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Origins, Ecology and Epidemiology of Pandemic and Seasonal Influenza A virus

Justin Bahl

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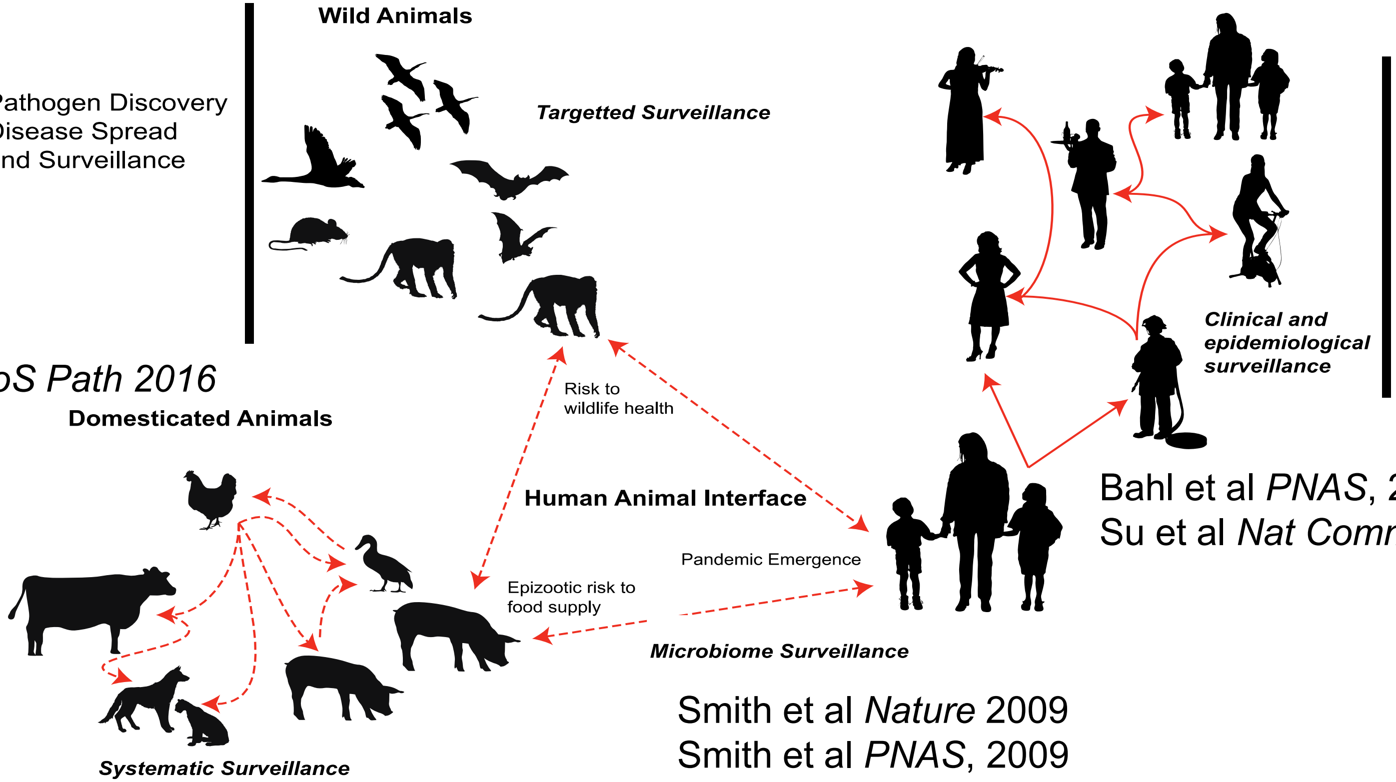
Ecology, Epidemiology and Evolution of Infectious Disease research group

Systems Ecology and scale of response



Bahl et al *Virol*, 2009
Bahl et al *PLoS Path 2013*

Wild Animals

Pathogen Discovery
Disease Spread
and Surveillance

Targetted Surveillance

Human Population

Transmission
Persistence,
Epidemic
Dynamics
and
Population
Genetics

Clinical and epidemiological surveillance

Bahl et al *PNAS*, 2009
Su et al *Nat Comm*, 2016

Bahl et al *PLoS Path 2016*

Domesticated Animals

Interspecies
Transmission
Dynamics,
Adaptation
and
Molecular
Diversity

Risk to
wildlife health

Human Animal Interface

Pandemic Emergence

Epizootic risk to
food supply

Microbiome Surveillance

Systematic Surveillance

Vijaykrishna et al *Nature* 2011
Vijaykrishna et al *PLoS Path*, 2008

Smith et al *Nature* 2009
Smith et al *PNAS*, 2009
Zaraket et al *Nat Comm*, 2015

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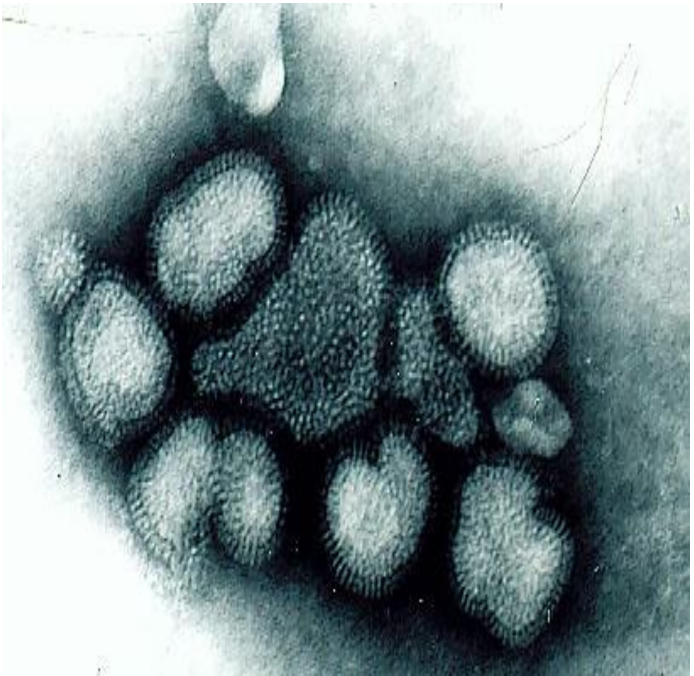
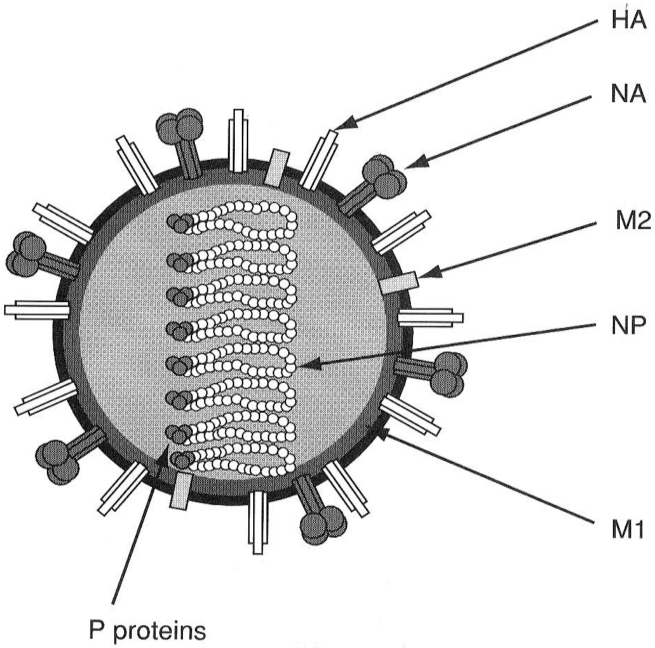
1. Influenza virus

- Types A, B and C
- Segmented genome
- *ss RNA (single stranded, negative sense)*





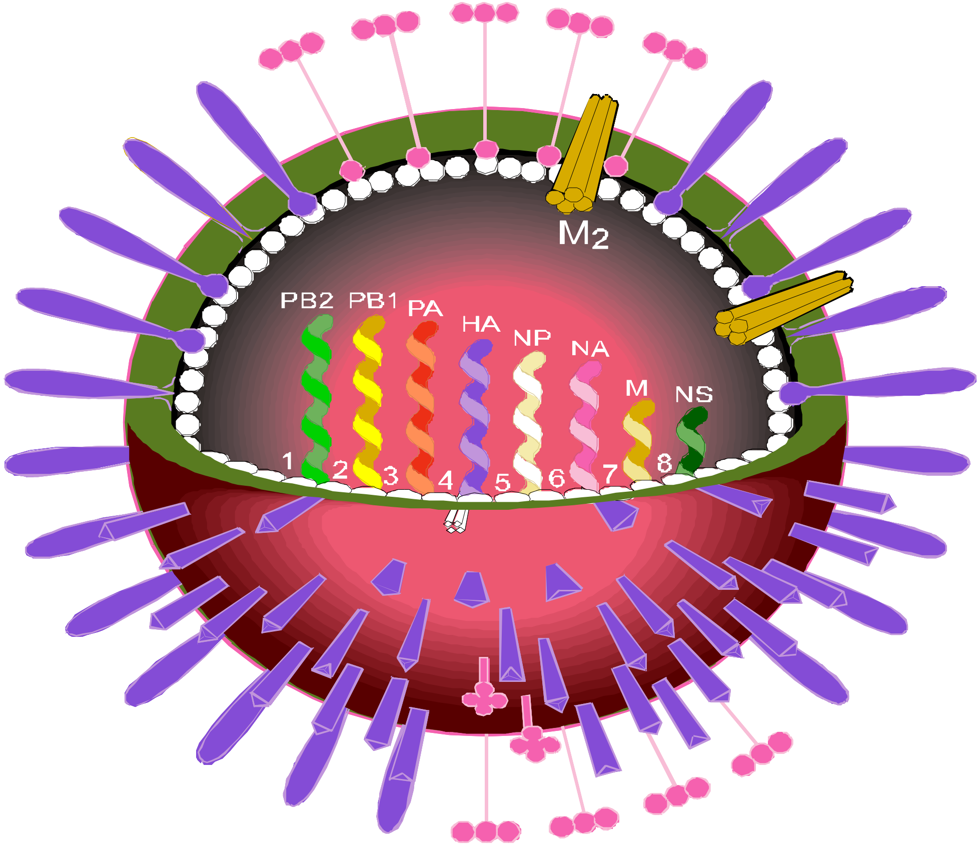
SUBTYPES

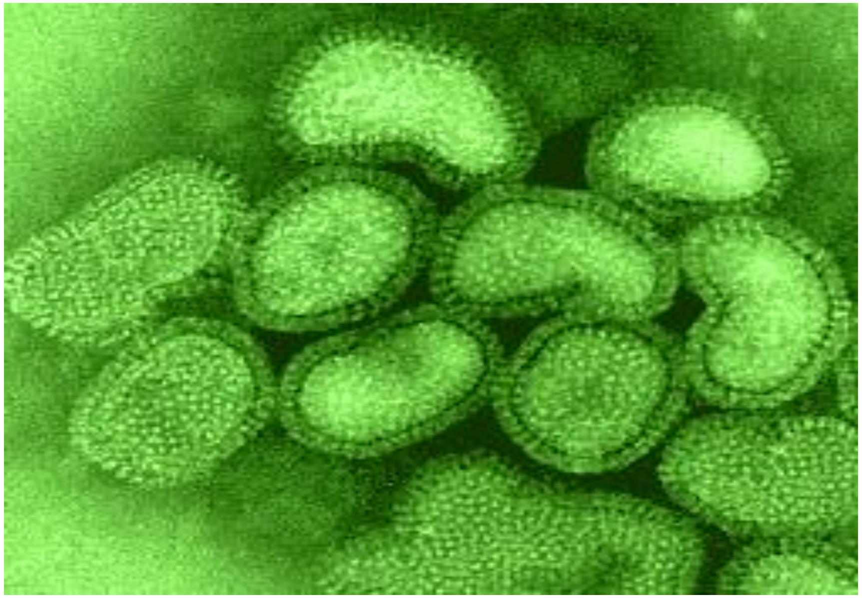
Haemagglutinin (HA)

