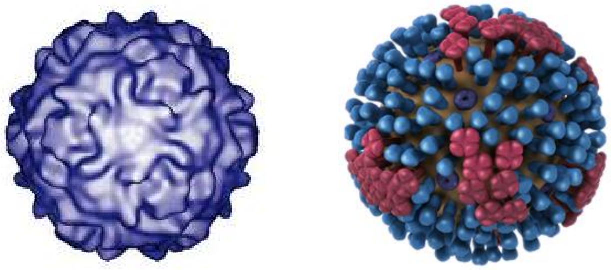
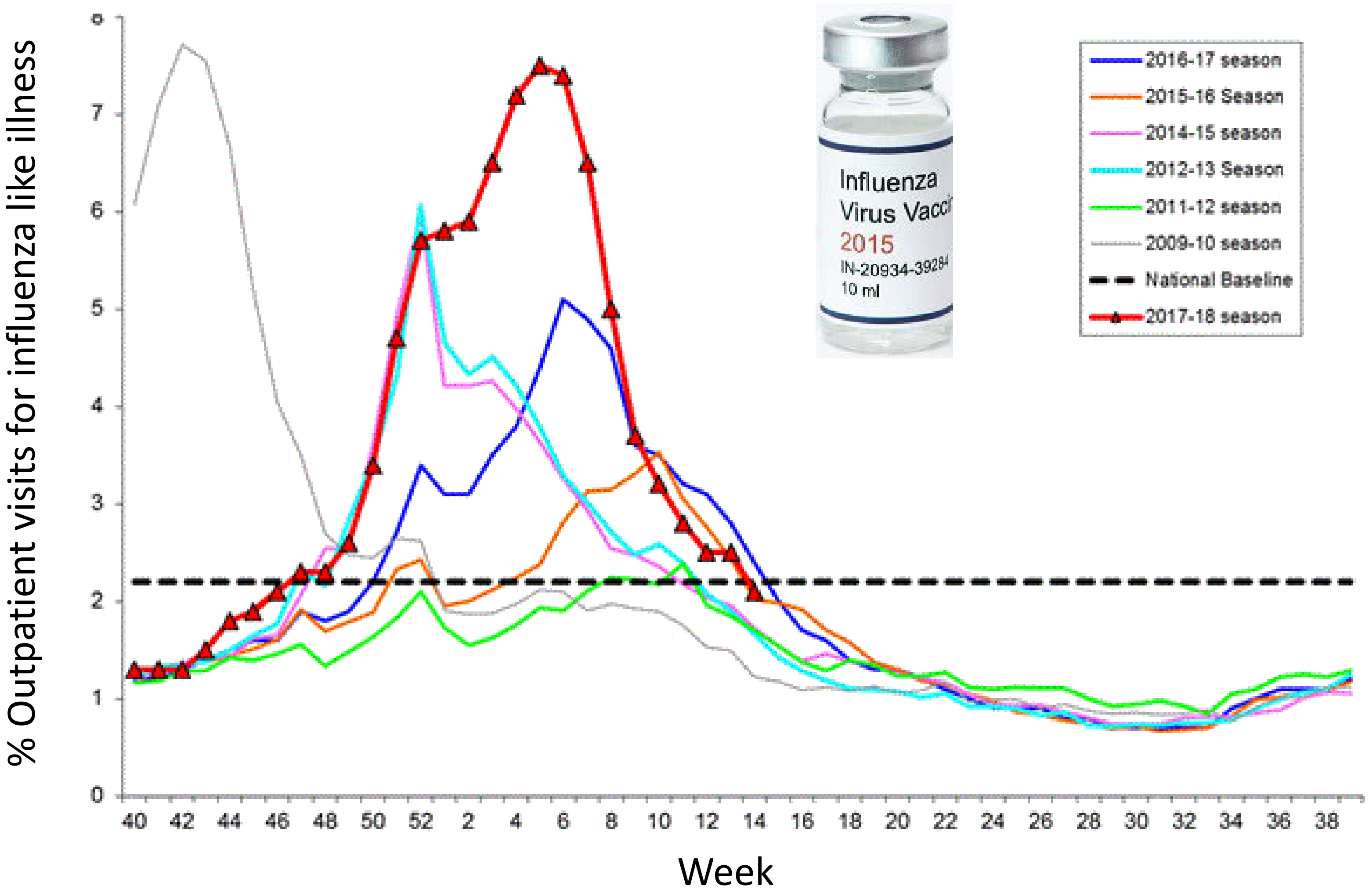
Influenza virus evolution…getting personal

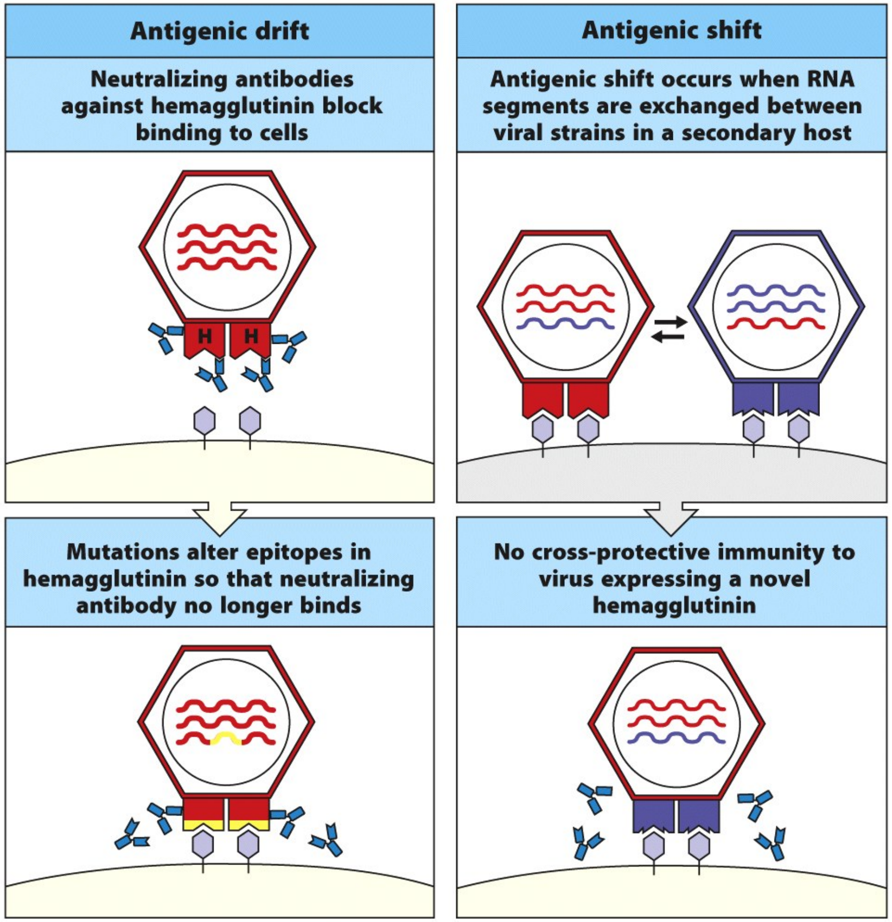


Adam Lauring
Department of Medicine, Infectious Diseases
Department of Microbiology and Immunology
University of Michigan

Why influenza evolution?



Engines of genetic diversity



Antigenic drift

Neutralizing antibodies against hemagglutinin block binding to cells

Mutations alter epitopes in hemagglutinin so that neutralizing antibody no longer binds

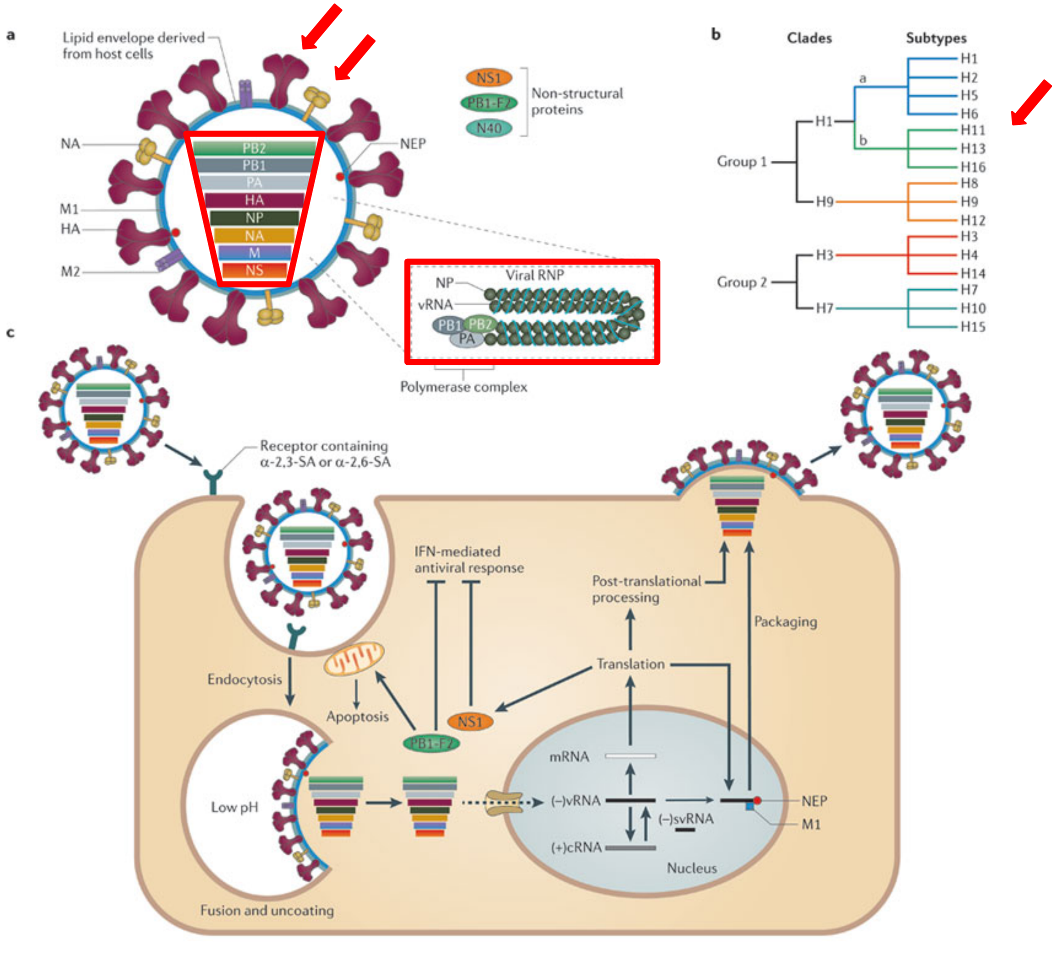
Antigenic shift

Antigenic shift occurs when RNA segments are exchanged between viral strains in a secondary host

No cross-protective immunity to virus expressing a novel hemagglutinin

Figure 13.2 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

Influenza A virus, what you need to know



Nature Reviews | Microbiology

Medina and Garcia-Sastre, Nat Rev Micro 2011

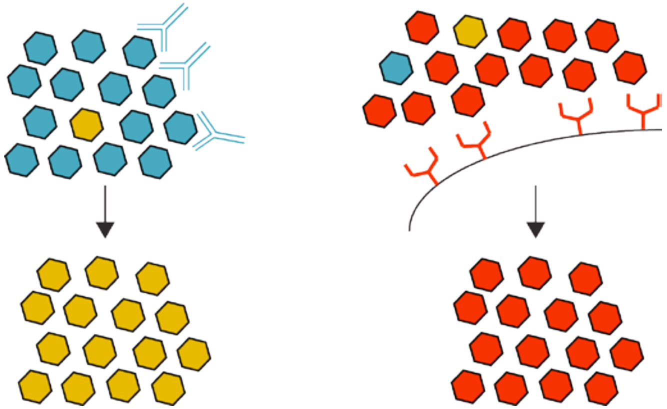
A review of some population genetics

Mutation

A review of some population genetics

Mutation

Selection



A review of some population genetics

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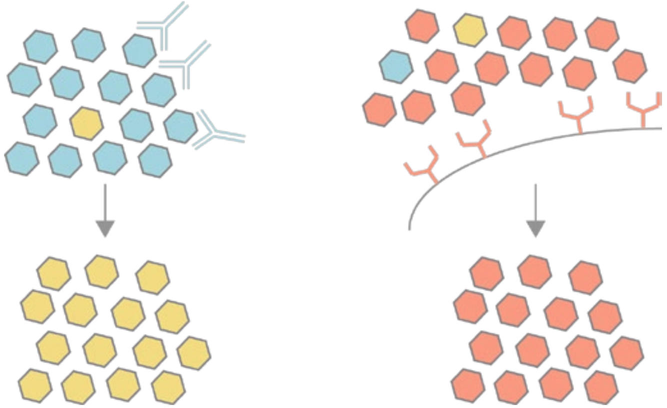
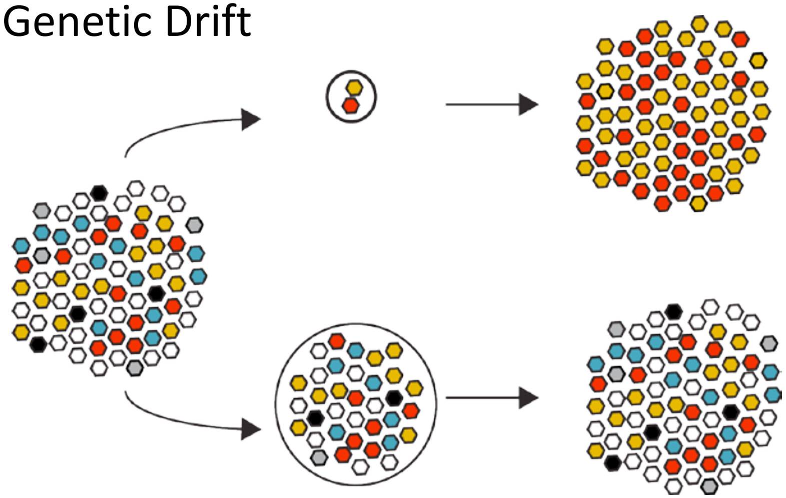


