



DukeNUS
Medical School

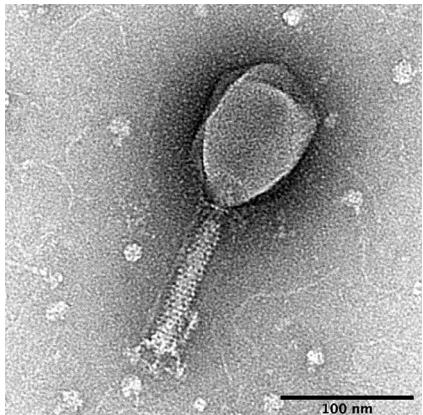
Virus Evolution

Michael Zeller in lieu of Gavin Smith
Laboratory of Virus Evolution

What is a virus?

- Obligate parasites
 - Rely on host for replication
 - Lack metabolic machinery
- Without a host, infectious particles cannot replicate

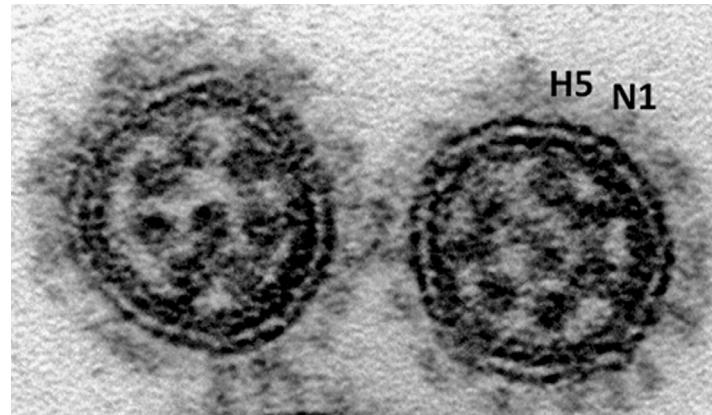
T4 phage



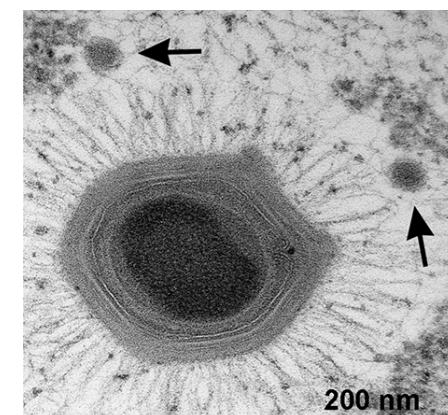
smallpox



H5N1 Influenza

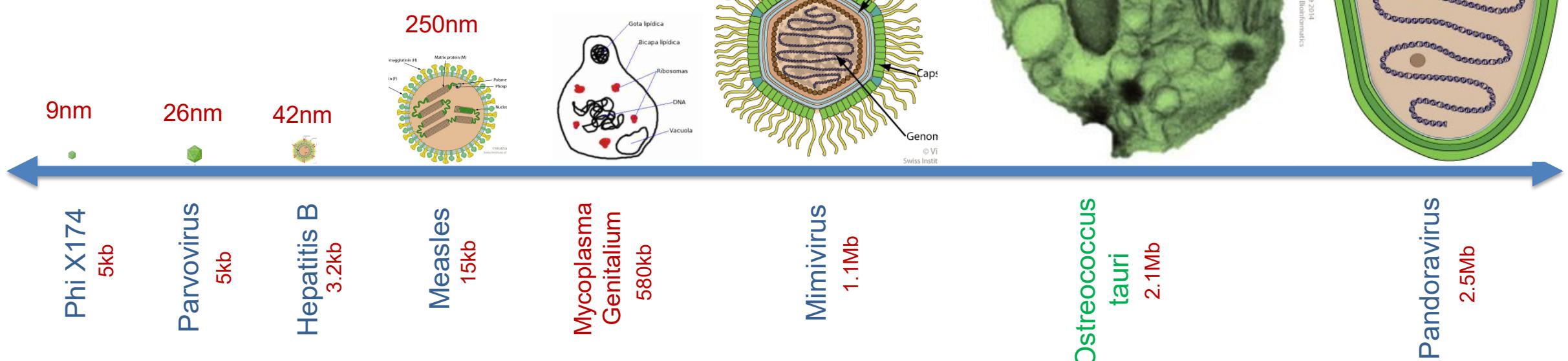


Mimivirus



(H5N1 influenza viruses: Facts, not fear", Peter Palese, 2012)

- Virus size comparable to smallest bacteria
- Primary difference is metabolic activity
 - Contains ribosomes



Ostreococcus from "Plastic plankton prosper", D. A. Hutchins 2013

Bacterial depictions from Wikimedia commons

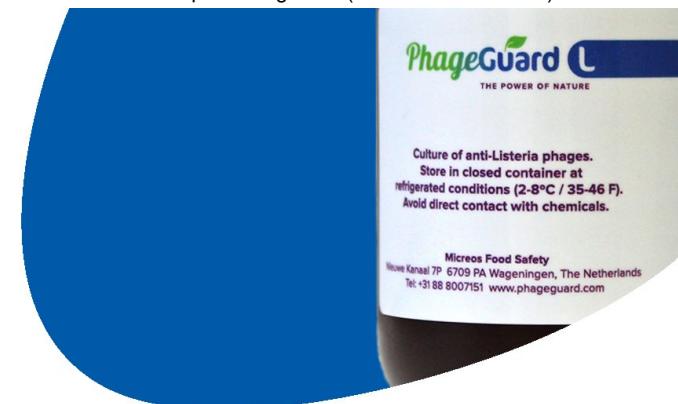
Viral depictions from the Swiss Institute of Bioinformatics

Commercially important viruses

- Tulip breaking virus (TBV)
 - Historically important
 - Caused 1637 market crash in Holland
- Bacteriophages
 - Used to fight listeria in luncheon meat
 - Studied by USSR (Georgia)
- Viral therapeutics
 - Lentiviral vectors



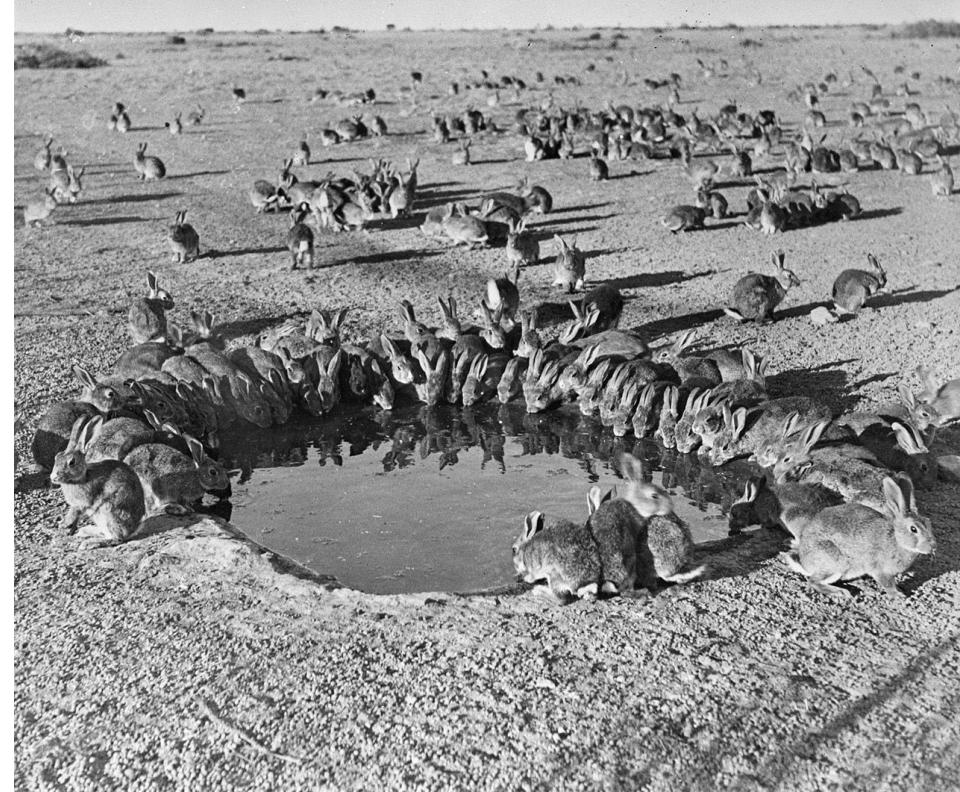
Tulip breaking virus (Wikimedia commons)



PhageGuard (<https://phageguard.com/listeria-solution/>)

Commercially important viruses

- 13 rabbits introduced to Australia for sport hunting (1859)
- Rapidly bred, destroyed local fauna and damaged crops
- 1937 - Myxoma virus wiped out 99.8%
- 1995 - Calcivirus used to control rabbit populations



Myxoma virus trial on Wardang Island in 1938
(Wikimedia commons)

Where do viruses come from?

1. Regressive evolution (parasitism)
 - Pathogens degenerated from previously independent life forms
 - Retain only what they needed for parasitic lifestyle
2. Cellular origins
 - Pathogens derived from sub-cellular functional assemblies of macromolecules that gained the capacity to move from cell to cell
3. Independent entities
 - Evolved from primitive, pre-biotic self-replicating molecules

Recent discoveries blur the lines of what is a virus

- Pandoravirus
 - 2.5MB, 1uM capsule
- Megavirus
 - 1.2 Mb 440 nm capsule
- Mimivirus
 - 1.1 Mb 440 nm capsule
- All dsDNA
- All have aminoacyl tRNA synthetases
 - Can produce tRNA for some amino acids
 - Contains lipid and sugar metabolism genes

Science

A Giant Virus in Amoebae

BERNARD LA SCOLA, STÉPHANE AUDIC, CATHERINE ROBERT, LIANG JUNGANG, XAVIER DE LAMBALLERIE, MICHEL DRANCOURT, RICHARD BIRTLES, JEAN-MICHEL CLAVERIE,
AND DIDIER RAOUlt [Authors Info & Affiliations](#)

SCIENCE • 28 Mar 2003 • Vol 299, Issue 5615 • p. 2033 • DOI: 10.1126/science.1081867

Pandoraviruses: Amoeba Viruses with Genomes Up to 2.5 Mb Reaching That of Parasitic Eukaryotes

NADÈGE PHILIPPE, MATHIEU LEGENDRE, GABRIEL DOUTRE, YOHANN COUTÉ, OLIVIER POIROT, MAGALI LESCOT, DEFNE ARSLAN, VIRGINIE SELTZER, LIONEL BERTAUX, [...]
CHANTAL ABERGEL [+4 authors](#) [Authors Info & Affiliations](#)

SCIENCE • 19 Jul 2013 • Vol 341, Issue 6143 • pp. 281-286 • DOI: 10.1126/science.1239181

PNAS

Distant Mimivirus relative with a larger genome highlights the fundamental features of Megaviridae

Defne Arslan, Matthieu Legendre, Virginie Seltzer, [+1](#), and Jean-Michel Claverie [Email](#) [Authors Info & Affiliations](#)

October 10, 2011 | 108 (42) | <https://doi.org/10.1073/pnas.1110889108>

Viruses in the fossil record

– more or less non-existent



Viruses from ice cores

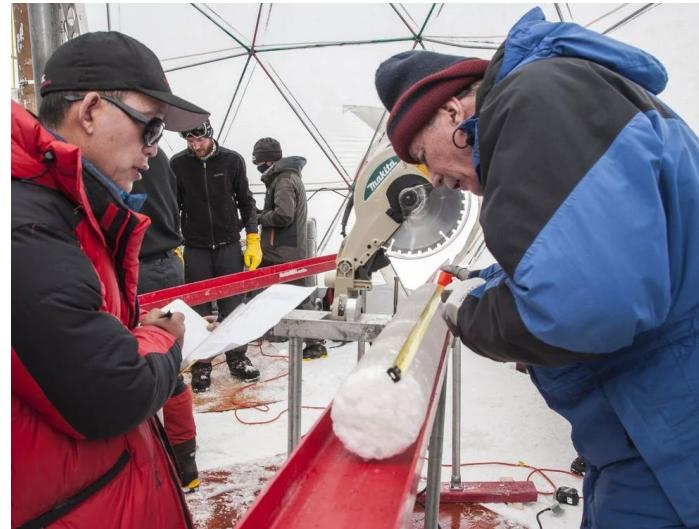
- Ice cores from permafrost Gulia, Tibetan Plateaus
- Viruses expected to be ancient phages
- Estimated 15,000yo
- Concerns about resurrection with global warming

 **BMC** Part of Springer Nature

Microbiome

Glacier ice archives nearly 15,000-year-old microbes and phages

Zhi-Ping Zhong, Funing Tian, Simon Roux, M. Consuelo Gazitúa, Natalie E. Solonenko, Yueh-Fen Li, Mary E. Davis, James L. Van Etten, Ellen Mosley-Thompson, Virginia I. Rich, Matthew B. Sullivan  & Lonnie G. Thompson 



Smithsonian magazine, (Lonnie Thompson / Ohio State University)

Other evidence



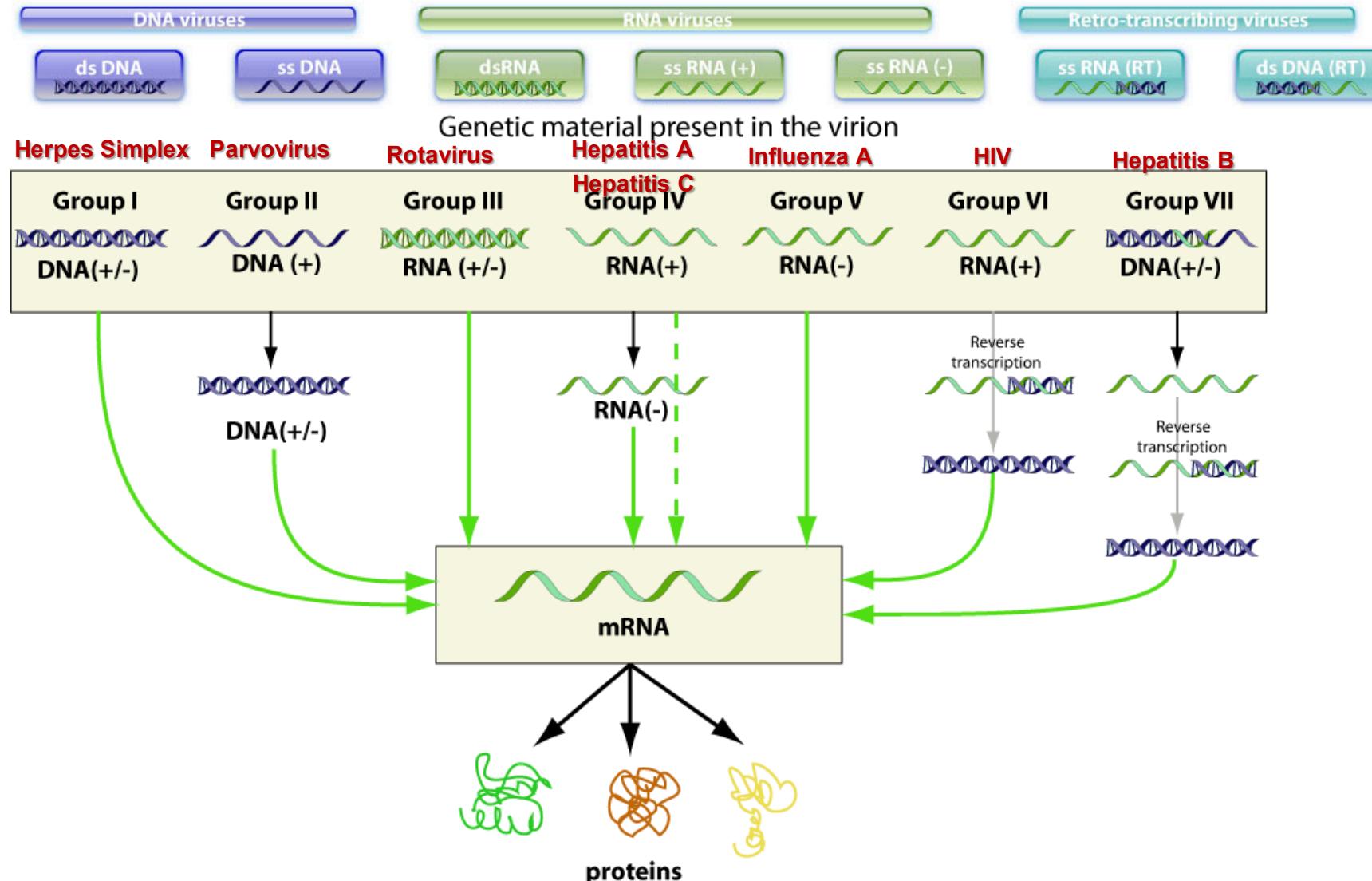
Poliomyelitis 1403–1365 BC



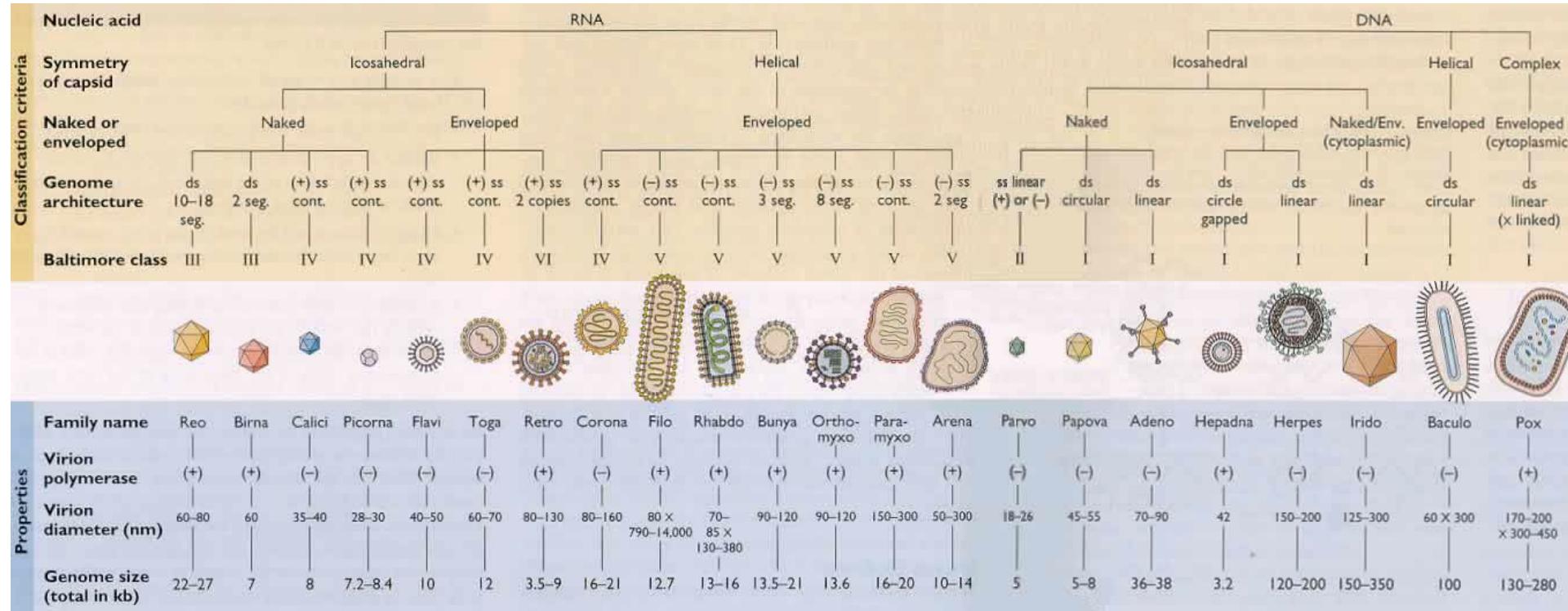
Smallpox Ramses V 1145 BC

Types of viruses

Baltimore classification



Baltimore classification



<https://talk.ictvonline.org/taxonomy/>

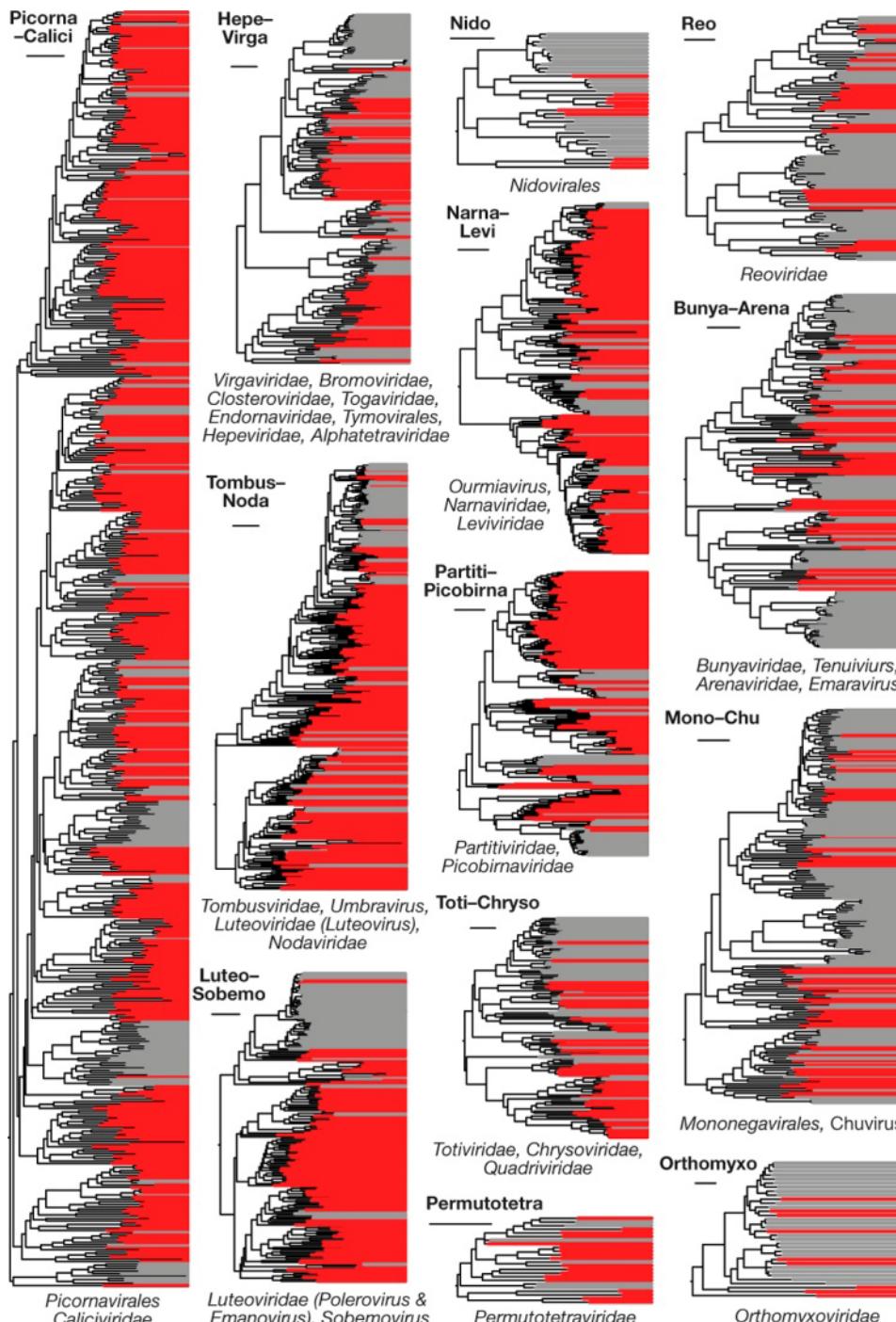
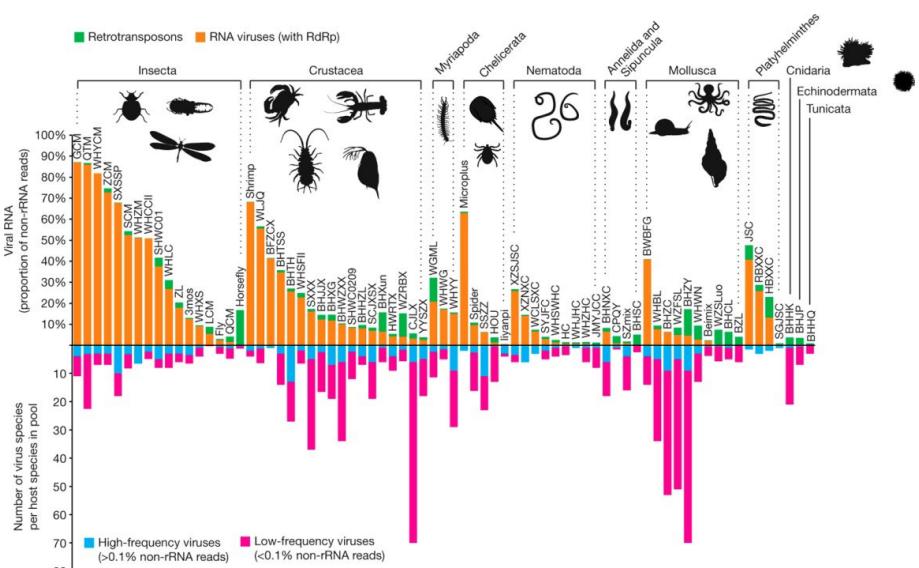
Published: 23 November 2016