Neuraminidase (NA)

Influenza A virus

- Each segment encodes a different protein
- Smallest RNAs encode 2 proteins
- PB1-F2 in alternative reading frame of PB1

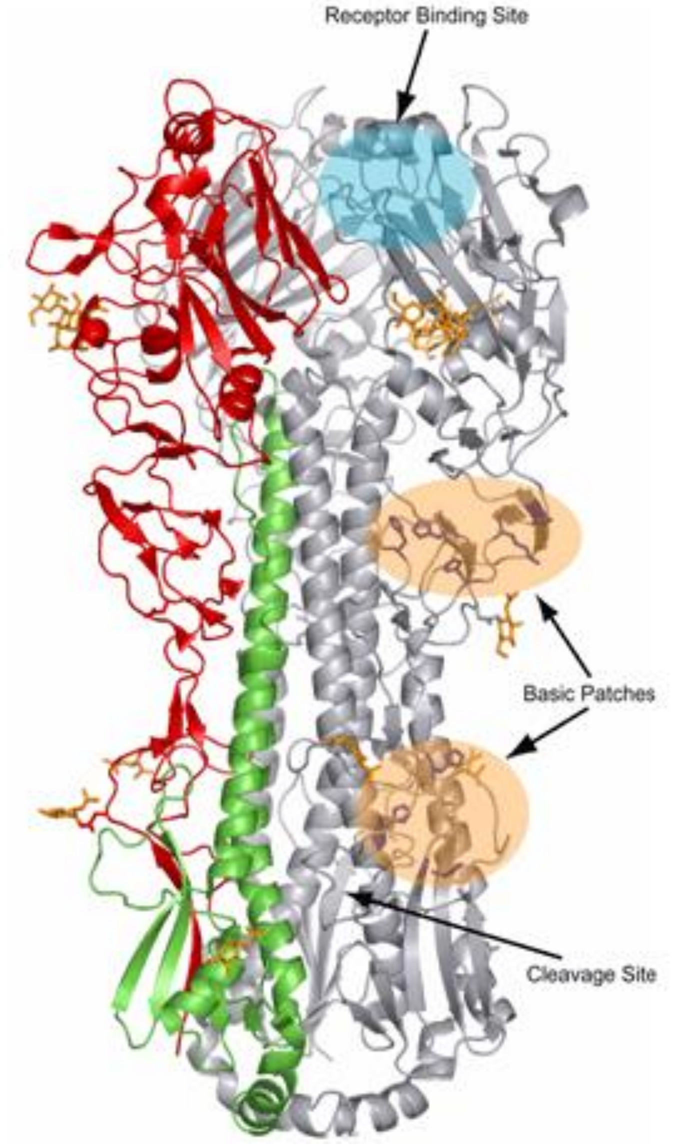




Influenza A proteins

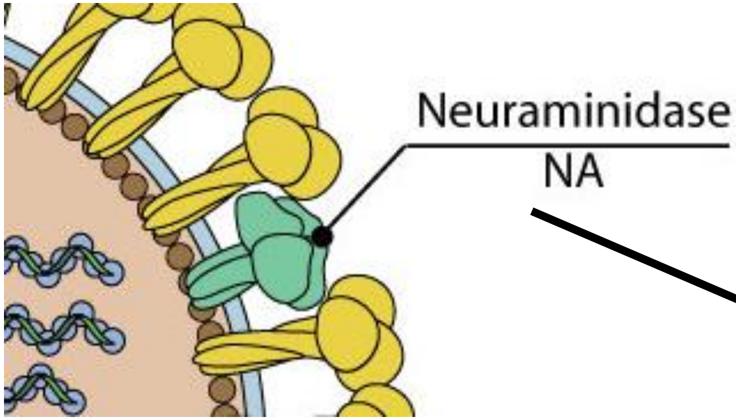
Segment	Size (nucleotides)	Polypeptide	Function
1	2341	**PB2**	Subunit of polymerase: Host cap binding and endonuclease
2	2341	**PB1**	Catalytic subunit of polymerase
		PB1-F2	Genomic RNA nuclear export
3	2233	**PA**	Subunit of polymerase, plays a role in replication
4	1778	**HA**	Haemagglutinin: cell receptor binding, membrane fusions.
5	1565	**NP**	Nucleoprotein: protects the RNA genome.
6	1413	**NA**	Neuraminidase: release of virus
7	1027	**M1**	Matrix protein: Budding, virion structure.
		M2	Ion channel: virus entry.
8	890	**NS1**	Anti-interferon protein. Effects on cellular RNA transport
		NS2	Genomic RNA nuclear export

Hemagglutinin: cell entry

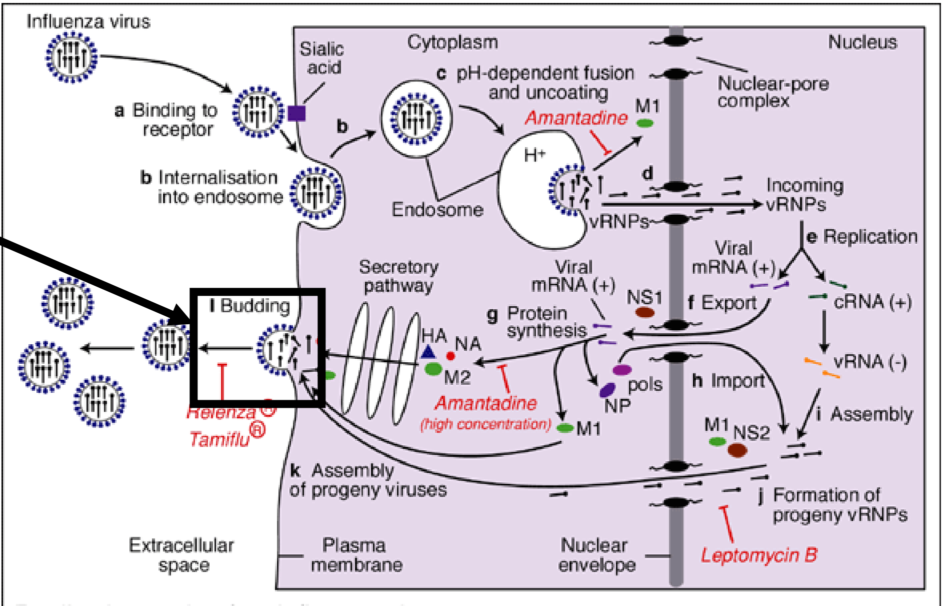


- Binds to sialic acid-containing receptors on the cell surface
- The major antigenic site of influenza virus

Neuraminidase: virion budding



zanamivir & oseltamivir (Tamiflu)



Replication cycle of an influenza virus

Expert Reviews in Molecular Medicine ©2001 Cambridge University Press

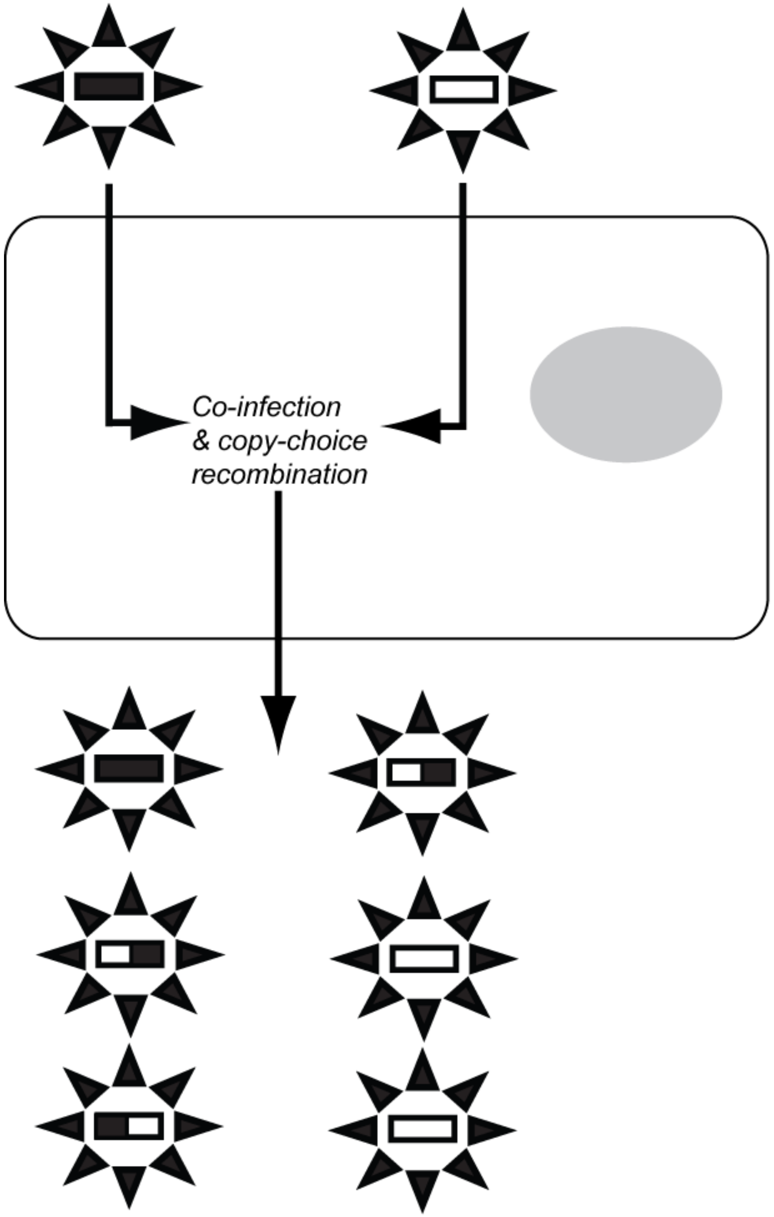
Evolutionary processes give rise to diversity

There are four basic mechanisms by which biological evolution takes place. These include mutation, migration, genetic drift, and natural selection.