Genetic Drift



A review of some population genetics

Mutation

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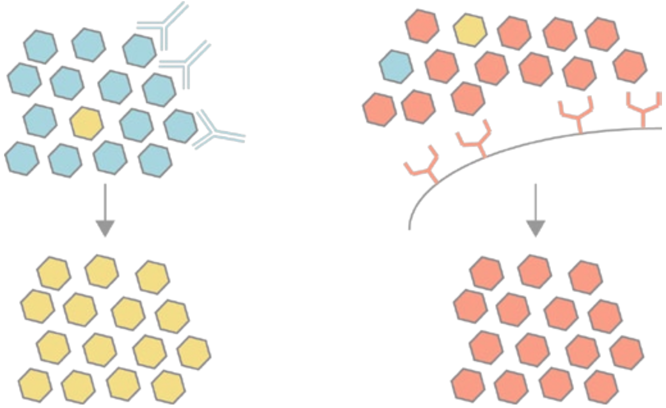
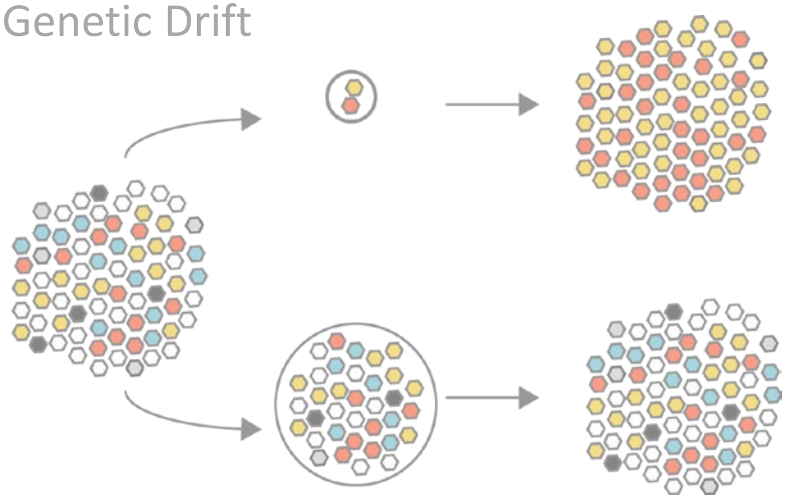
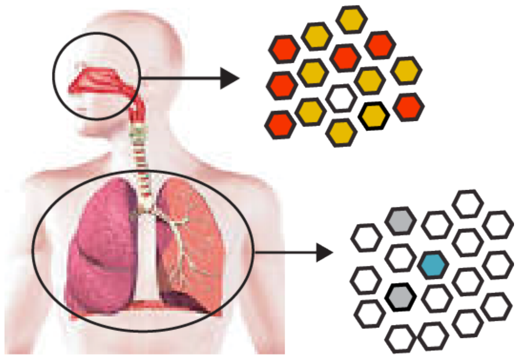


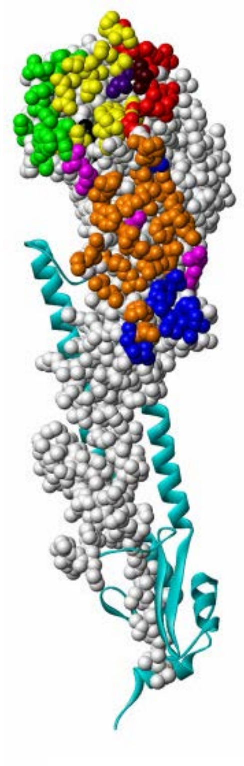
Genetic Drift

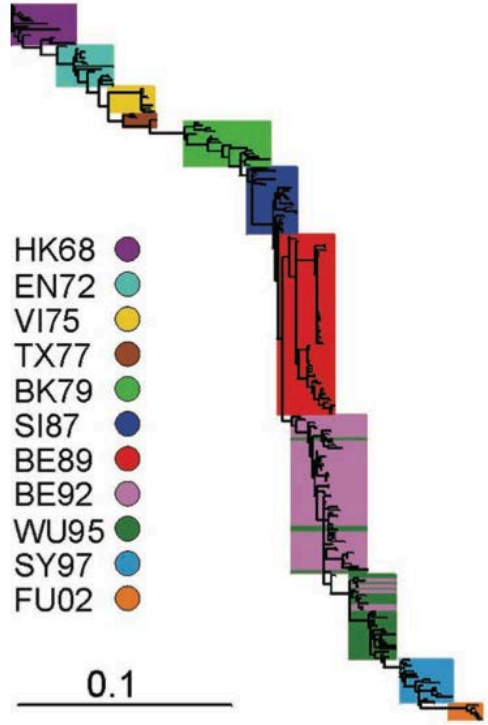


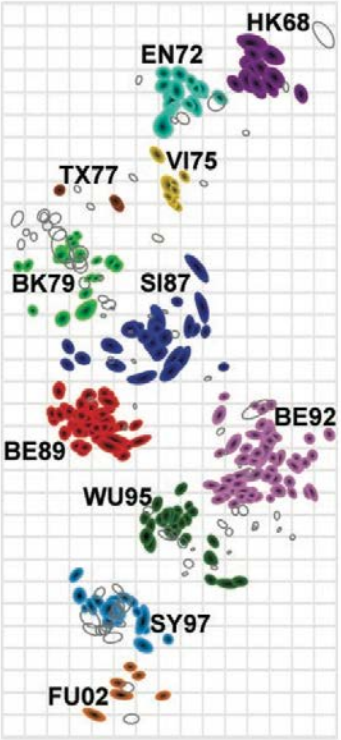
Migration



Antigenic drift and seasonal influenza

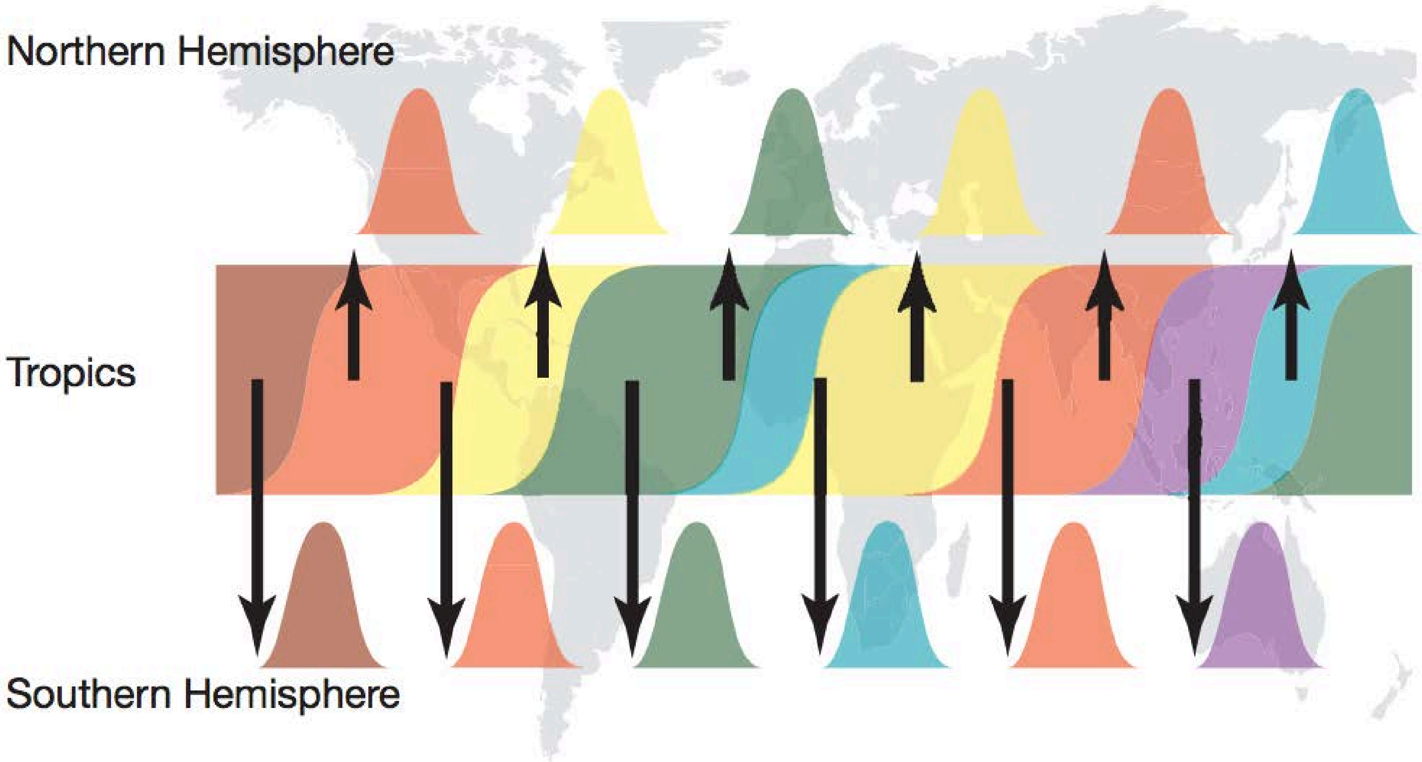






Smith et al. Science 2004

Global evolution of seasonal influenza viruses



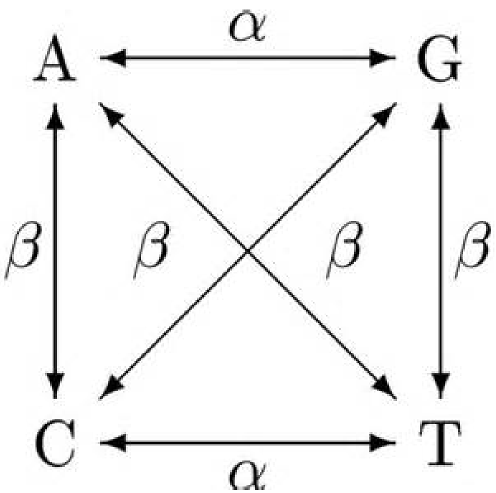
Rambaut et al. Nature 2008

Understanding evolution across scales

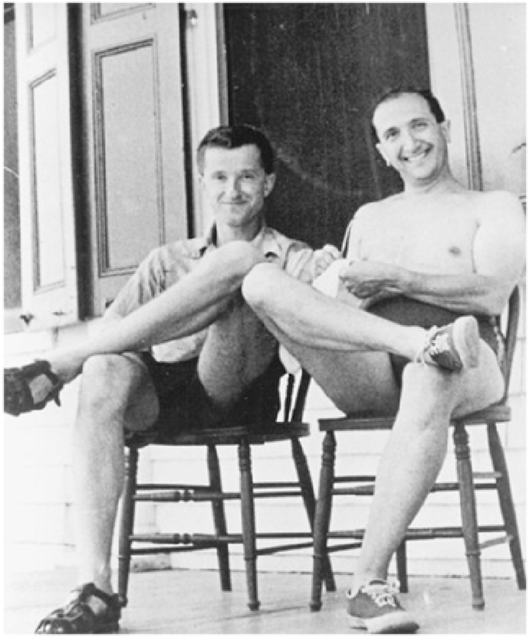
What is the influenza virus mutation rate?

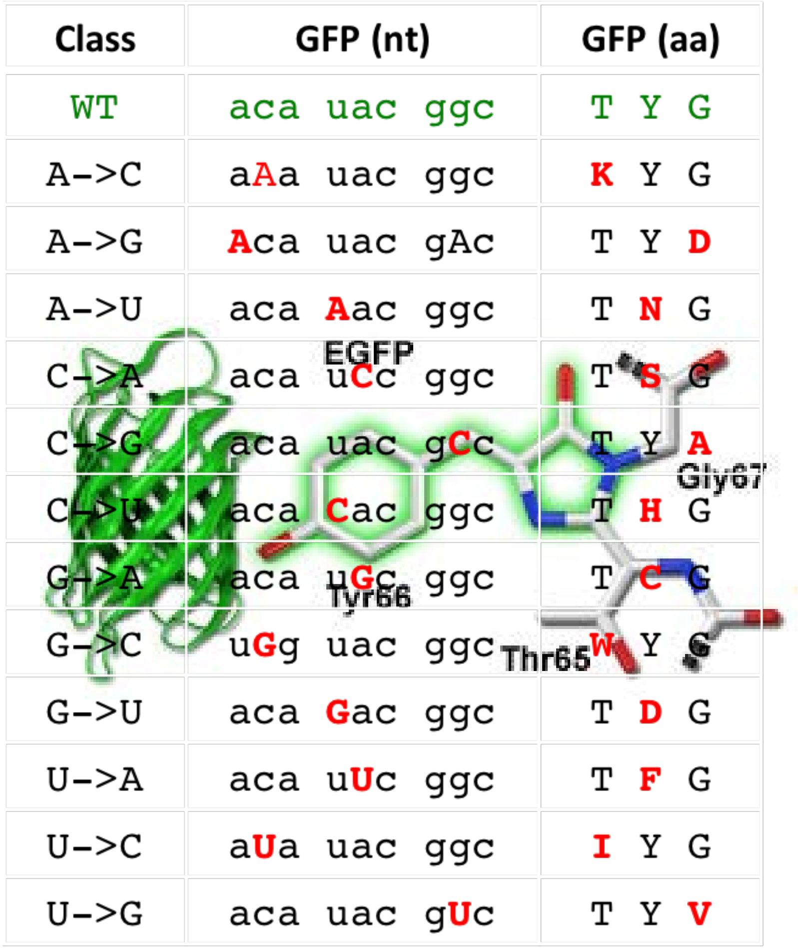
- Sequencing assays
  - Bias
  - Lack of power

- Genetic markers
  - Only measure some classes



What is the influenza virus mutation rate?