Redefining the invertebrate RNA virosphere

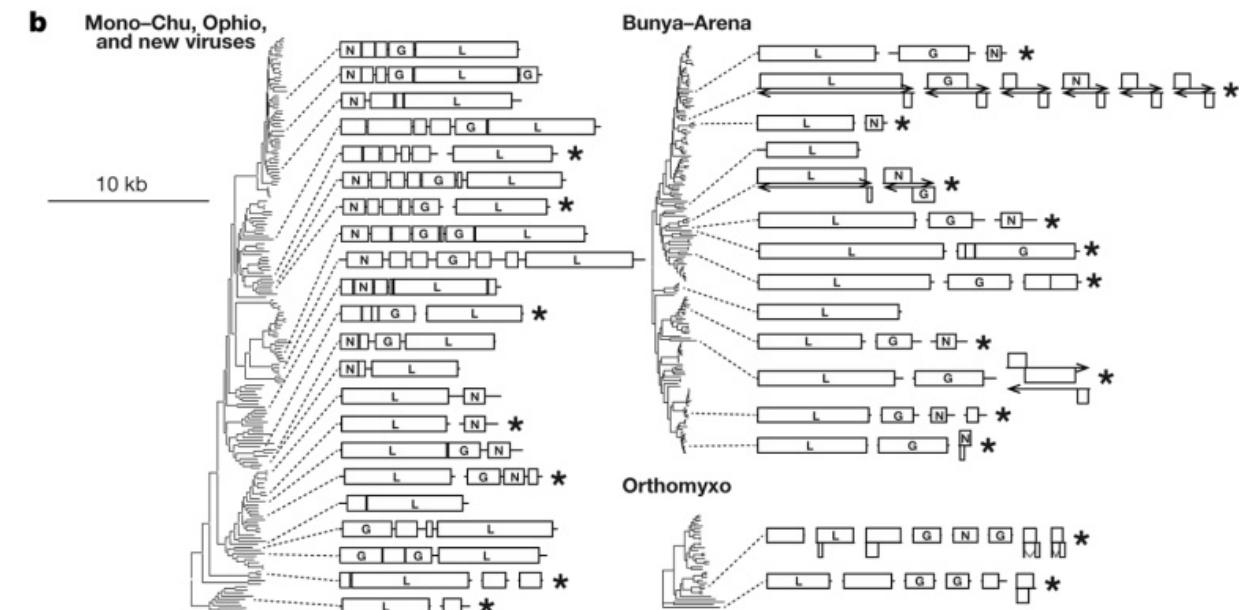
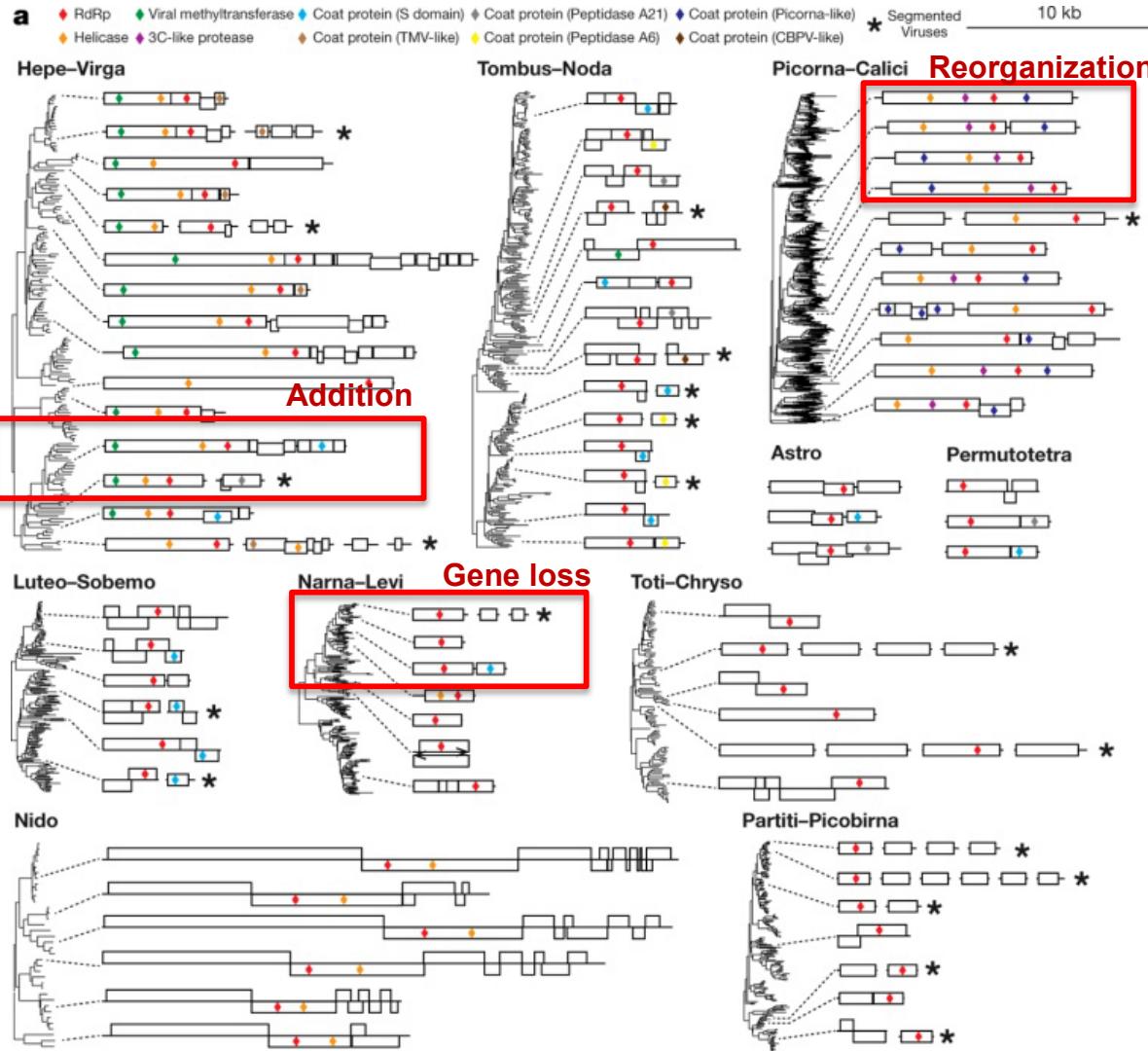
Mang Shi, Xian-Dan Lin, Jun-Hua Tian, Liang-Jun Chen, Xiao Chen, Ci-Xiu Li, Xin-Cheng Qin, Jun Li, Jian-Ping Cao, John-Sebastian Eden, Jan Buchmann, Wen Wang, Jianguo Xu, Edward C. Holmes & Yong-Zhen Zhang 

Nature 540, 539–543 (2016) | [Cite this article](#)

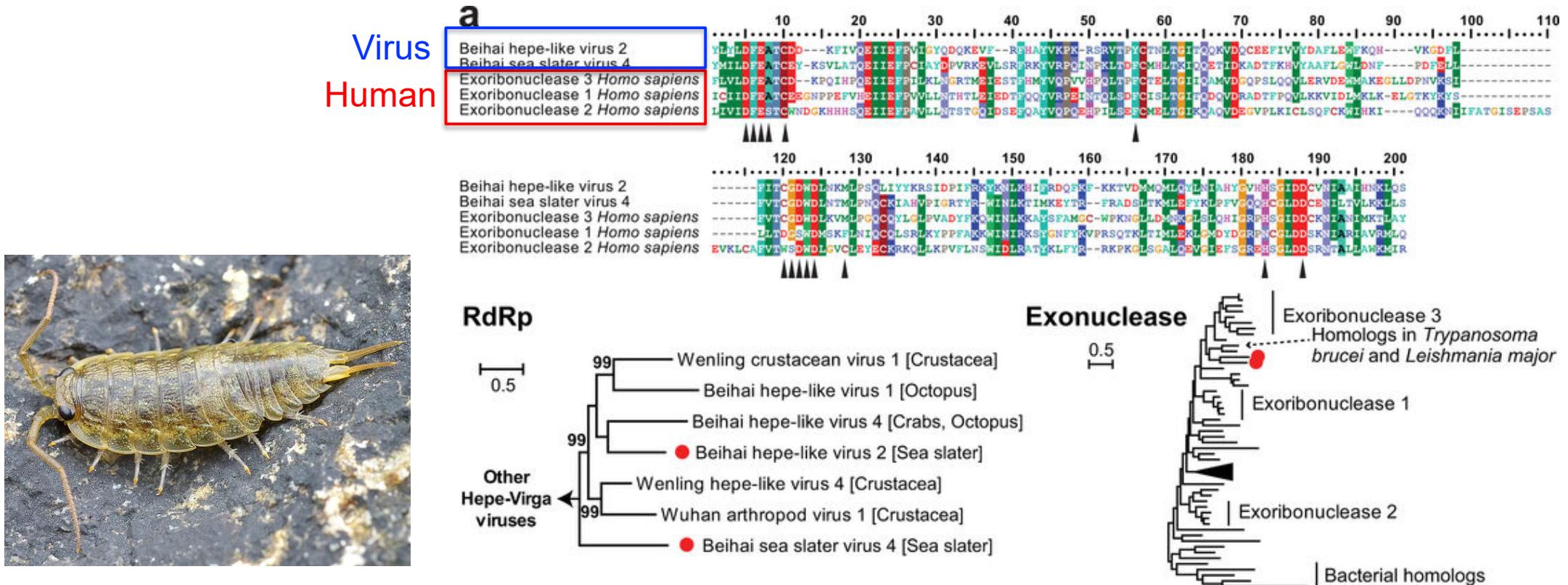
35k Accesses | 711 Citations | 606 Altmetric | [Metrics](#)



Evolution of genome organization in RNA viruses

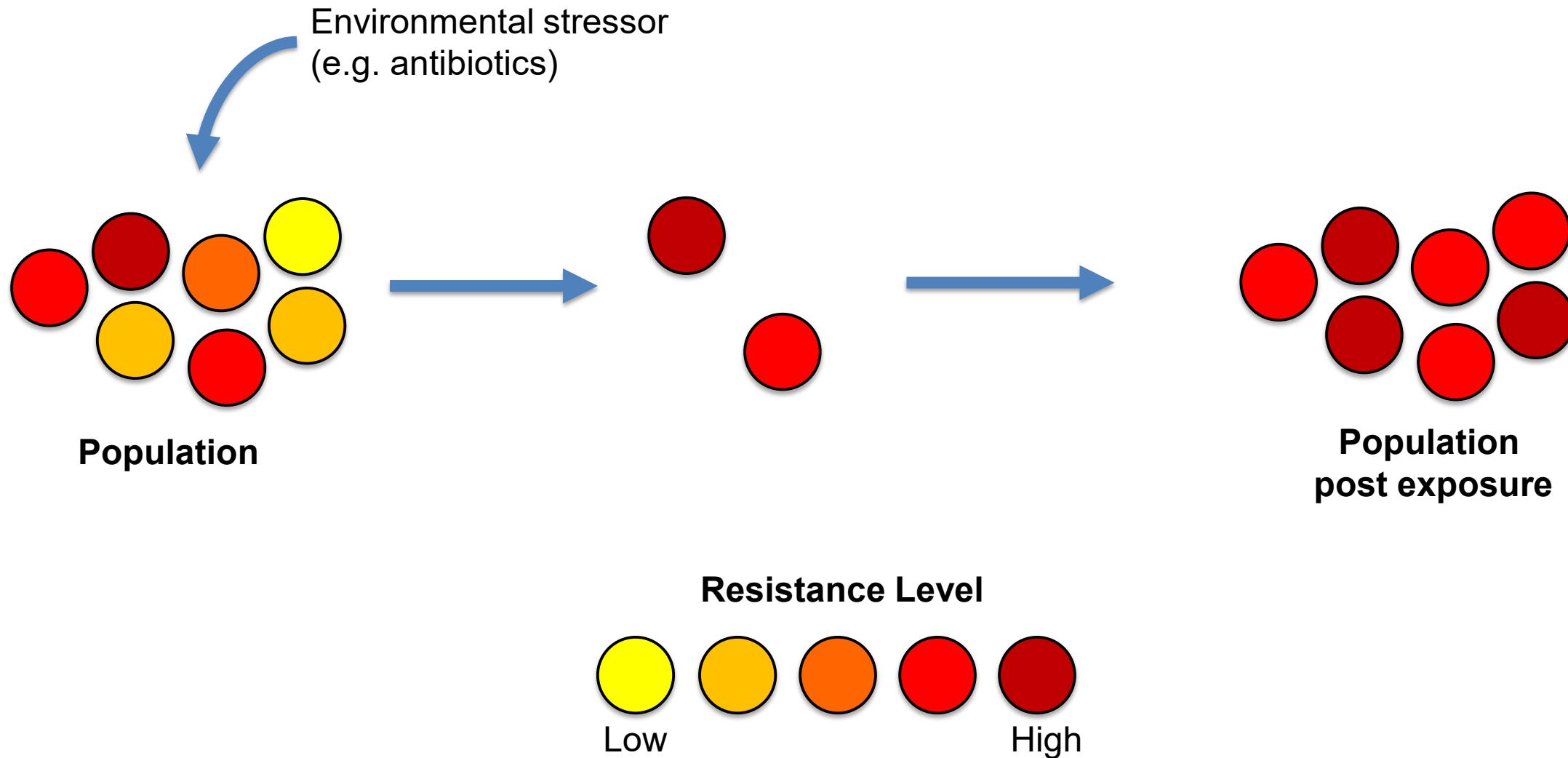


Lateral gene transfer between RNA viruses and cellular organisms



Evolution & diversity

Evolution – Natural selection



Measuring natural selection

$d_N = \# \text{ nonsynonymous substitutions} / \# \text{ nonsynonymous sites}$

$d_S = \# \text{ synonymous substitutions} / \# \text{ synonymous sites}$

Test for selection by comparing d_N and d_S

$d_N / d_S = 1$: Neutral evolution

$d_N / d_S < 1$: Purifying selection

$d_N / d_S > 1$: Positive selection



RESEARCH ARTICLE | BIOLOGICAL SCIENCES | FREE ACCESS



Weak selection on synonymous codons substantially inflates dN/dS estimates in bacteria

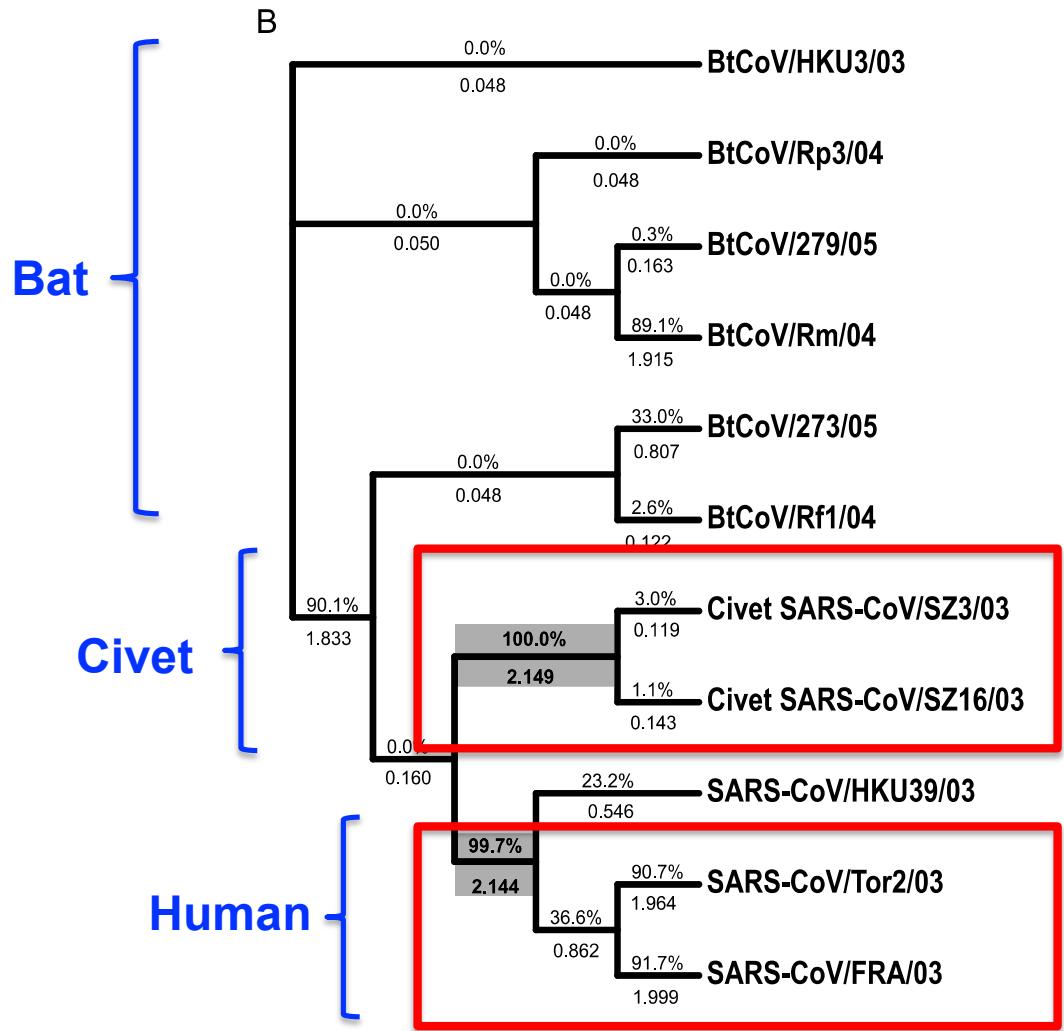
Shakibur Rahman , Sergei L. Kosakovsky Pond , Andrew Webb, and Jody Hey [Authors Info & Affiliations](#)

May 10, 2021 | 118 (20) e2023575118 | <https://doi.org/10.1073/pnas.2023575118>

The d_N/d_S ratio (ω) measures the selective pressure

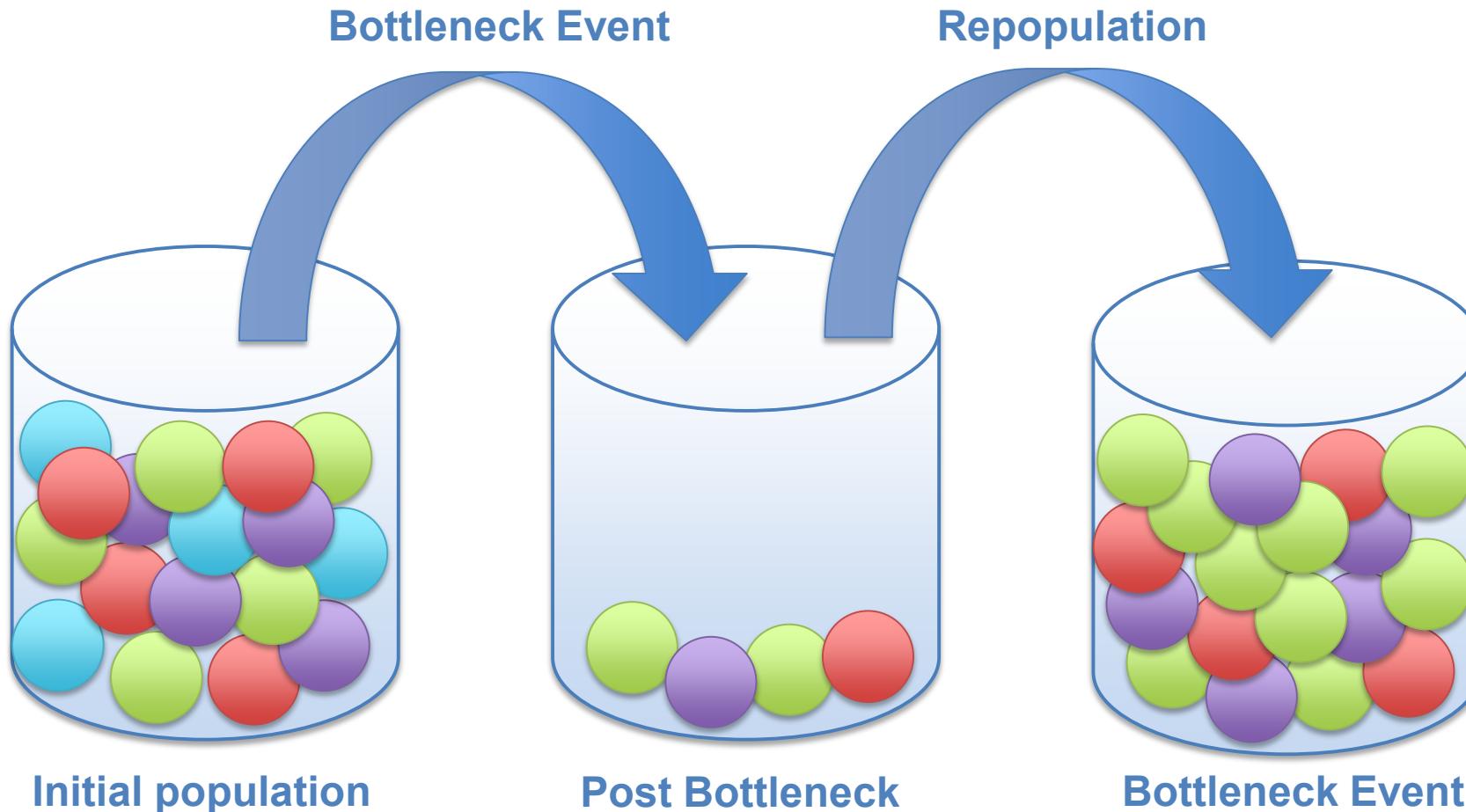
Using natural selection

Lineage-specific selection pressure (B) on the spike genes of SARS-CoVs. Mean ω values calculated using GA are presented **above** branches, with ω values of 1 shown in bold. Numbers below branches indicate an averaged model probability of 1 along specific lineages. Branches with confidence > 95% are highlighted in gray.



