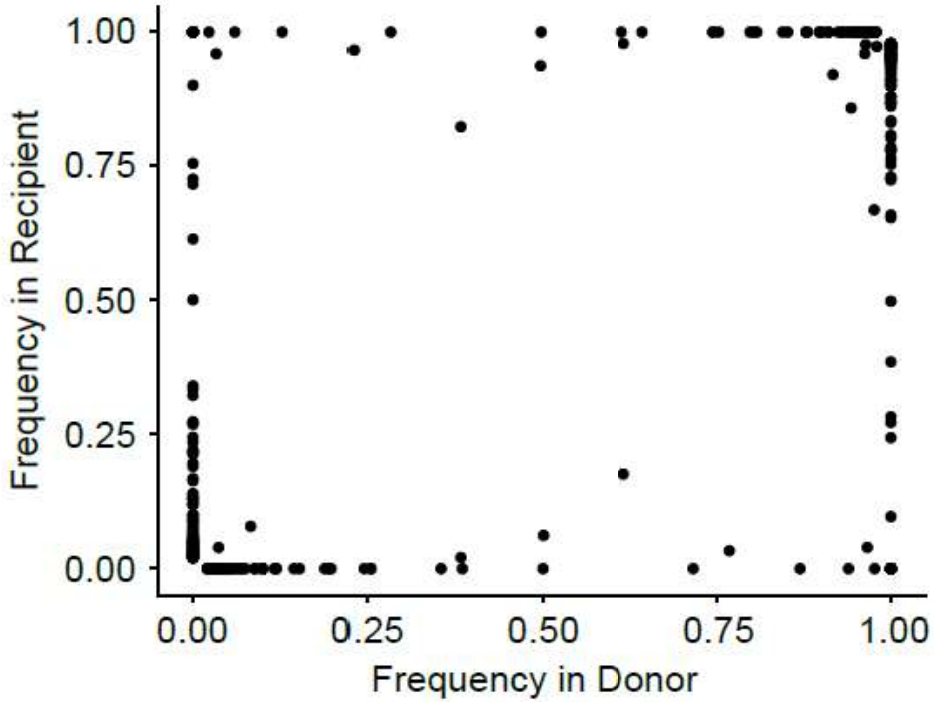
Mean number of subclonal mutations that exceed threshold value of α: $\overline{m}_s = \frac{u(1-\alpha)}{(1-\delta)\alpha}$

Table 1. Expected number of subclonal and clonal mutations for different values of $\delta = d/b$.

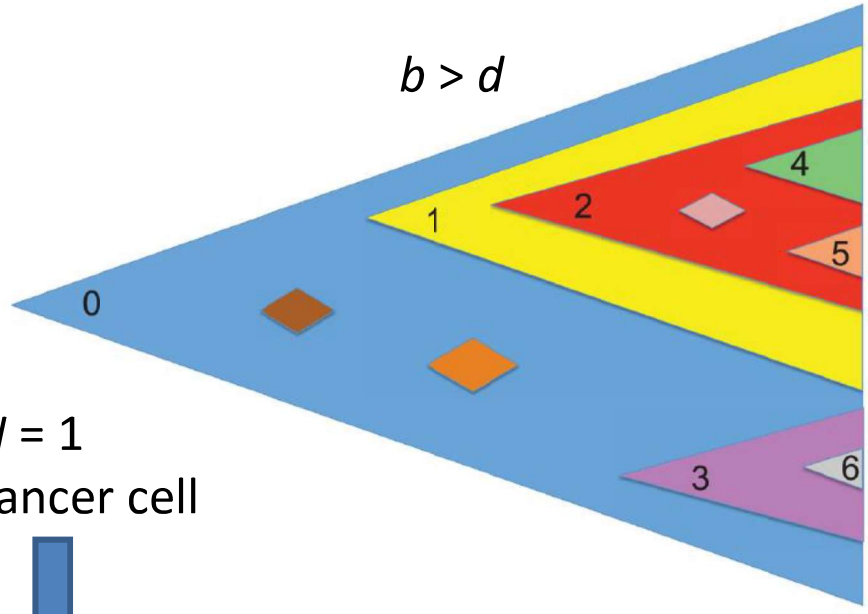
δ	> 0.1%	> 1%	> 10%	> 50%	Clonal
0	15.0	1.5	0.14	0.015	0
0.72	53.5	5.3	0.48	0.05	0.04
0.96	374.6	37.1	3.37	0.38	0.36
0.99	1498.5	148.5	13.5	1.5	1.48
0.999	14985	1485	135	15	15

with $u = 0.015$

Application of Bozic et al. (2016) to transmission bottleneck size estimation

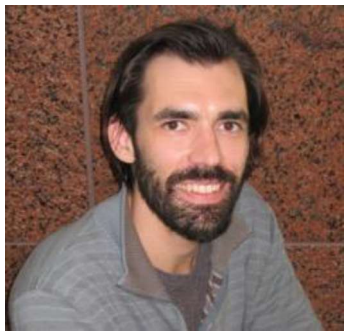


McCrone et al. (2018)



$N = 1$ cancer cell

Generalize for N_b starting "cells"/virions



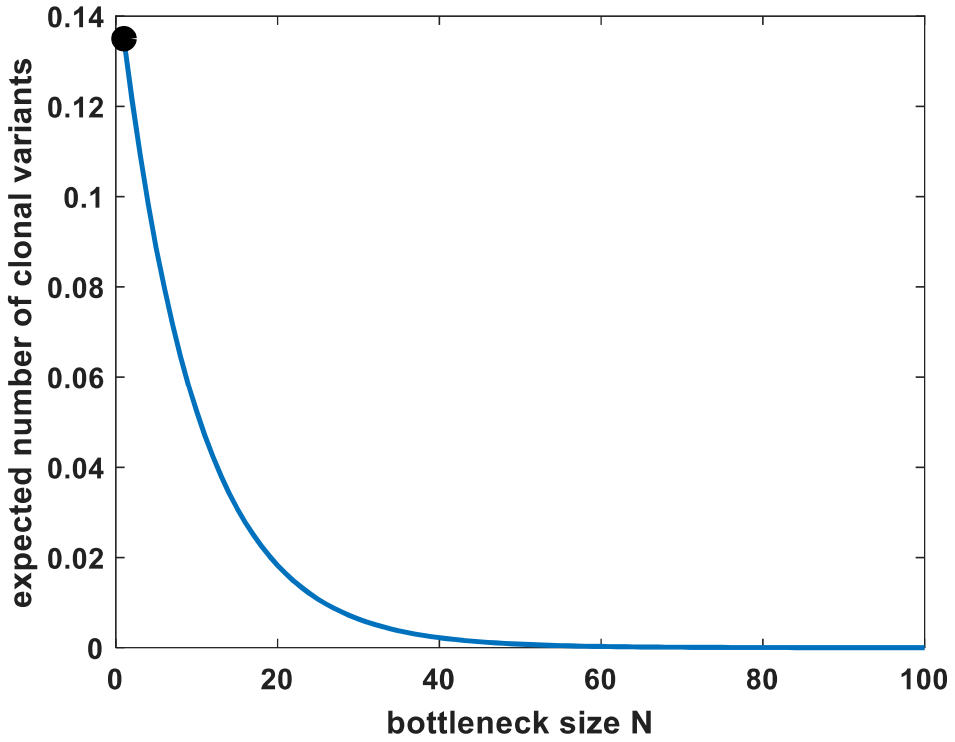
Harris & Koelle (*in prep*.)

Generalizing Bozic et al. (2016)

Expected number of clonal mutations:

N = 1 → $\overline{m}_c = \frac{\delta u}{1-\delta}$

Arbitrary *N* → $\bar{m}_c(N) = \frac{\delta^N u}{(1-\delta)}$



u = 0.015
δ = 0.9

Generalizing Bozic et al. (2016)

Expected number of subclonal mutations:

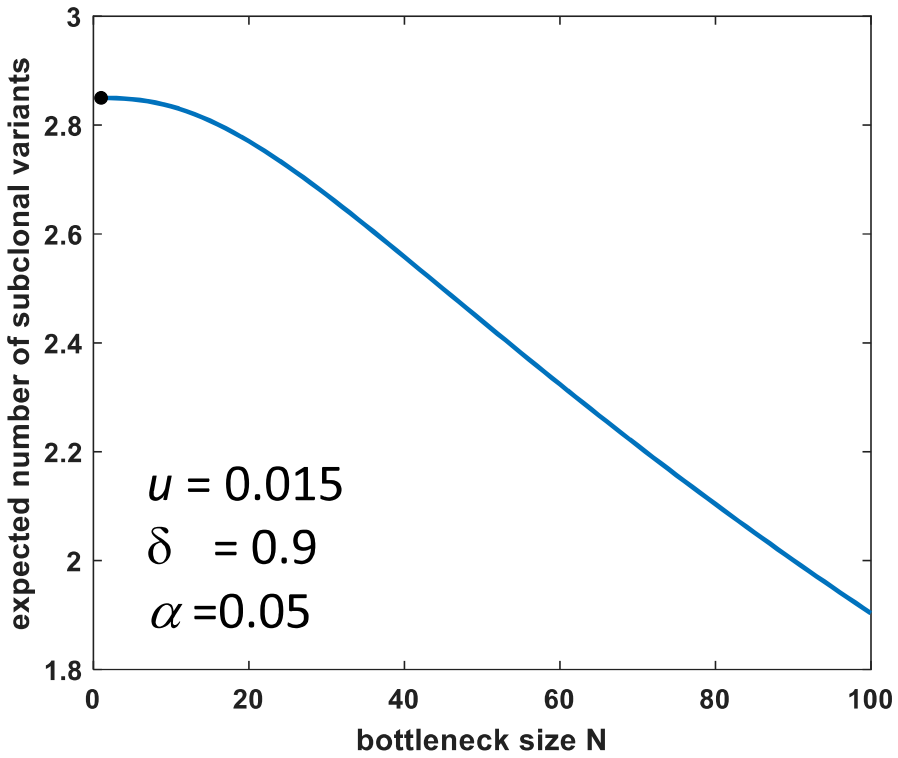
N = 1

$$\overline{m}_s = \frac{u(1-\alpha)}{(1-\delta)\alpha}$$

Arbitrary *N*

$$\overline{m}_s = u\,\delta^N\,(r-1) + u\,\delta^N\left(r^2\left(\frac{r^{N-1}-1}{r-1}\right)+1-N\right) + u\left(\frac{\delta^{N+1}\,r^{N+1}}{\alpha\,(1-\delta)} - \frac{\delta^{N+1}}{1-\delta}\right)$$