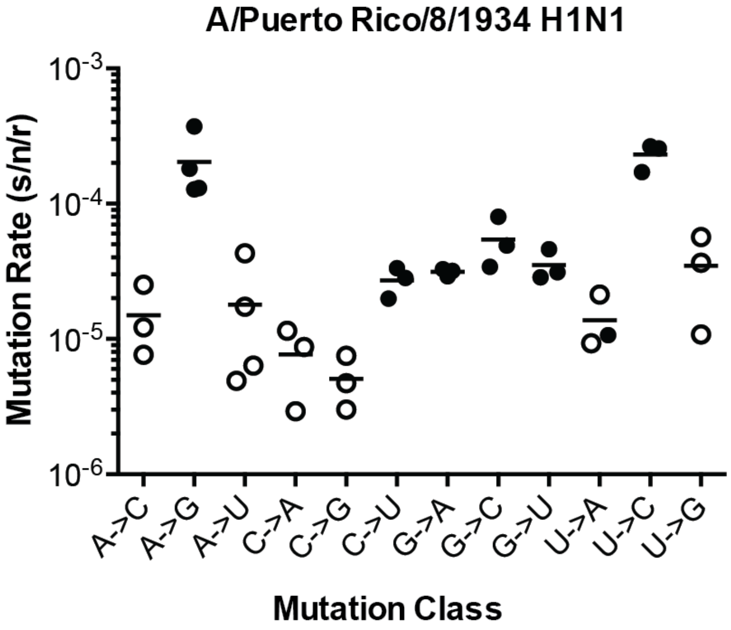




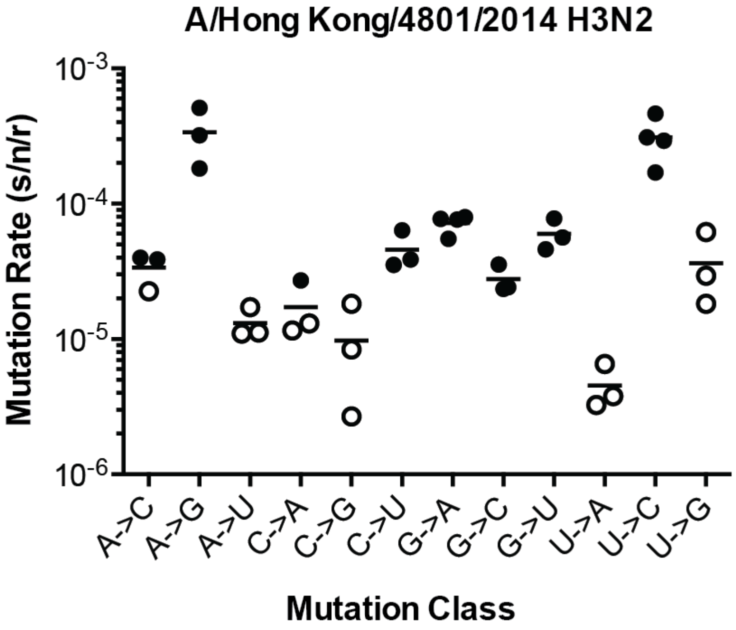
Class	GFP (nt)	GFP (aa)
WT	aca uac ggc	T Y G
A->C	aAa uac ggc	K Y G
A->G	Aca uac gAc	T Y D
A->U	aca Aac ggc	T N G
C->A	aca uCc ggc	T S G
C->G	aca uac gCc	T Y A
C->U	aca Cac ggc	T H G
G->A	aca uGc ggc	T C G
G->C	uGg uac ggc	W Y G
G->U	aca Gac ggc	T D G
U->A	aca uUc ggc	T F G
U->C	aUa uac ggc	I Y G
U->G	aca uac gUc	T Y V

Pauly et al. eLife 2017

Influenza mutation rate…2-3 per replicated genome


A/Puerto Rico/8/1934 H1N1

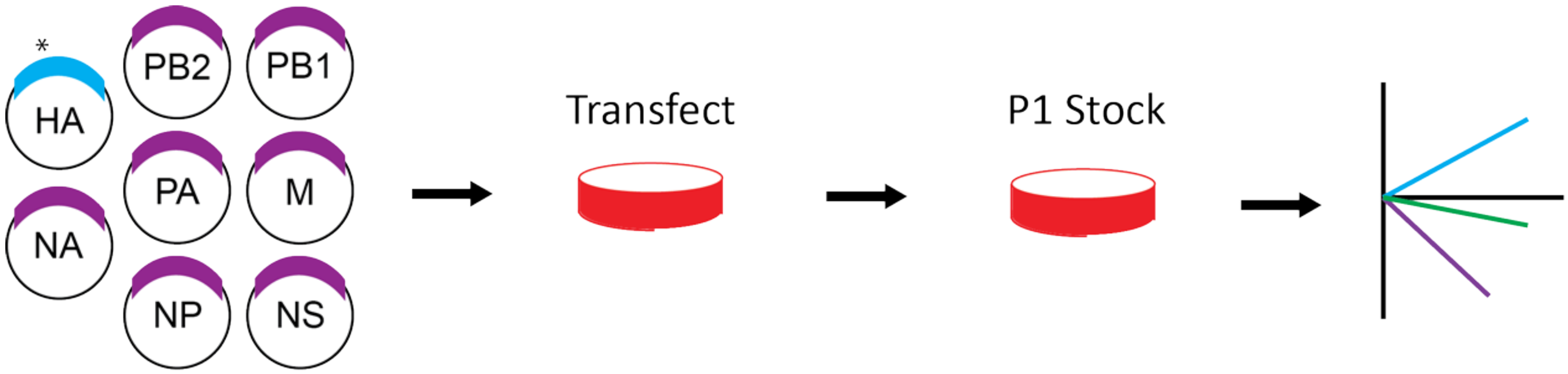
1.8 x 10^{-4}


A/Hong Kong/4801/2014 H3N2

2.5 x 10^{-4}

Pauly et al. eLife 2017

Are mutations generally good or bad?



Transfect

P1 Stock

128 plasmid clones

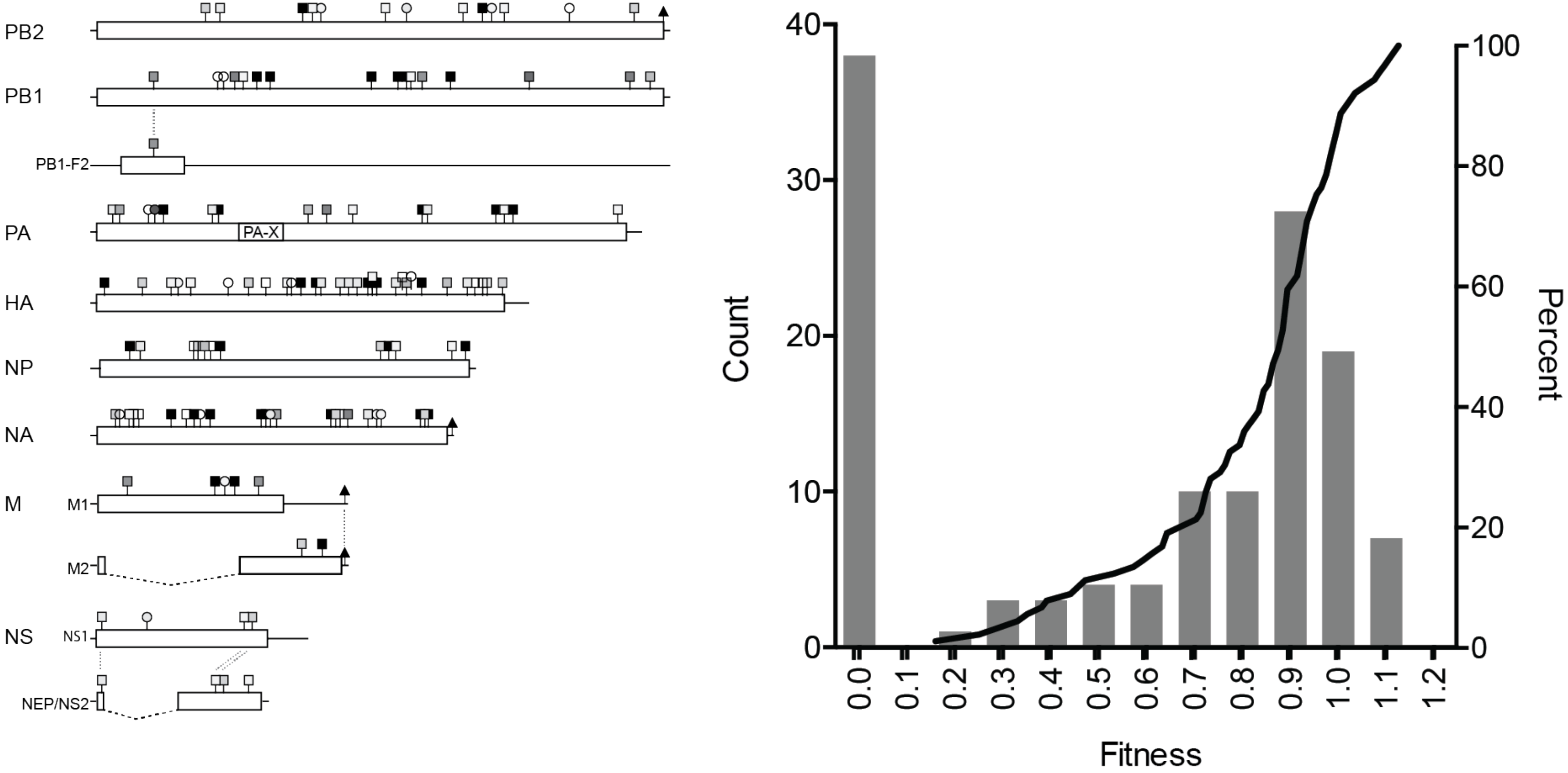
3x failure = lethal

NGS sequence

Competition qPCR

Visher et al. PLoS Pathogens 2016

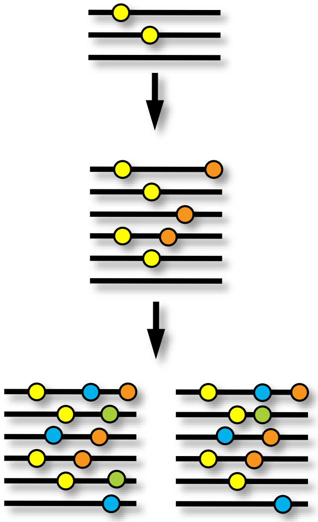
The distribution of fitness effects in influenza A



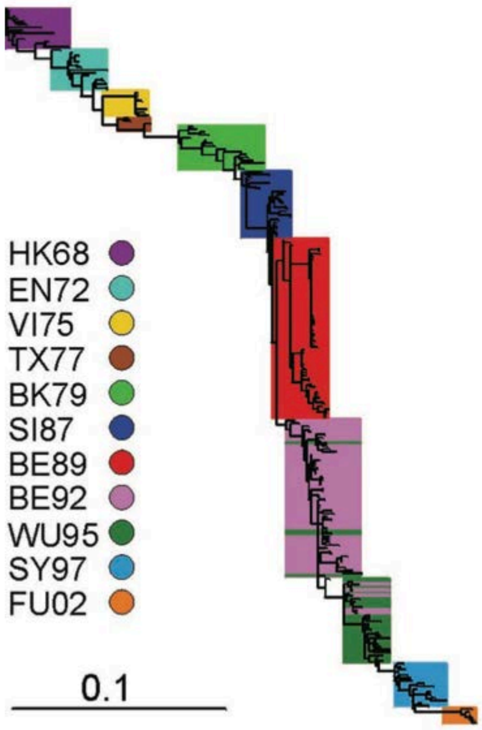
Visher et al. PLoS Pathogens 2016

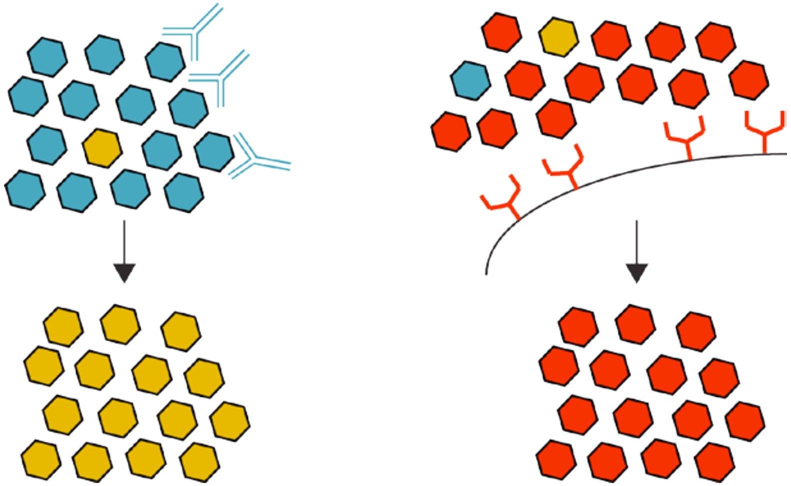
Understanding evolution across scales

How does influenza evolve in people?



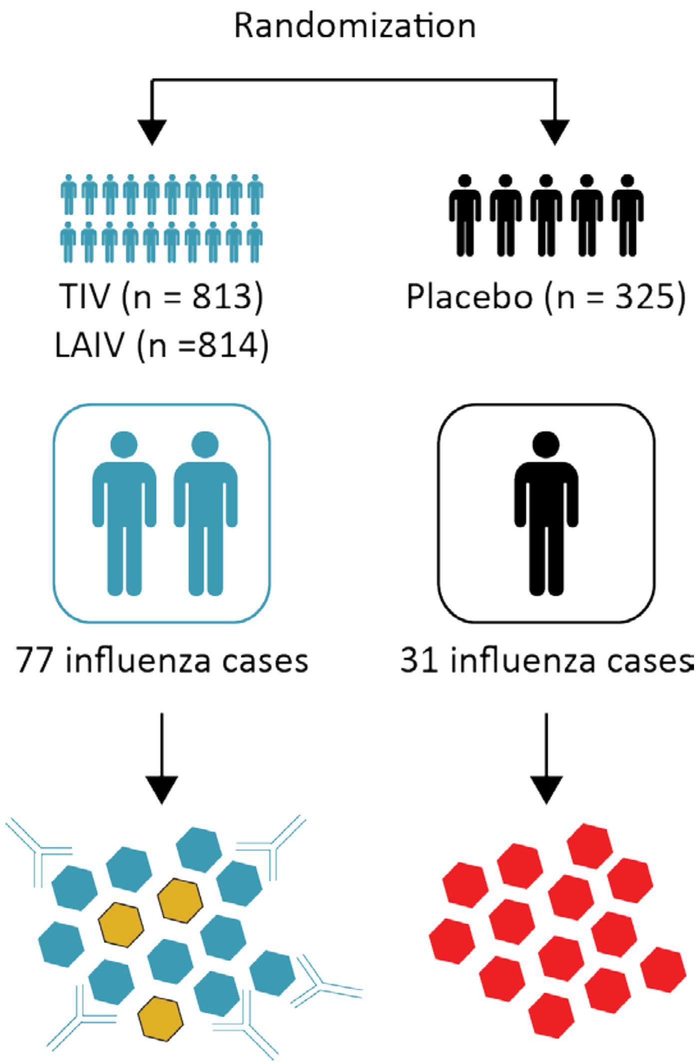
Within host selection?