Intra-host population diversity

And evolution via bottlenecks



Evolutionary strategies

Bacteria & DNA viruses

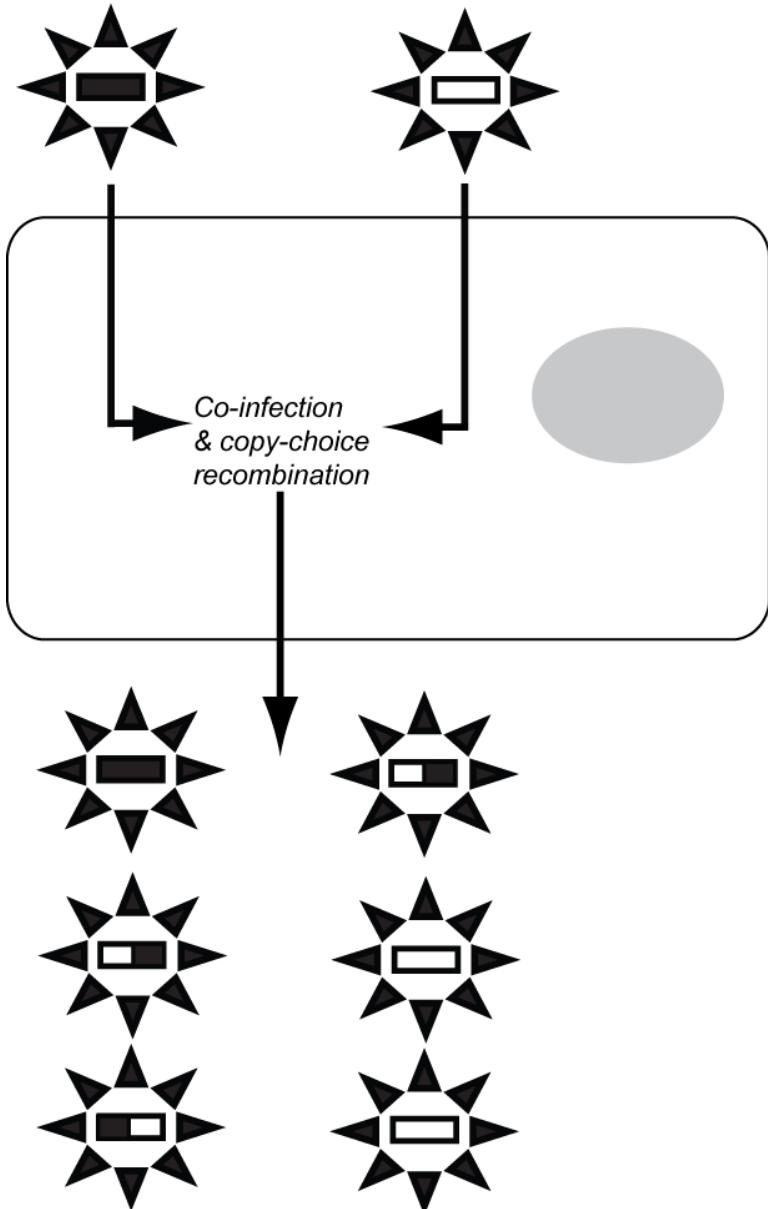
- Mutation
- Recombination

RNA viruses

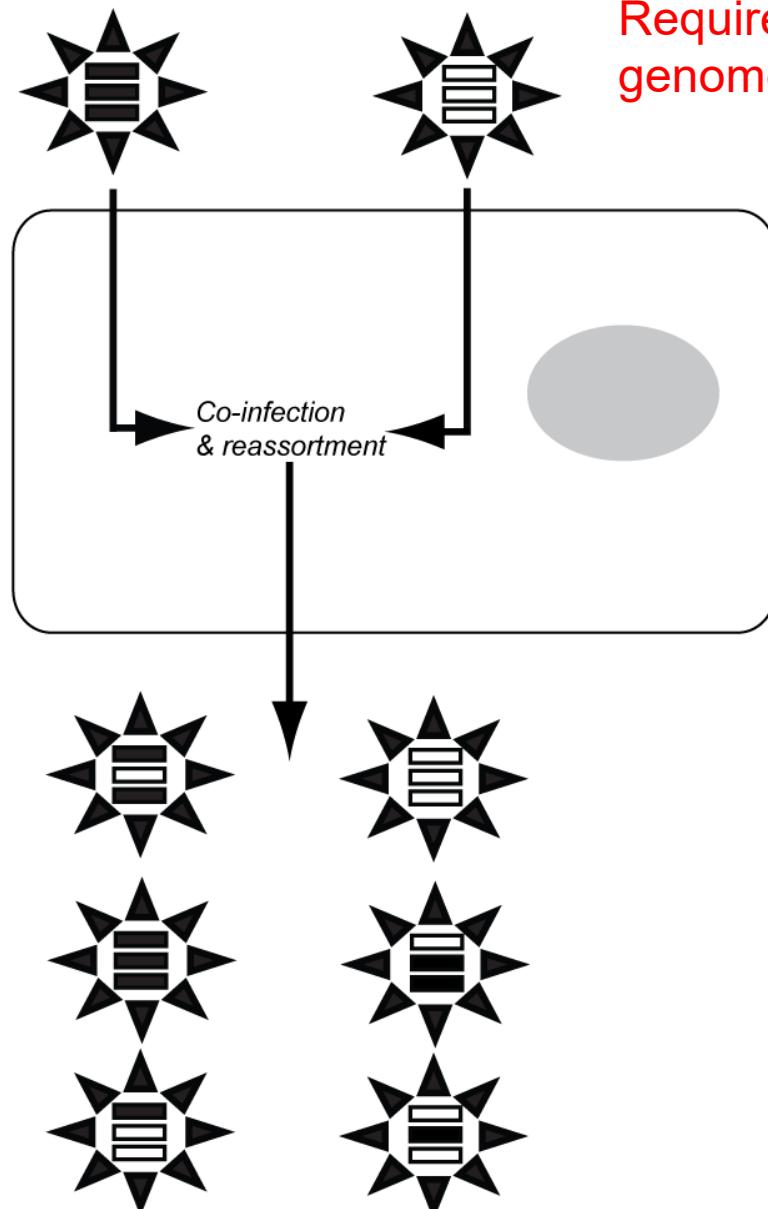
- Mutation
- Reassortment
- Recombination

These mechanisms generate genetic diversity within a population & lead to divergence between populations

(a) RNA recombination



(b) Reassortment



Requires a segmented genome

Mutation rates

- Error rate of human DNA polymerase is approximately 10^{-9}
 - 3 mutations per replication of the human genome
 - Error correction machinery lowers this to 10^{-11}
- Virus RNA and DNA polymerases are much more error prone
 - RNA dependent RNA pol error rates: $10^{-4} - 10^{-5}$
 - DNA polymerases: $10^{-6} - 10^{-7}$

Generating virus diversity

Given:

- An RNA virus with a genome of 10 kb
- RDRP error rate of 10^{-4} $10,000nt \times 10^{-4} = 1nt$
- 1 mutant in 1 position for every virion produced

If 10^9 viral particles produced in a person per day, then 10^9 mutant progeny are being produced in that one individual each day of infection!

Also many multiple mutations will be present

Rates of viral evolution

Group	Virus	Mutation Rates (Mutations per Nucleotide per Replication Cycle)	Evolutionary Rate (Substitutions per Nucleotide Site per Year)
Positive-stranded RNA	Poliovirus 1	2.2×10^{-5} – 3×10^{-4}	1.17×10^{-2}
Negative-stranded RNA	Influenza A virus	7.1×10^{-6} – 3.9×10^{-5}	9×10^{-4} – 7.84×10^{-3}
Retrovirus	Human immunodeficiency virus 1	7.3×10^{-7} – 1.0×10^{-4}	1.13×10^{-3} – 1.08×10^{-2}
Single-stranded DNA	Bacteriophage phiX174	1×10^{-6} – 1.3×10^{-6}	Unknown
Double-stranded DNA	Herpes simplex 1	5.9×10^{-8}	8.21×10^{-5}

Units are important

Viral evolution

Problem: Two strains of H1 subtype swine influenza have 21nt differences along the HA gene. How long did it take these two strains to diverge?

rate = 4.6×10^{-3} substitutions per site per year (ssy)

length = 1701 nucleotide sites

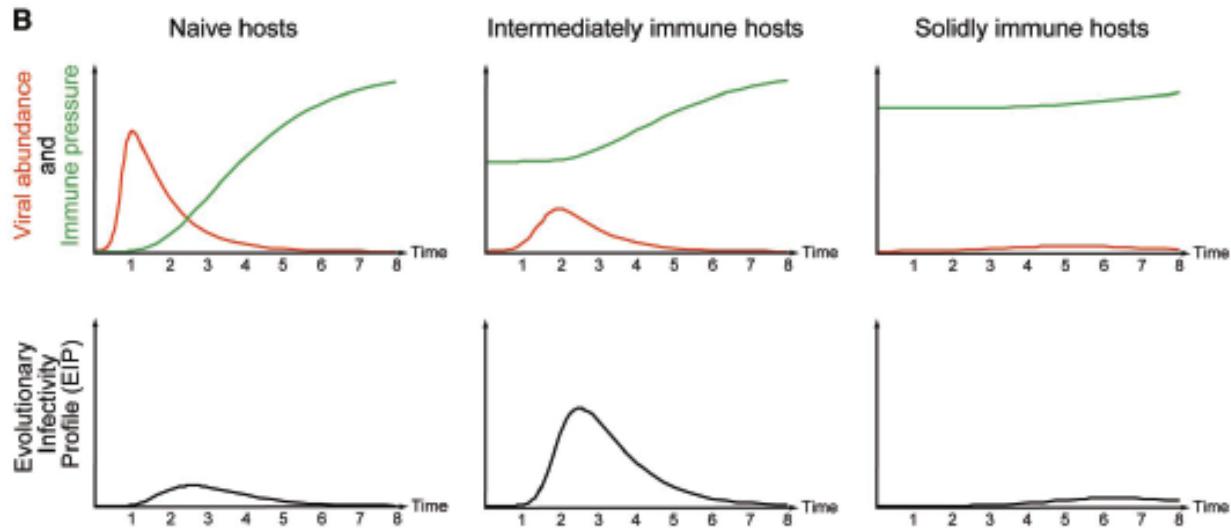
$1701\text{nt} \times 4.6 \times 10^{-3} \text{ ssy} = 7.8$ substitutions per year (sy)

$21 \text{ substitutions} \div 7.8 \text{ sy} \approx 2.69 \text{ years}$

Analytical approaches

Phyldynamics

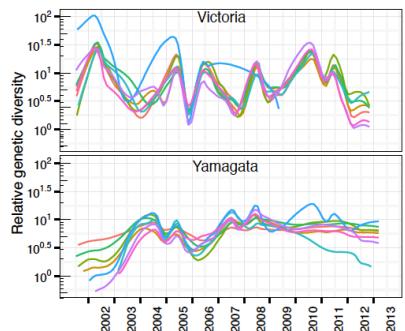
- Behavior of genetic change that arises from the combined effects of evolutionary and ecological processes



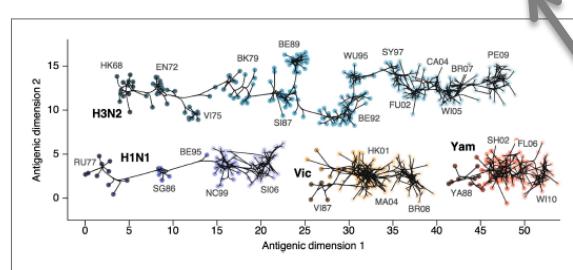
Continual Immune Selection	Weak or Absent Immune Selection		
	Tree shape controlled by non-selective population dynamic processes		
Population size dynamics	Exponential growth	Strong spatial structure	
Constant size	Weak spatial structure	Weak spatial structure	
Examples	Human influenza A virus intra-host HIV	inter-host HIV inter-host HCV	Measles, rabies inter-host HIV
Tree Inferences	Detection of antigenic escape mutations	Estimation of population growth rates	Estimation of population migration rates

VIRAL PHYLOGENETIC DYNAMICS

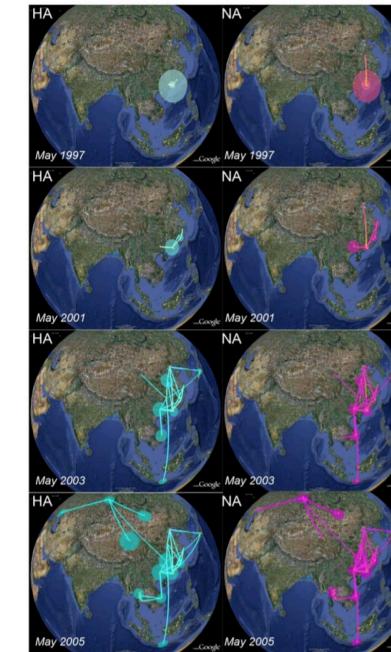
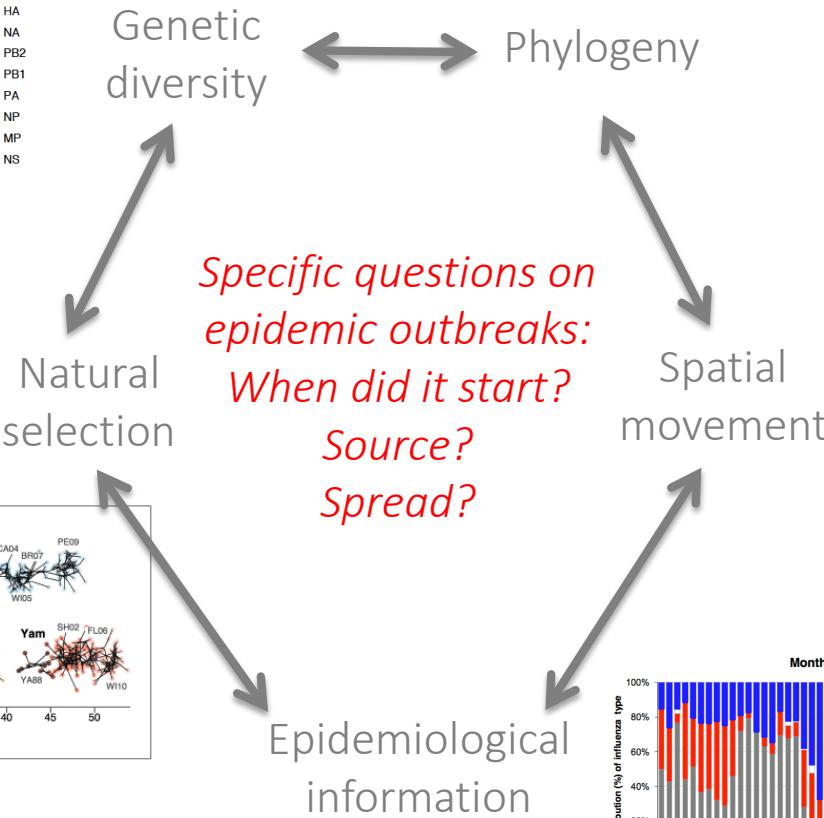
“the study of how epidemiology, immunological, and evolutionary processes act and potentially interact to shape viral phylogenies (Volz, Koelle and Bedford, 2013)”



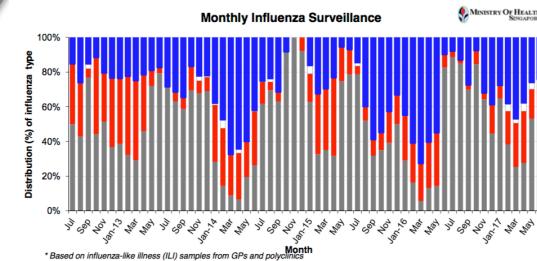
Vijaykrishna et al. (2015).
eLife 4: e05055.



Bedford et al. 2014 eLife 3:
e01914



Lemey (2009). *PLoS Computational Biology*.



Analytical developments (1)

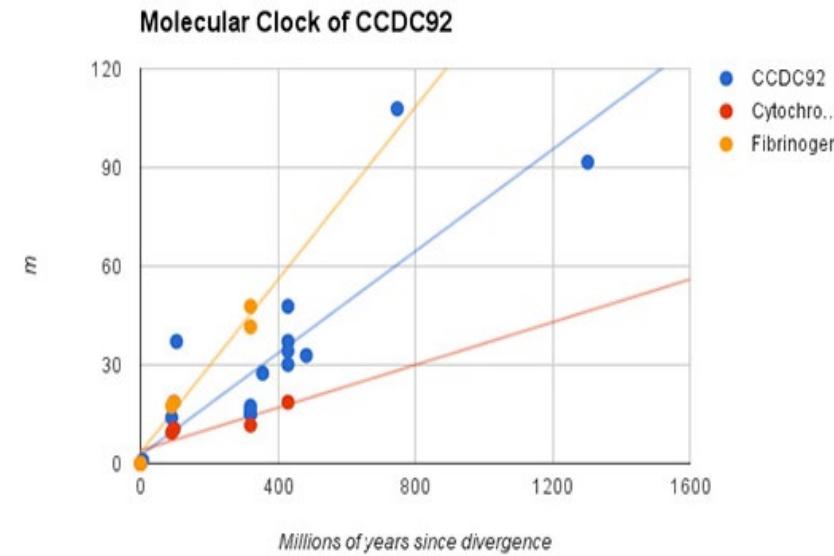
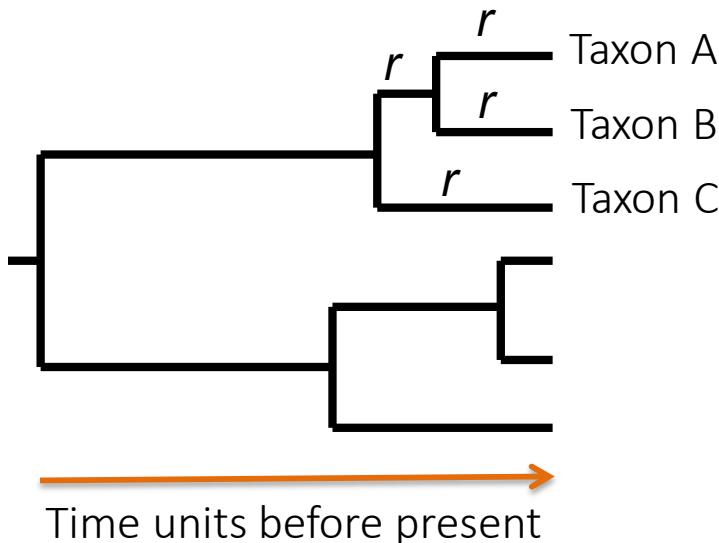
- Greatly aided by increased computer processing power
- More robust statistical methods available
 - **Molecular clocks:** A statistical model that describes the relationship between time & the genetic distance among nucleotide sequences
 - **Coalescent theory:** Describes the shape & size of genealogies over time, allowing detection of changes in population diversity over time

Analytical developments (2)

- Allows us to address key biological questions, such as:
 - When did a newly emergent epidemic begin?
 - From which population/species did it emerge?
 - The order & timing of transmission events & viral adaptations (natural selection)
 - Changes in virus population behavior/diversity following interspecies transmission

Molecular clock hypothesis

- Introduced by Pauling and Zuckerkandl in 1962
- Based on fossil evidence, they proposed that the rate of evolution in a given protein is nearly constant over time and among different lineages
- All branches in the tree evolve at the same rate (i.e. constant), and this will give a **linear** relationship between genetic distance and time since divergence