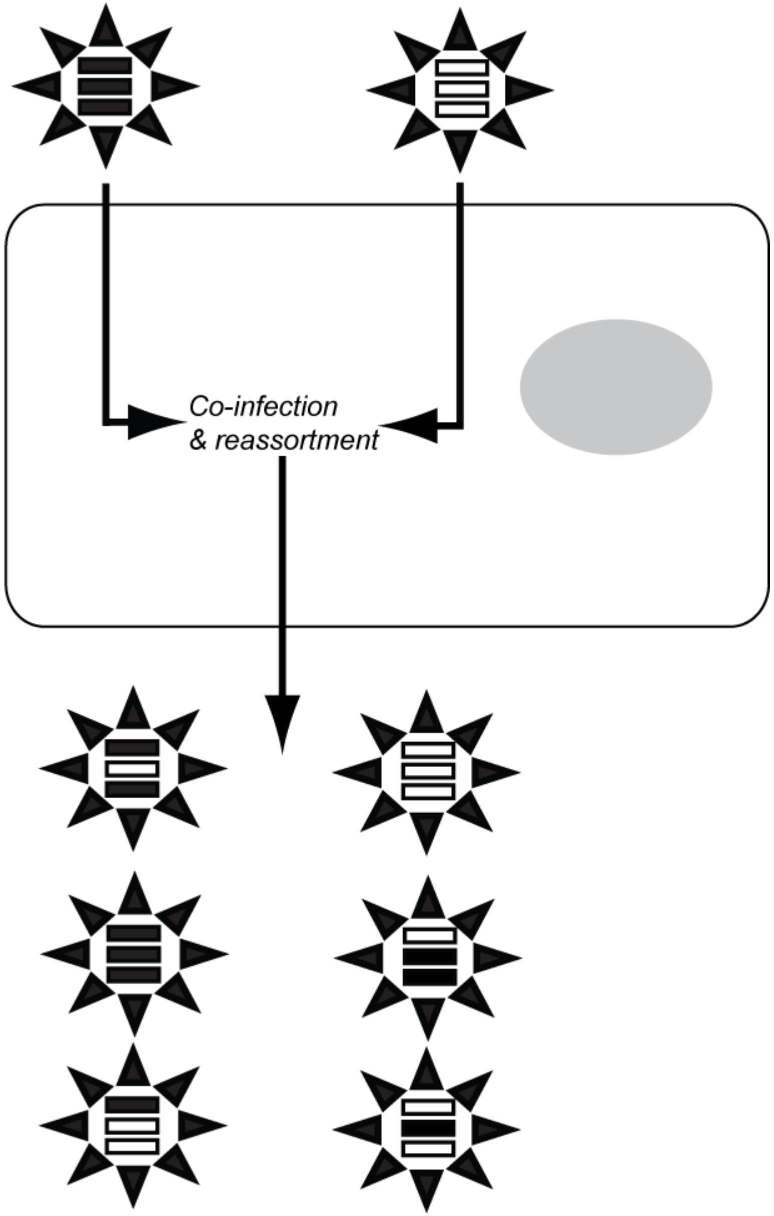
Evolutionary processes give rise to diversity

There are four basic mechanisms by which biological evolution takes place. These include mutation, migration, genetic drift, and natural selection.

When we sequence a population of viruses we are observing genetic diversity resulting from those processes



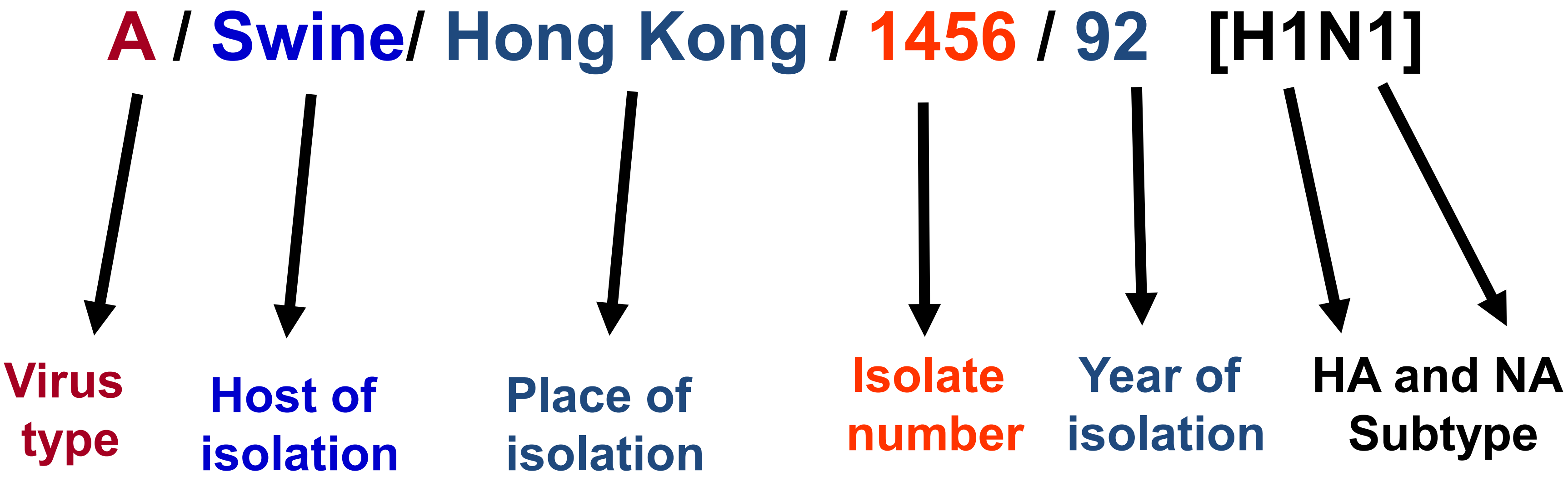
(a) RNA recombination



(b) Reassortment

Courtesy of Eddie Holmes

Virus nomenclature



A / Swine/ Hong Kong / 1456 / 92 [H1N1]

Virus type

Host of isolation

Place of isolation

Isolate number

Year of isolation

HA and NA Subtype

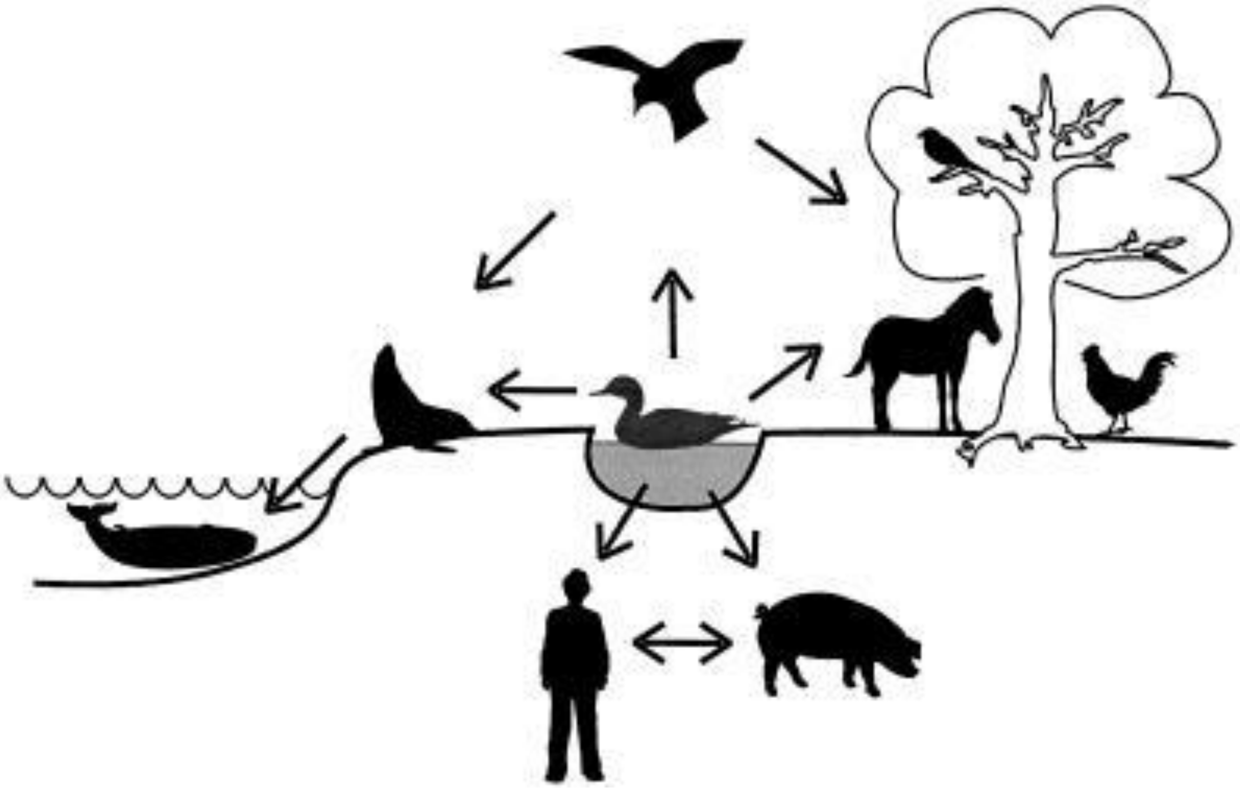
A / Chicken / NY / 14009 / 93 [H5N2] - Chicken isolate
A / Hong Kong / 156 / 97 [H5N1] - Human isolate
A/Sydney/05/97 [H3N2] - Human isolate