Do vaccine-induced antibodies select for escape variants?

- FLUVACS study, 2004-2008
- Last placebo controlled RCT
- 60-70% efficacy
- 5119 person years of observation
- 166 samples analyzed



Randomization

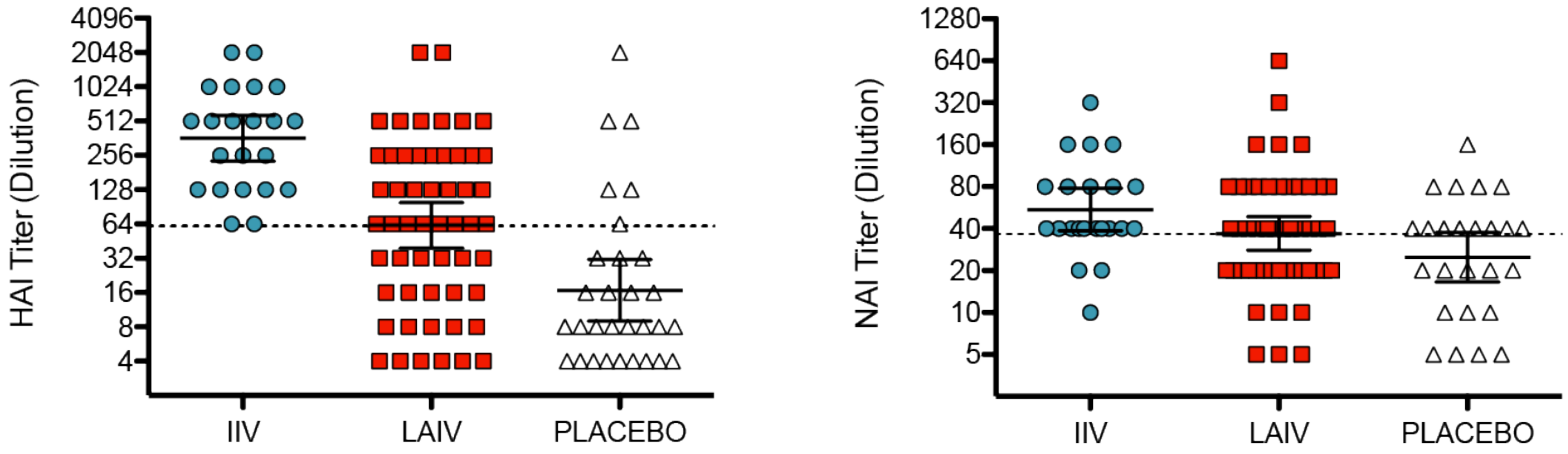
TIV (n = 813)
LAIV (n =814)

Placebo (n = 325)

77 influenza cases

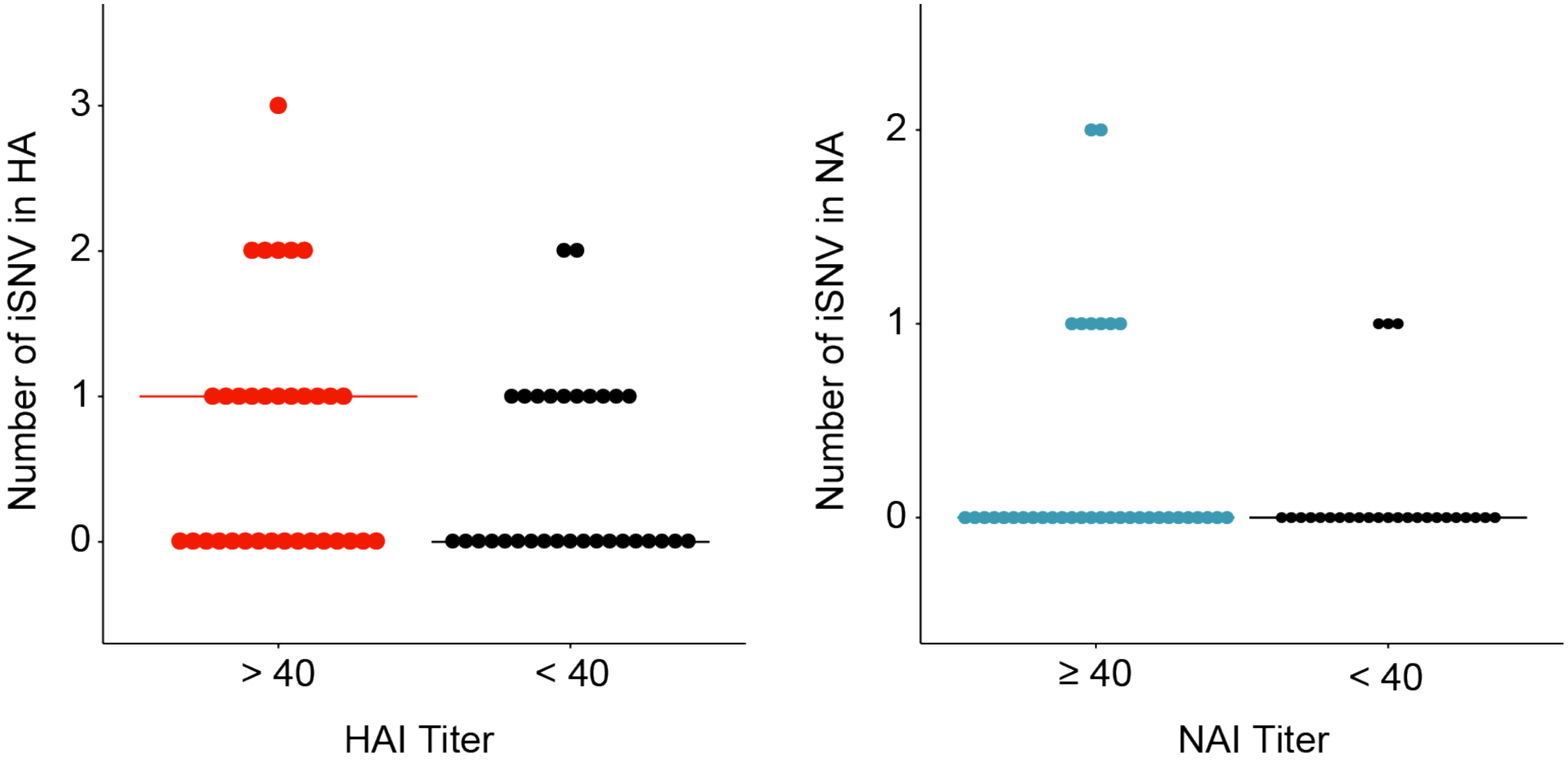
31 influenza cases

Pre-season serostatus of sampled individuals



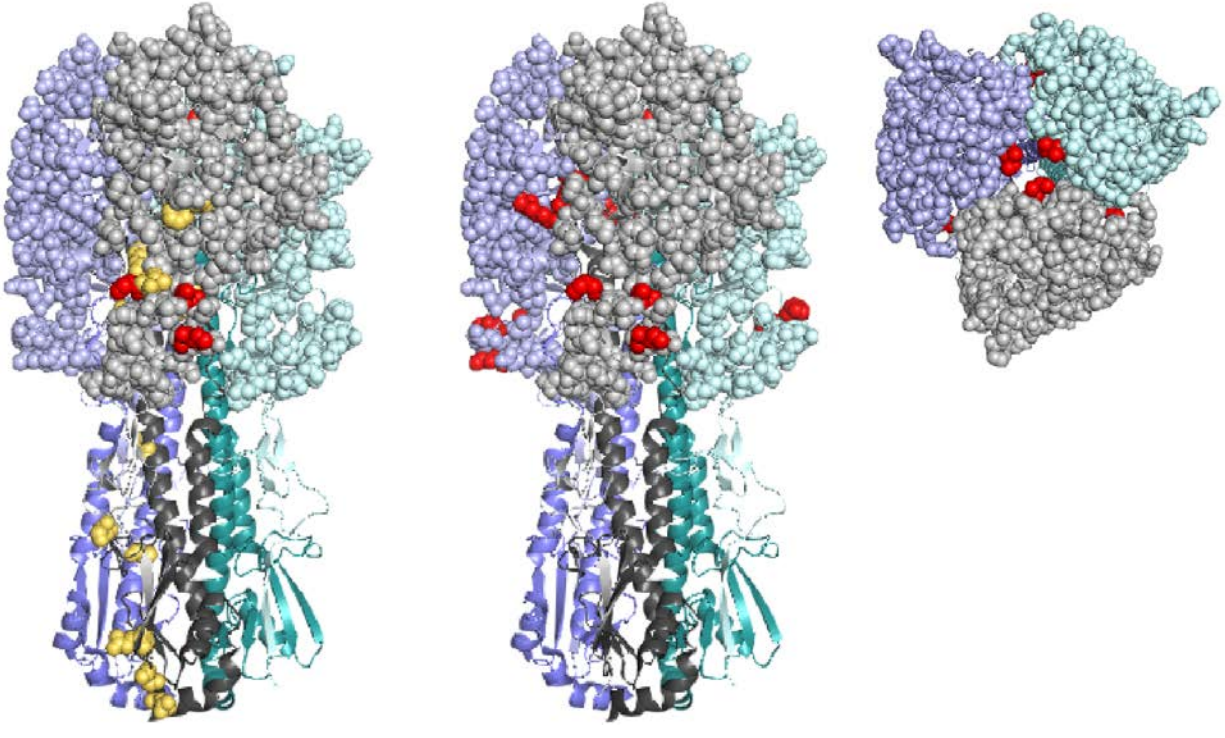
Debbink et al, PLoS Pathogens 2017

Pre-existing antibodies do not impact within host diversity



Debbink et al, PLoS Pathogens 2017

No evidence for positive selection of antigenic variants

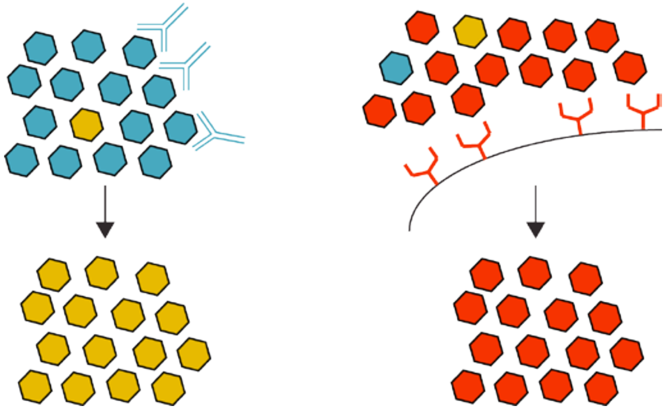


Debbink et al, PLoS Pathogens 2017

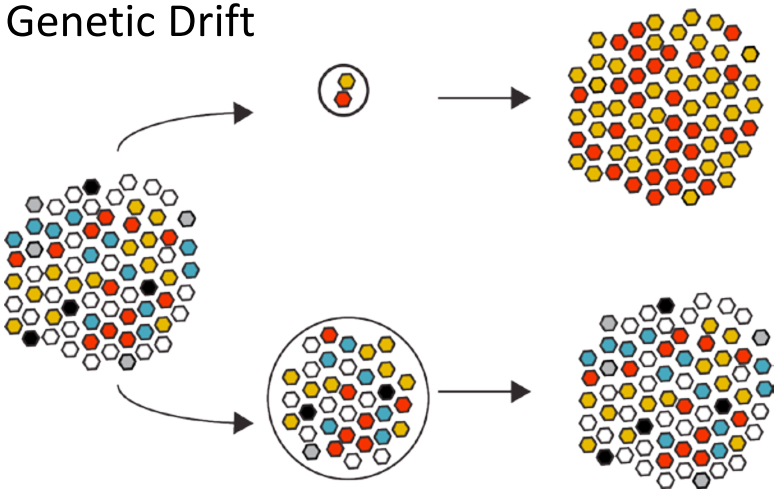
Evolutionary forces

Mutation

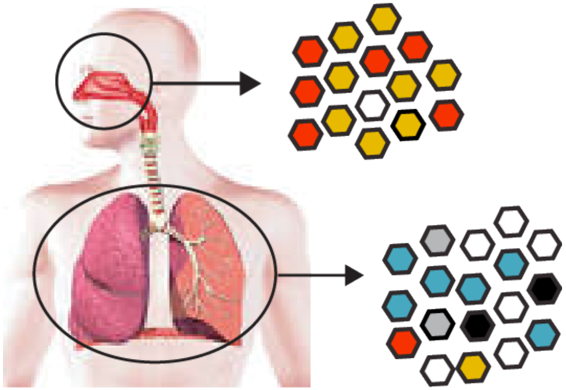
Selection



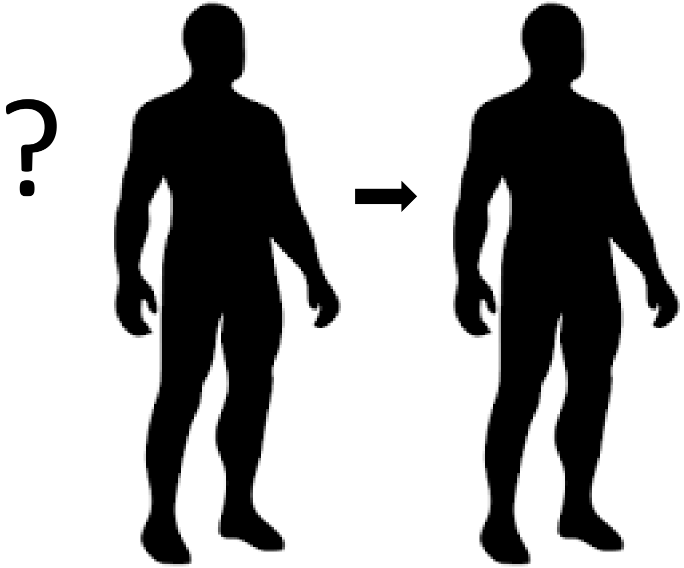
Genetic Drift



Migration



How does influenza virus evolve in people?