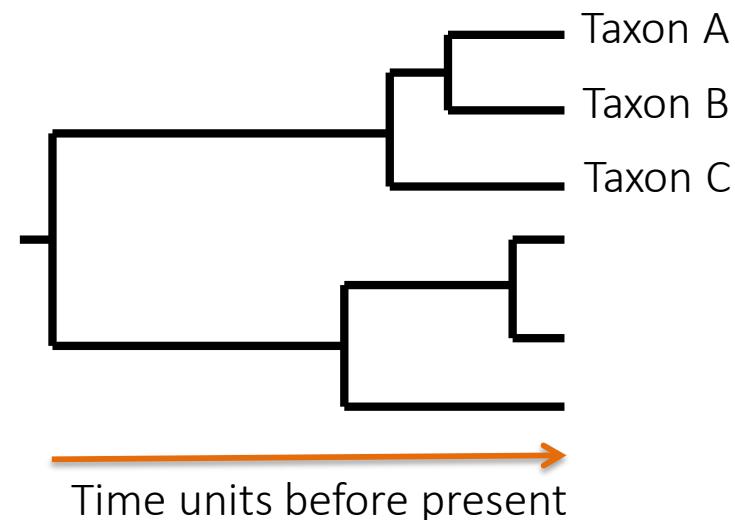
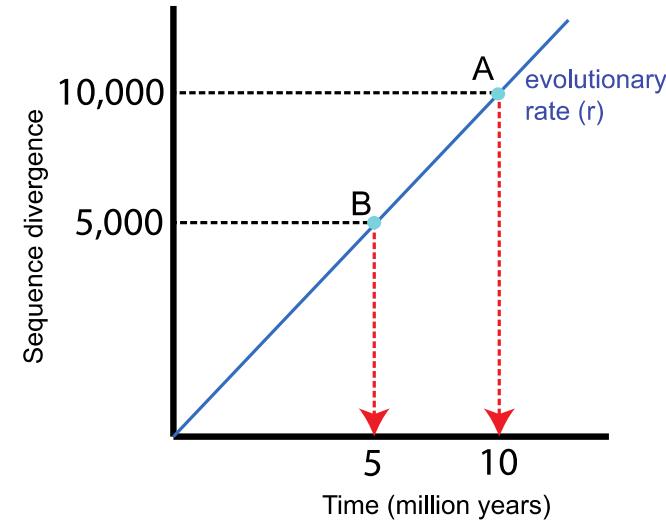


Constant (strict) molecular clock

Nucleotide substitutions accumulate at a fixed (constant) rate over time, this can be used to estimate divergence times between sequences

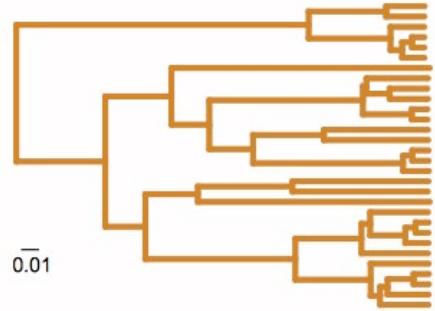
For example:

If the genes of two taxon A and B differ in 5,000 mutations (=genetic distance), and we know the mutation rate (1,000 mutations for every million years), then the two diverged about 5 million years ago



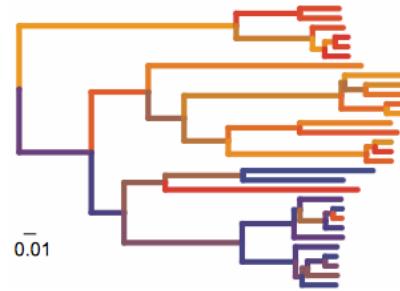
Strict versus relaxed clocks

(b) Strict clock



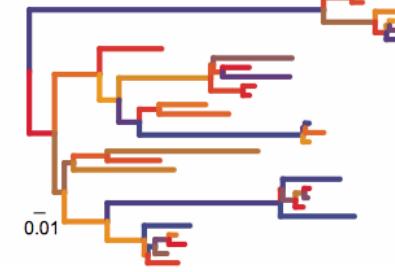
Constant rate among branches

(e) Autocorrelated relaxed clock



A distinct rate along each branch that is correlated with the rate along its parent branch

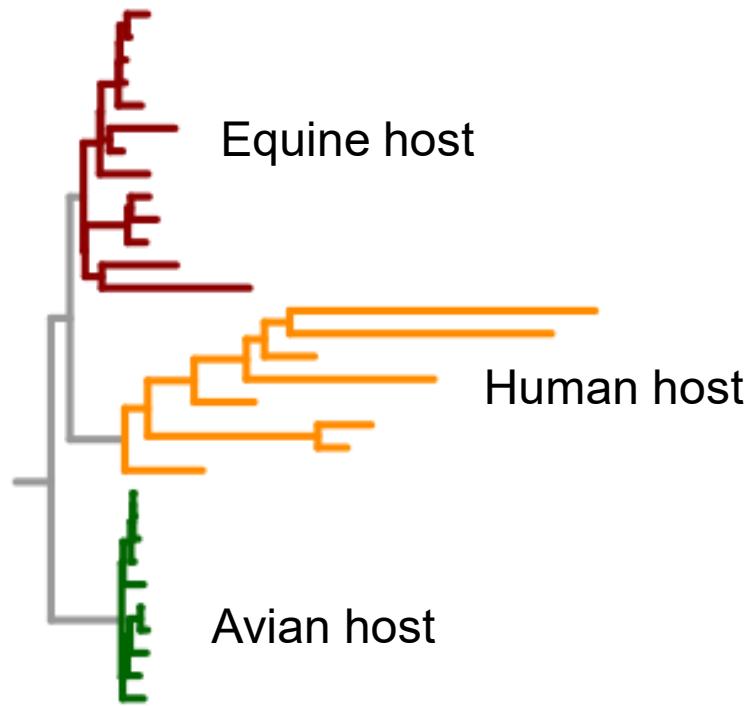
(f) Uncorrelated relaxed clock



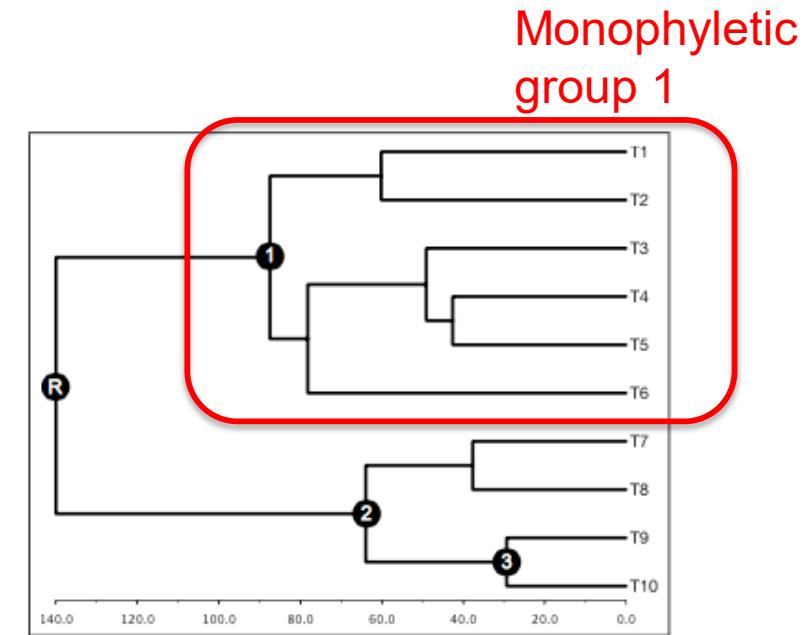
A distinct rate along each branch

Fixed local clock

- Allows different regions in the tree to have different rates, but within each region the rate must be the same
- Creates local rate for each taxon set with enforced monophyly

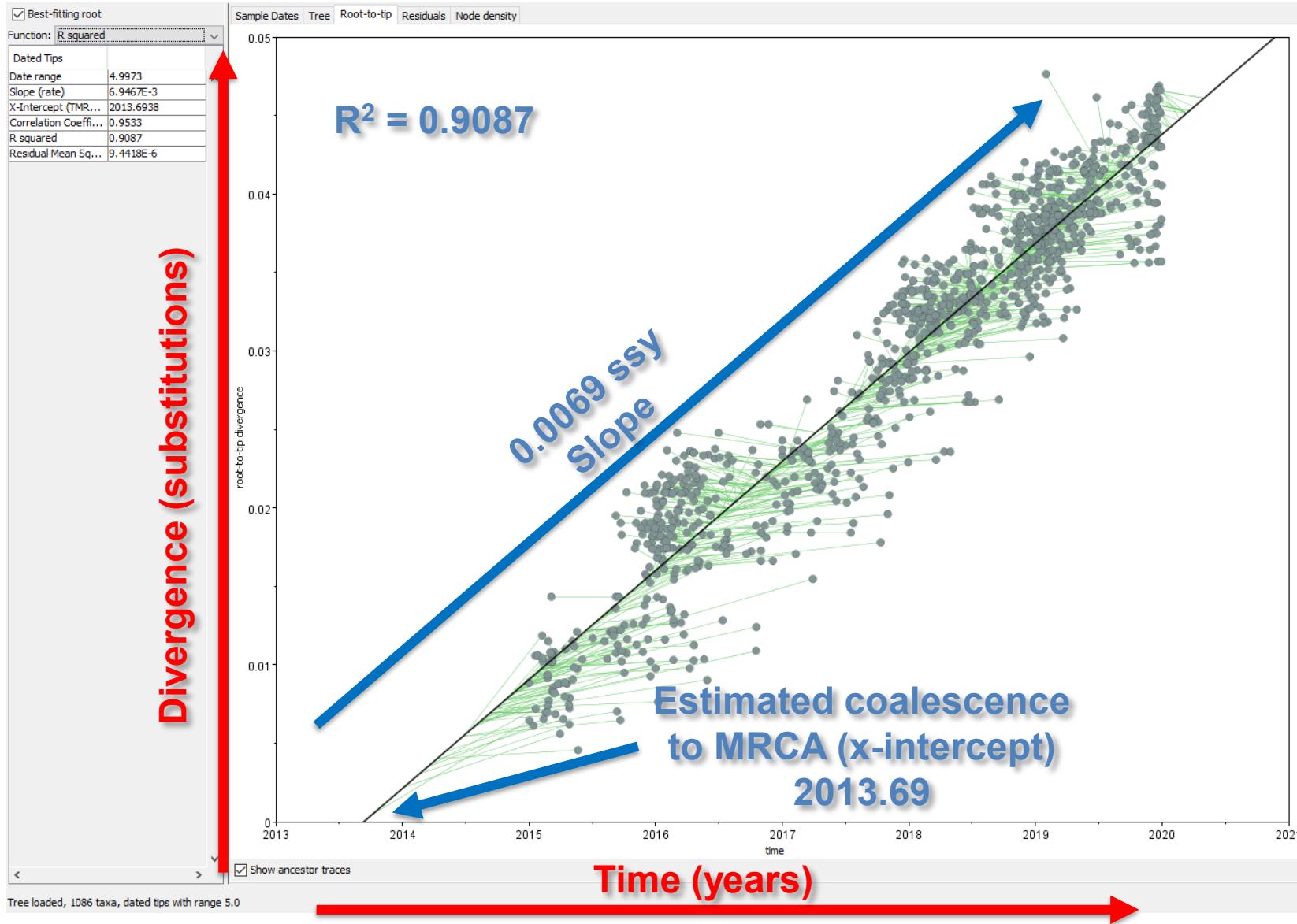


Host specific local clock



Lineage specific local clock

Root-to-tip regression



Some DNA viruses have no temporal structure

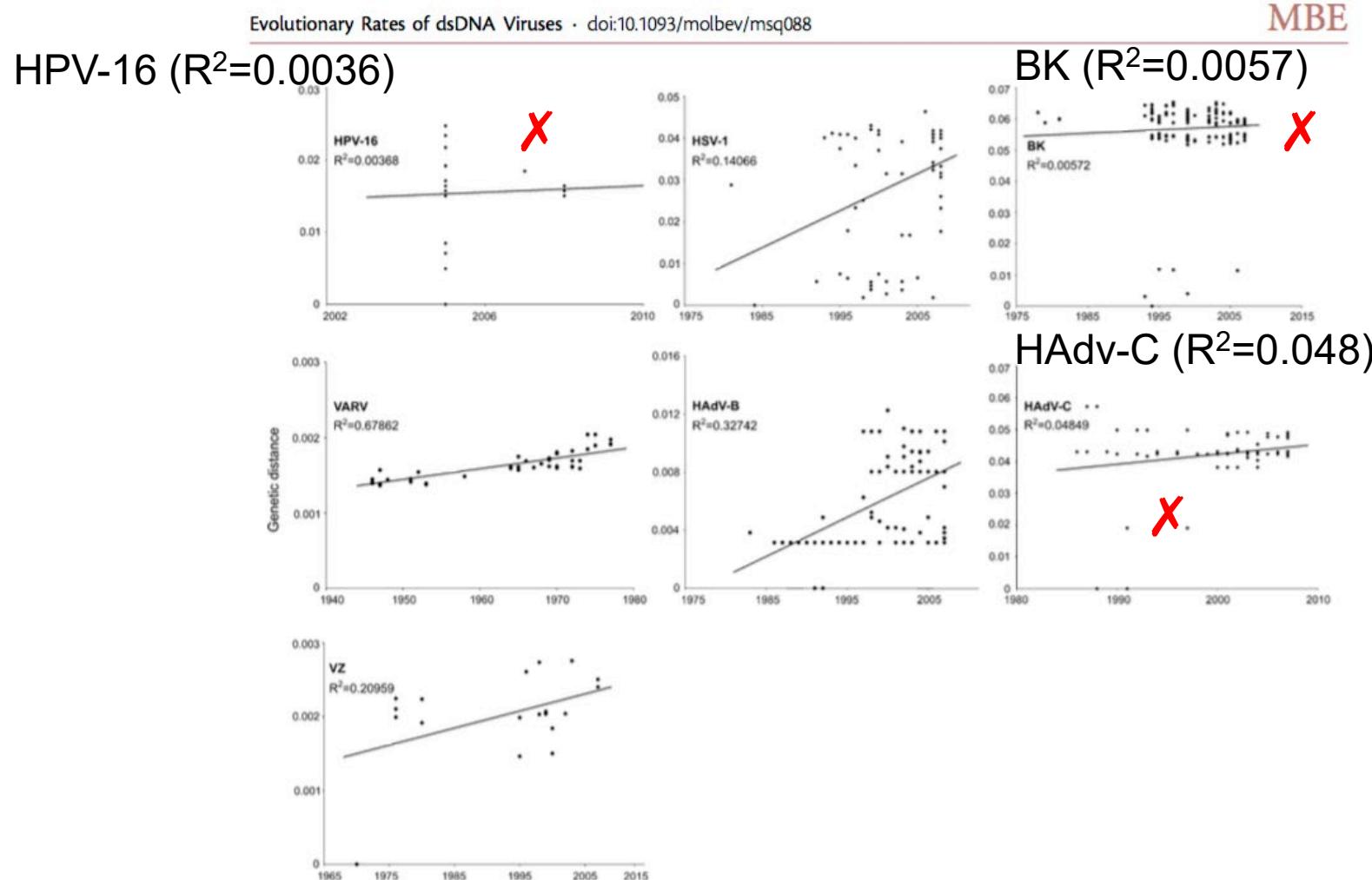


FIG. 3. Genetic distance versus sampling year for the dsDNA viruses (clockwise from top left): HPV-16, HSV-1, BK virus (BK), VARV, HAdV-B, HAdV-C, and VZ virus (VZ). The regression coefficient (R^2) estimates the fit of the data to a strict molecular clock by testing the degree of influence sampling time has over the amount of pairwise diversity in the data. This analysis supports the presence of temporal structure in the data for VARV and HAdV-B, while suggesting the presence of temporal structure for HSV-1 and VZ. No evidence for temporal structure within the sampled period was found for the HPV-16, BK, and HAdV-C data sets using this method.

Case studies in influenza

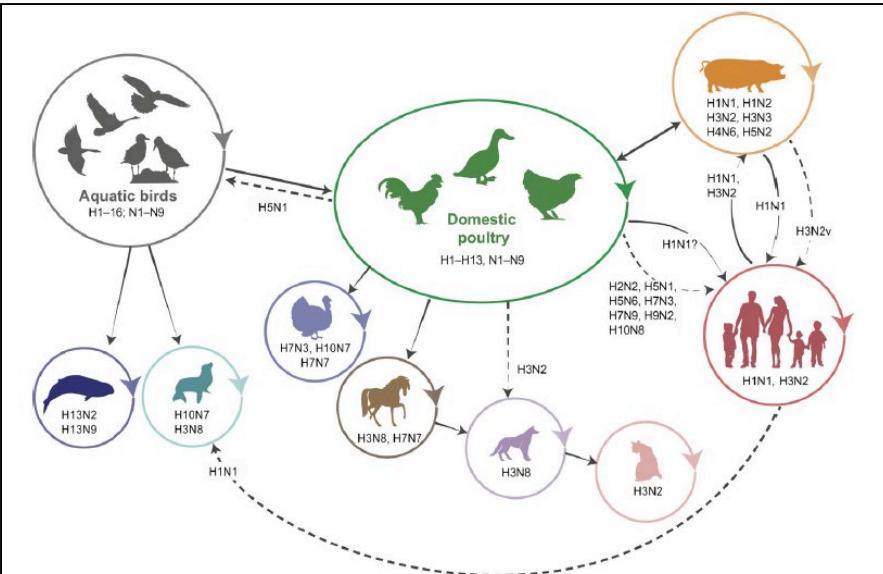
Natural history of influenza & pandemic emergence

ROLE OF WILD BIRDS IN INFLUENZA ECOLOGY

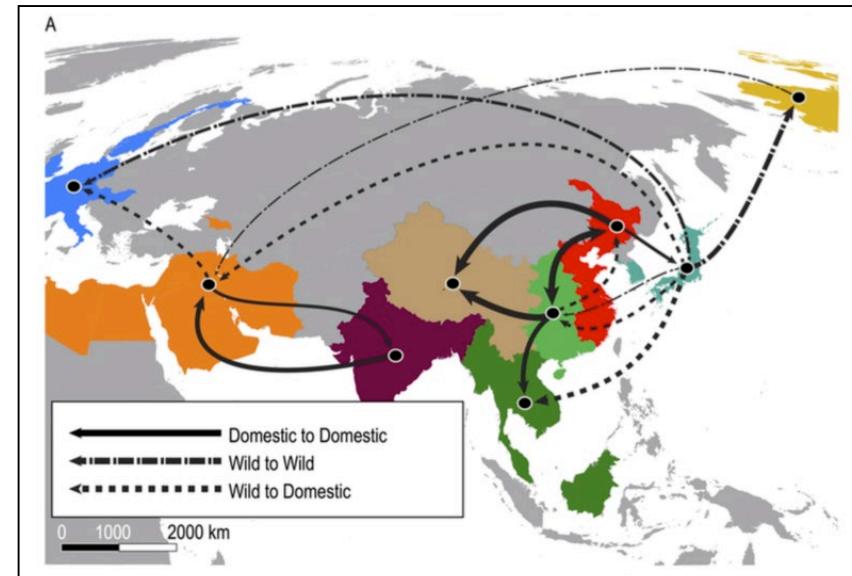


Photo: Yvonne Su

- **Wild aquatic birds** (e.g. ducks, geese, shorebirds, gulls etc.) are the primary reservoir of avian influenza viruses
- Wild birds can infect a range of hosts, but direct transmission of IAV from a wild bird source to humans is rare
- Migratory nature of the bird species plays an important role in IAV transmission and persistence

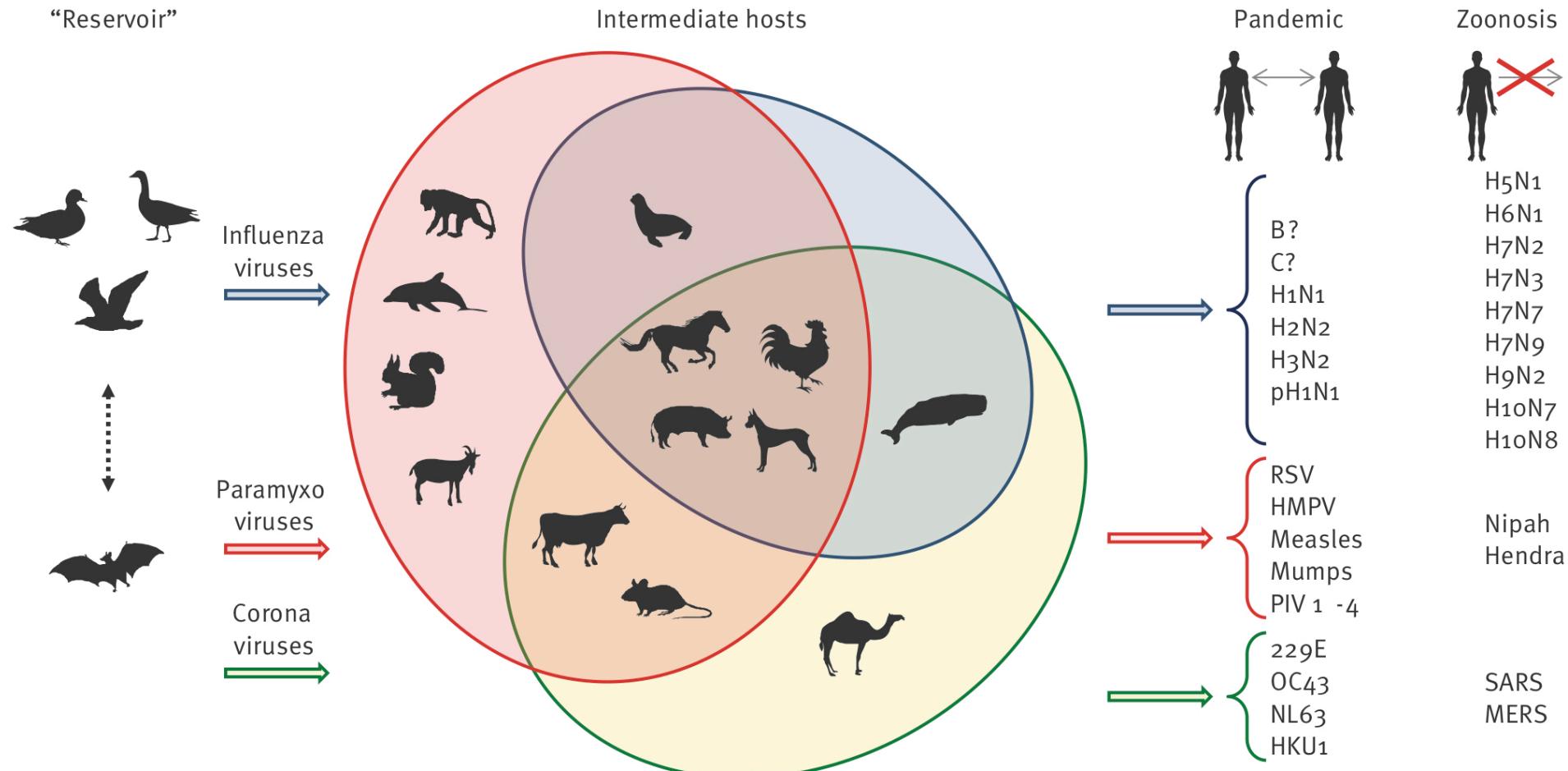


Joseph U, Su YCF, Vijaykrishna & Smith GJD (2017).
Influenza and other respiratory viruses 11: 74–84.