2. Ecology – Avian origin viruses with pandemic potential

High Risk: H5N1 & H7N9

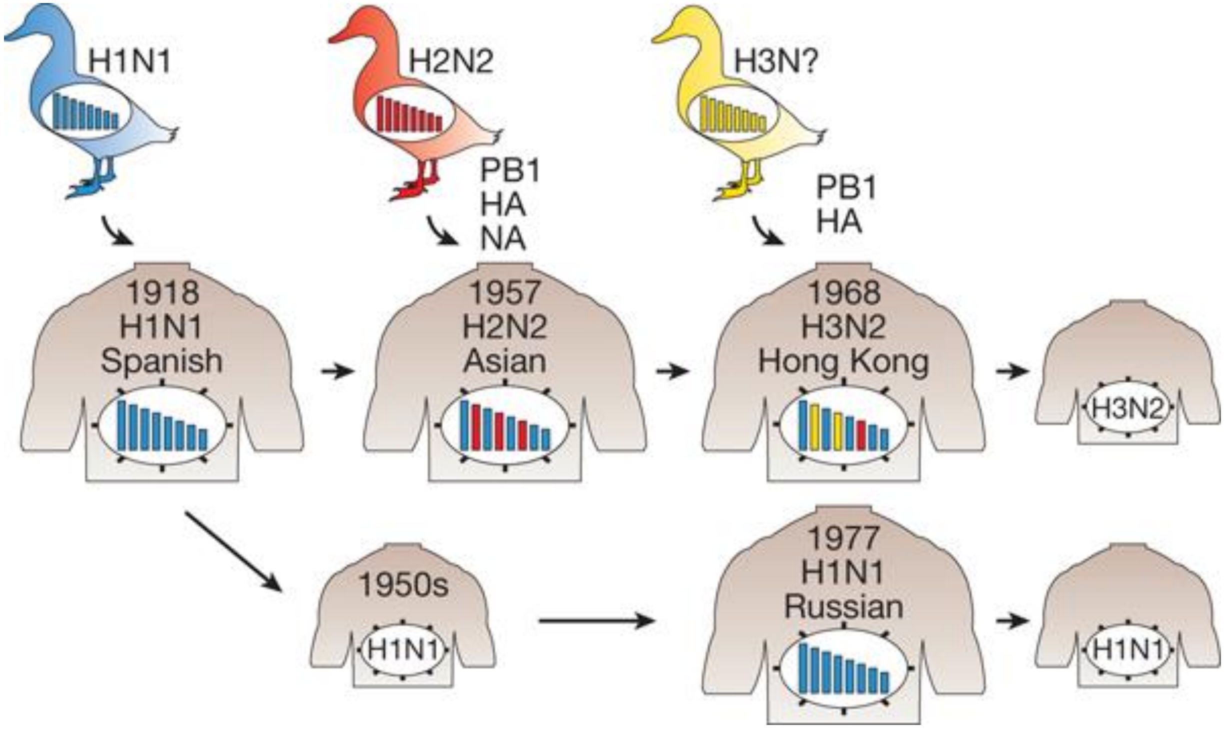
Intermediate Risk: H6 & H9

Influenza Ecology



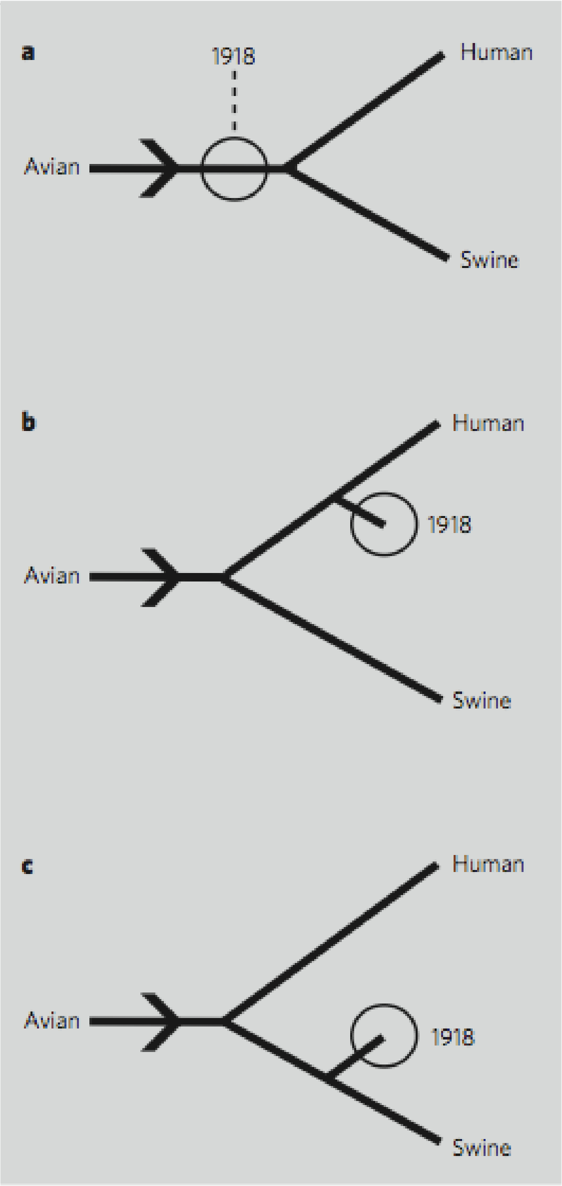
Source: Horimoto & Kawaka (2001). *Clin Microbiol Rev.; 14(1): 129-149.*

Emergence of pandemic influenza viruses



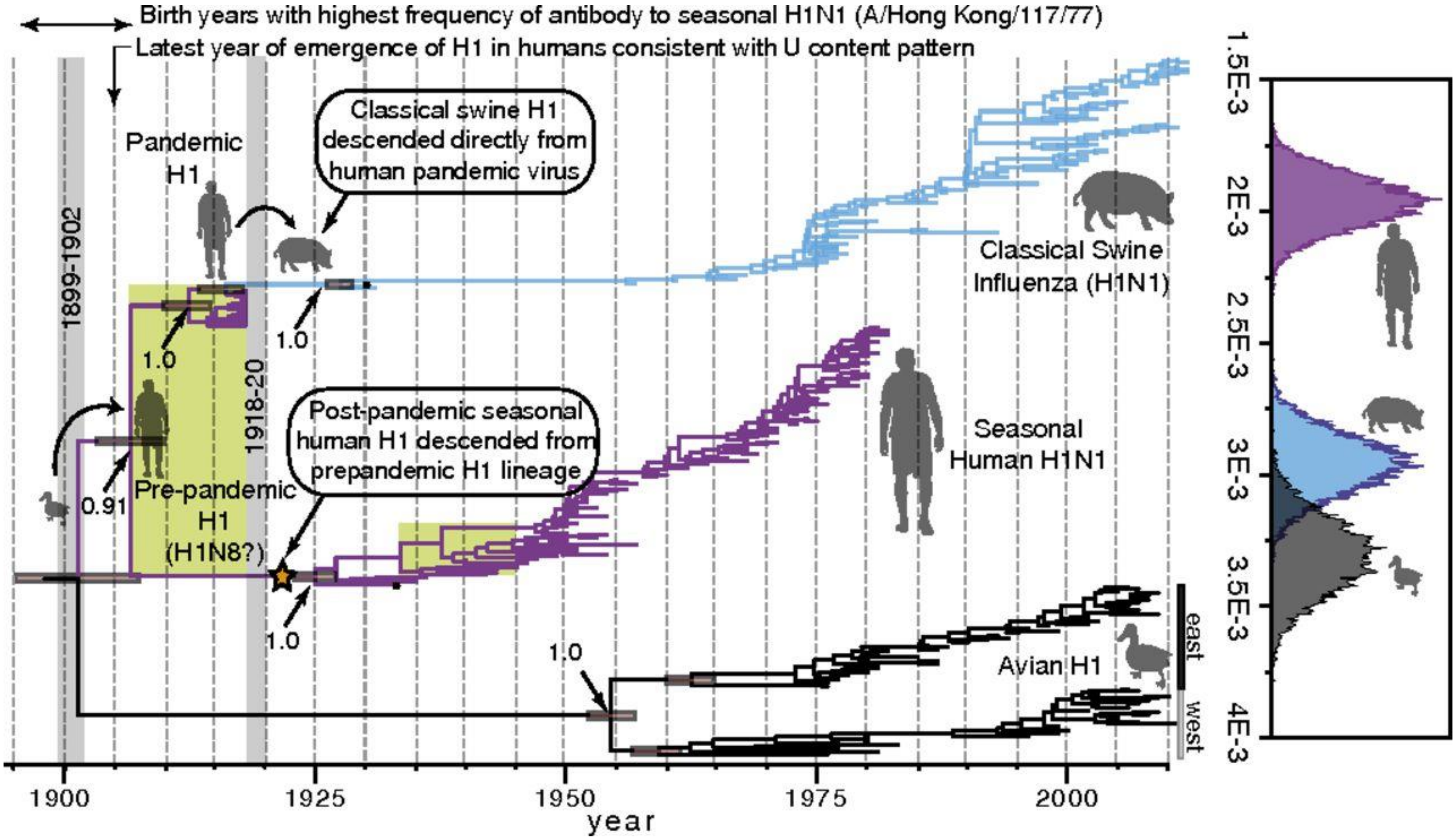
G Neumann *et al.* *Nature* **000**, 1-9 (2009) doi:10.1038/nature08157

Was the 1918 flu avian in origin?



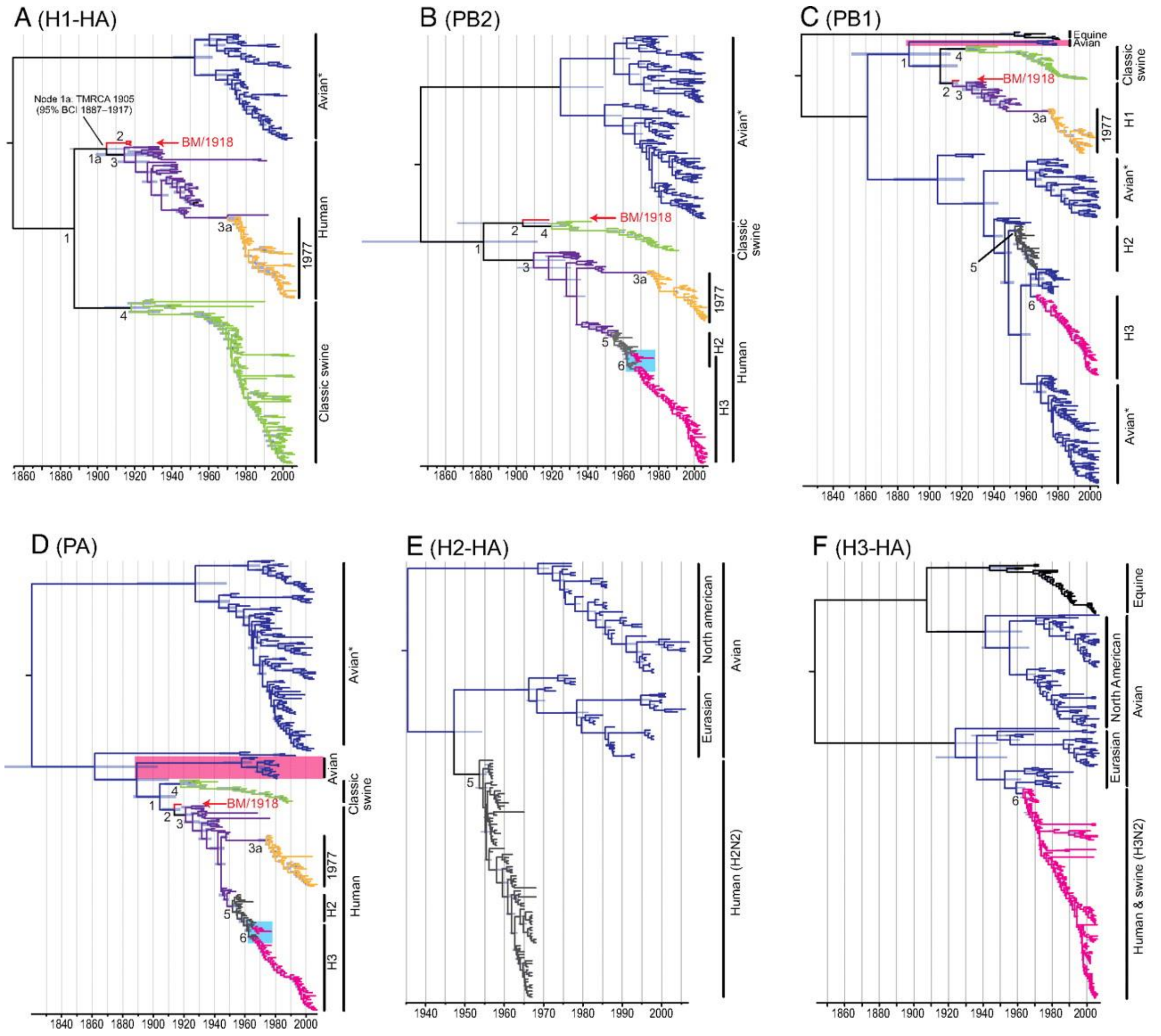
Gibbs and Gibbs, Nature 2005

Maximum clade credibility (MCC) tree of the H1 subtype of HA. (Right) Clade-specific rate distributions (in substitutions per site per year).