where $r = (1-(1-\delta)\,\alpha)/\delta$

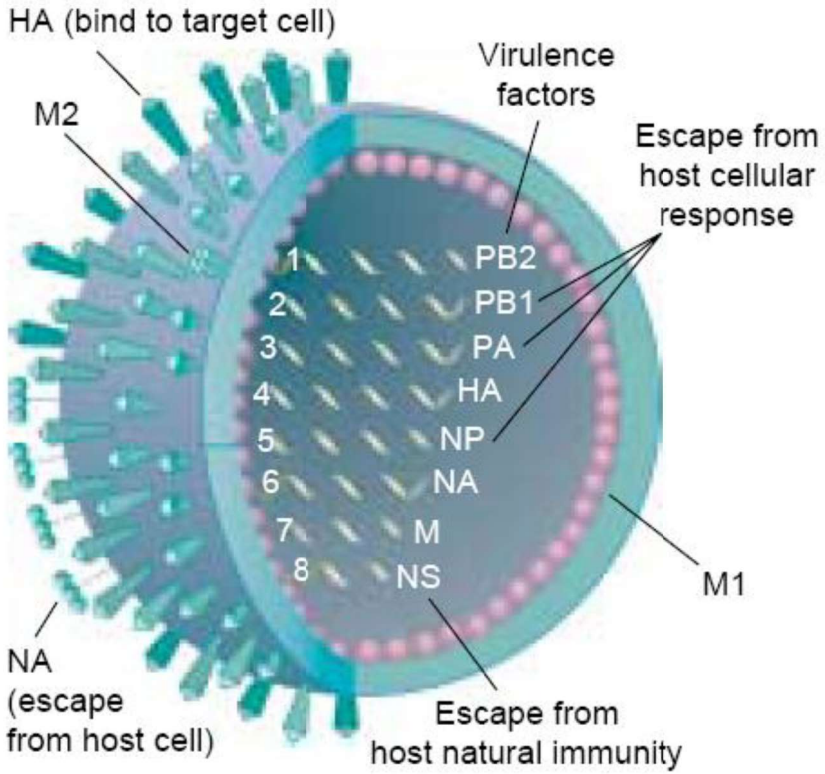


Estimating *N*, *u*, and δ from flu data is work in progress

What are some evolutionary constraints to influenza adaptation?

1. **Transmission bottlenecks between donors and recipients**
   - ✓ Influenza transmission bottleneck size

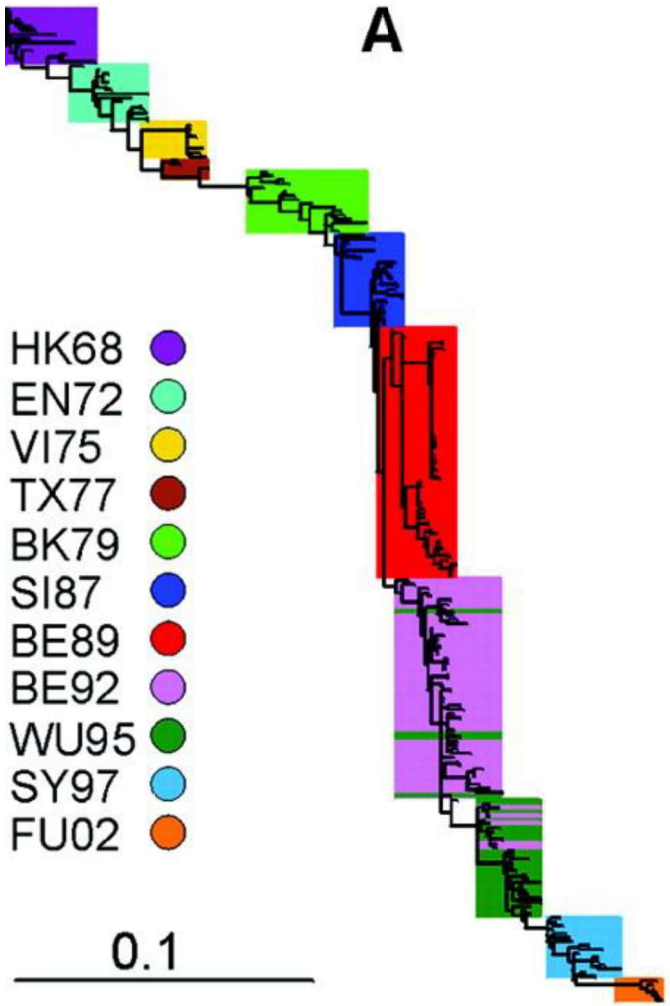
2. **Genetic linkage**
   - ↳ Deleterious mutations shaping influenza's antigenic evolution



Influenza A/H3N2:

- Present in humans since 1968
- Segmented RNA virus (8 segments)
- Hemagglutinin HA = 'H' of H3N2
- Importance of HA for antigenic evolution

Cluster transitions precipitated by very few amino acid changes



Smith et al. (2004) *Science*

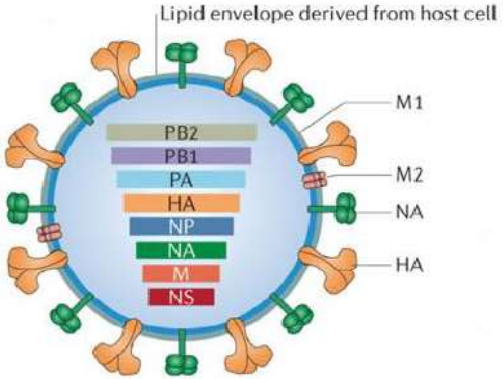
Occur every 2-8 years

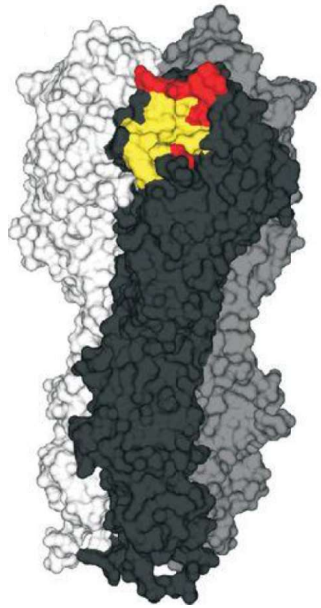
Cluster transitions caused by:

- a single amino acid change (7 out of 10 instances)
- two amino acid changes (2 out of 10 instances)
- three amino acid changes (1 out of 10 instances)

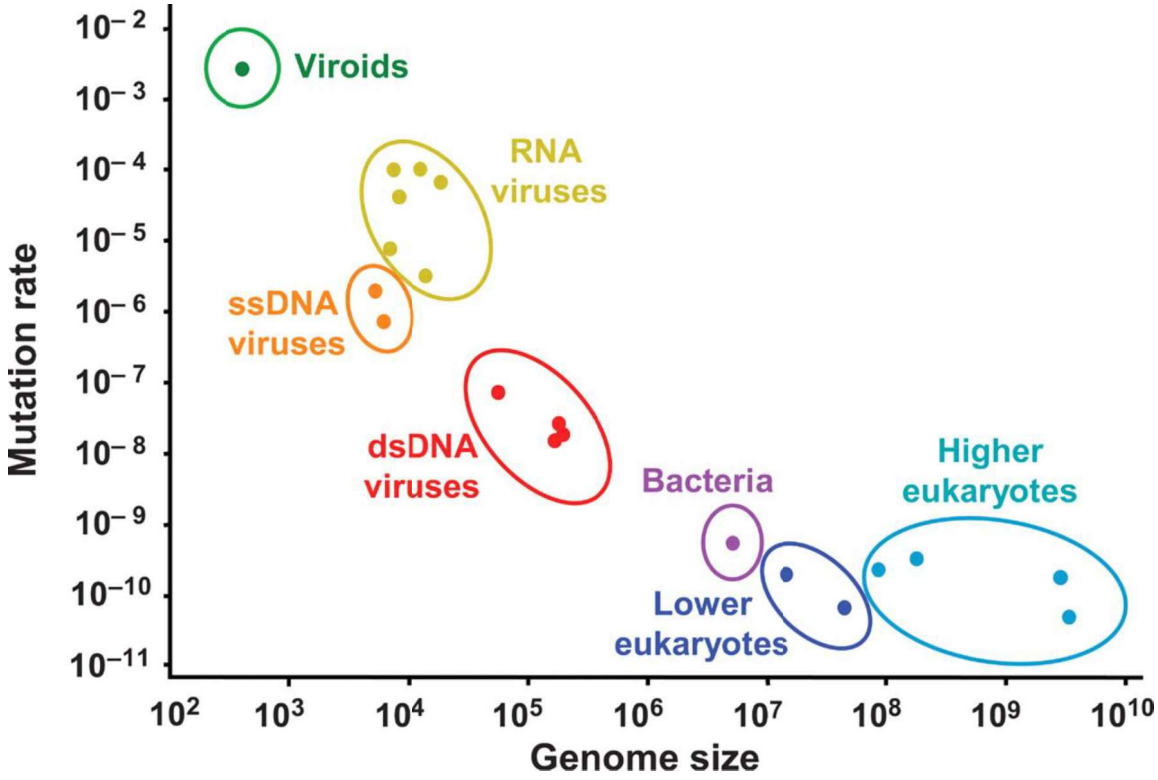
Koel et al. (2013) *Science*

(no, I didn't misspell my last name – this is not me)