Bahl J et al. (2016). *PLoS Pathogens*
12:e1005620.

Ecology of pandemic emergence

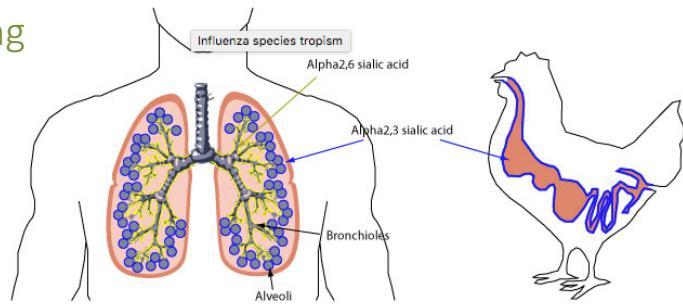


INTERSPECIES TRANSMISSION

Influenza key host species: aquatic birds, poultry (chicken & duck), pigs and humans

- Swine regarded as mixing vessels or intermediate host
- Little synergy to understand the ecology of interspecies adaptation and transmission

Receptor binding preference



Transmission modes



Roche et al. (2014). PLoS Biol.
12: e1001931.

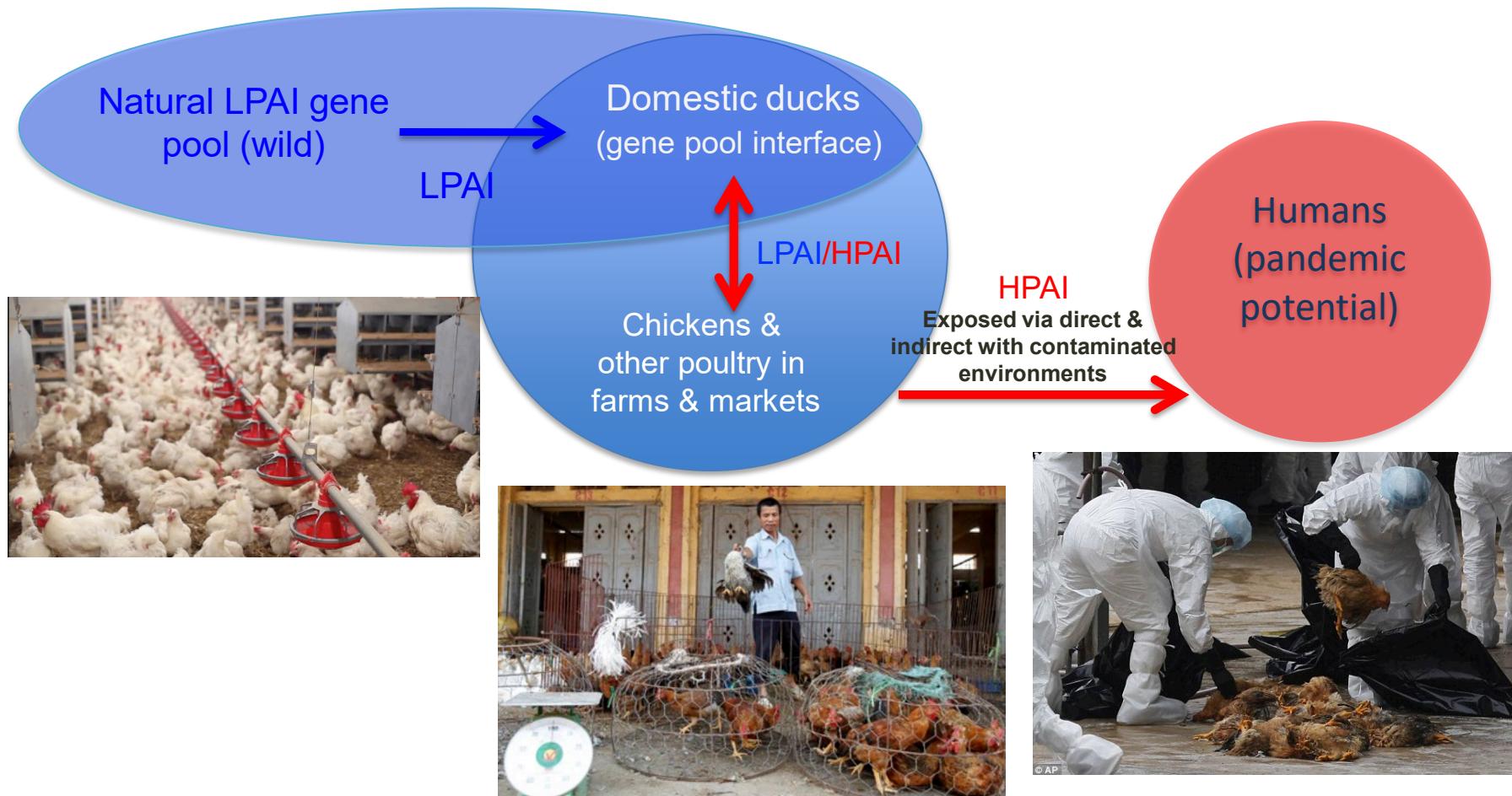
Increased human consumption of meat



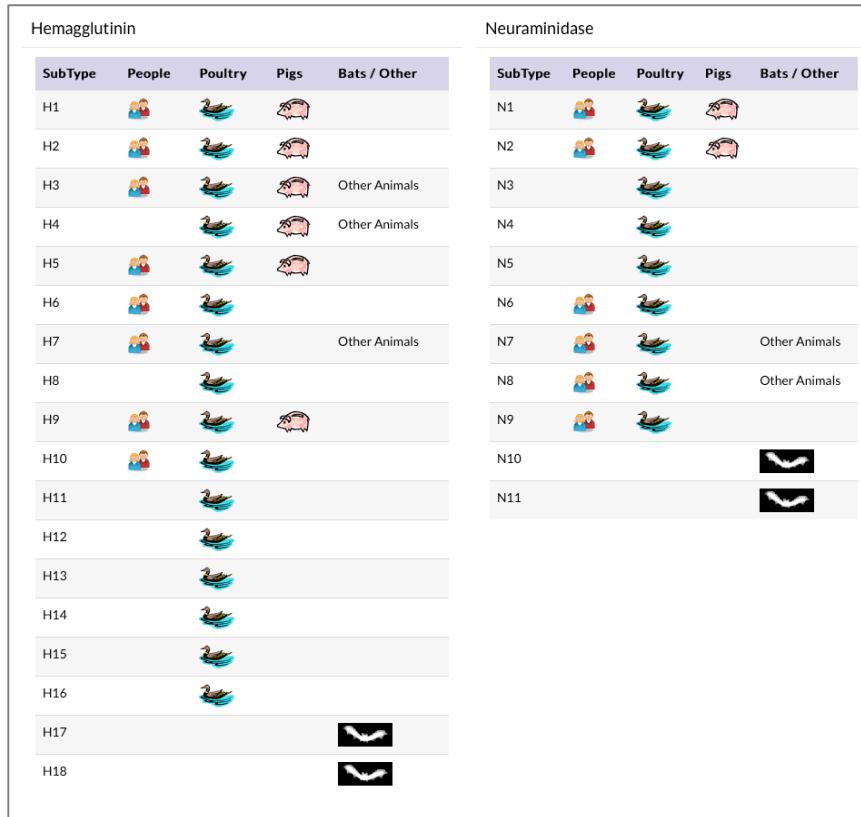
Live poultry market

PANDEMIC POTENTIAL

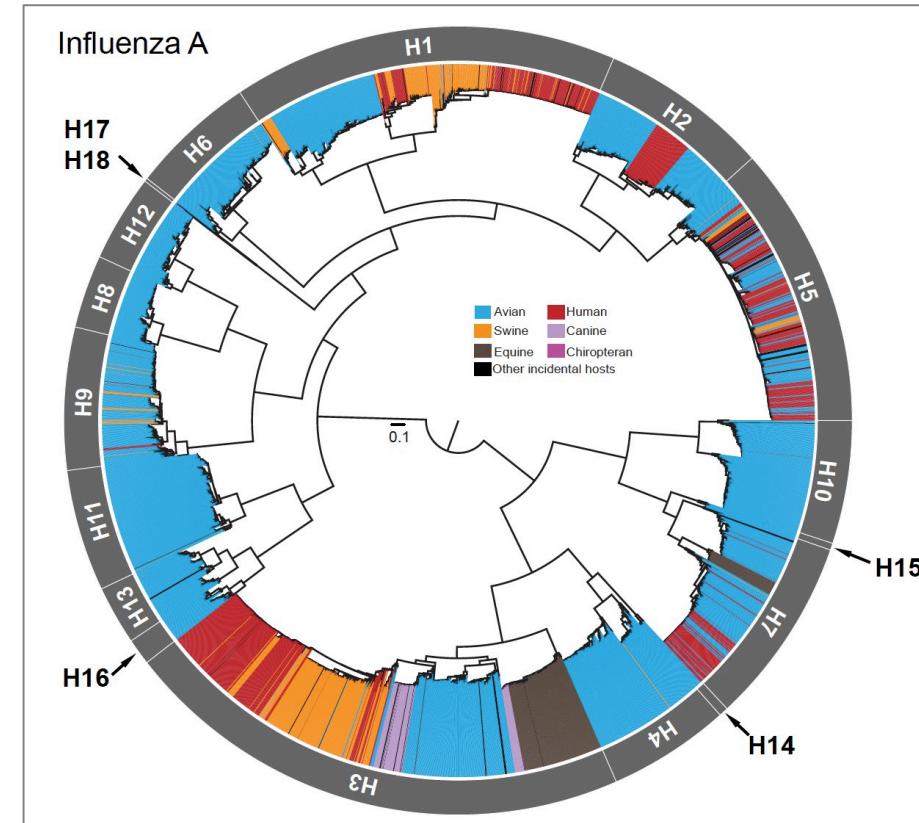
Avian influenza viruses exhibit complex pattern of population dynamics and continue to evolve, therefore active surveillance is crucial for monitoring HPAI outbreaks especially in H5N1, H7N9 and H10N8.



DIVERSITY OF INFLUENZA VIRUSES



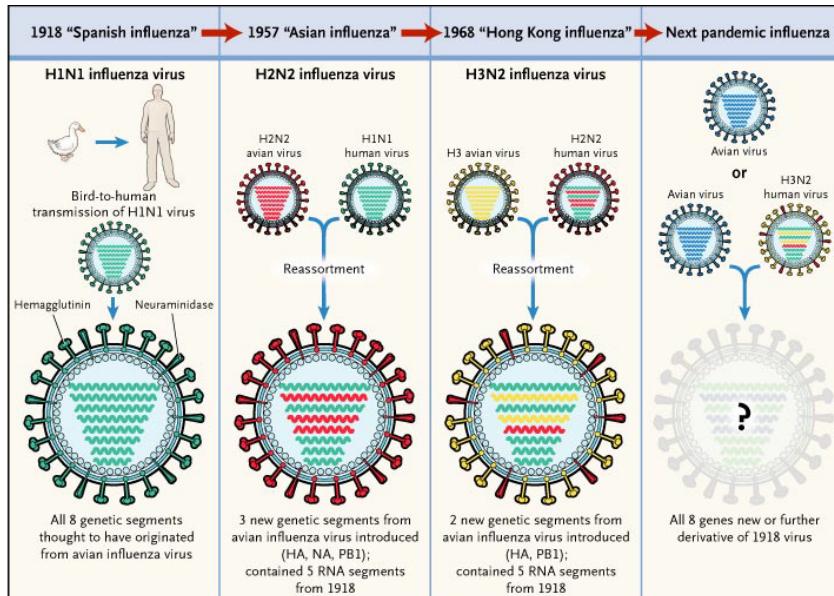
<https://www.cdc.gov/flu/about/viruses/transmission.htm>



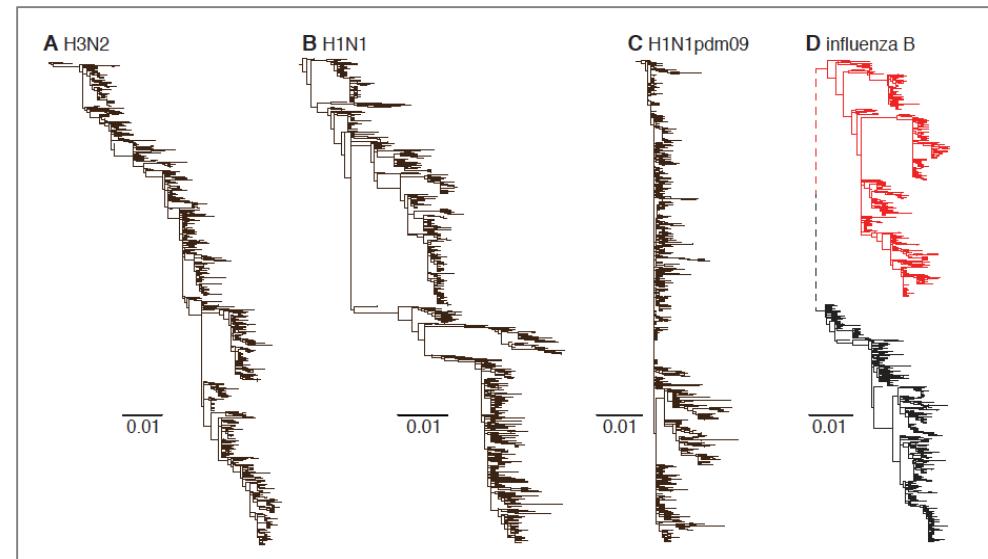
Joseph U, Su YCF, Vijaykrishna & Smith GJD
(2017). *Influenza and other respiratory viruses*
11: 74–84.

EVOLUTIONARY MECHANISMS

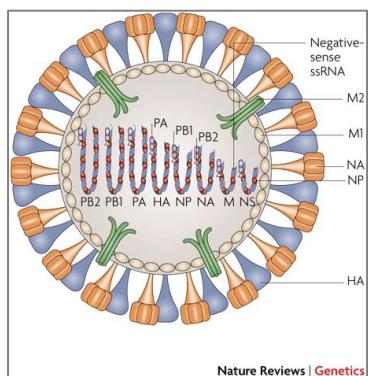
Antigenic **SHIFT**: reassortment



Antigenic **DRIFT**: mutation



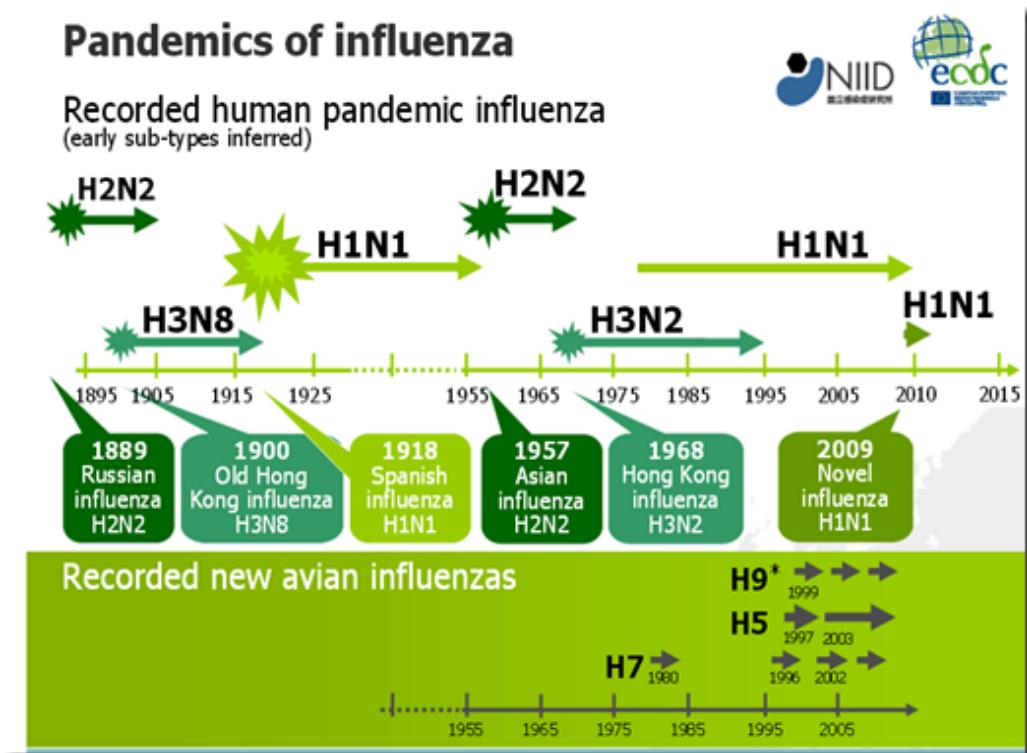
Webster et al. (2013).
Textbook of influenza.



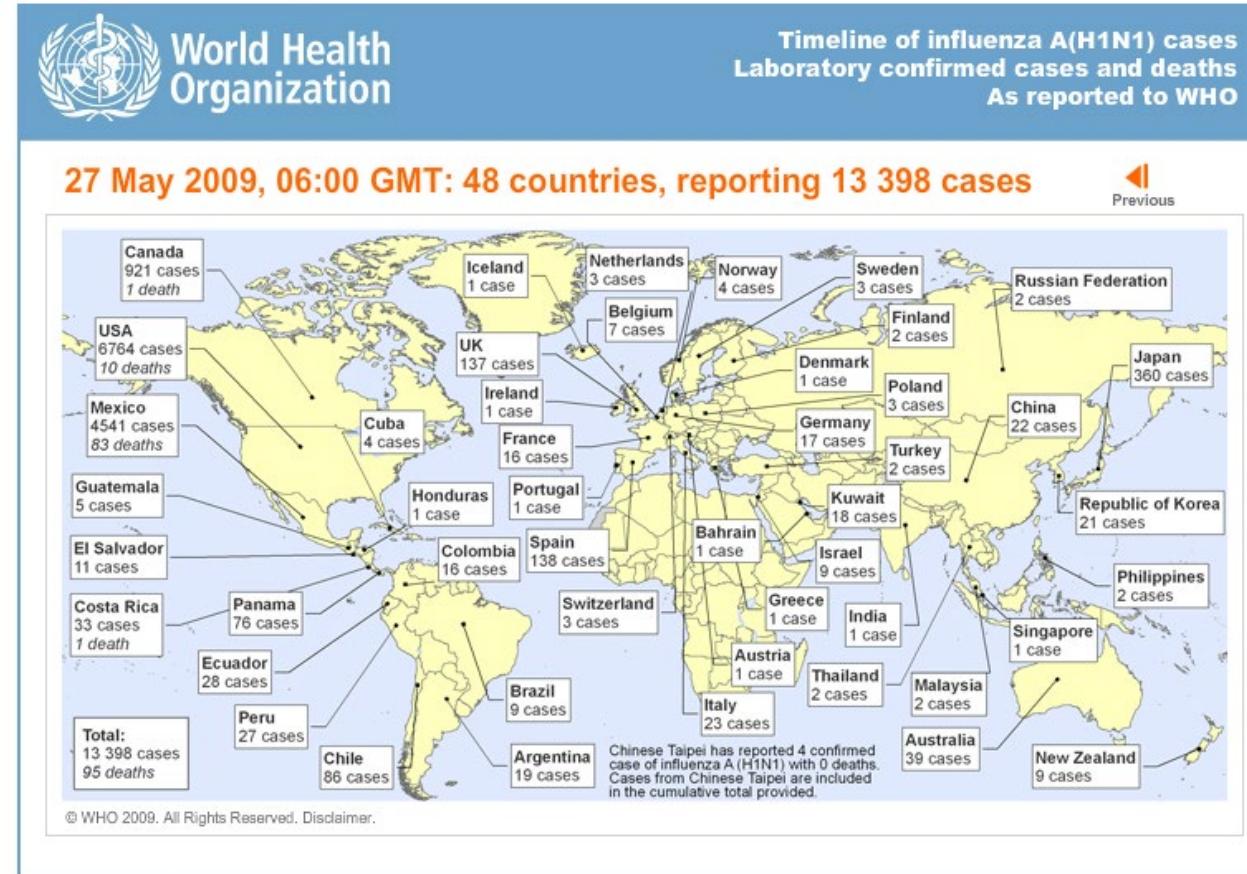
Nelson & Holmes (2007). *Nature Reviews Genetics* 8, 196-205.

Vijaykrishna et al. (2015). *eLife* 4:
e05055.