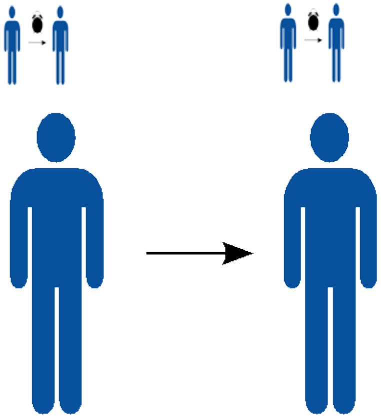


- Household influenza vaccine effectiveness
  - Prospective household cohort
  - Families ≥ 4, 2 children
  - > 6000 person years of observation
  - 249 samples, 200 individuals
- Transmission pairs within households
- Next generation sequencing
  - Infer transmission and bottleneck
  - Define within host dynamics

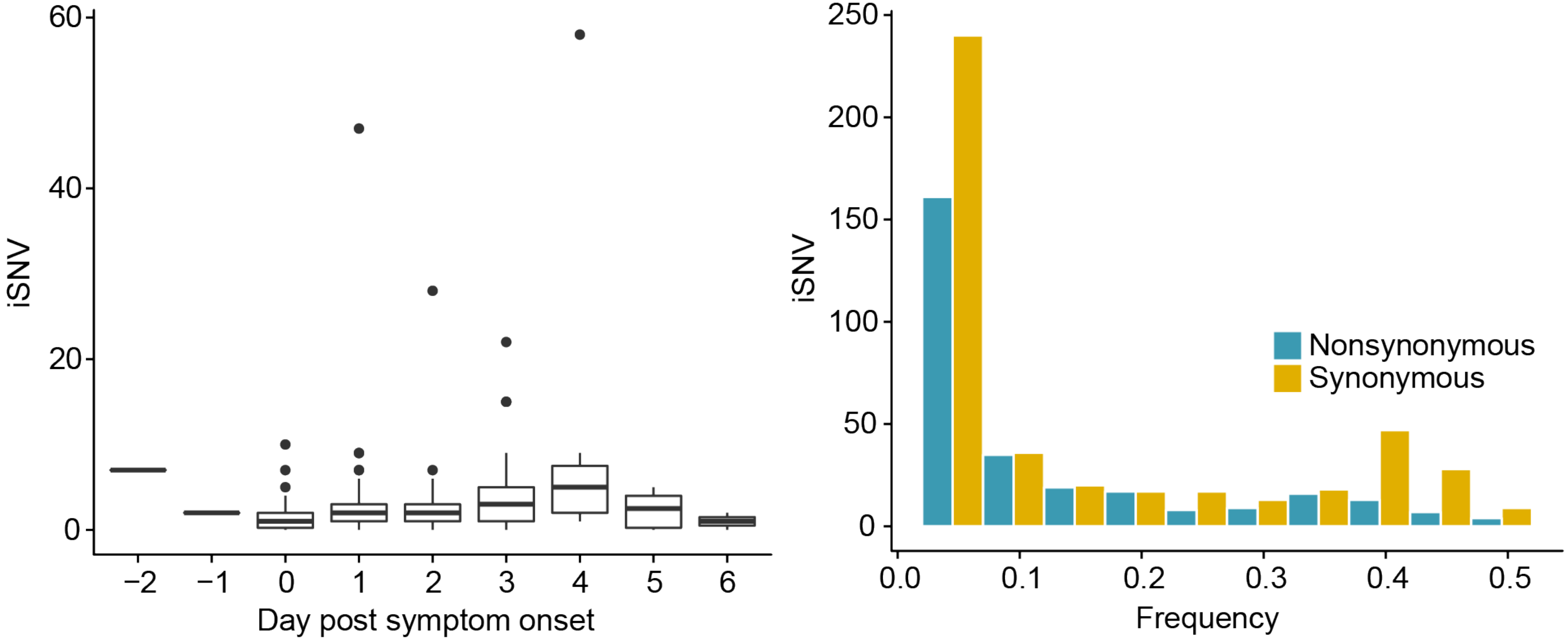
Study design

Cross-sectional study

Longitudinal study

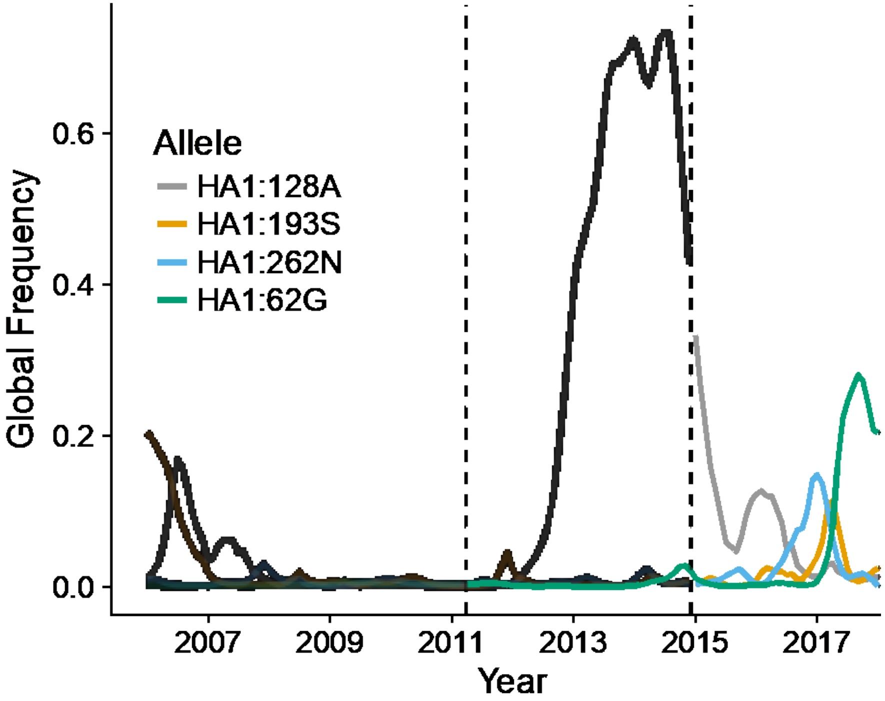


Within host genetic diversity is low



Little evidence for positive selection

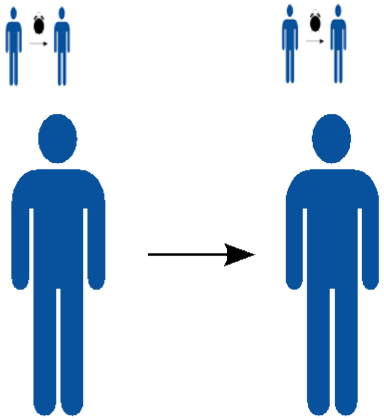
Season	Subtype	Frequency	Mutation	Site
10/11	H3N2	0.07	E62G	E
10/11	H3N2	0.09	L86I	E
11/12	H3N2	0.07	V297A	C
12/13	H3N2	0.07	I214T	D
13/14	H1N1	0.02	R208K	Ca
14/15	H3N2	0.18	F193S	B
14/15	H3N2	0.11	T128A	B
14/15	H3N2	0.41	I260V	E
14/15	H3N2	0.03	S262N	E
14/15	H3N2	0.36	G208R	D
14/15	H3N2	0.03	A163T	B
14/15	H3N2	0.12	I307R	C
14/15	H3N2	0.04	K189N	B
14/15	H3N2	0.03	D53E	C
14/15	H3N2	0.02	S312G	C
14/15	H3N2	0.03	I242T	D
14/15	H3N2	0.16	I242L	D
14/15	H3N2	0.16	I307R	C