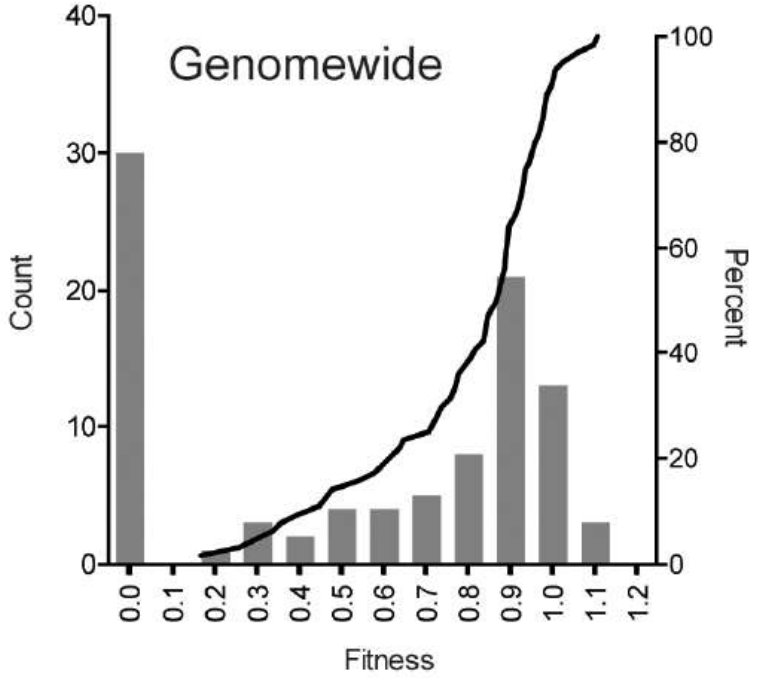
Unsolved mysteries of antigenic evolution



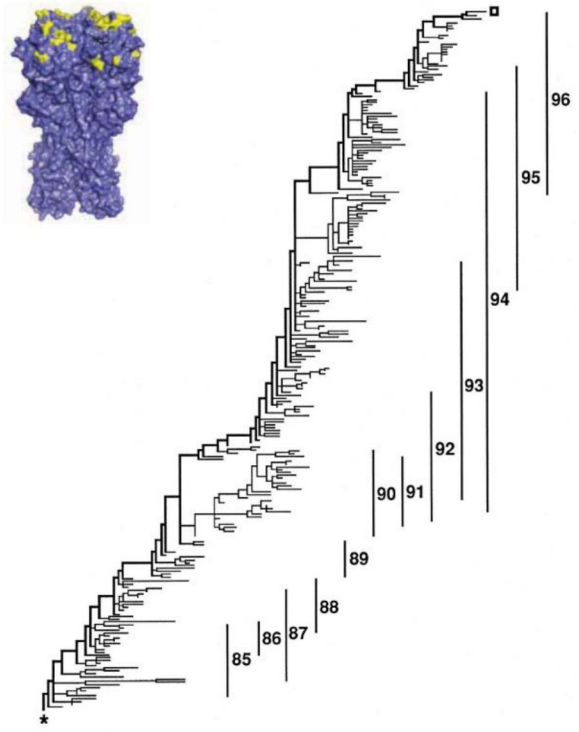
Gago et al. (2009) *Science*

- Why don't cluster transitions happen more quickly?
- Why don't we see explosive antigenic diversification?
- Why is antigenic evolution so punctuated?

Deleterious mutations?



Visher et al. (2016) *PLoS Pathogens*



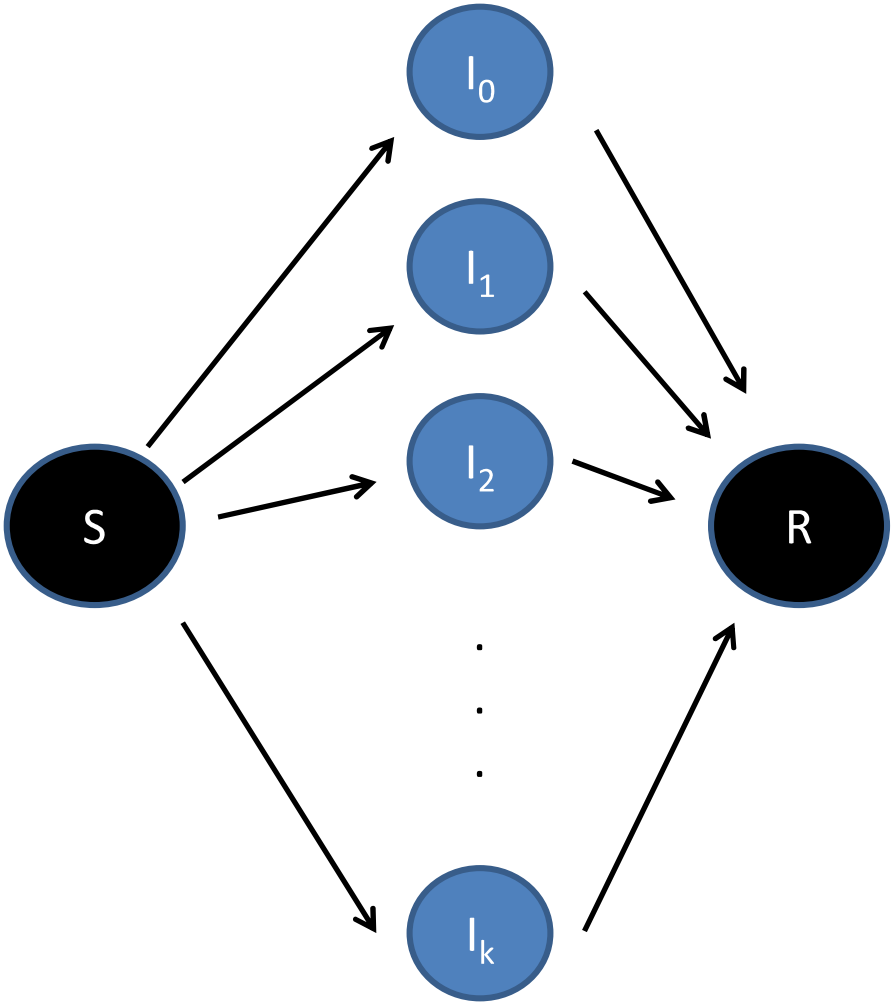
Fitch et al. (1997) *PNAS*

Phylogenetic Evidence for Deleterious Mutation Load in RNA Viruses and Its Contribution to Viral Evolution

Pybus et al. (2007) *MBE*

Load also present in other influenza gene segments

Simple model: Viral population subject to only deleterious mutations

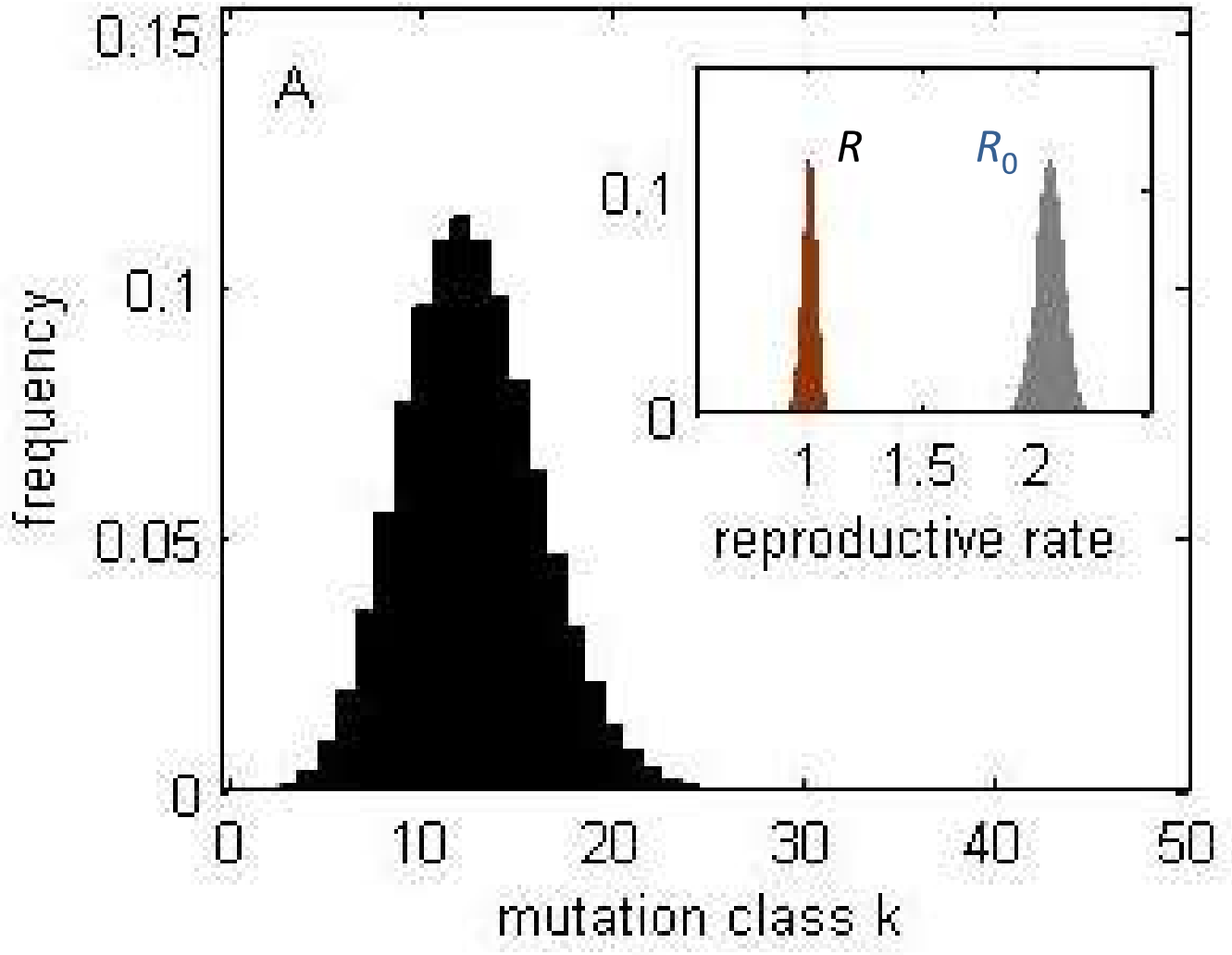


λ = per-genome per-transmission deleterious mutation rate

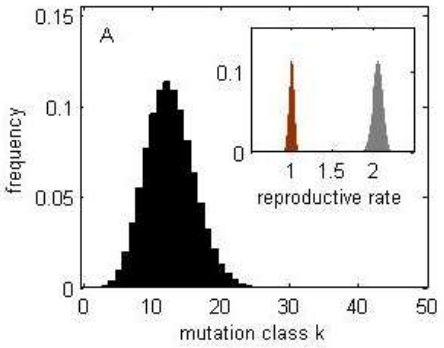
s_d = fitness effect of deleterious mutations