INFLUENZA PANDEMICS AND MAJOR OUTBREAKS



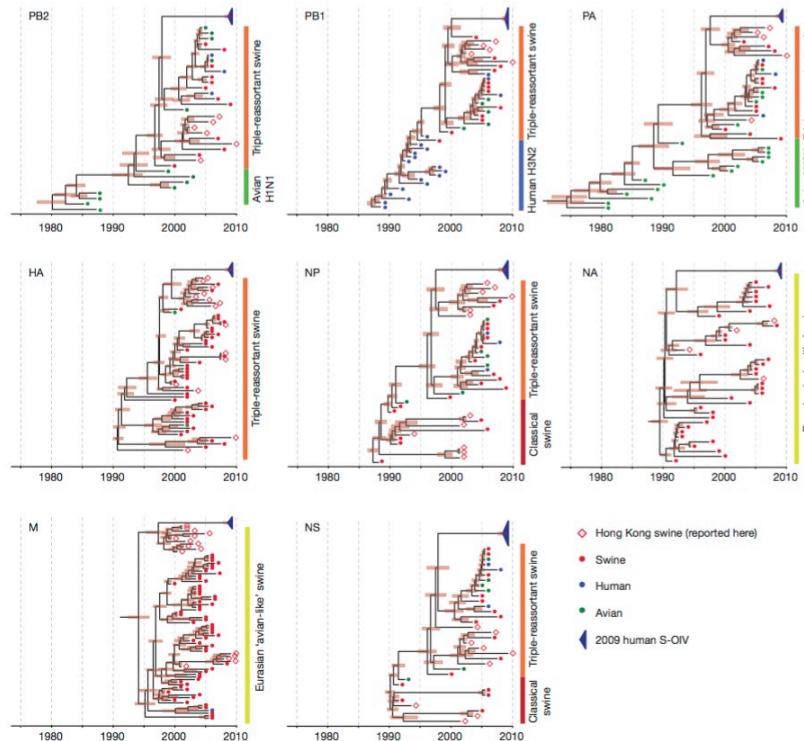
EMERGENCE OF H1N1/2009 VIRUS



First human H1N1/2009 infection detected on Mar 17, 2009 in Mexico.

EMERGENCE OF H1N1/2009 VIRUS

TMRCAs (time to the most recent common ancestor) of the swine-origin H1N1 virus



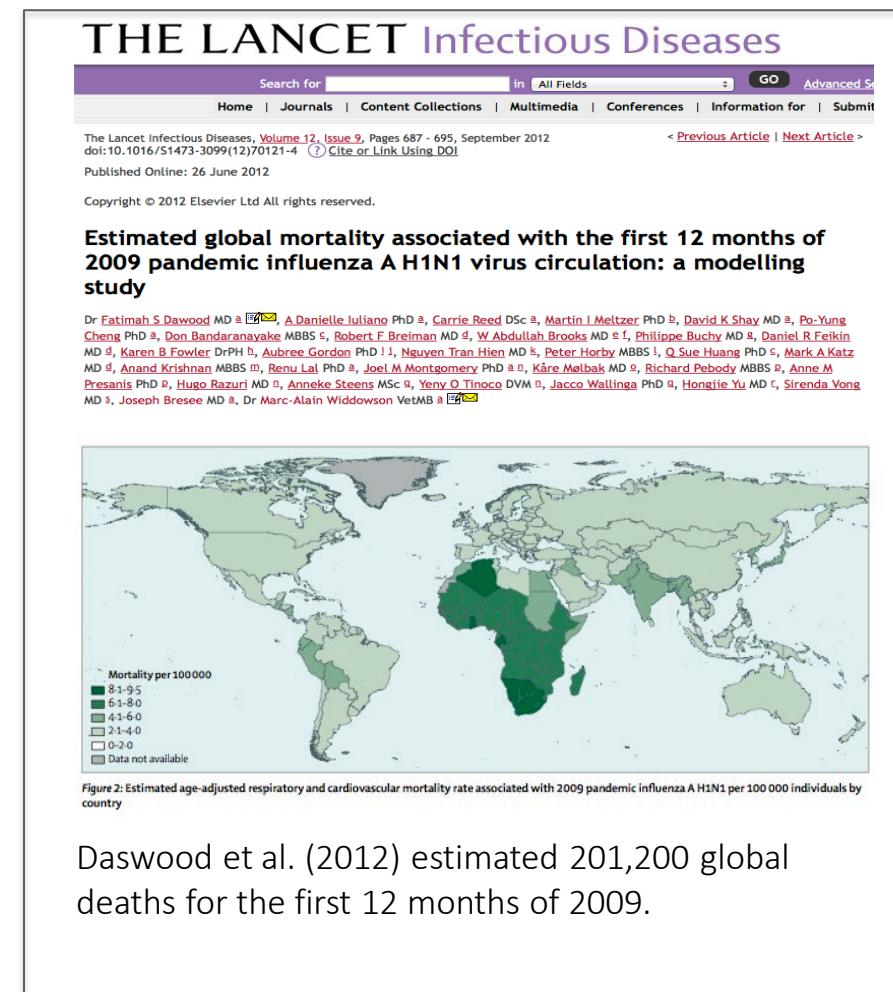
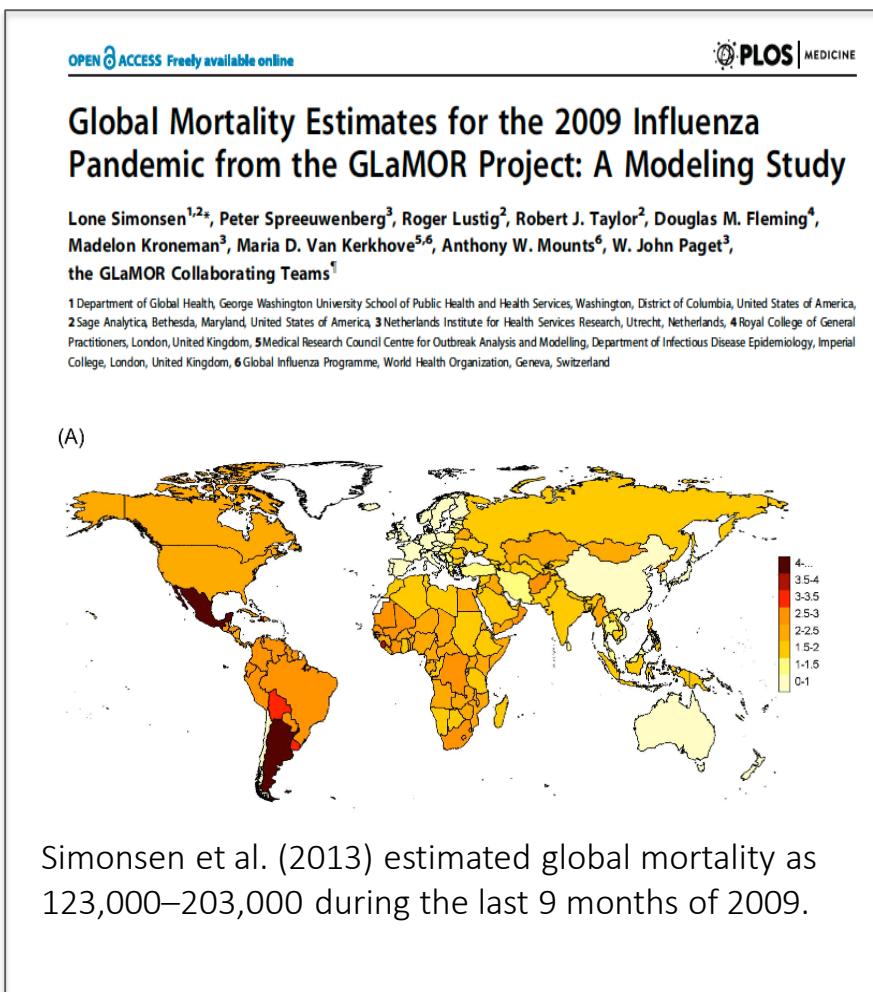
Smith et al. (2009) Nature 459:1122-1125

Reconstruction of the sequence of reassortment events leading up to the emergence of H1N1

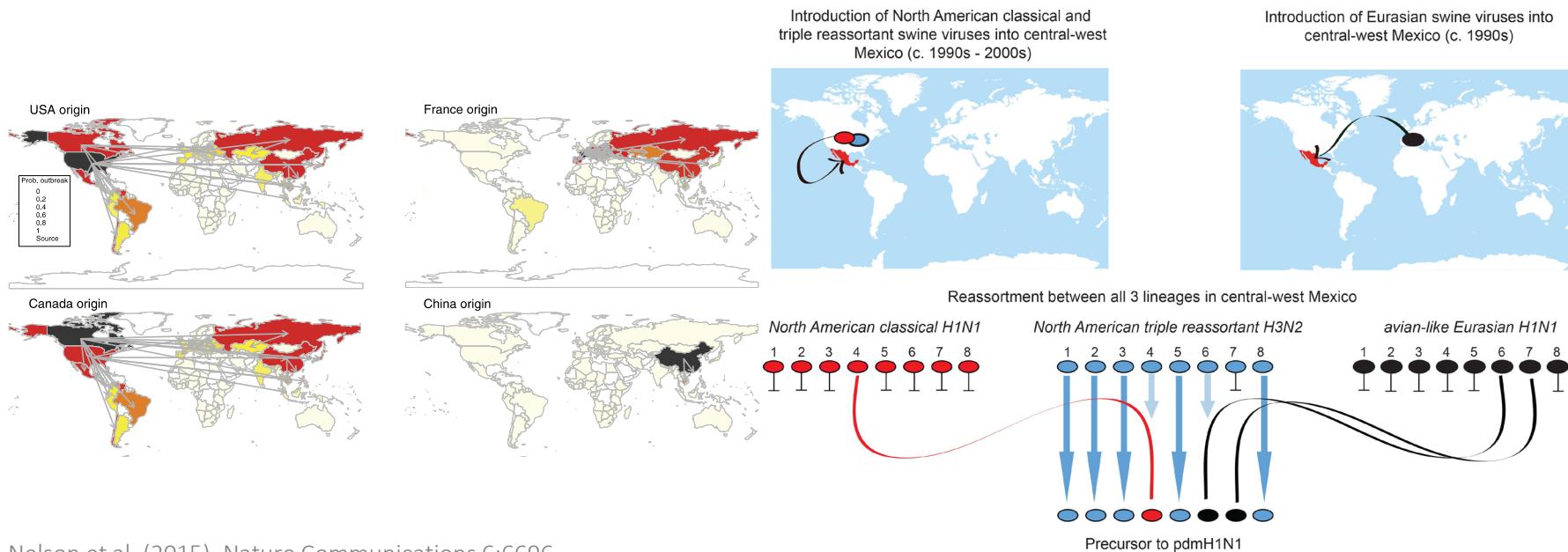


- The segments have been circulating undetected in swine for a decade or more but only emerged in humans several months before the outbreak

Global estimates of H1N1/2009 deaths



RECENT WORK IN SWINE INFLUENZA



Nelson et al. (2015). Nature Communications 6:6696.

- Europe and North America acting as sources of viruses
- Asia is net importer of swine from Europe and North America

Mena et al. (2016). eLife 5:e16777.

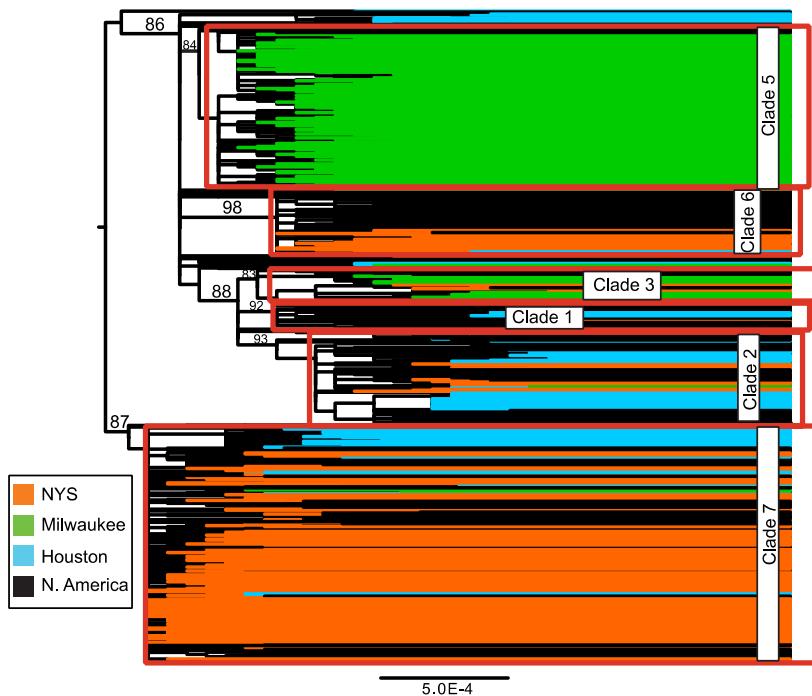
Long-distance trade of pigs allowed divergent Eurasian and North American viruses to co-circulate and reassort in central Mexico

Evolution of H1N1/2009 viruses since entering humans

EARLY DIVERSIFICATION OF H1N1/2009 VIRUS

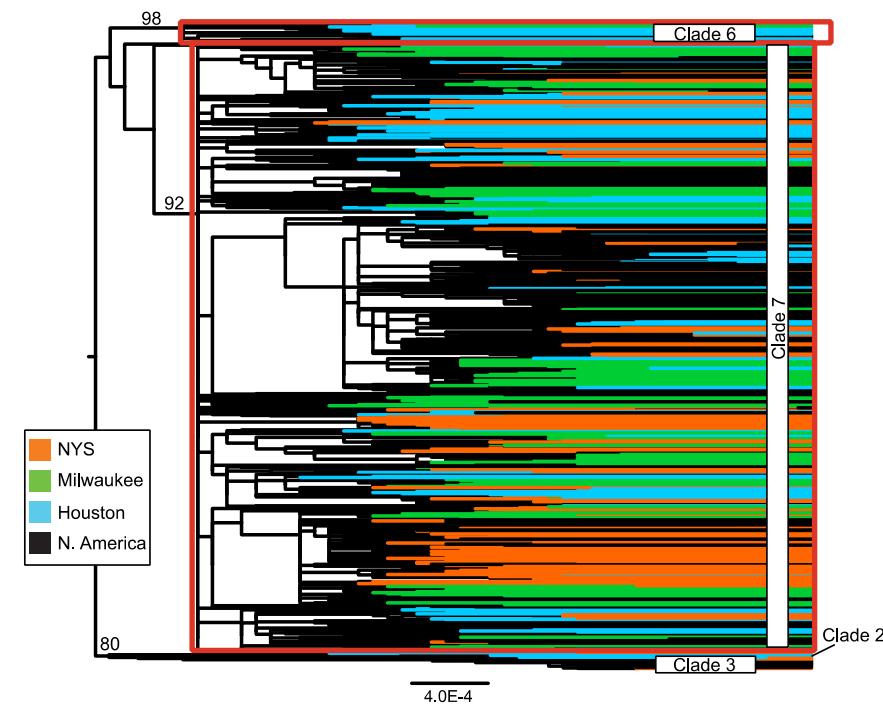
First pandemic wave

(1 April 2009 to 29 July 2009)



Second pandemic wave

(5 August 2009 to 3 March 2010)



Nelson et al. (2009). PloS Curr. 12, 1: RRN1126

EARLY LOCALITY-SPECIFIC EVOLUTIONARY STUDIES

2009–2010: ~54100 sequences
2011–2014: ~11000 sequences

RAPID COMMUNICATIONS

Evolution of the haemagglutinin gene of the influenza A(H1N1)2009 virus isolated in Hong Kong, 2009–2011

G C Mak¹, C K Leung¹, K C Cheng¹, K Y Wong¹, W Lim (wlim@pacific.net.hk)¹

1. Virology Division, Public Health Laboratory Services Branch, Centre for Health Protection, Department of Health, Kowloon, Hong Kong

The screenshot shows the Journal of Virology website. The article title is 'Evolutionary Dynamics of Local Pandemic H1N1/09 Influenza Lineages Revealed by Whole Genome Analysis'. The authors listed are Gregory J. Baillie, Monica Galiano, Paul-Michael Agapow, Richard Myers, Rachael Chiam, Astrid Gall, Anne L. Palser, Simon J. Watson, Jessica Hedge, Anthony Underwood, Steven Platt, Estelle McLean, Richard G. Pebody, Andrew Rambaut, Jonathan Green, Rod Daniels, Oliver G. Pybus, Paul Kellam, and Maria Zambon.

Whole Genome Characterization, Phylogenetic and Genome Signature Analysis of Human Pandemic H1N1 Virus in Thailand, 2009–2012

Jarika Makkoch¹, Kamol Suwanakarn¹, Sunchai Payungporn², Sliporn Prachayangprecha¹, Thaweesak Cheiocharnsri¹, Piyada Linsuwanon¹, Apiradee Theamboonlers¹, Yong Poovorawan^{1*}

¹ Center of Excellence in Clinical Virology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand, ² Department of Biochemistry, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

Multi-year persistence of two pandemic A/H1N1 influenza virus lineages in West Africa

Martha I. Nelson¹, Richard Njouom², Cecile Viboud⁴, Mbayame N.D. Niang³, Hervé Kadjo⁴, William Ampofo⁵, Adedeji Adebayo⁶, Zekiba Tarnagda⁷, Mark A. Miller¹, Edward C. Holmes^{1,8} and Ousmane M. Diop³

+ Author Affiliations

Correspondence: Martha I. Nelson, PhD, Division of International Epidemiology and Population Studies, Fogarty International Center, National Institutes of Health, 16 Center Drive, Room 202, Bethesda, MD 20892 (nelsonma@mail.nih.gov).

Abstract

JOURNAL OF VIROLOGY, July 2011, p. 6923–6929
0022-538X/11/116923-07 \$15.00 doi:10.1128/JVI.00438-11
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Vol. 85, No. 14

EMERIT ARTICLE

Evolutionary Dynamics of 2009 Pandemic Influenza A Virus Subtype H1N1 in South Africa During 2009–2010

Marietjie Venter,^{1,2} Dhamari Naidoo,¹ Marth Pretorius,¹ Amelia Buys,¹ Johanna McAnerney,¹ Lucille Blumberg,³ Shabir A. Madhi,^{1,3} Cheryl Cohen,¹ and Barry Schoub¹

¹National Institute for Communicable Diseases Division, National Health Laboratory Service, and ²Department of Medical Virology, University of Pretoria, and ³Department of Science and Technology/National Research Foundation: Vaccine-Preventable Diseases, University of the Witwatersrand, Johannesburg

Background: The 2009 pandemic influenza A virus subtype H1N1 (A/H1N1/Ind/09) was first detected in June

Molecular evolution of the hemagglutinin and neuraminidase genes of pandemic (H1N1) 2009 influenza viruses in Sendai, Japan, during 2009–2011

Iirona Khandaker · Akira Suzuki · Taro Kamigaki · Kentaro Tohma · Takashi Odagiri · Takashi Okada · Ayumu Ohno · Kanako Otani · Rumi Sawayama · Kazuhisa Kawamura · Michiko Okamoto · Hitoshi Oshitani

OPEN ACCESS Freely available online



Nationwide Molecular Surveillance of Pandemic H1N1 Influenza A Virus Genomes: Canada, 2009

Morag Graham^{1,2*}, Binhu Liang¹, Gary Van Domselaar^{1,2,3}, Nathalie Bastien¹, Carole Beaudoin^{1,4}, Shaun Tyler¹, Brynn Kaplen¹, Erika Landry¹, the National Influenza A/H1N1pdm Genomics Study Team (NIGST)¹, Yan Li^{1,2}

Genetic Diversity of the 2009 Pandemic Influenza A(H1N1) Viruses in Finland

Niina Ikonen^{1*}, Minna Haanpää¹, Esa Rönkkö¹, Outi Lyytikäinen², Markku Kuusi², Petri Ruutu², Hannimari Kalio-Kokko³, Laura Mannonen³, Maija Lappalainen³, Thedi Ziegler¹, Ilkka Julkunen¹

¹Viral Infections Unit, Department of Vaccination and Immune Protection, National Institute for Health and Welfare (THL), Helsinki, Finland, ²Department of Infectious Disease Surveillance and Control, National Institute for Health and Welfare (THL), Helsinki, Finland, ³Department of Virology, Helsinki University Hospital, Laboratory Services (HUSLAB), Helsinki, Finland

Phylogenetic analysis of H1N1 sequences from pandemic infections during 2009 in India

Guntupally Balaswamy Arti Flavia & Kalimuthusamy Natarajaseenivasan*

Division of Medical Microbiology, Department of Microbiology, School of Life Sciences, Bharathidasan University, Tiruchirappalli – 620 024, Tamilnadu, India; Kalimuthusamy Natarajaseenivasan - Email: natarajaseenivasan@rediffmail.com; Phone: +914312407082; Fax: +914312407045;

*Corresponding author

EXPERIMENTAL WORK AND SEQUENCING

SAMPLES FROM HUMAN INDIVIDUALS
WITH COLLECTION DATE



VIRUS CULTURE AND REAL-TIME PCR

FULL GENOME SEQUENCING
AND CONTIG ASSEMBLY

DATA SET CURATION AND MULTIPLE
SEQUENCE ALIGNMENT



EVOLUTIONARY DYNAMICS

Molecular
clock

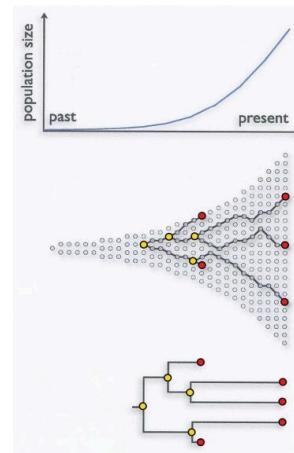
Bayesian
coalescent
inference

HI
assays

Phylogeographic
analysis

1

TEMPORAL GENE
PHYLOGENIES

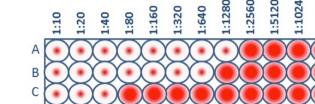


2

PAST
POPULATION
DYNAMICS

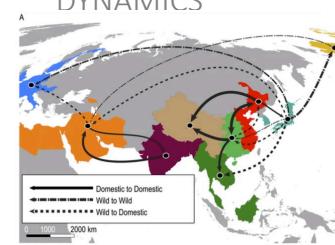
3

ANTIGENIC
EVOLUTION



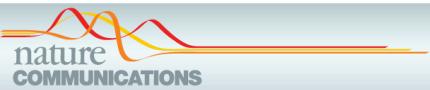
4

SPATIAL
DYNAMICS



PHYLODYNAMICS OF HUMAN H1N1/2009 VIRUSES

SU ET AL. NATURE COMM. (2015)