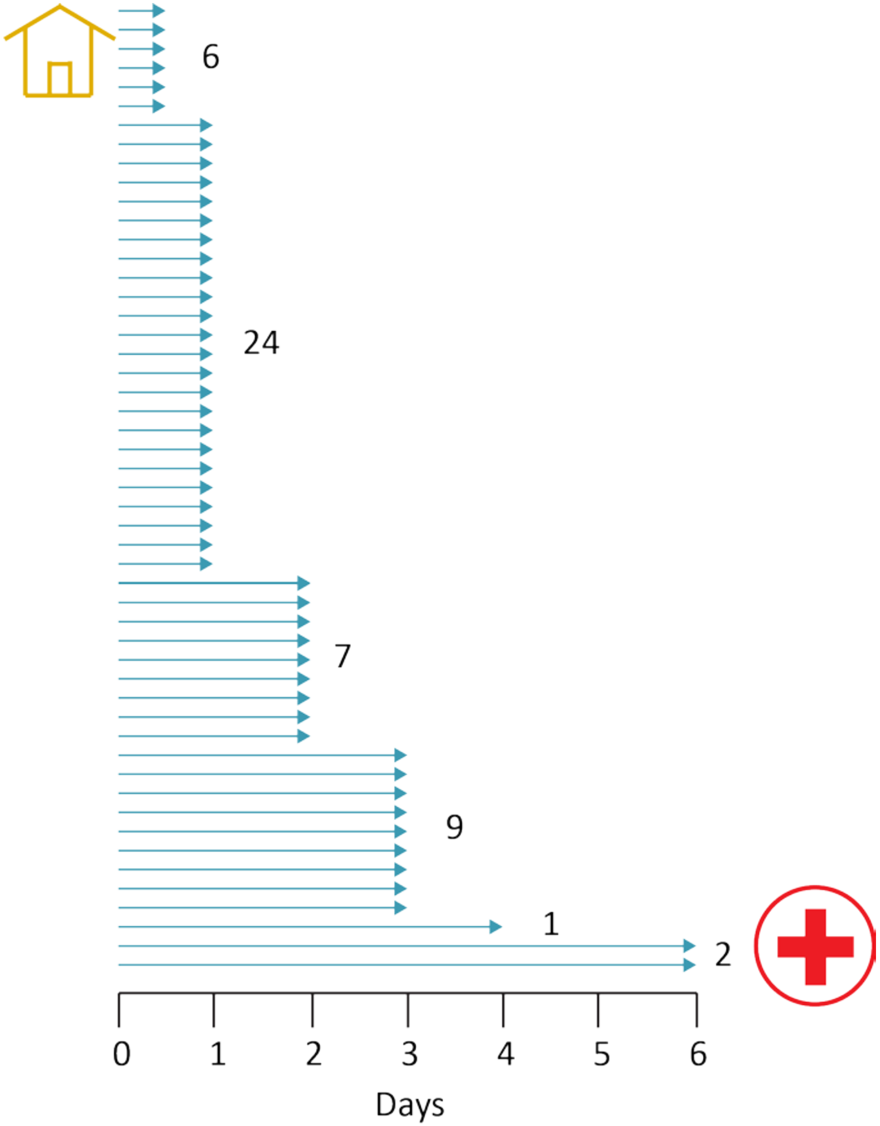
Study design

Cross-sectional study

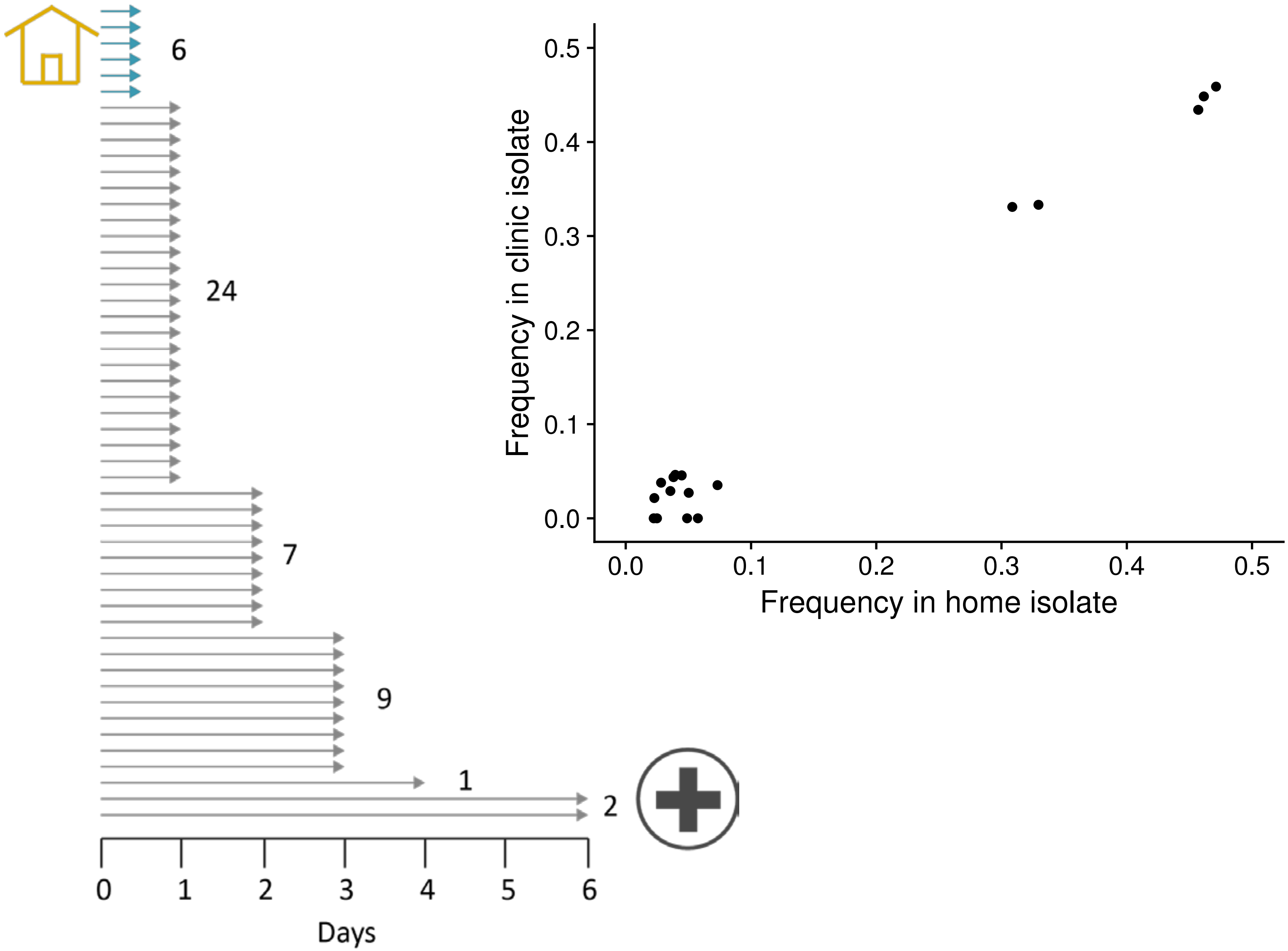
Longitudinal study



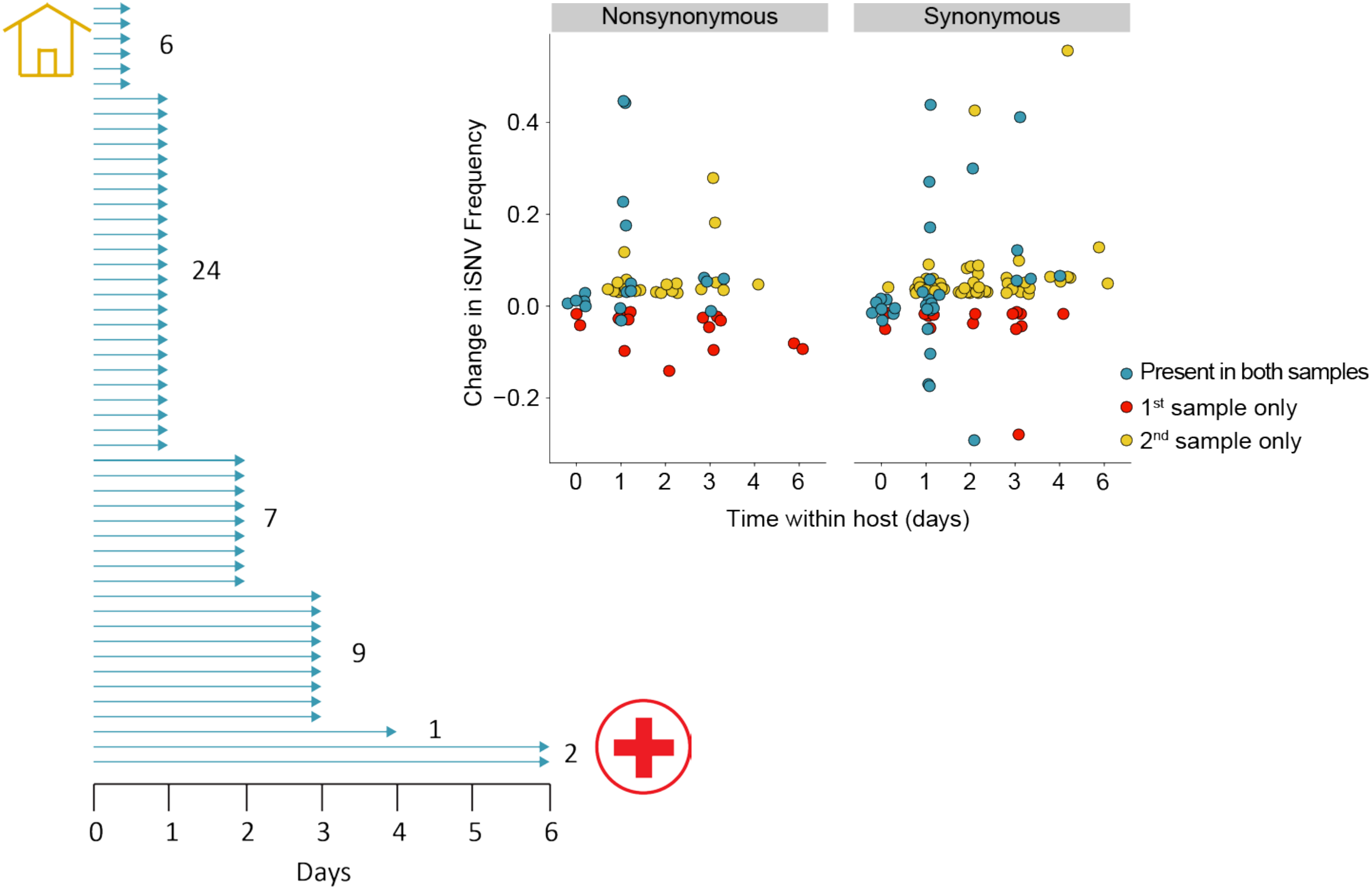
Within host dynamics, 2014-2015 season



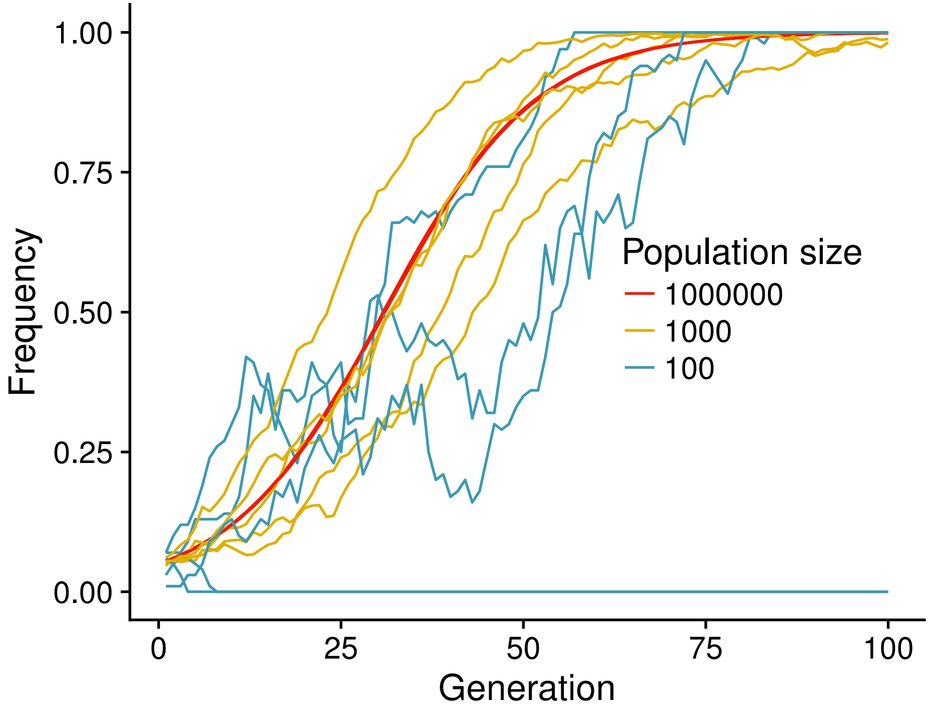
Within host dynamics, 2014-2015 season



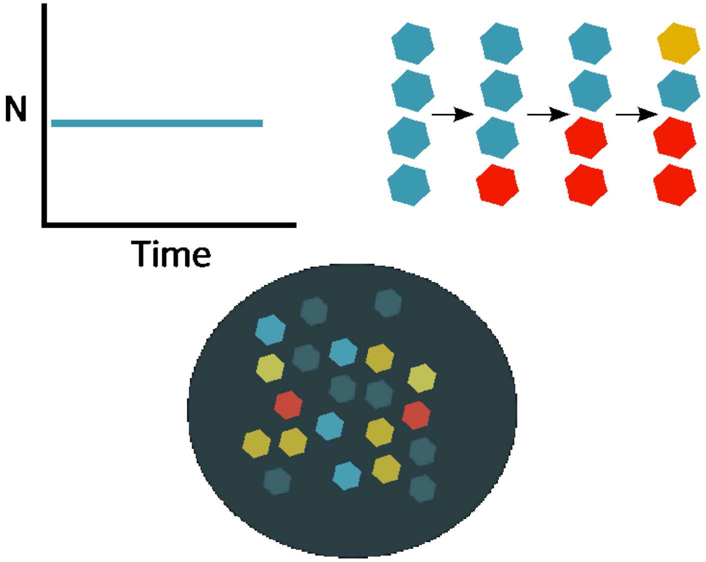
Within host dynamics, 2014-2015 season



Selection vs. Drift….depends on population size



Ideal Population



Describing within host processes – effective population size

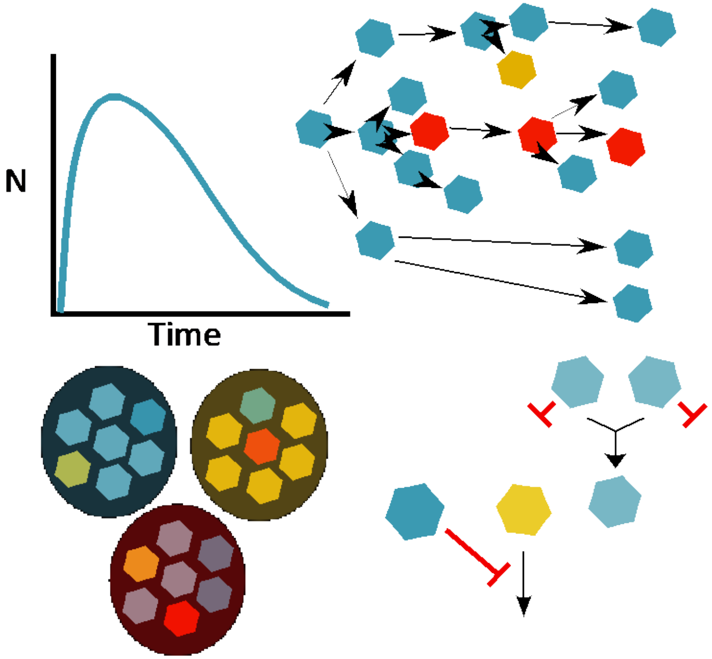
999,999 Hens ; 1 Cock

Census Population : 1,000,000