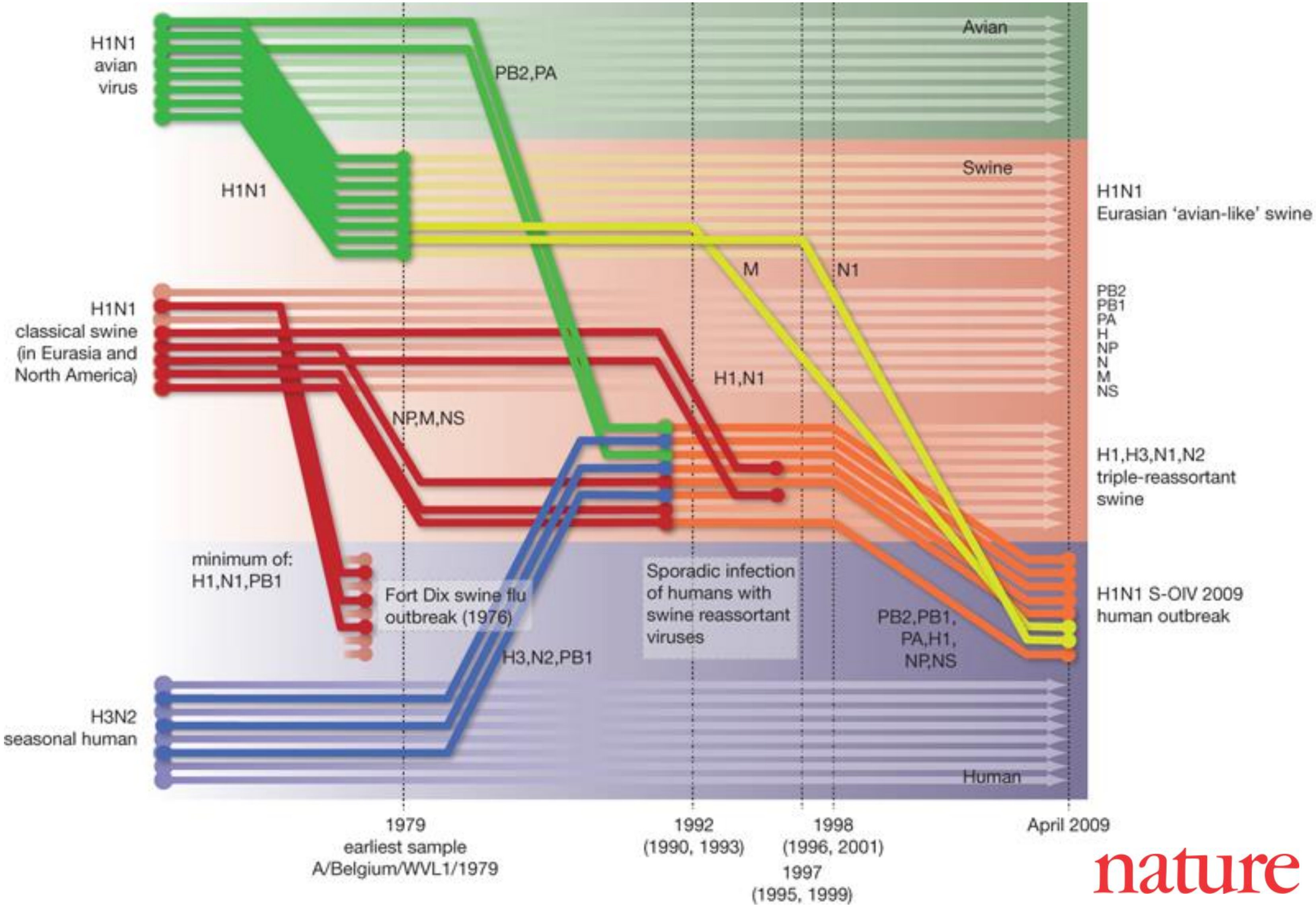
Michael Worobey et al. PNAS 2014;111:8107-8112

Dated phylogenies of influenza A virus genes.



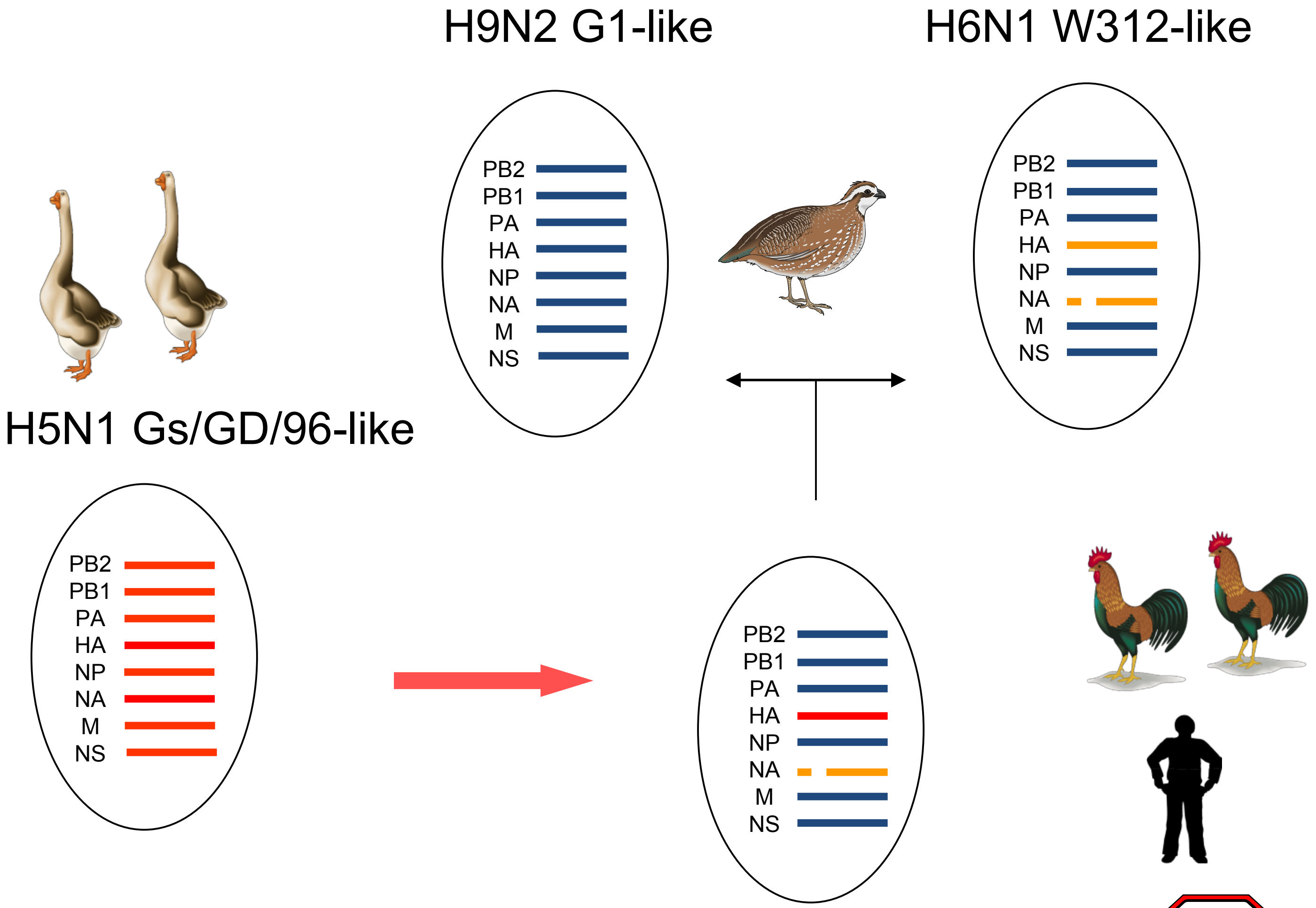
Gavin J. D. Smith et al. PNAS 2009;106:11709-11712

Reconstruction of the sequence of reassortment events leading up to the emergence of S-OIV.



GJD Smith *et al.* *Nature* **459**, 1122-1125 (2009) doi:10.1038/nature08182

1997 H5N1 incident of HK



H9N2 G1-like

PB2
PB1
PA
HA
NP
NA
M
NS

H6N1 W312-like

PB2
PB1
PA
HA
NP
NA
M
NS

H5N1 Gs/GD/96-like

PB2
PB1
PA
HA
NP
NA
M
NS

PB2
PB1
PA
HA
NP
NA
M
NS

Xu et al. Virology, 1999.
Guan et al. PNAS, 1999.
Hoffmann et al. J Virol 2000.