ARTICLE

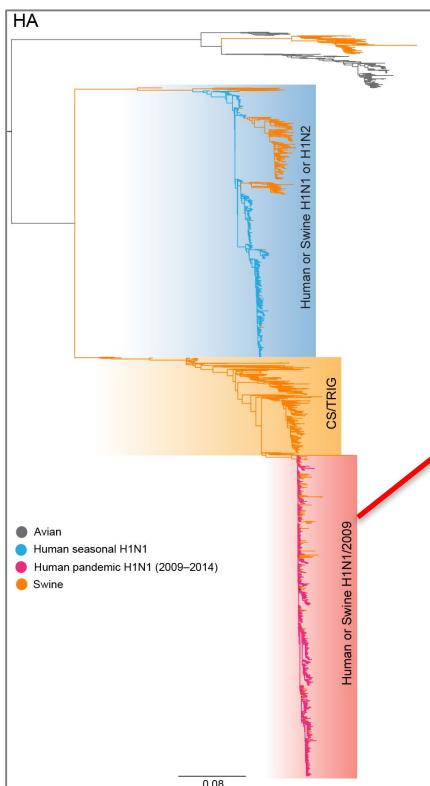
Received 27 Nov 2014 | Accepted 30 Jun 2015 | Published 6 Aug 2015

DOI: 10.1038/ncomms8952

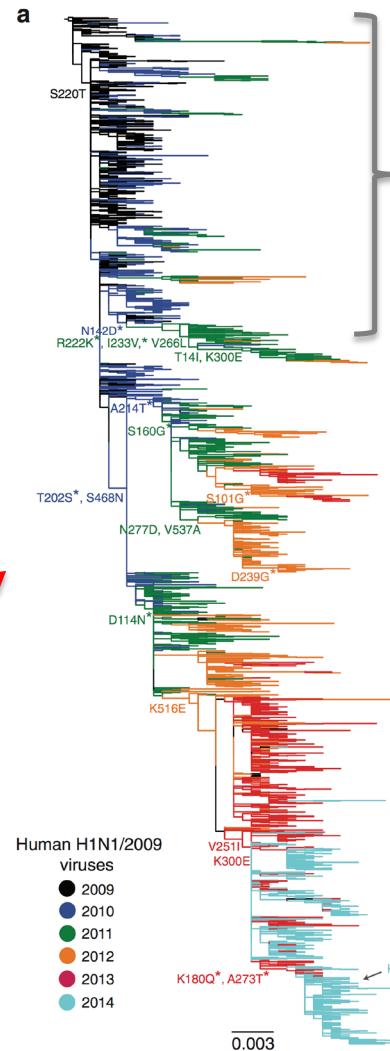
OPEN

Phylogeny of H1N1/2009 influenza reveals the transition from host adaptation to immune-driven selection

Yvonne C.F. Su¹, Justin Bahl^{1,2}, Udayan Joseph¹, Ka Man Butt¹, Heidi A. Peck³, Evelyn S.C. Koay⁴, Lynette L.E. Oon⁵, Ian G. Barr³, Dhanasekaran Vijaykrishna^{1,3,6} & Gavin J.D. Smith^{1,3,7}



Global H1N1/2009 phylogeny (2009–2014)



1

Comb-like appearance during the early phase of the pandemic (2009–2010): indicative of a rapid increase in genetic diversity in the absence of strong selective pressures

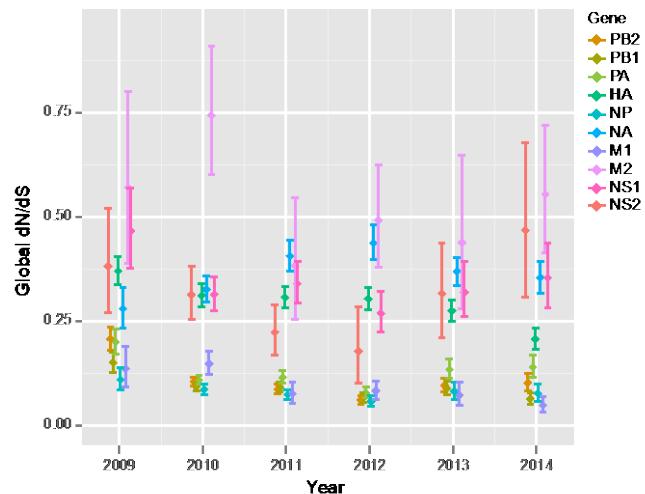
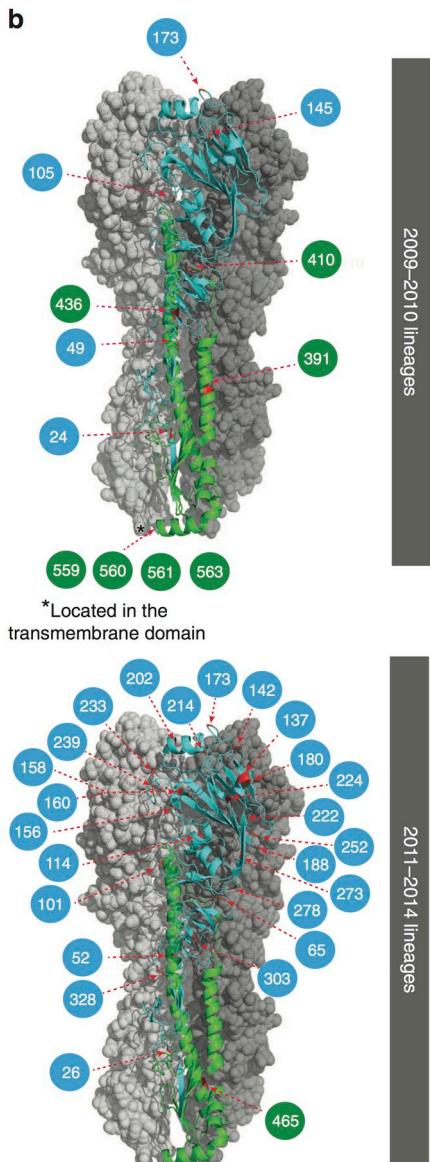
2

Ladder-like phylogeny (2011–2014)
Characteristic of viruses subject to continuous antigenic drift, typical of human seasonal influenza viruses

3

By 2014 a single dominant lineage in circulation

CHANGES IN SELECTION PRESSURE OVER TIME



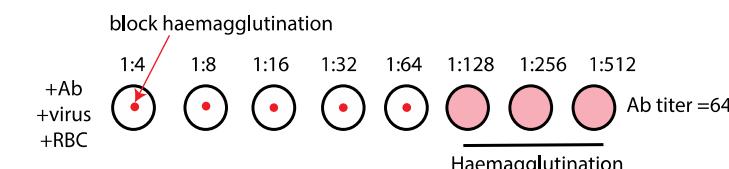
- Global dN/dS estimates were generally higher during the pandemic than in the post-pandemic period
 - Evidence of higher **relaxed selection** in 2009-2010
- **2009-2010:** HA2 & polymerase genes showed amino acids under positive selection pressure
- **2011-2014:** HA1 sites under positive selection, selection in other genes reduced or absent
- Positive selection in the pandemic period predominantly driven by adaptation to the new human host
- Post-pandemic period, positive selection directed towards escaping host immune response

ANTIGENIC EVOLUTION

Supplementary Table 3. Antigenic characterization of H1N1/2009 viruses using haemagglutinin inhibition (HI) assays. used to test against a range of H1H1/2009 viruses from Australia collected from 2009–2014.

Virus strains	Antisera					
	CAL/7	ILLINOIS/9	CHIC/16	BRIS/70	VIC/63/7	STH AUS/17
A/CALIFORNIA/07/2009 (reference)	5120	5120	2560	2560	5120	640
A/PERTH/2/09/2009	2560	2560	1280	2560	2560	640
A/SOUTH AUSTRALIA/2001/2009	2560	2560	1280	2560	2560	640
A/VICTORIA/21/2/2009	2560	2560	1280	2560	2560	640
A/BRISBANE/12/2010	5120	2560	10240	5120	5120	1280
A/BRISBANE/23/2010	5120	2560	10240	5120	5120	1280
A/SOUTH AUSTRALIA/19/2010	5120	2560	10240	5120	5120	1280
A/SYDNEY/2/2010	2560	1280	5120	1280	2560	640
A/SYDNEY/4/2010	5120	2560	10240	10240	5120	1280
A/TOWNSVILLE/7/2/2010	320	80	320	160	640	160
A/VICTORIA/670/2010	2560	2560	1280	2560	2560	640
A/BRISBANE/139/2011	320	320	640	160	640	160
A/BRISBANE/154/2011	320	80	320	160	320	160
A/BRISBANE/18/2011	160	80	320	160	640	160
A/BRISBANE/210/2011	320	160	320	160	640	160
A/BRISBANE/409/2011	2560	640	1280	1280	1280	320
A/BRISBANE/77/2011	2560	2560	2560	5120	2560	640
A/BRISBANE/76/2011	160	80	160	160	320	80
A/DARWIN/74/2011	5120	5120	5120	5120	5120	1280
A/SYDNEY/100/2011	2560	1280	1280	2560	2560	640

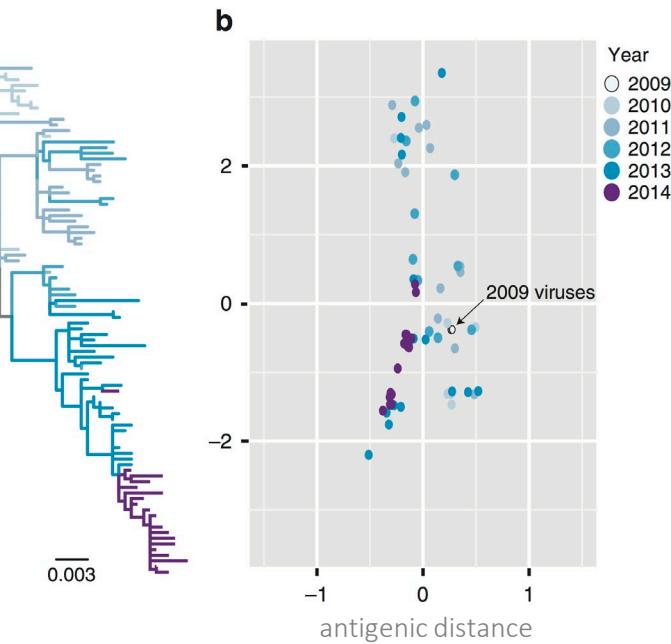
HI table (partial)



Antibodies (Ab) to influenza virus will prevent attachment of virus to red blood cells, the RBC will sink and form a button

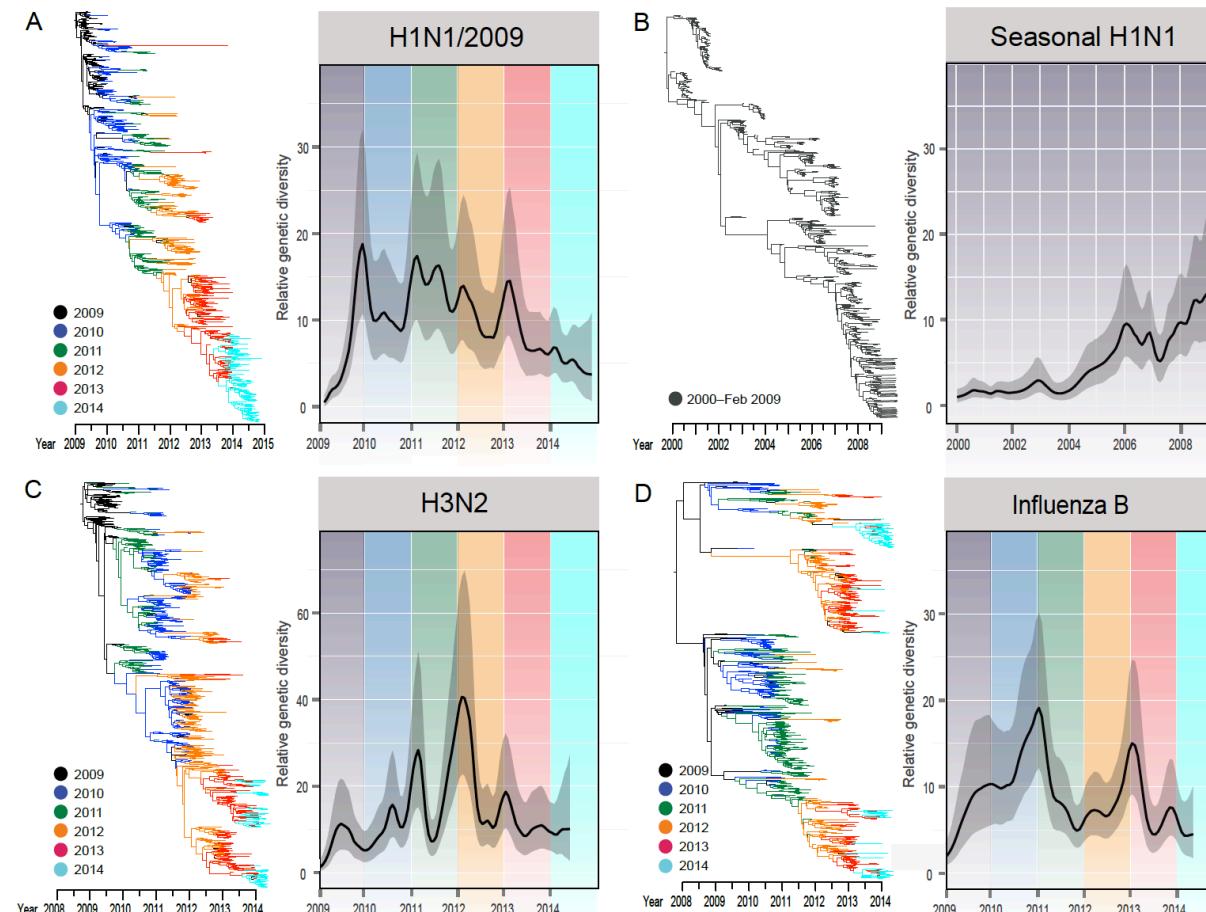
If the serum contains no Abs that react with the virus, then haemagglutination will be observed

- HA inhibition (HI) assays of 66 H1N1/2009 viruses using a panel of ferret polyclonal antisera
- Viruses from the pandemic period & early post-pandemic period showed a broader antigenic diversity in comparison to viruses from the late post-pandemic period
- The restricted antigenic diversity of later viruses corresponds to the period when selection was directed to escape host immune response

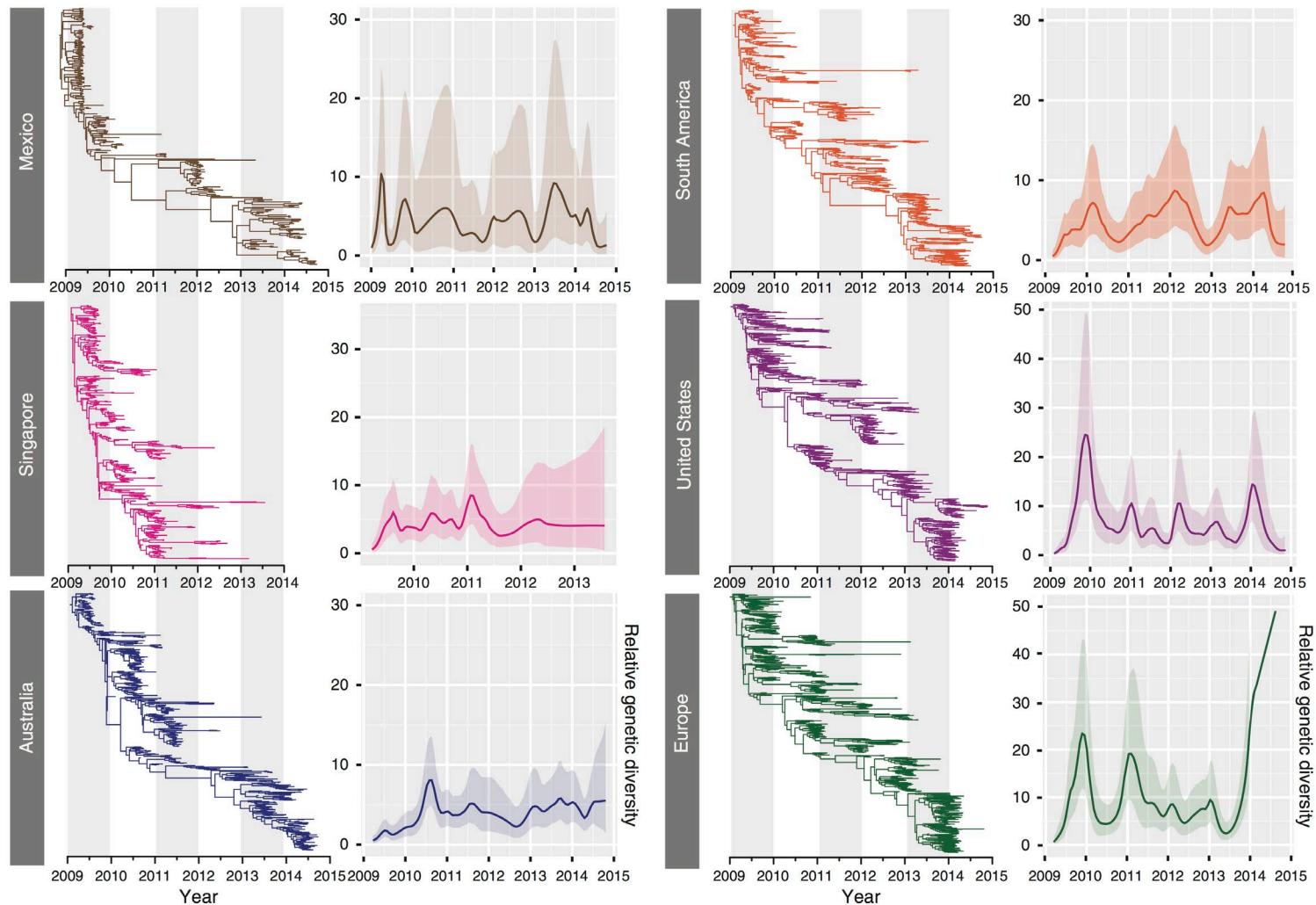


EPIDEMIC PATTERNS OF HUMAN INFLUENZA VIRUS SUBTYPES

Evidence of strong seasonal bottlenecks in contrast to seasonal H1N1



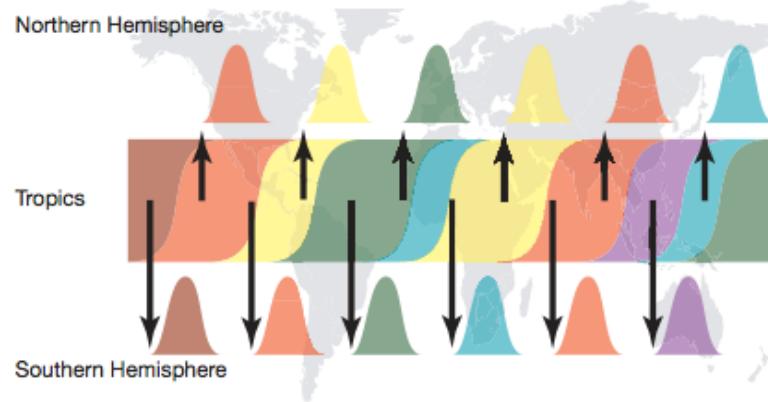
REGIONAL COMPARISON OF RELATIVE GENETIC DIVERSITY



GLOBAL TRANSMISSION OF SEASONAL INFLUENZA A

Two proposed theories:

(A) Source-sink model



Rambaut et al. (2008) Nature 453: 615–619.

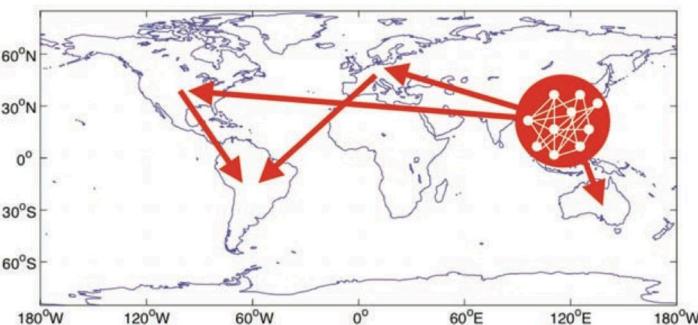
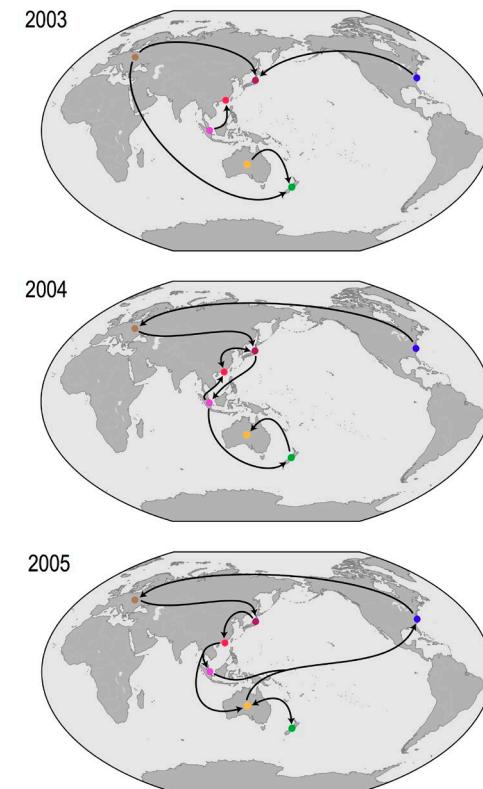


Fig. 5. Schematic of the dominant seeding hierarchy of seasonal influenza A (H3N2) viruses. The structure of the network within E-SE Asia is unknown.

Russell et al. (2008) Science 320: 340–346.