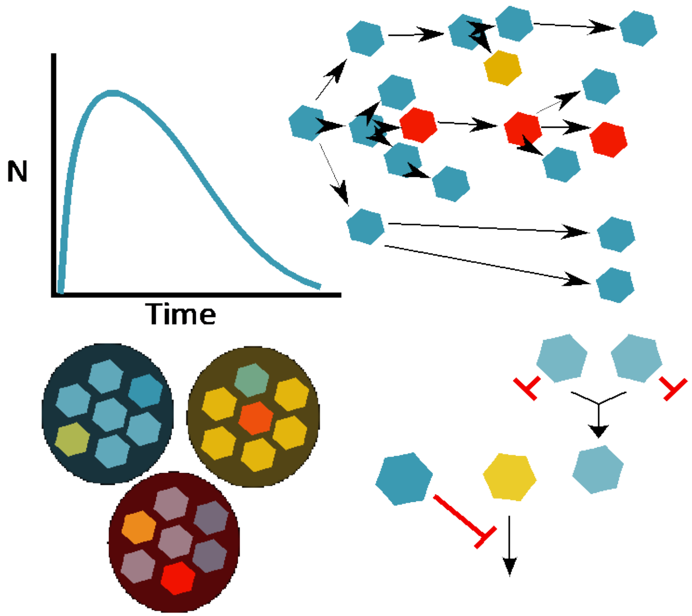
Effective Population: 4

Describing within host processes – effective population size

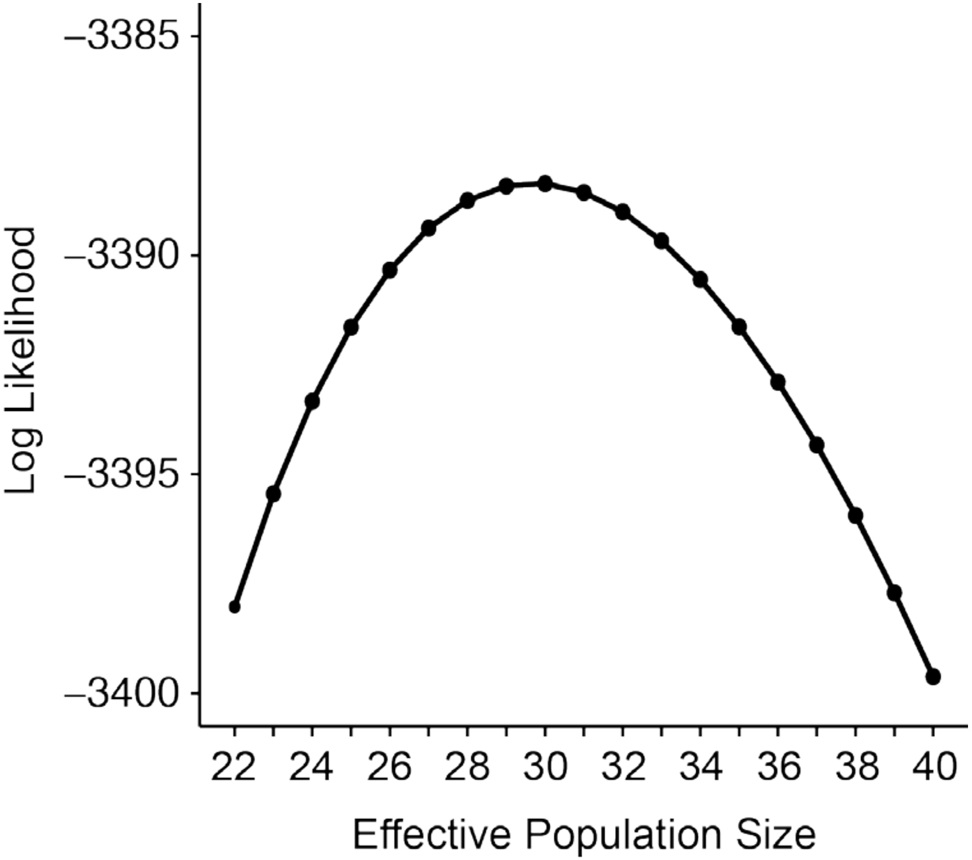
Real Populations



Real Populations

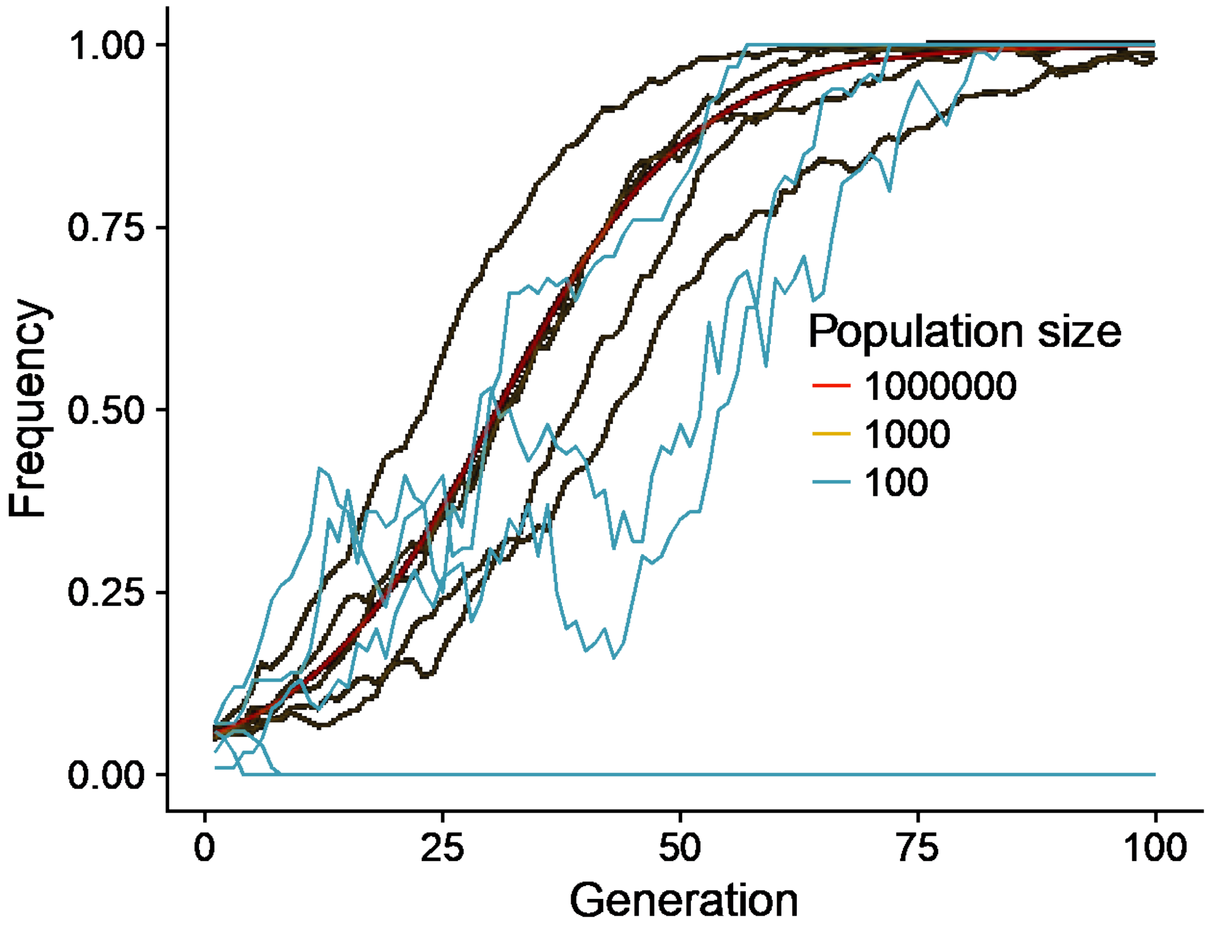


Diffusion models to estimate effective population size

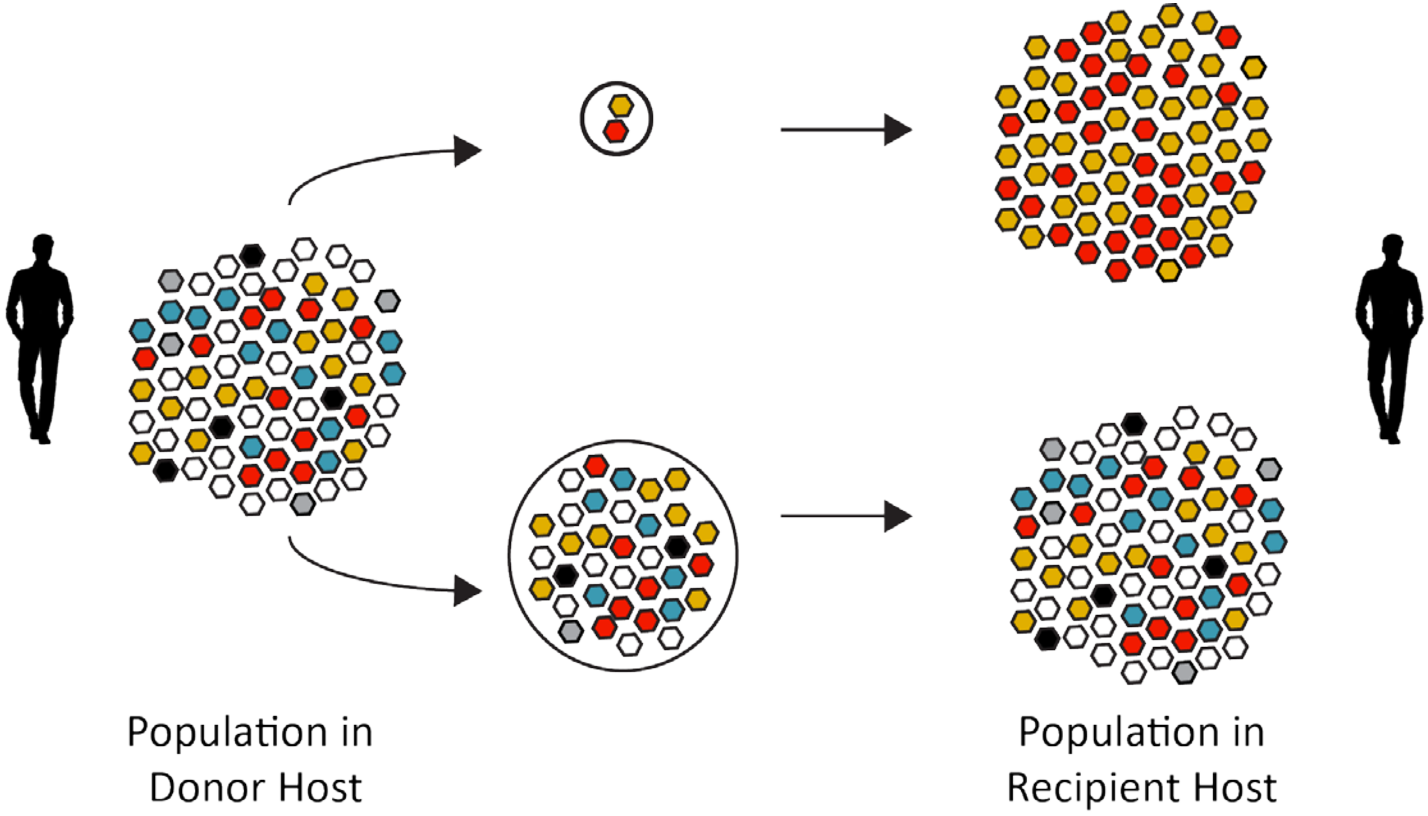


$$P(p_0, p_t, t|N_e) = \sum_{i=1}^{\infty} p_0(1-p_0)(i+1)(2i+1)F(1-i, i+2, 2, p_0) \times F(1-i, i+2, 2, p_t)e^{-[i(i+1)/2N_e]t}$$

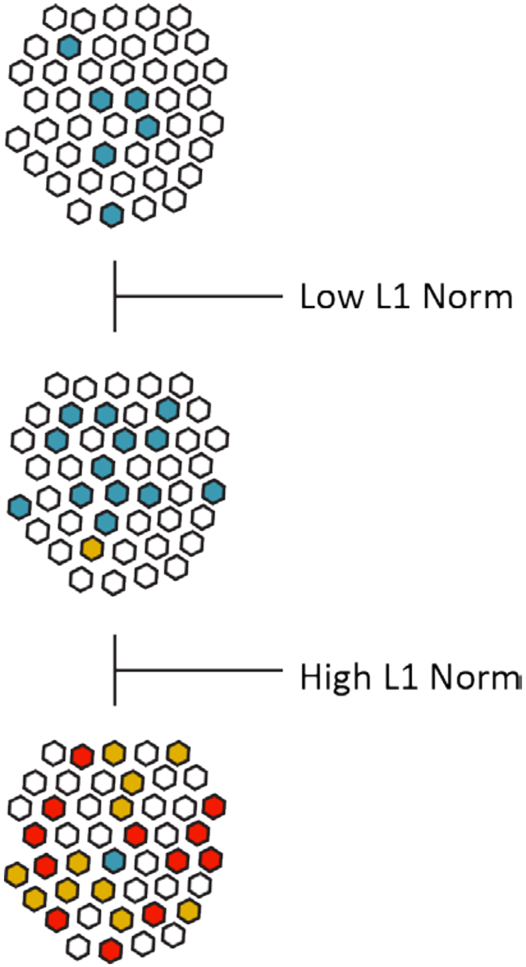
Genetic drift is a dominant force in small populations

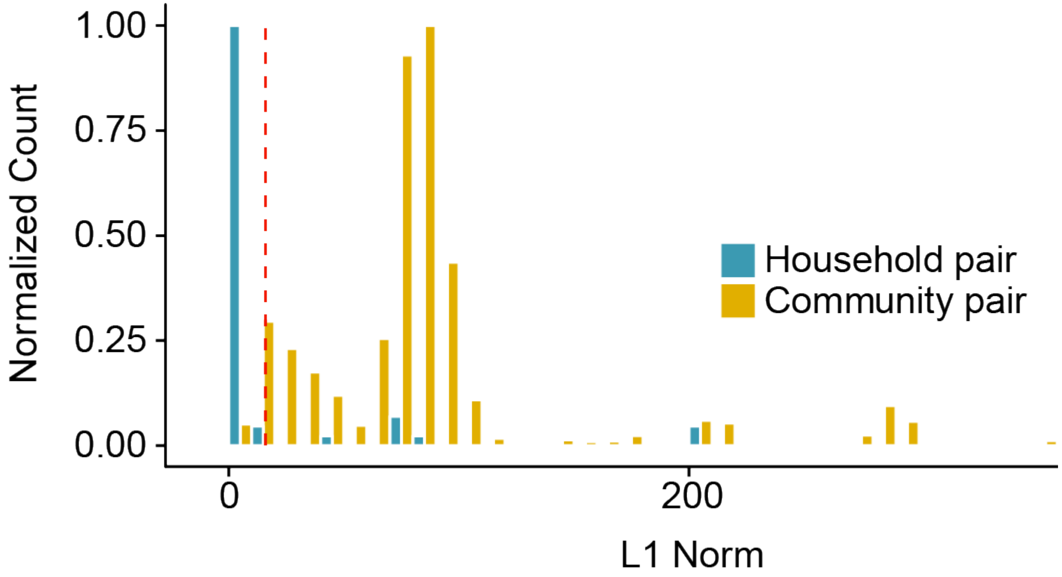


What happens between hosts?