$$\beta_i = \beta_0(1 - s_d)^k$$

Deleterious mutation-selection balance



Fates of antigenic mutants

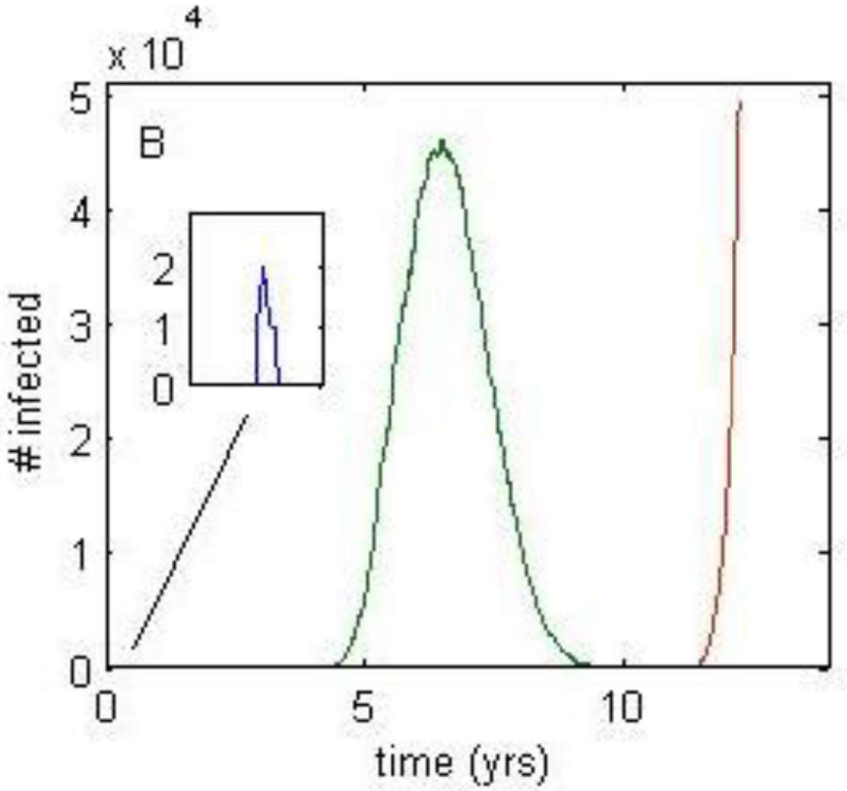


Antigenic mutations occur in a certain genetic background with *i* deleterious mutations

Antigenic mutations are of a certain size σ, which quantifies the degree of immune escape

Calculate reproductive rates R of invading mutant strain: $R_m(t = 0)$ and $R_m(t = \infty)$

THREE POSSIBLE FATES
(based on Peck (1994) *Genetics*)

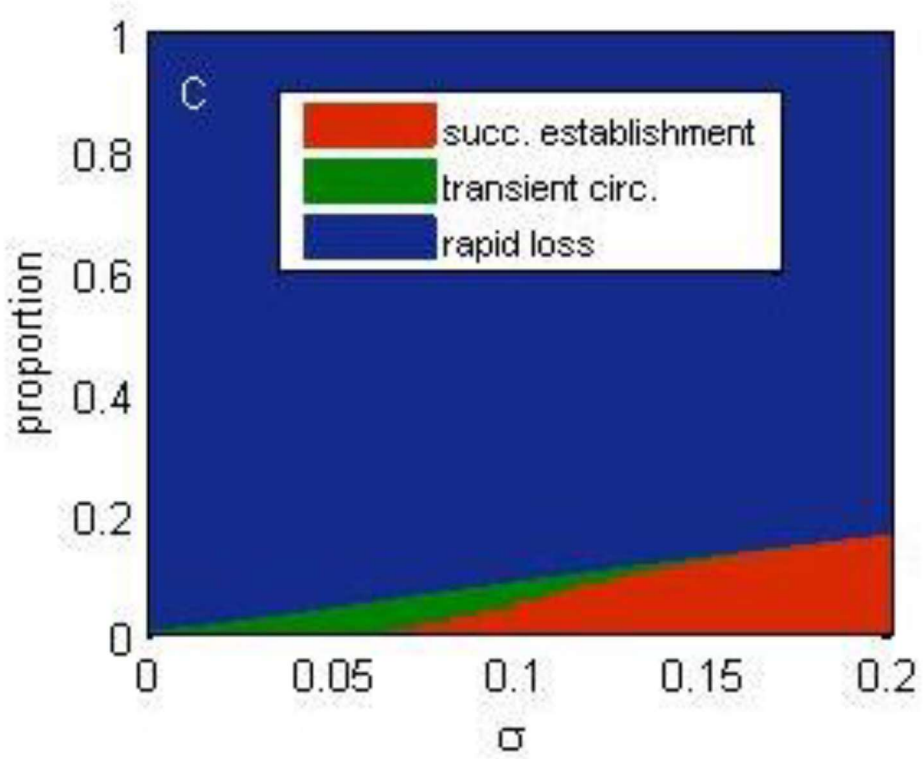


Probabilities of fates

Which fate occurs depends on:

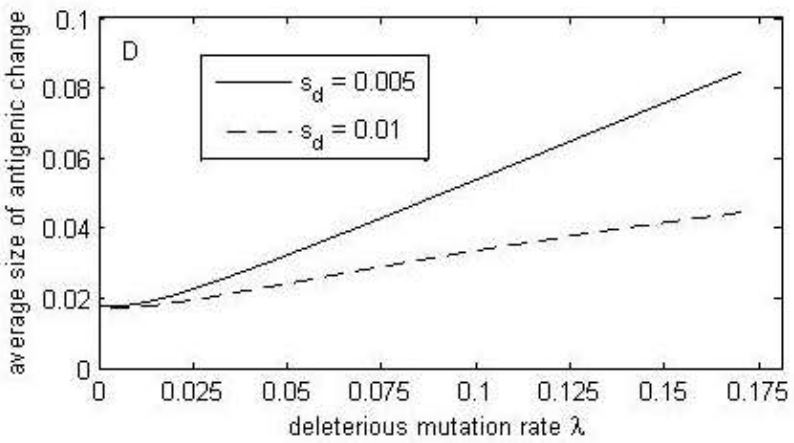
- the antigenic mutant's # of deleterious mutations:
  lower # deleterious mutations → higher reproductive rates

- the antigenic mutant's degree of immune escape σ:
  higher σ → higher reproductive rates

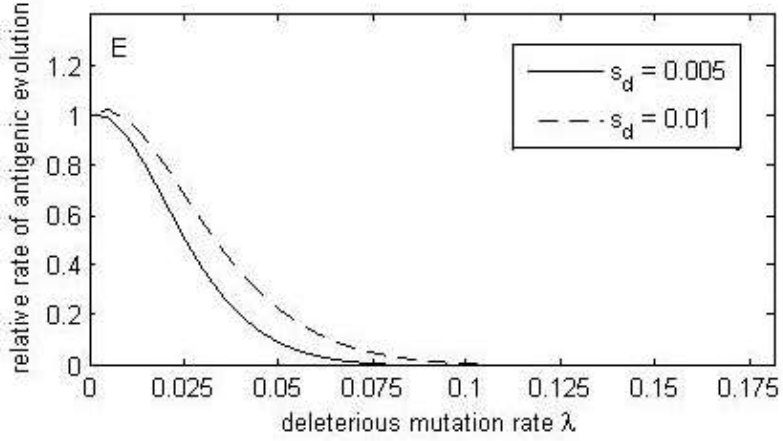


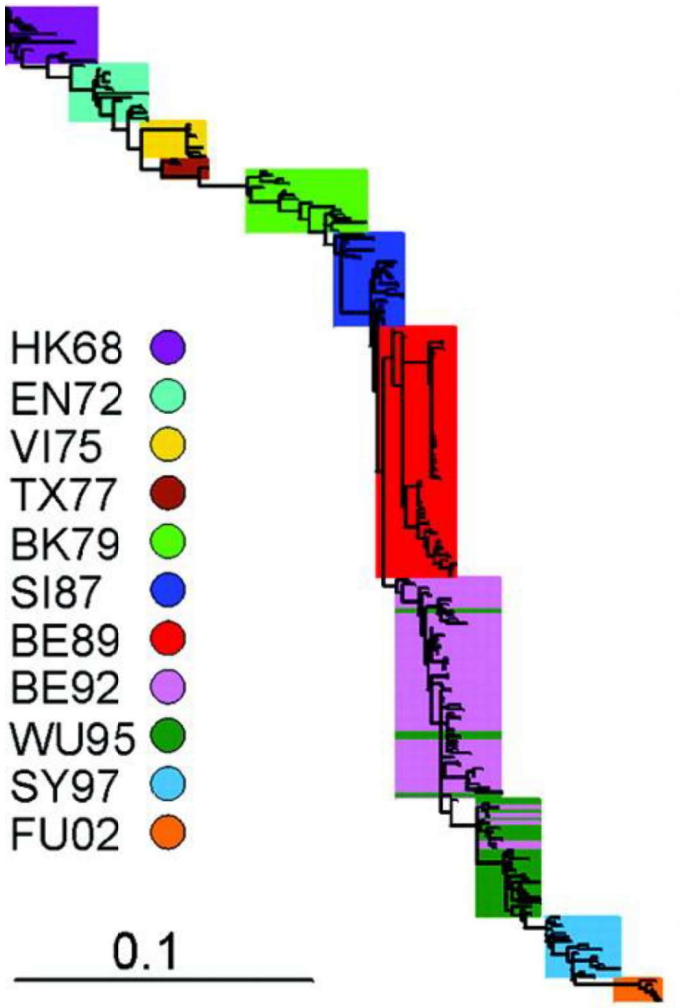
Effect on influenza evolution

Expectation of "punctuated" antigenic evolution…



occurring rarely.