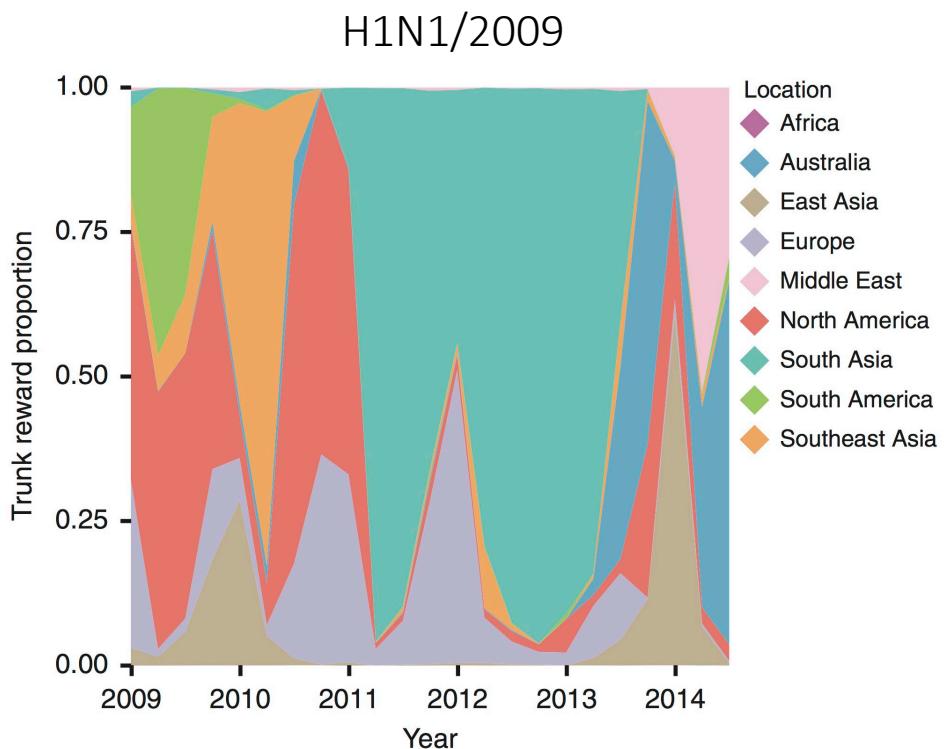
(B) Metapopulation dynamics



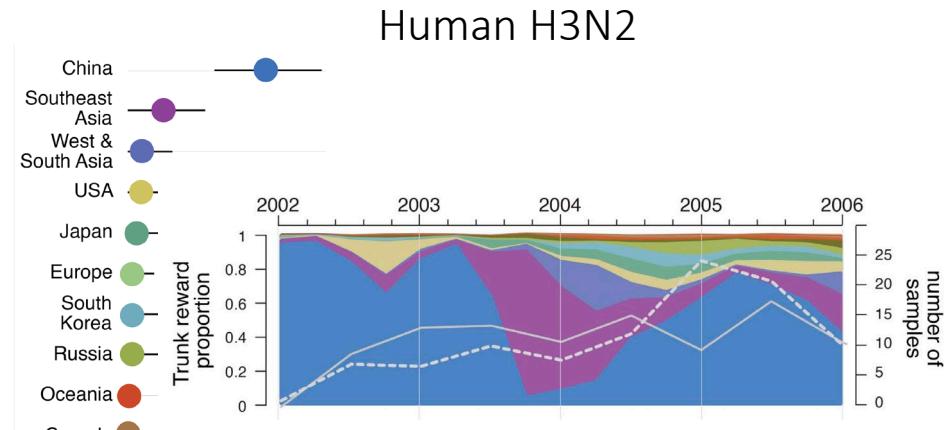
Bahl et al. (2011) PNAS 108: 19359–19364.

SPATIAL TRANSMISSION

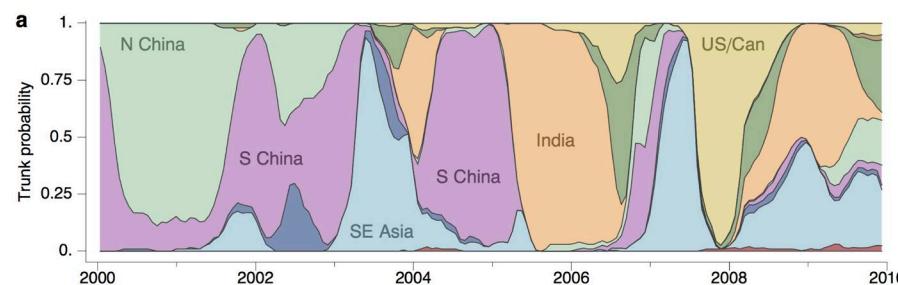
Trunk location over time:



Su et al. (2015) Nature Communications 6:7952



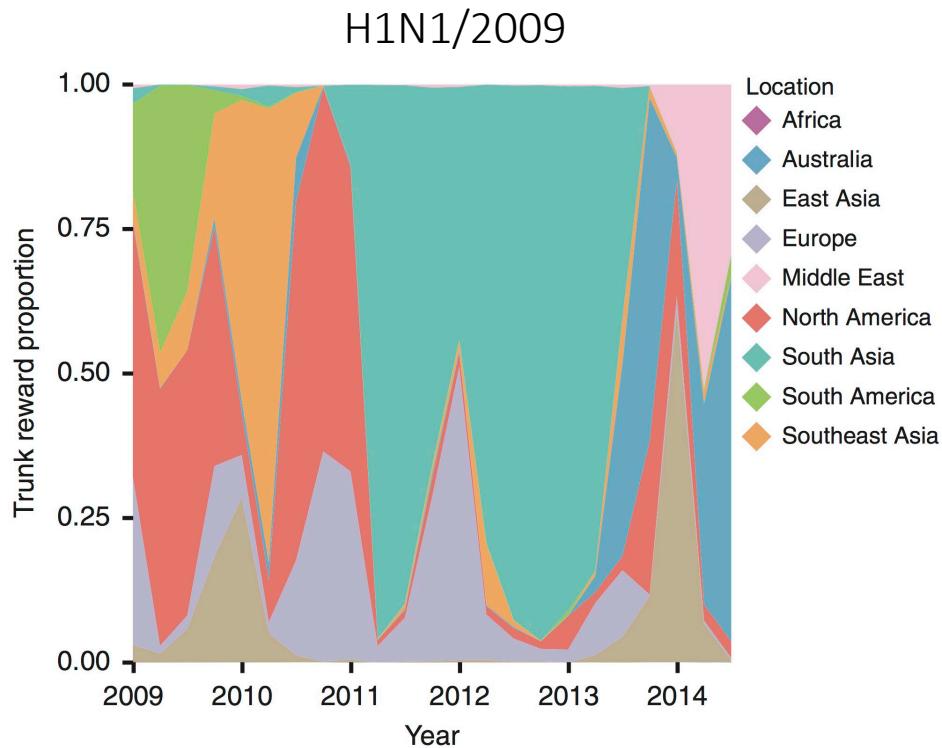
Lemey et al. (2014) PLoS Pathog 10(2): e1003932



Bedford et al. (2015) Nature 523: 217–220

SPATIAL TRANSMISSION

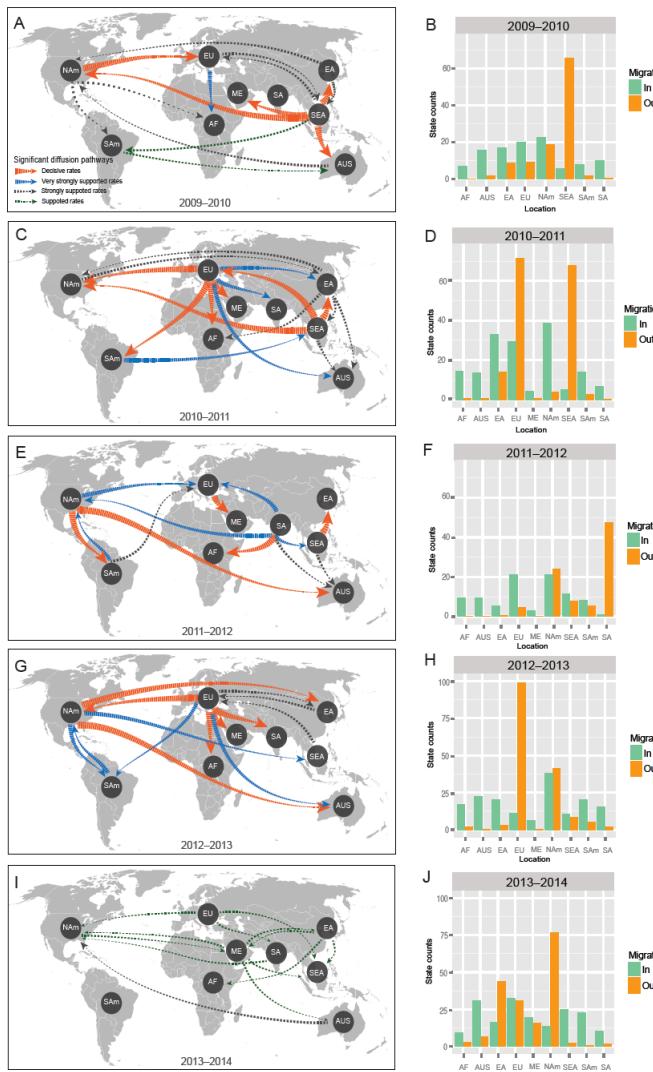
Trunk location over time:



- Annual epidemics emerge from a globally migrating population
- The ancestral population occupies multiple locations on the phylogenetic tree trunk
- For example, Southeast Asia and North America occupied the trunk in early and late 2010, respectively
- South Asia had the strongest signal of any region with >50% support from early 2011 to mid-2013
- After which Australia was identified as the dominant trunk location

Su et al. (2015) Nature Communications 6:7952

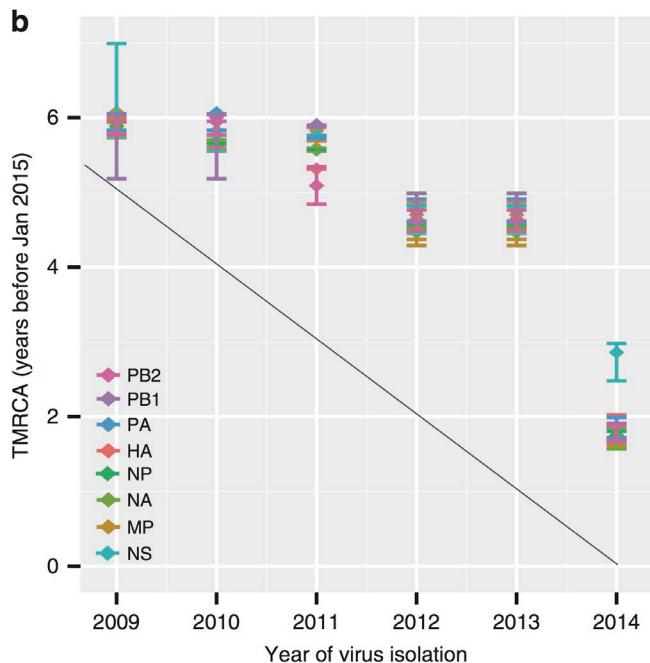
TEMPORAL DYNAMICS OF SPATIAL H1N1/2009 DIFFUSION



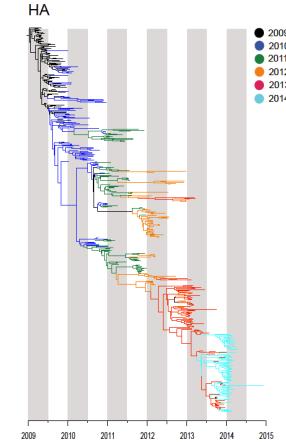
- Results of trunk reward analysis is supported by migration patterns
- No single region acts as a consistent source for seeding local epidemics in other regions, therefore our results support the metapopulation model
- Increasing evidence that H3N2, for which these hypotheses were first generated, may behave differently from other influenza A subtypes & influenza B

GLOBAL REDUCTION IN VIRUS DIVERSITY IN 2014

Genomic diversity



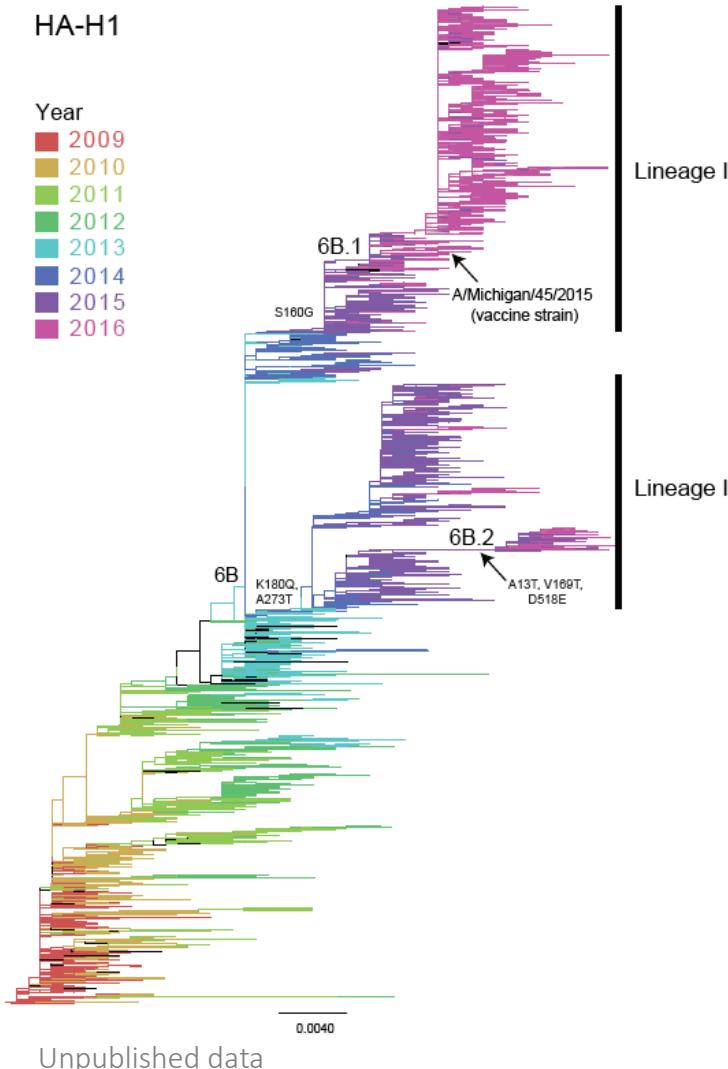
- Analysis of the mean TMRCA age of each genomic segment for each epidemic year
- Viruses isolated in 2009–2013 fell progressively further before the start of the epidemic season
 - shows diversification of H1N1/2009 as it circulated in humans
 - also indicates that diversity persisted across multiple epidemics
- In 2014 the TMRCAs of all genes except the NS fell very close to the start of the year
 - indicative of a global reduction in virus genetic diversity circulating in humans



SUMMARY

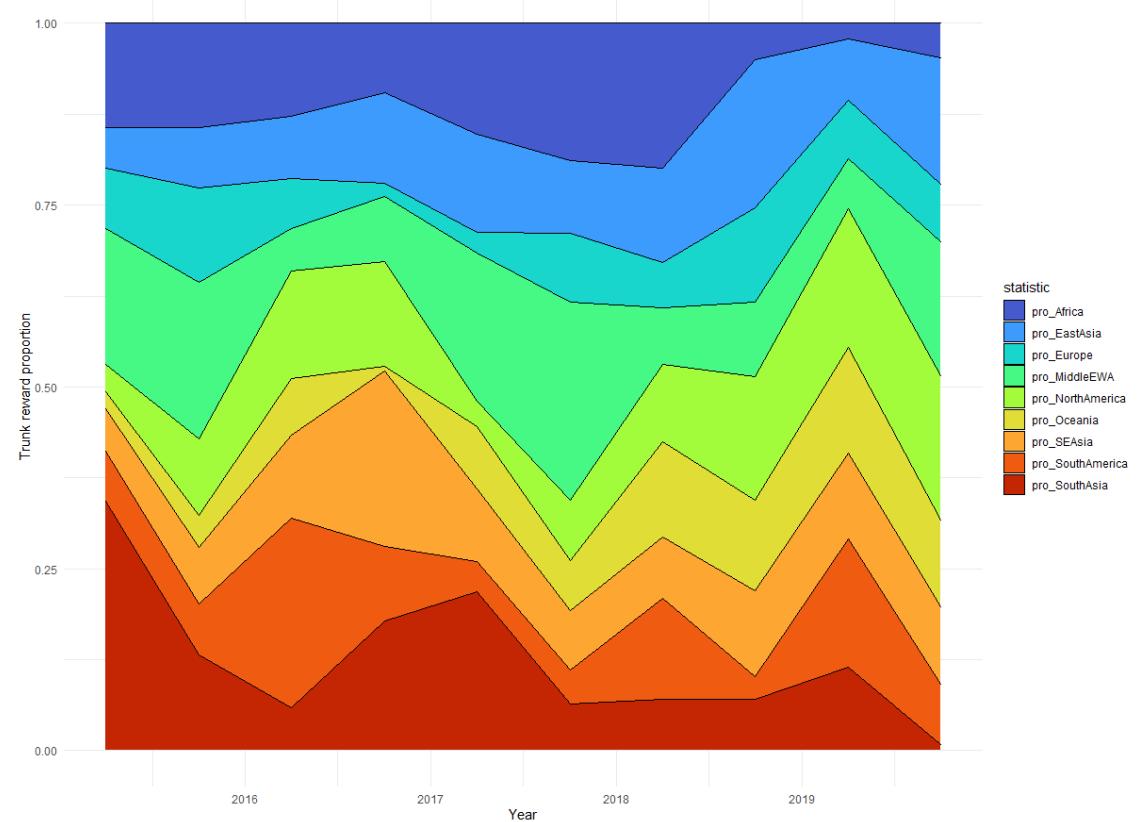
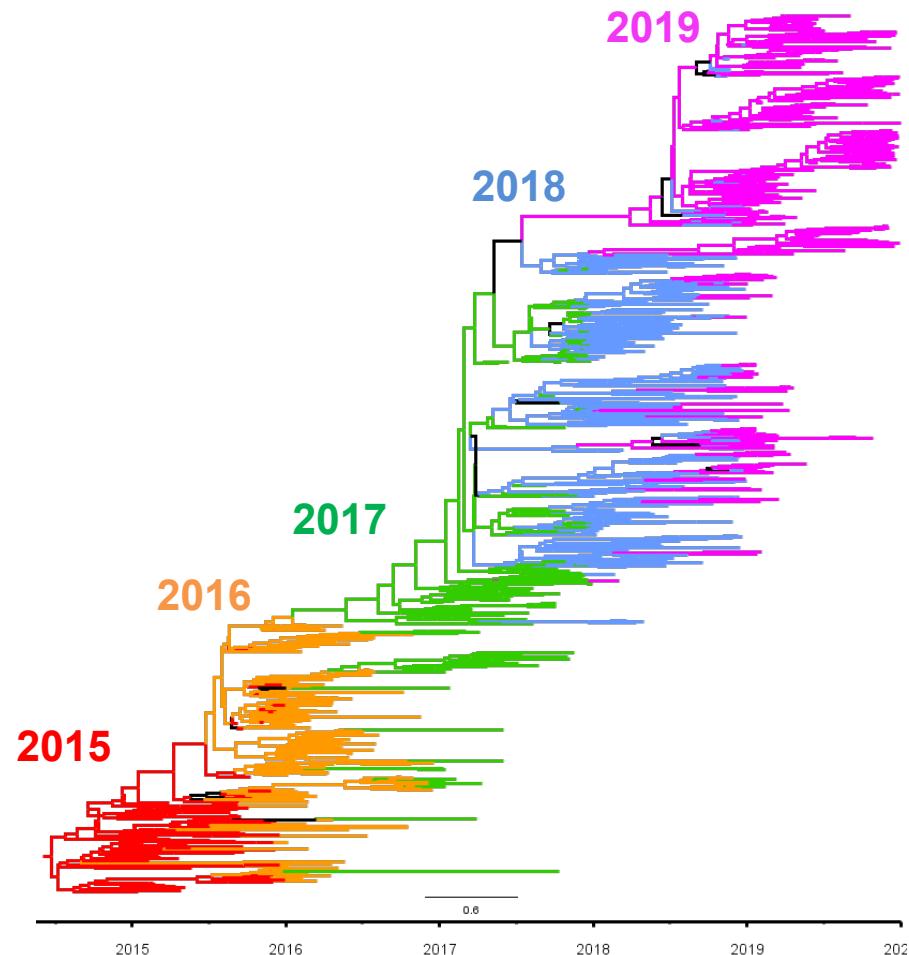
1. We show that natural selection acting on H1N1/2009 directly after introduction into humans was driven by the adaptation to new host; but since then, selection has been driven by immunological escape, resulting in restricted antigenic diversity
2. Recent population dynamics of H1N1/2009 viruses more closely resemble that of human H3N2, displaying seasonal bottlenecks
3. H1N1/2009 viruses have been subject to regular seasonal bottlenecks and a global selective sweep in 2014. These dynamics may lead to greater lineage turnover, complicating control efforts

CURRENT EVOLUTION OF H1N1/2009 VIRUS



- Older A/California/07/2009 WHO vaccine strain is being replaced by the A/Michigan/45/2015 strain
- H1N1 virus has diverged into two monophyletic lineages
- According to WHO, the updated influenza vaccine strain matches with the majority of the circulating viruses and remains similar to the A/California/07/09 virus

Even more current evolution of pdm09



Future challenges

- “The explosion in viral genomic data is outpacing our ability to develop methods that fully exploit the potential of these data”
 - Pybus & Rambaut 2009. *Nat Rev Gen* **10**:540–550
- New generation sequencing methodologies will exacerbate this problem
 - Newer technologies far outstrip current outputs, with constant updates in technology

End

Aux – dN/dS

- Syn & Nonsyn sites per codon
- Syn & Nonsyn sites per gene
- Syn & Nonsyn sites per sequence
- Proportion Syn/Nonsyn

$$n = \sum_{i=1}^3 f_i \quad s = (3 - n)$$

$$N = \sum_{i=1}^r n_i \quad S = (3r - N)$$

$$N_d = \sum_{i=1}^r n_{di} \quad S_d = \sum_{i=1}^r s_{di}$$

$$p_N = \frac{N_d}{N} \quad p_S = \frac{S_d}{S}$$

$$d_N = -\frac{3}{4} \ln \left(1 - \frac{4p_N}{3} \right) \quad d_S = -\frac{3}{4} \ln \left(1 - \frac{4p_S}{3} \right)$$

$$dN/dS = \frac{d_N}{d_S}$$