H5N1/97

Human & Chicken

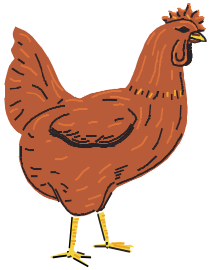
Market surveillance network

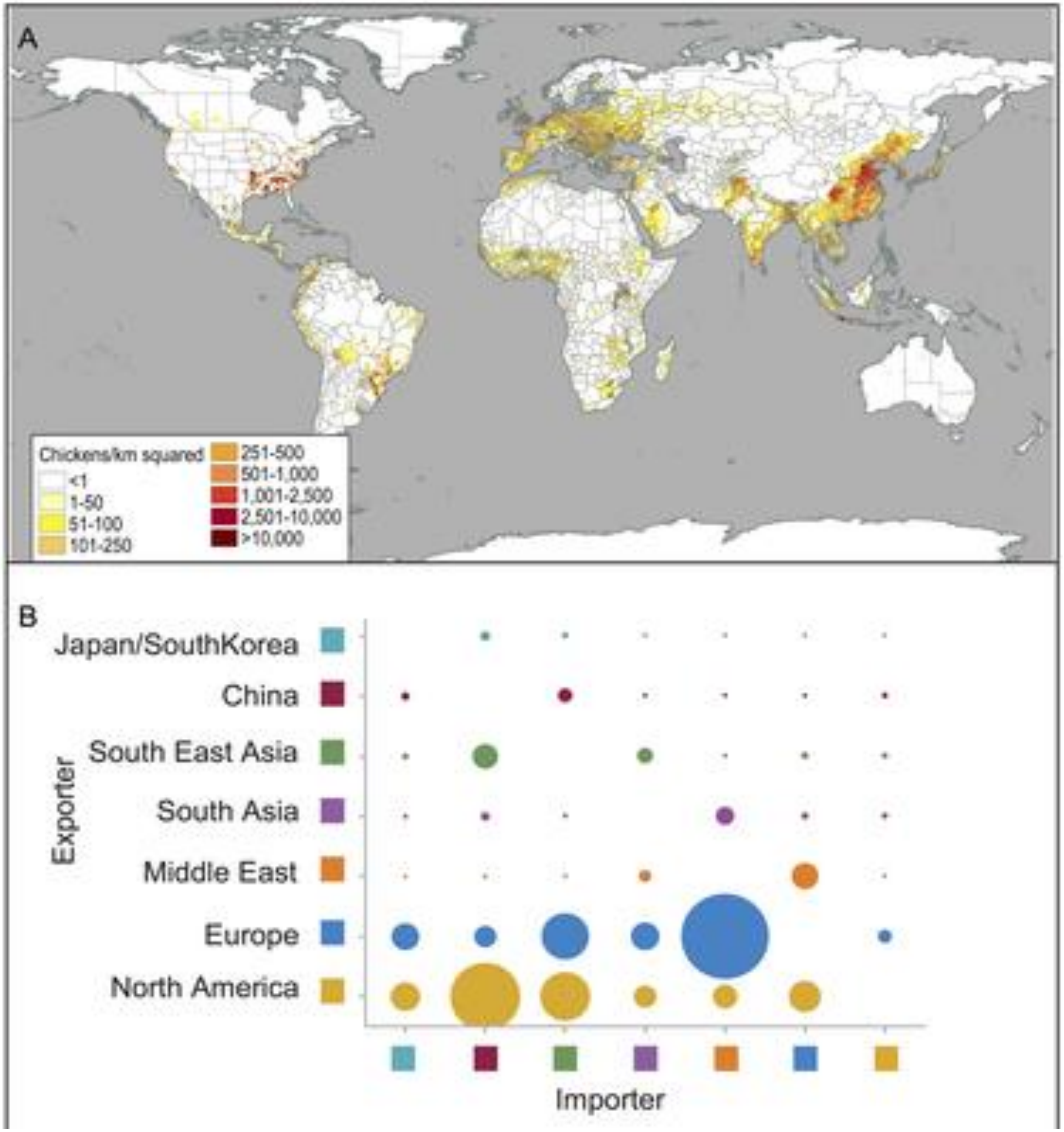
Surveillance in HK first started by Prof Ken Shortridge in 1976

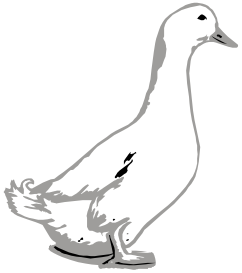
Expanded to mainland China in 2002 by Prof Yi Guan with 50-70,000 birds sampled each year

Hong Kong is a world leader in the surveillance & control of avian influenza

Poultry production, global trade intensity and location of viral sampling.





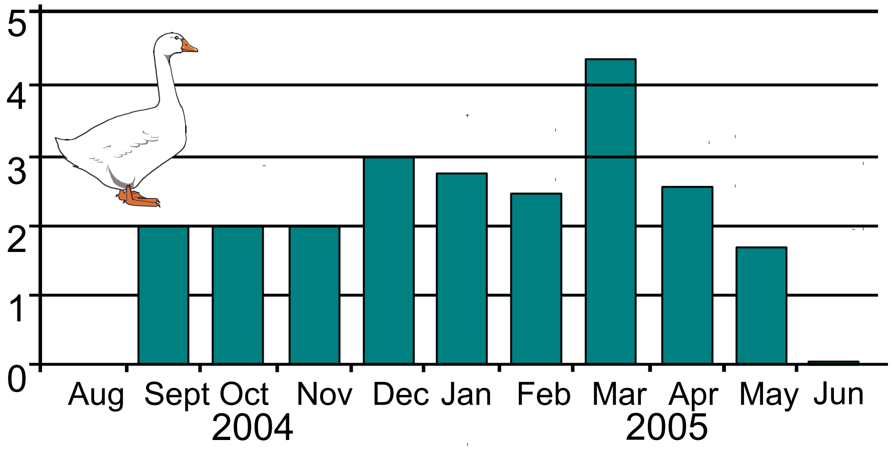


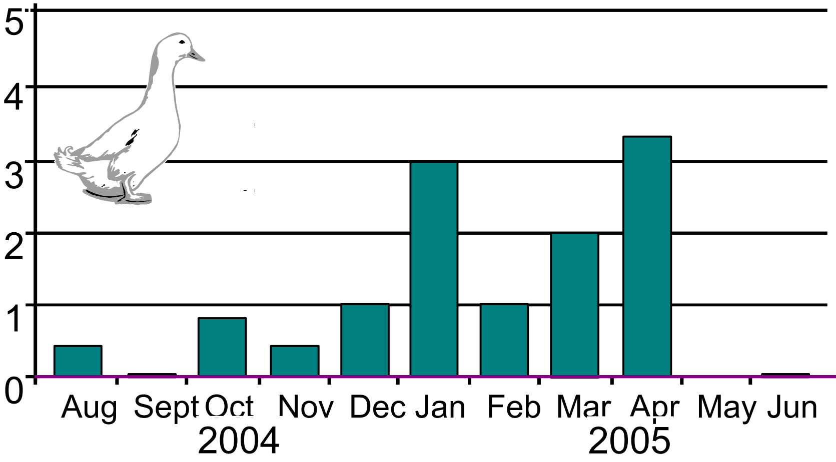
Bahl J, et al. (2016) Ecosystem Interactions Underlie the
Spread of Avian Influenza A Viruses with Pandemic Potential.

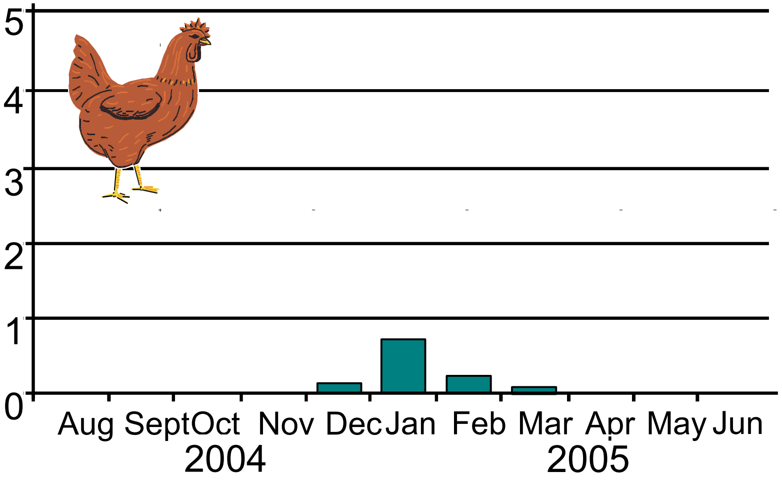
PLOS | PATHOGENS

Isolation of H5N1 Viruses From Live Poultry Markets in S. China

% Positive

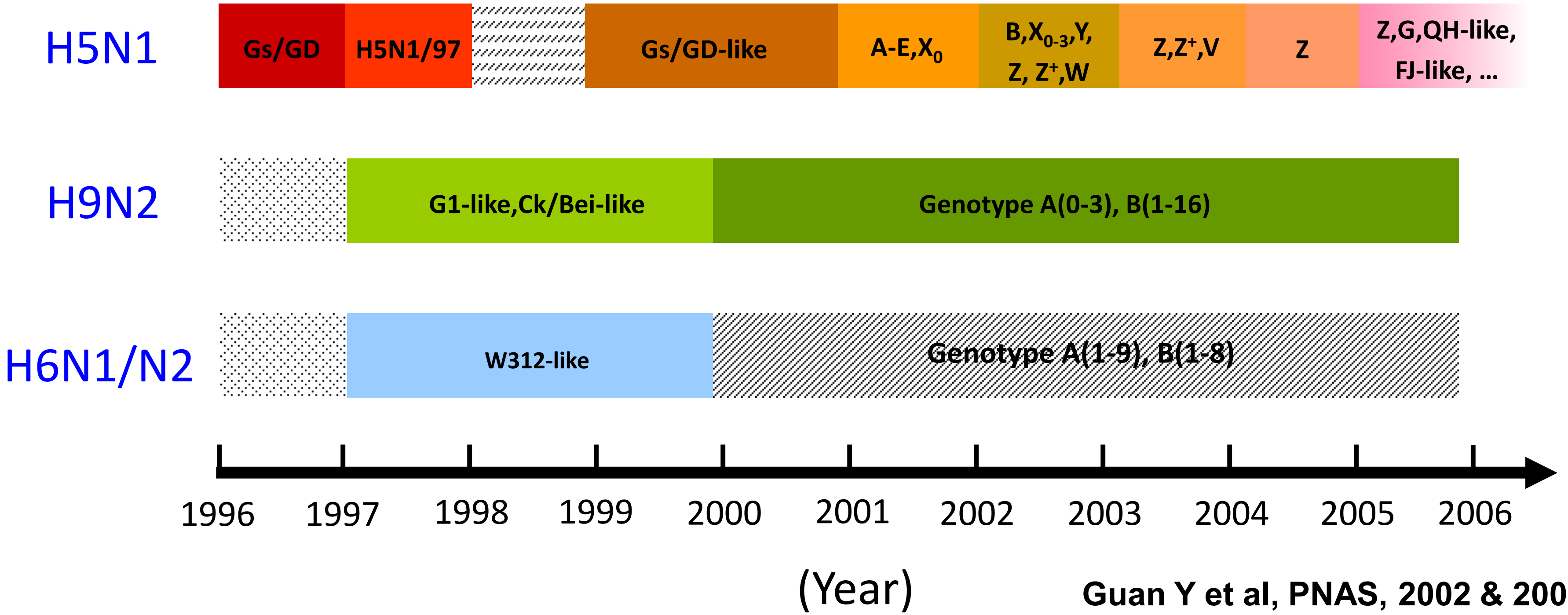






Based on numbers (%) from August 2004 to June 2005

Overview of 3 major subtypes of avian influenza A viruses in southern China



H5N1

Gs/GD

H5N1/97

Gs/GD-like

A-E,X_0

B,X_{0-3},Y, Z, Z^+,W

Z,Z^+,V

Z

Z,G,QH-like, FJ-like, …

H9N2

G1-like,Ck/Bei-like

Genotype A(0-3), B(1-16)