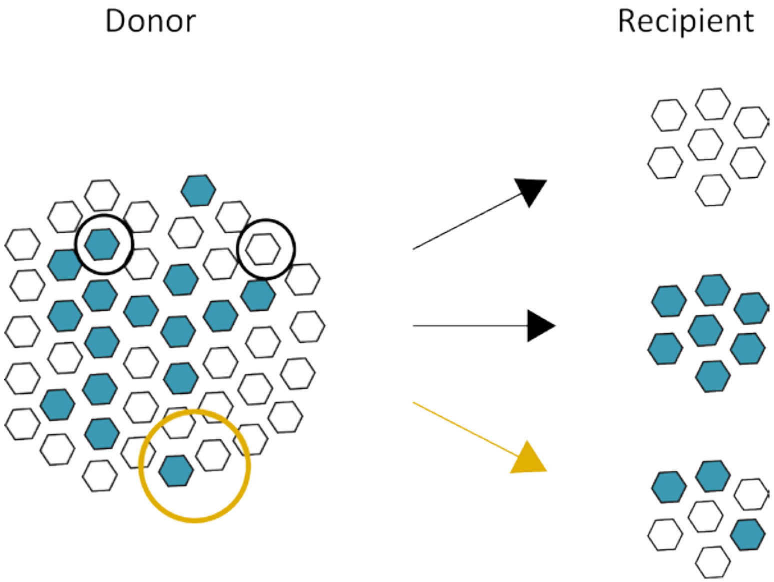


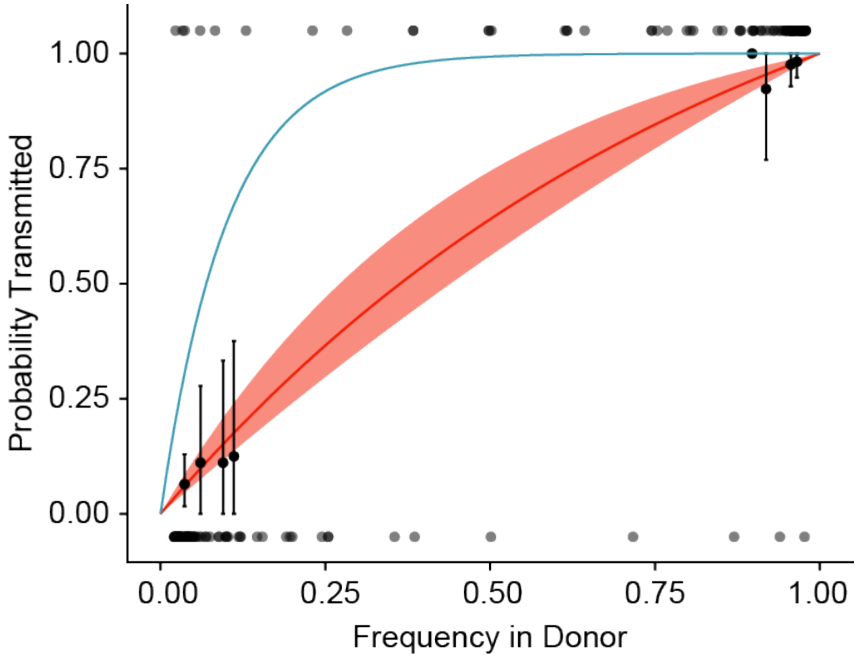
Sequence based inference of 41 transmission pairs





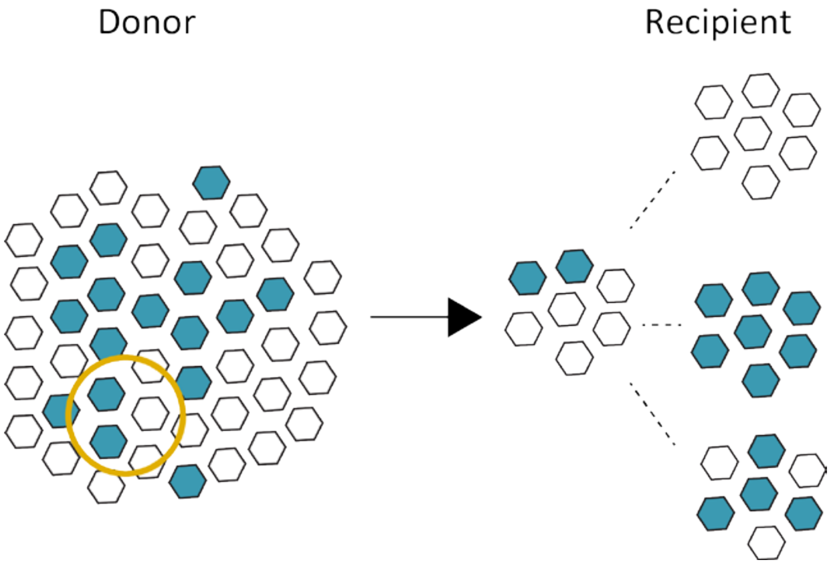
Bottleneck size – presence/absence model

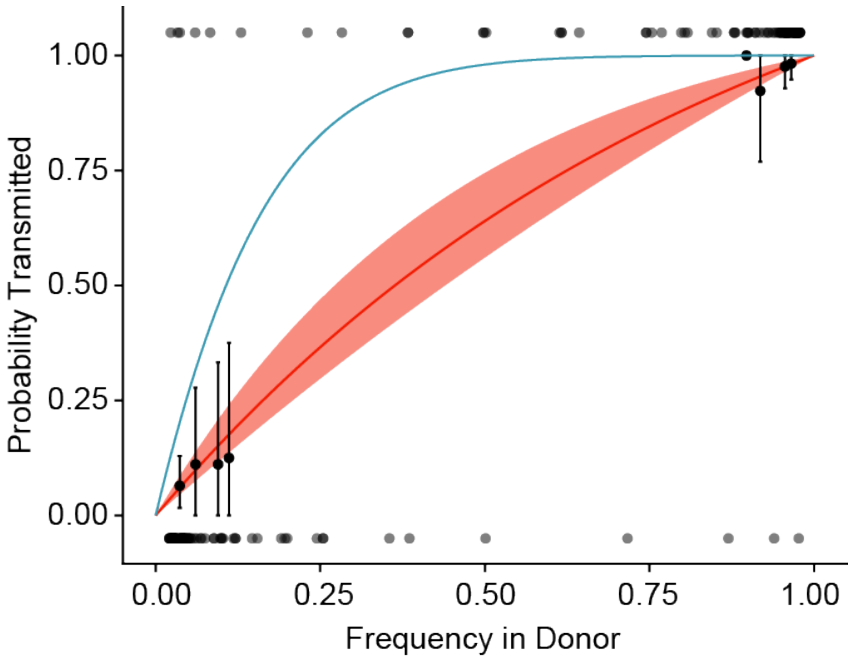




Mean Bottleneck = 1.68
Probability of >5 is 0.0017

Bottleneck size – beta binomial model

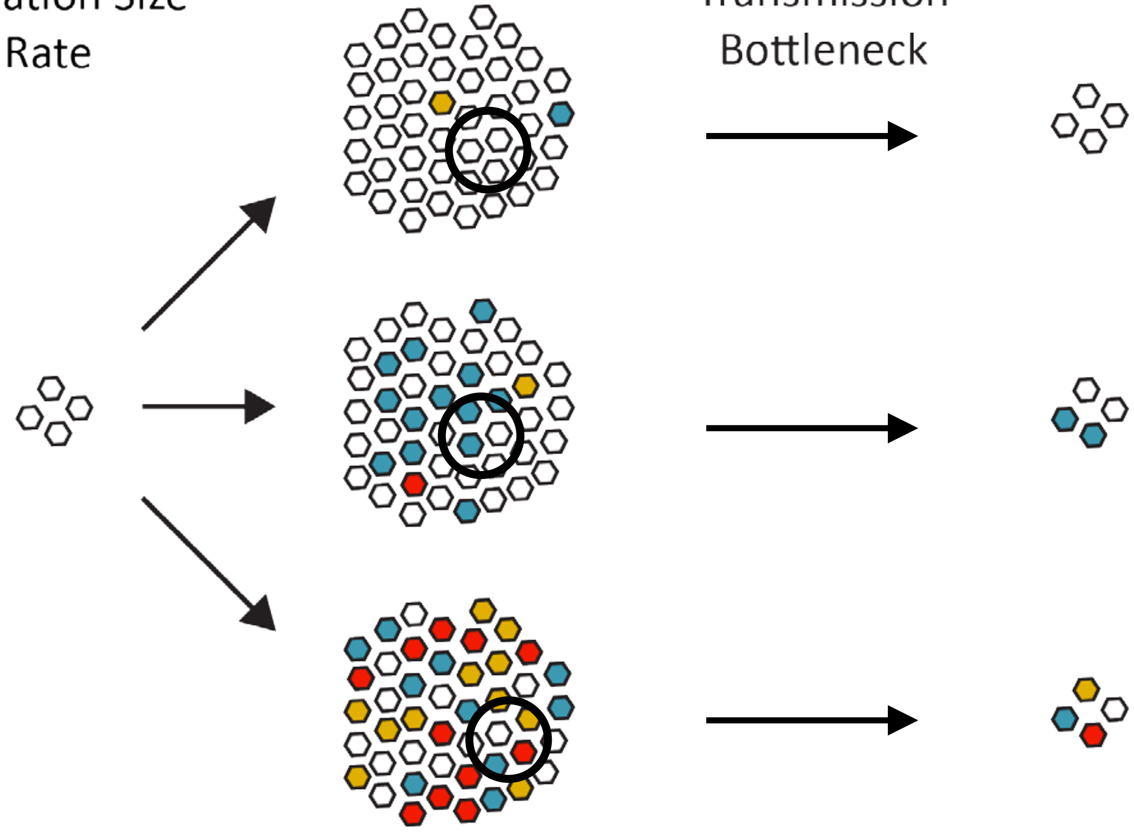




Mean Bottleneck 1.7

Sobel et al. JVI 2017

Evolution within and between hosts



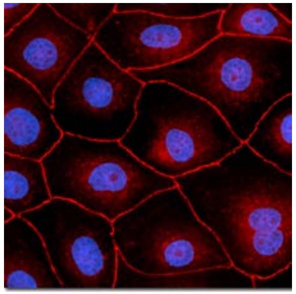
Effective Population Size
Mutation Rate

Transmission
Bottleneck

Within Host

Next Host

Evolution across scales



Very high mutation rates
Strong purifying selection

Very high mutation rates
Strong purifying selection
Lots of genetic drift
Migration?

Lots of genetic drift
Random fixation of deleterious alleles
Weak selection?

Influenza viruses are like compulsive gamblers

Acknowledgements

@lauringlab

Will Fitzsimmons
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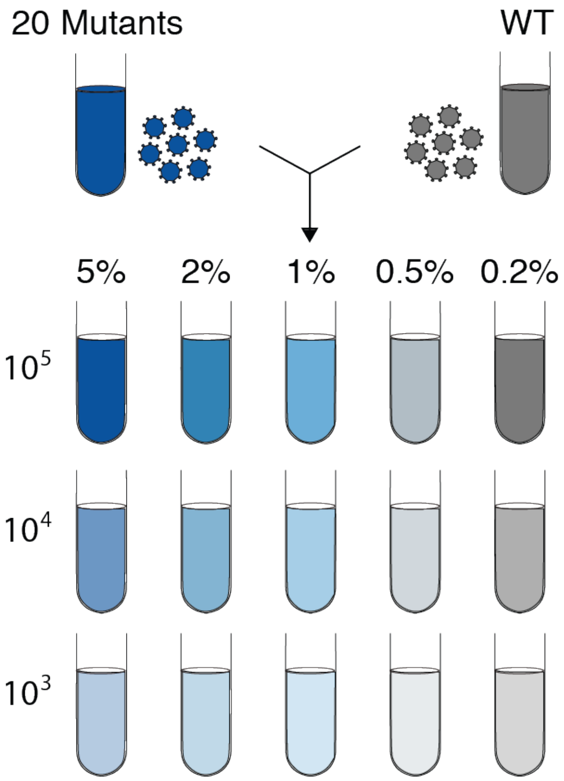
Kayla Peck

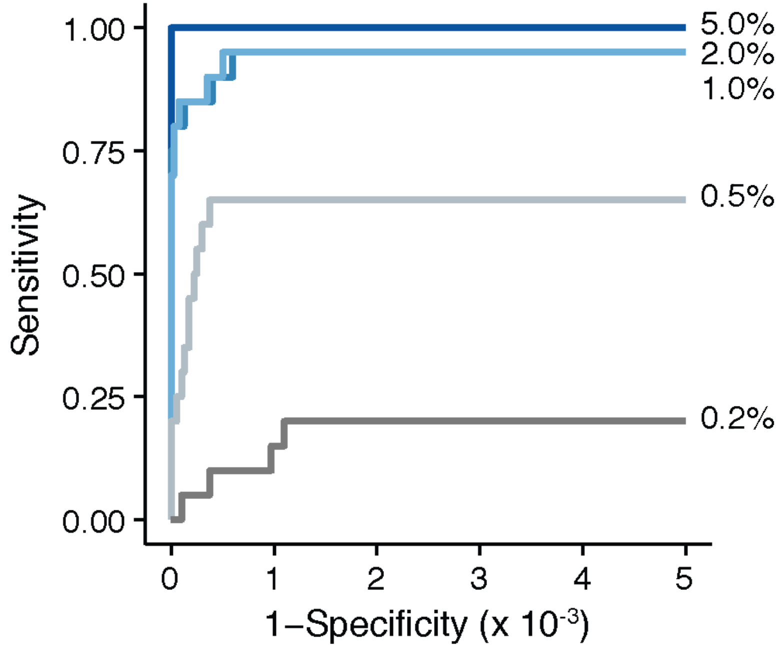
Former
Kari Debbink
Matt Pauly
Elisa Visher
Shawn Whitefield

UM School of Public Health
Arnold Monto
Emily Martin
Josh Petrie

Doris Duke Charitable Foundation
IDSA/Pfizer Young Investigator Award
NIAID R01 AI11886
Burroughs Wellcome Fund, PATH

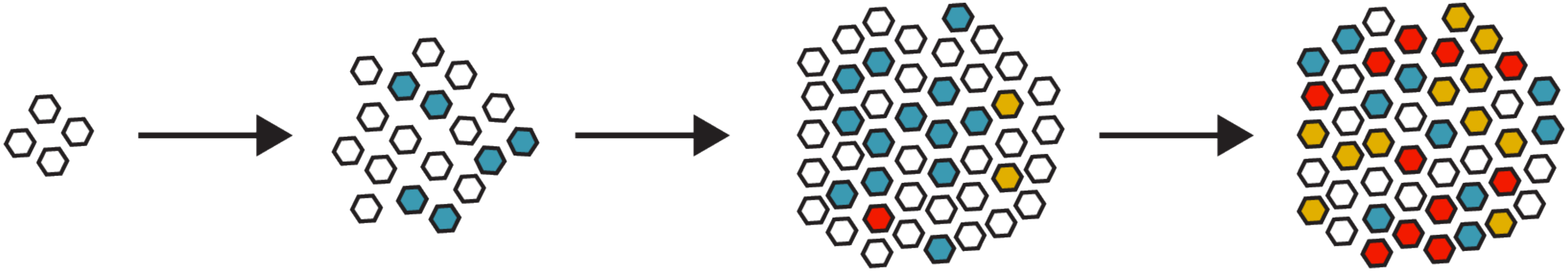
High quality variant calling is critical in deep sequencing





McCrone and Lauring, JVI 2016

Evolution within and between hosts



Mutation rate and effective population size (Ne)