Smith et al. (2004) *Science*

consistent with Barton (1995) *Genetics*

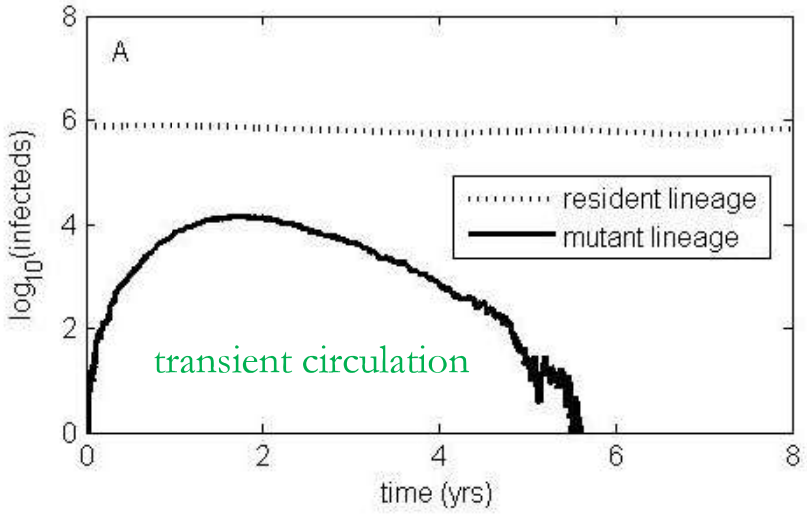
Simple model with explicit epidemiological dynamics

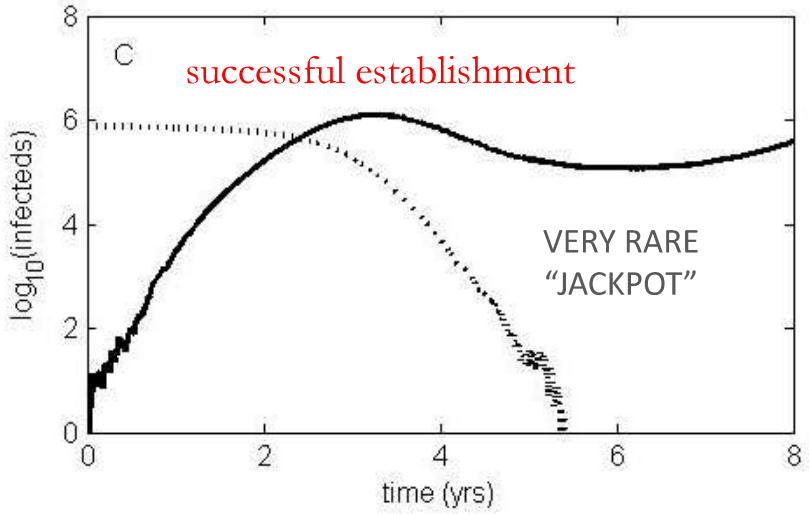
low immune escape σ

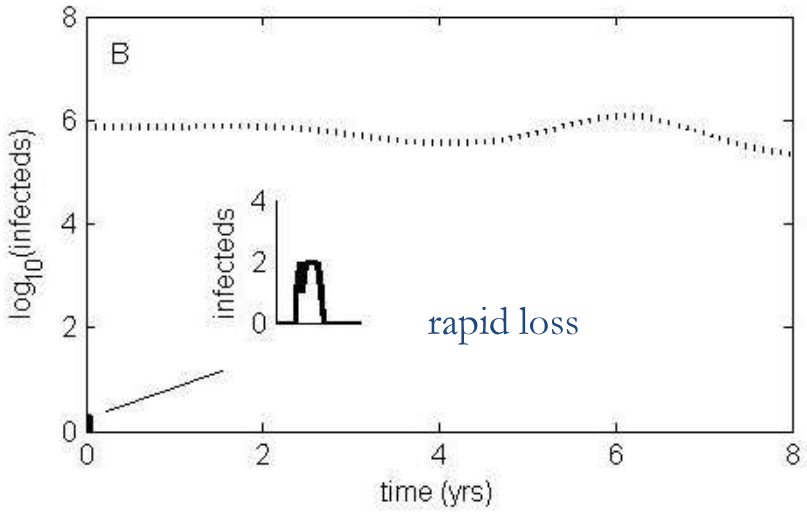
high immune escape σ

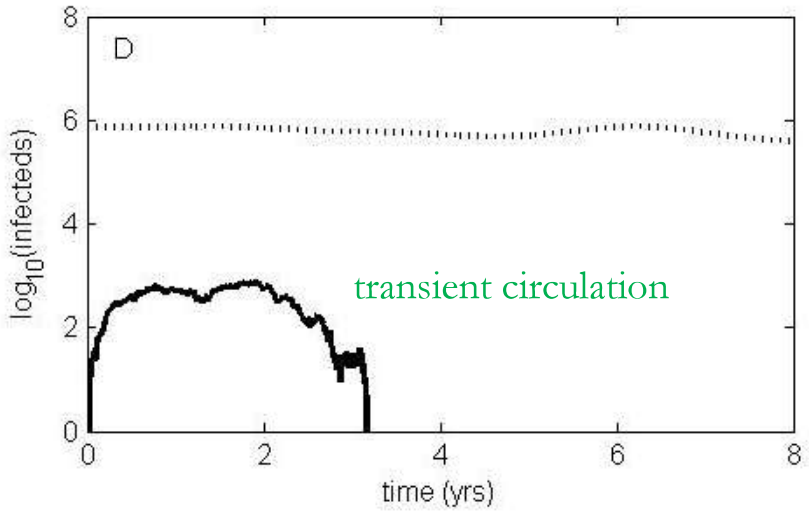
low # del. mut.

Avg # del. mut.