H6N1/N2

W312-like

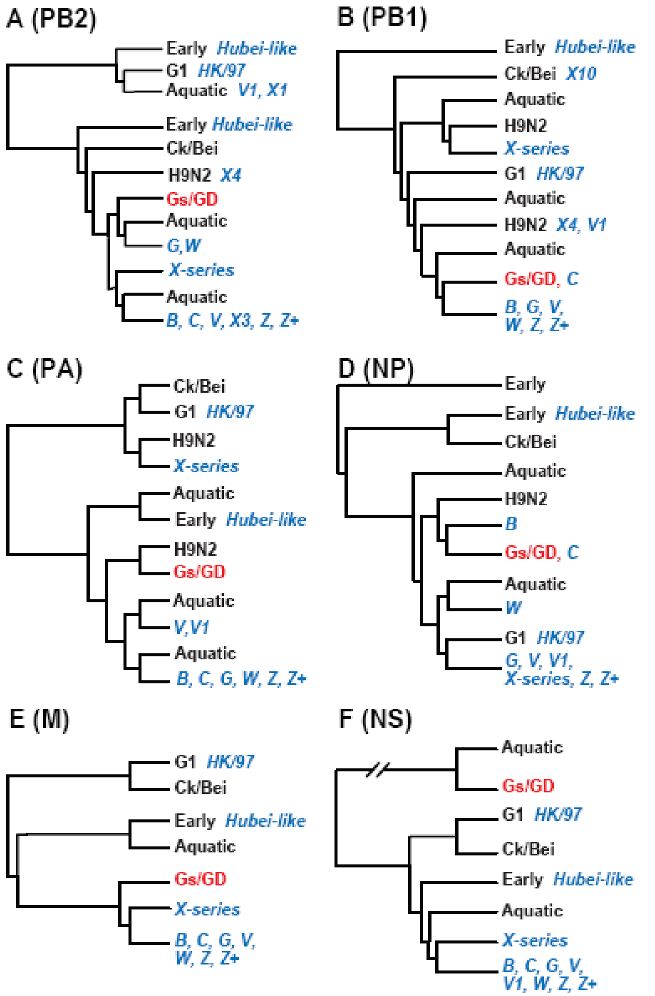
Genotype A(1-9), B(1-8)

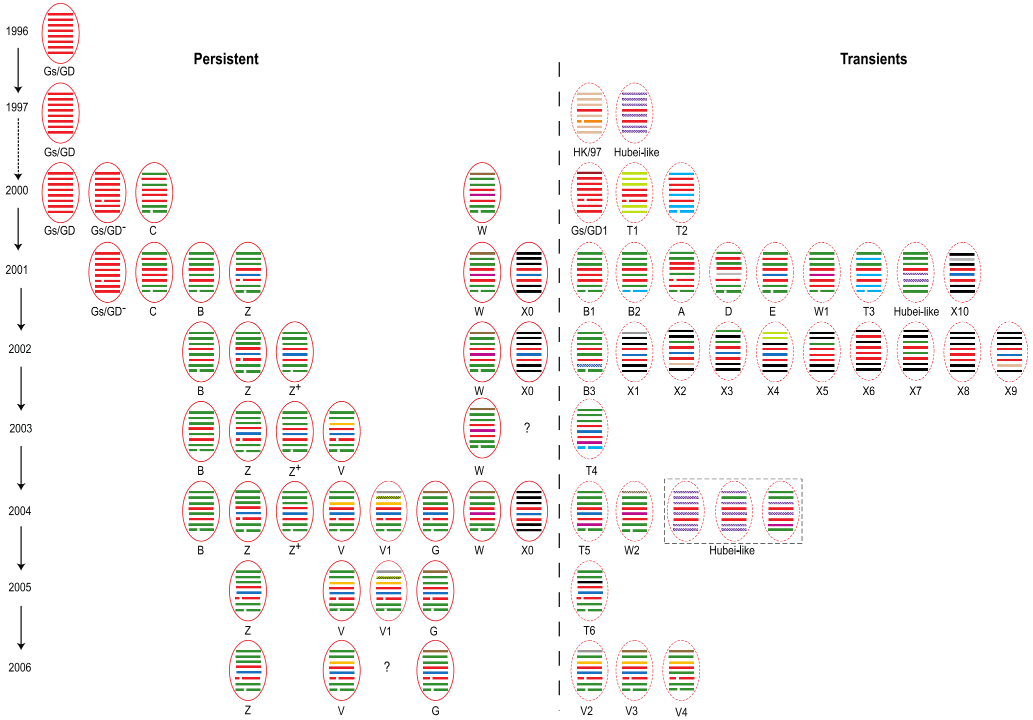
1996 1997 1998 1999 2000 2001 2002 2003 2004 2005 2006

(Year)

Guan Y et al, PNAS, 2002 & 2004
Li KS, et al, *Nature*, 2004
Chen H, et al, *PNAS*, 2006
Smith GJD, et al, *PNAS*, 2006
Xu KM, et al, *J. Virol.*, 2006 & 2007
Cheung CL et al, *J. Virol.* 2007