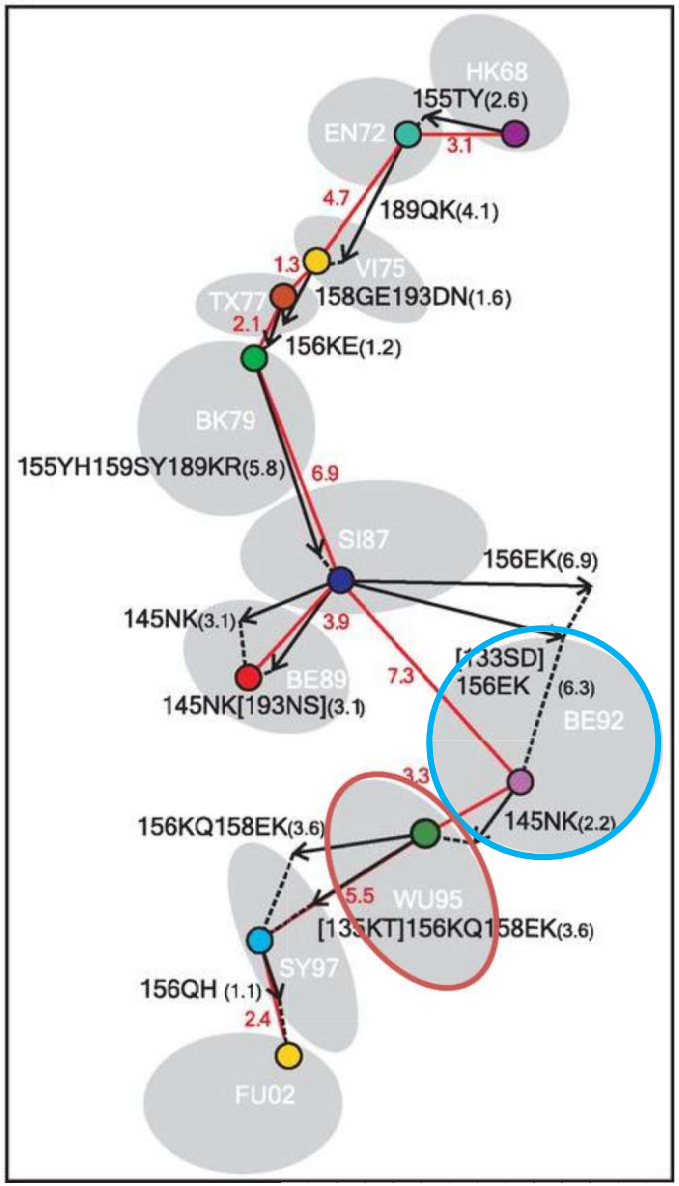




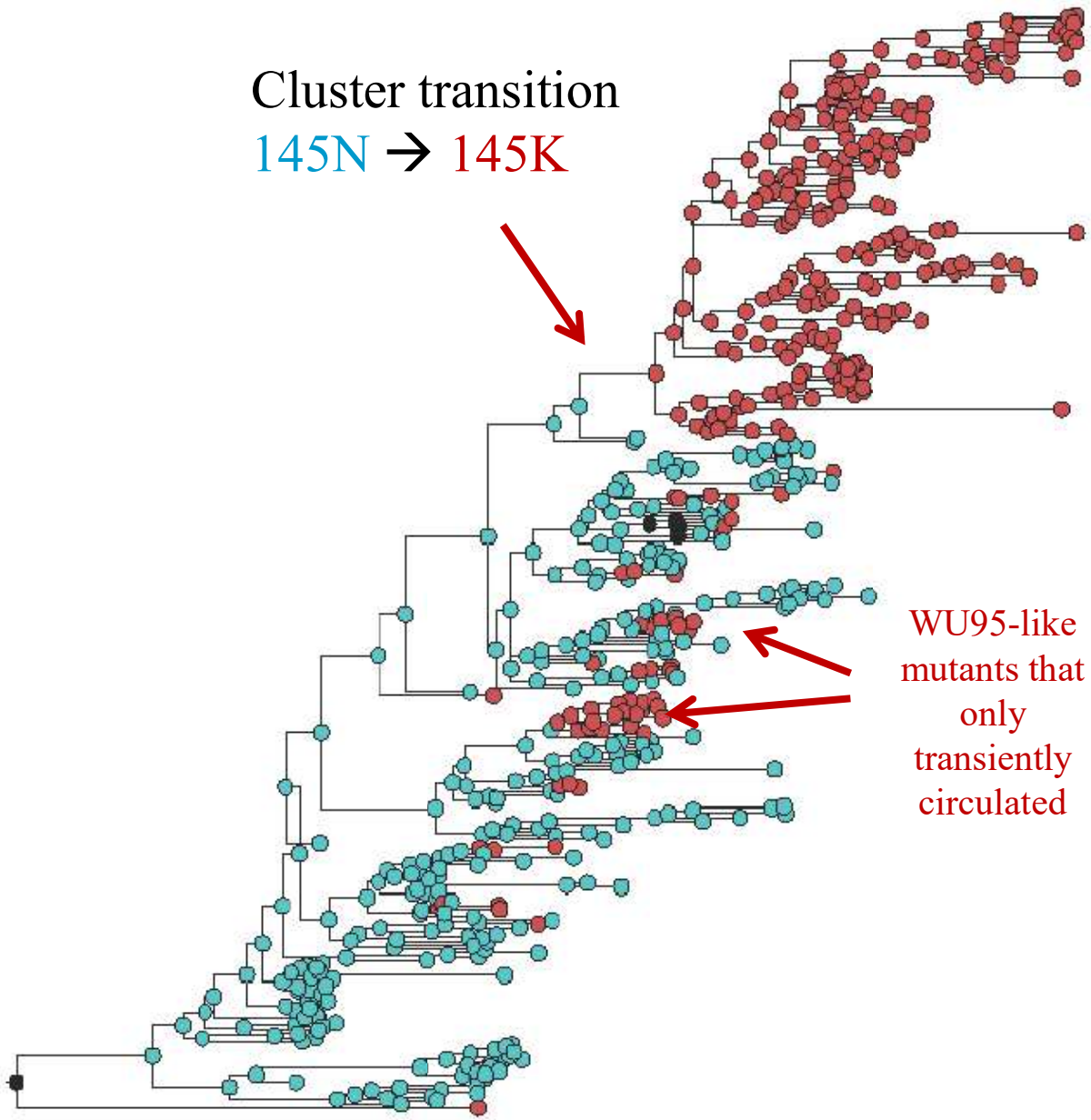




Cluster transition consistent with occurrence of the 'jackpot' strategy



Koel et al. (2013) *Science*

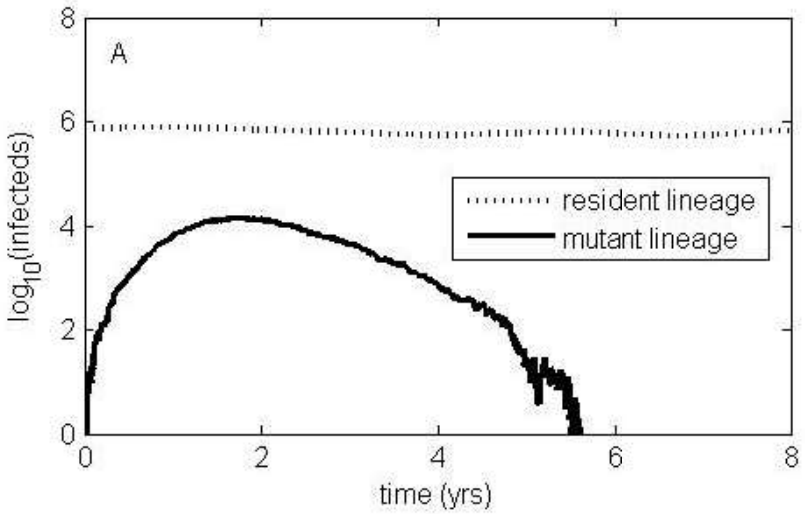


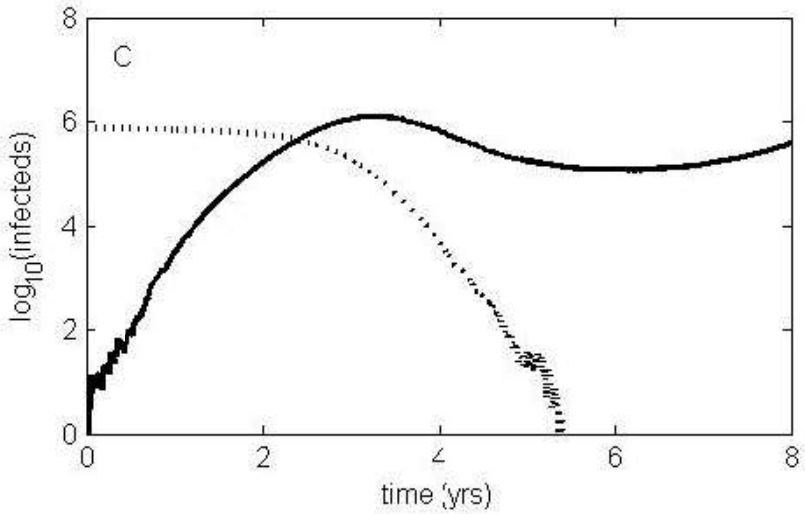
Alternative strategies for hitting the jackpot

low σ

high σ

low # del. mut.



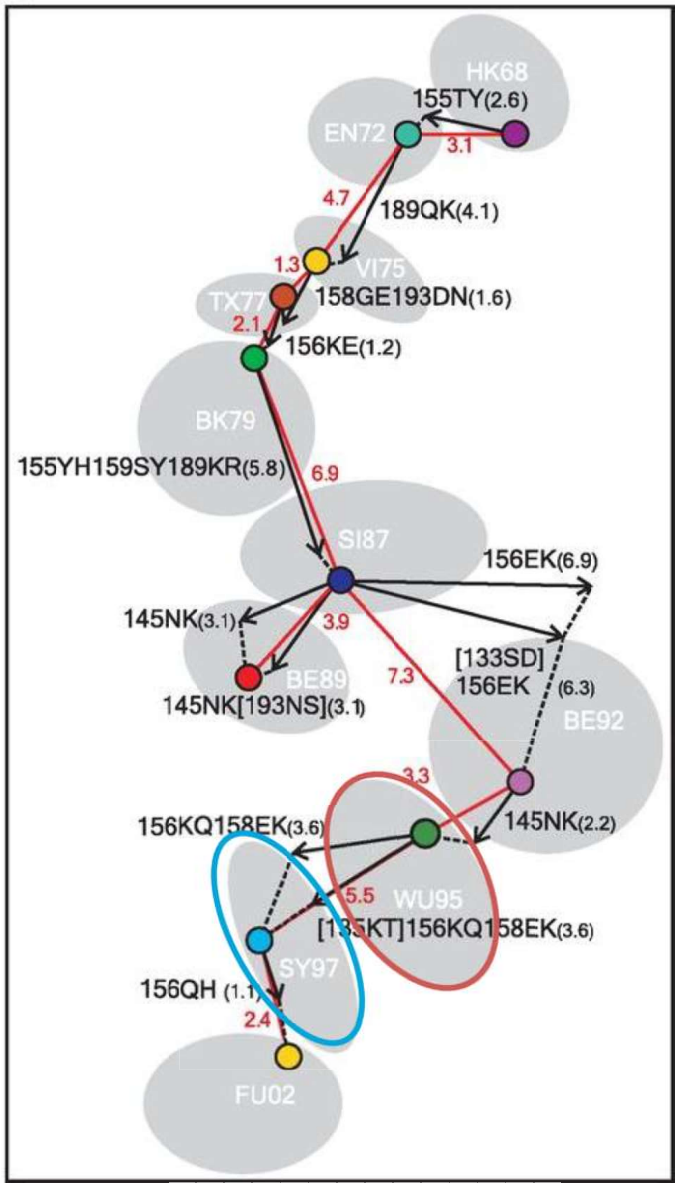


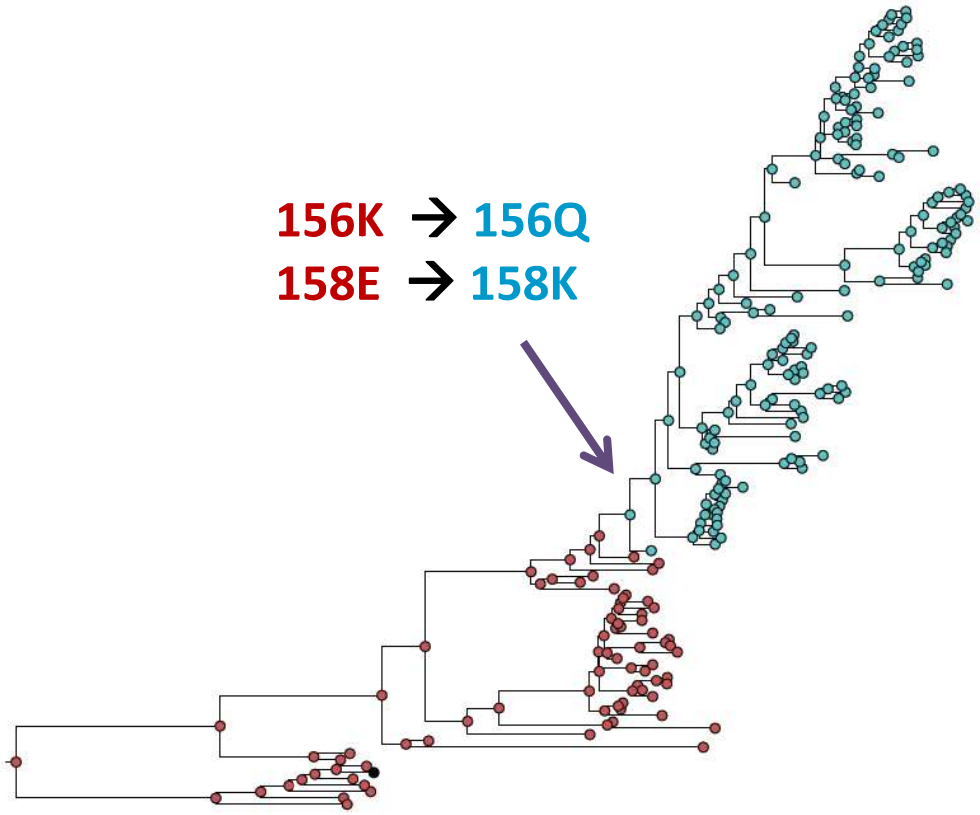
Step 1: More likely small- to moderate-sized mutation occurs first in 'good' background, transiently rises, thereby "inflating" low-*i* viral counts

Step 2: Less likely large antigenic mutation occurs shortly thereafter, resulting in an antigenic cluster transition

Will effectively appear as two simultaneous antigenic amino acid changes in a viral phylogeny

Cluster transition consistent with occurrence of this two-step antigenic change strategy





Koel et al. (2013) *Science*

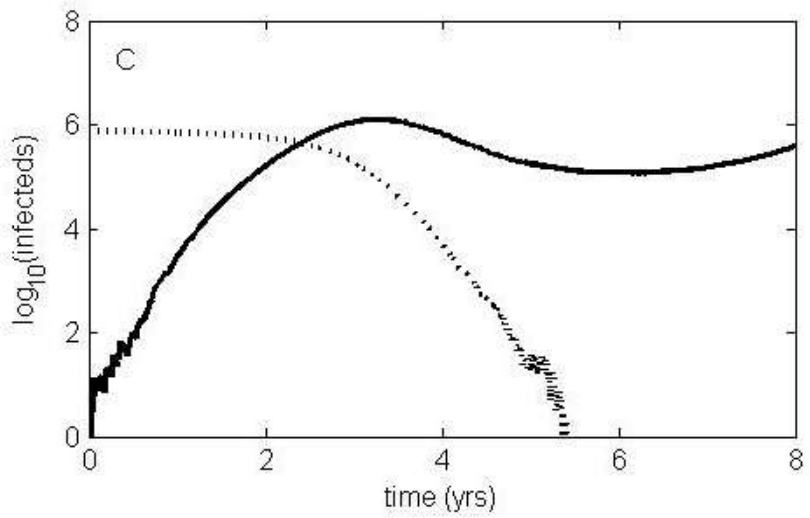
Alternative strategies for hitting the jackpot

Step 1: Large antigenic mutation occurs in average genetic background, and transiently rises.

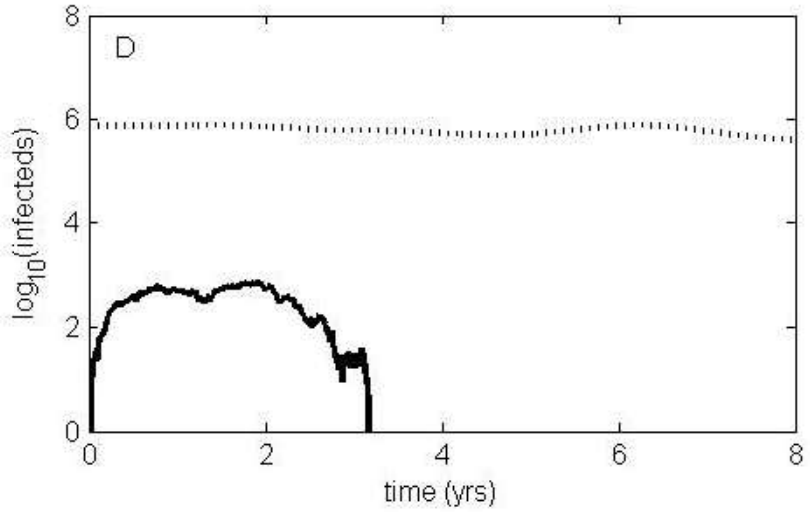
Step 2: Co-infection occurs (with resident strain, also likely with average genetic background). Reassortment leads to purging of deleterious mutations, and therewith an antigenic cluster transition.

high σ

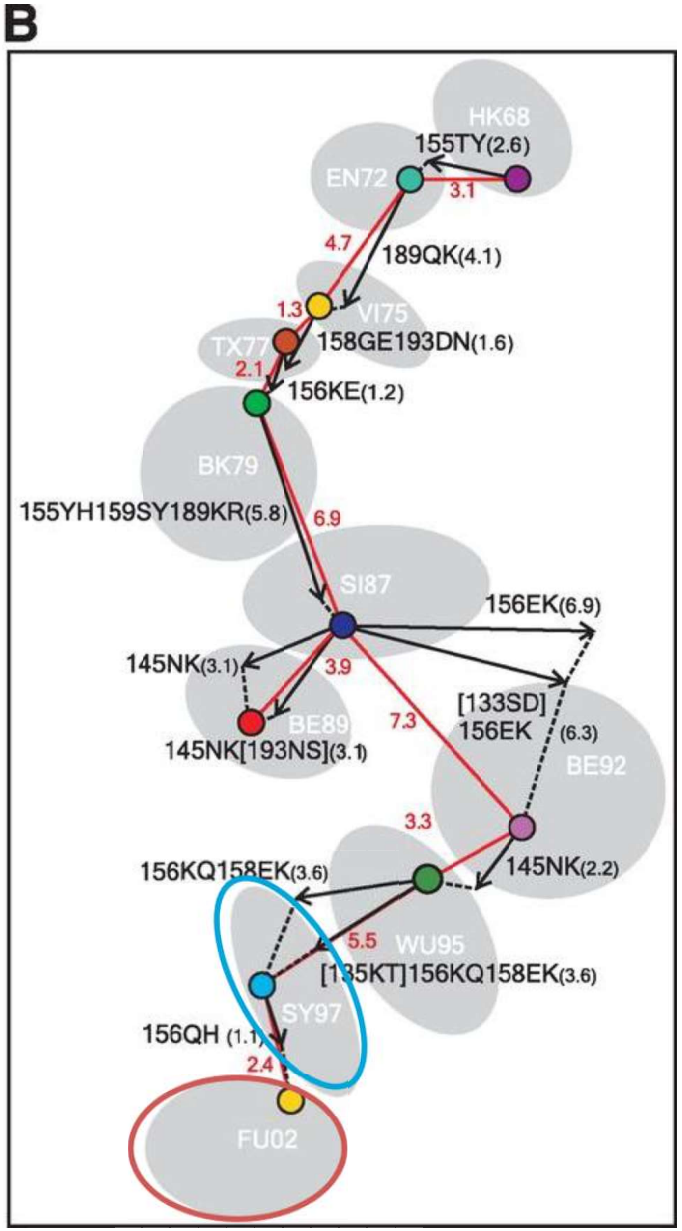
Low #. del mut.

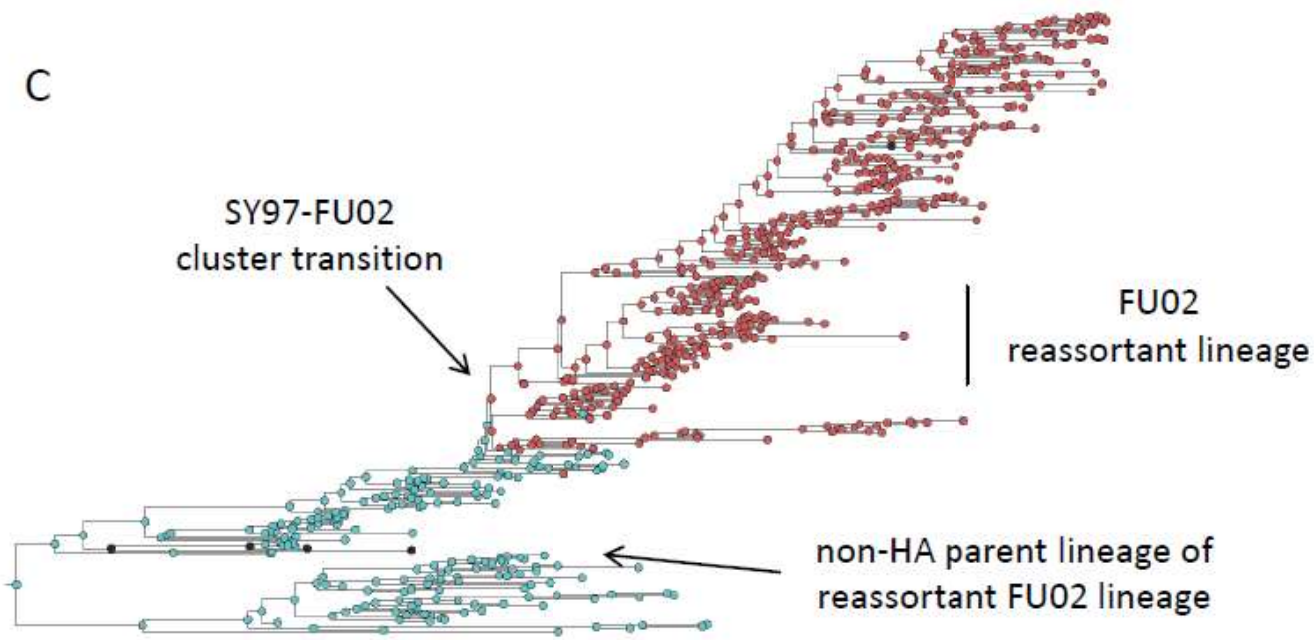


Avg # del. mut.



Cluster transition consistent with occurrence of this two-step reassortment strategy





Koel et al. (2013) *Science*

Epidemiological model

- Hosts have infection histories tracked

- Antigenic mutations (on HA)
- Antigenicity (+ host history of infection) determine susceptibility to infection

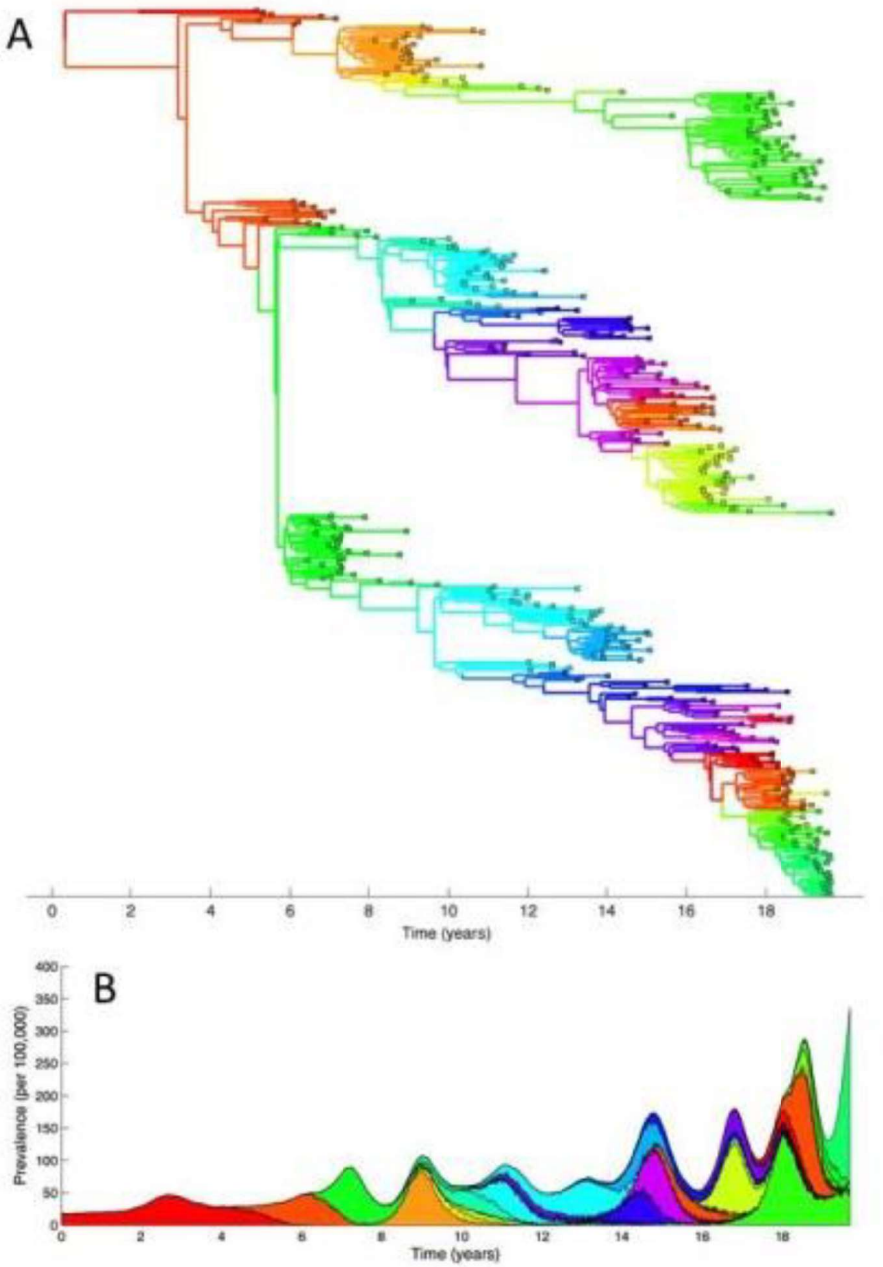
- Deleterious mutations (on all gene segments) – with constant fitness cost
- Deleterious mutations lower transmission rate

Population genetic theory predicts that deleterious mutations will act to slow the rate of adaptive evolution and to make it more punctuated

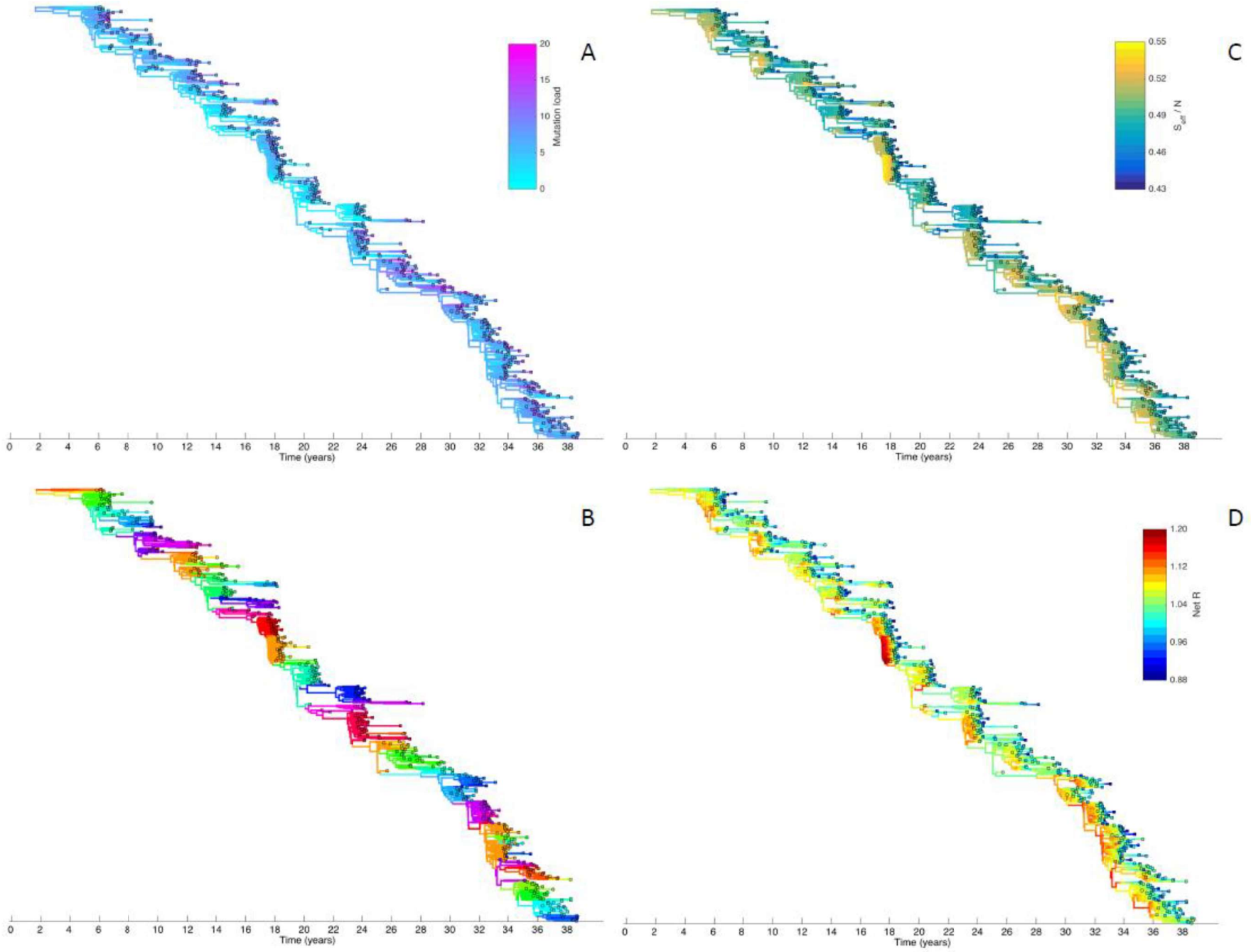
Full ‘phylodynamic’ simulations

No load simulation
(10 yrs)

Explosive genetic &
antigenic diversity



Full ‘phylodynamic’ simulations



What are some evolutionary constraints to influenza adaptation?

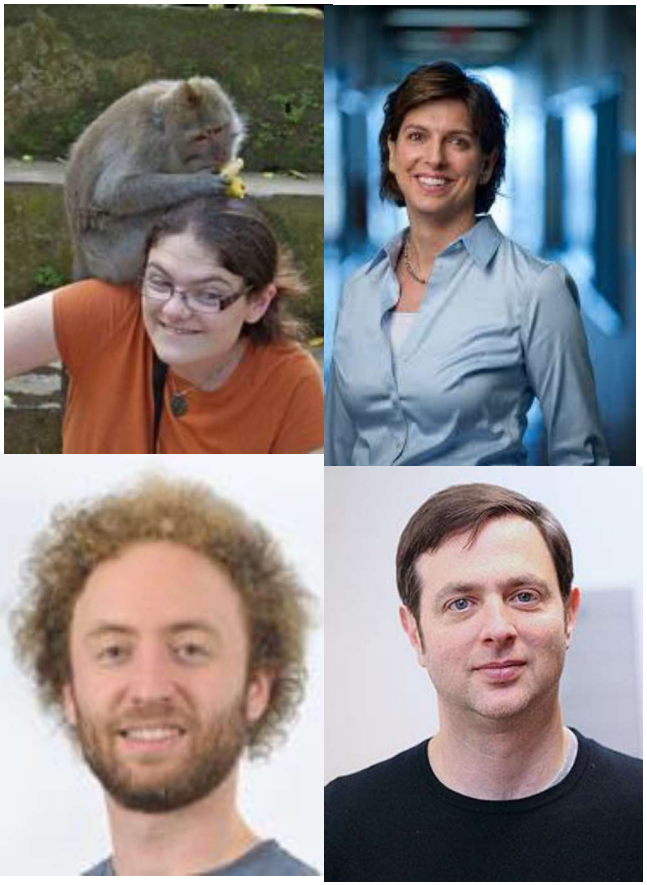
1. **Transmission bottlenecks between donors and recipients**
   - ✓ Influenza transmission bottleneck size

2. **Genetic linkage**
   - ✓ Deleterious mutations shaping influenza's antigenic evolution

These constraints are likely related… small bottleneck sizes allows for deleterious mutations to fix in individuals

Acknowledgements

DELETERIOUS MUTATIONS
Koelle & Rasmussen
(2015) *eLife*



TRANSMISSION BOTTLENECK – shared variants
Sobel Leonard et al. (2017) *Journal of Virology*

TRANSMISSION BOTTLENECK - *de novo* variants
Harris and Koelle (in prep.)

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