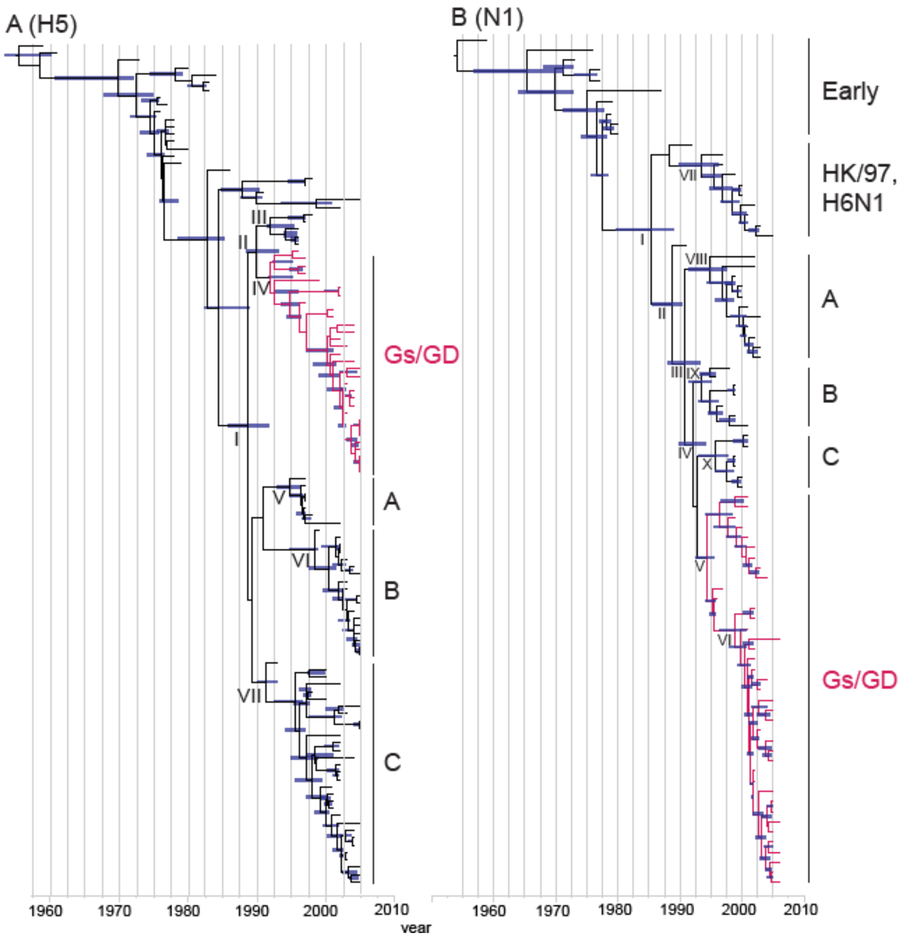
Genesis & evolution of H5N1

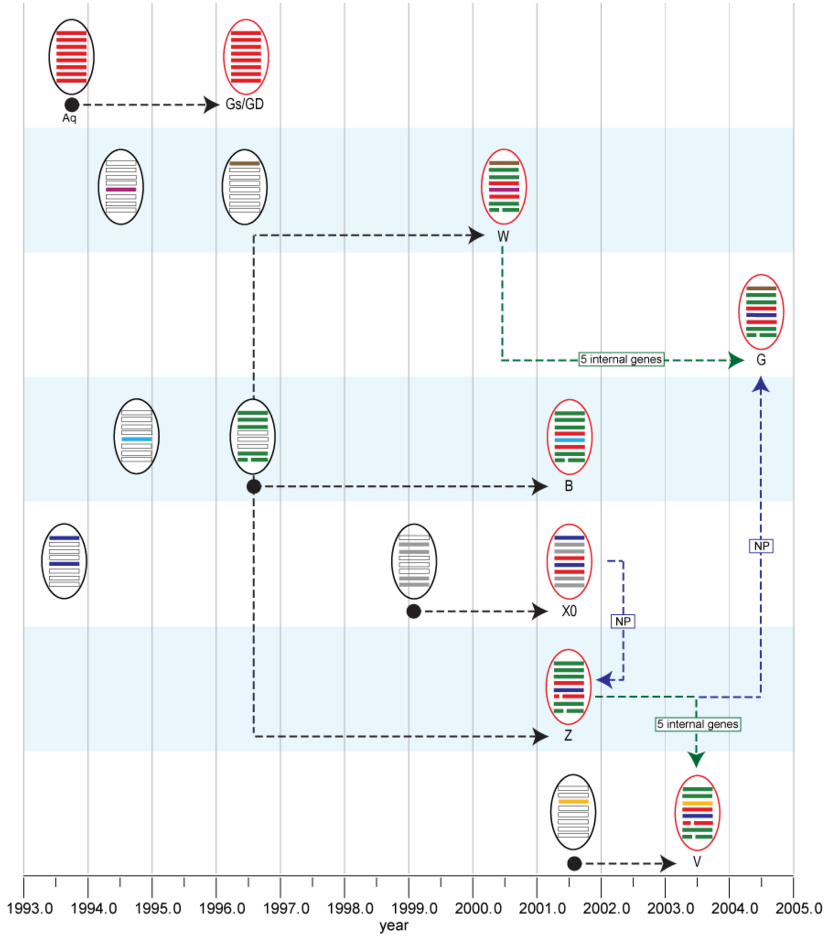




Duan et al Virology 2008

Evolutionary dynamics & emergence of H5N1





Vijaykrishna et al PLoS Pathogens 2008

H5N1 emergence

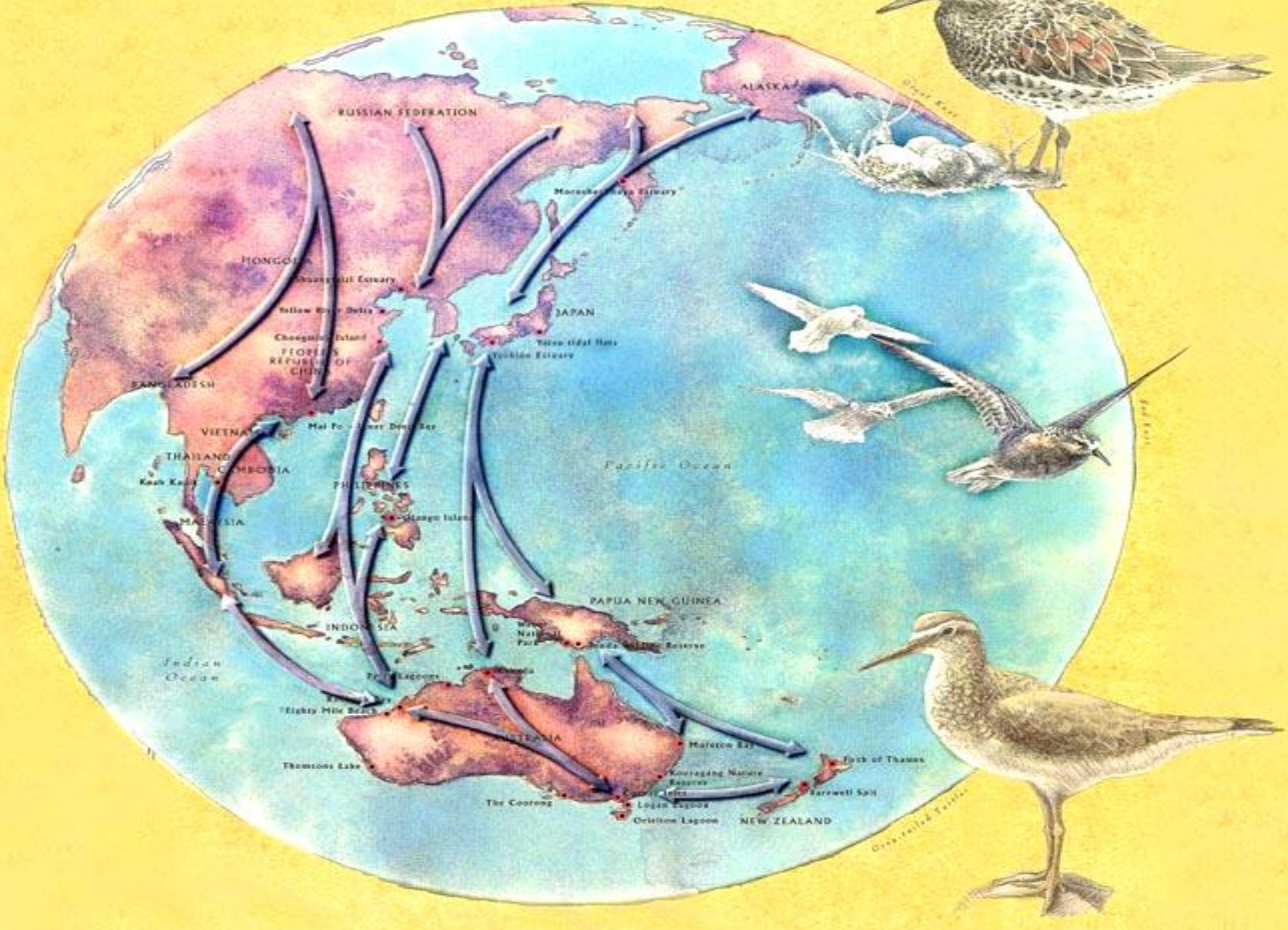
- Resulted from complex ecology of farms & live-poultry markets in southern China
- Different bird species harbor different influenza A subtypes
- Markets allow interaction between different bird species
  - Aquatic poultry (goose, duck)
  - Terrestrial poultry (chicken)
  - Minor poultry (especially quail)
- AND also allow close contact of humans & infected birds


2004 1 24


龍江雞
龍江雞
45
50
55
生


22
28
65
60
55
53
50
45
43
清遠雞
龍崗雞 60
龍崗雞 55
龍崗雞 50
芝麻
老
竹

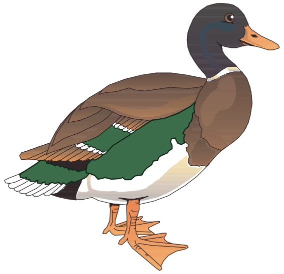
Migrating Bird Routes



Spread of H5N1 Influenza Viruses by Migratory Birds

May 2005
Outbreak of H.P. H5N1

Jan., March 2005
5 H.P. H5N1

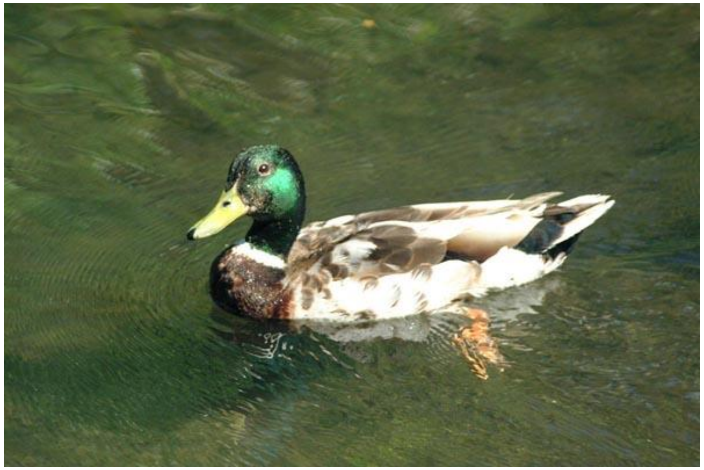


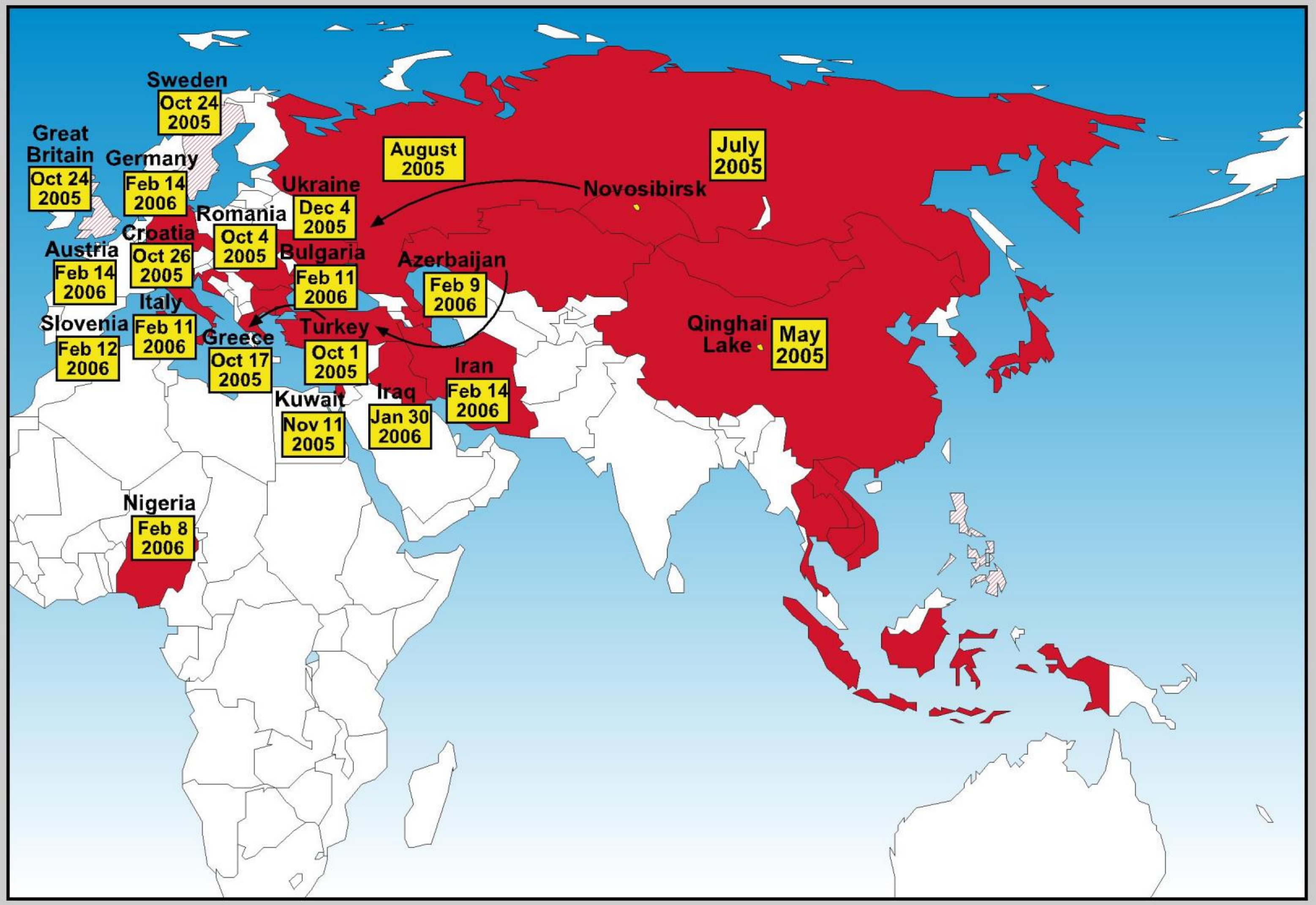
Outbreak of H5N1 in Migrating Birds in Western China 2005

April 30	**Dead geese, neurological signs**
May 4	**Daily mortality over 100 geese**
May 20	**~1,500 dead birds**
June	**~5,000 dead birds**

Pathogenicity of H5N1 Viruses for Ducks and Geese

		Lethality for:	
Virus	**Date of Isolation**	**Ducks**	**Geese**
Mallard duck/JX/05	**January/05**	**4/9**	**6/6**
Bar-headed goose/QH/05	**May/05**	**0/9**	**6/6**




Sweden
Oct 24 2005
Great Britain
Oct 24 2005
Germany
Feb 14 2006
Ukraine
Dec 4 2005
August 2005
July 2005
Novosibirsk
Romania
Oct 4 2005
Croatia
Oct 26 2005
Austria
Feb 14 2006
Bulgaria
Feb 11 2006
Azerbaijan
Feb 9 2006
Italy
Feb 11 2006
Slovenia
Feb 12 2006
Greece
Oct 17 2005
Turkey
Oct 1 2005
Qinghai Lake
May 2005
Iran
Feb 14 2006
Iraq
Jan 30 2006
Kuwait
Nov 11 2005
Nigeria
Feb 8 2006

H5N2 and H5N8 virus detections in poultry and wild birds in 2014.The almost simultaneous detection of closely related viruses in Asia, Europe, and North America suggests linkage with wild bird migration via a large region in Russia.

Josanne H. Verhagen et al. Science 2015;347:616-617

Published by AAAS


Black Diamond

3. Epidemiology – Transmission of seasonal influenza A virus

H3N2 & H1N1

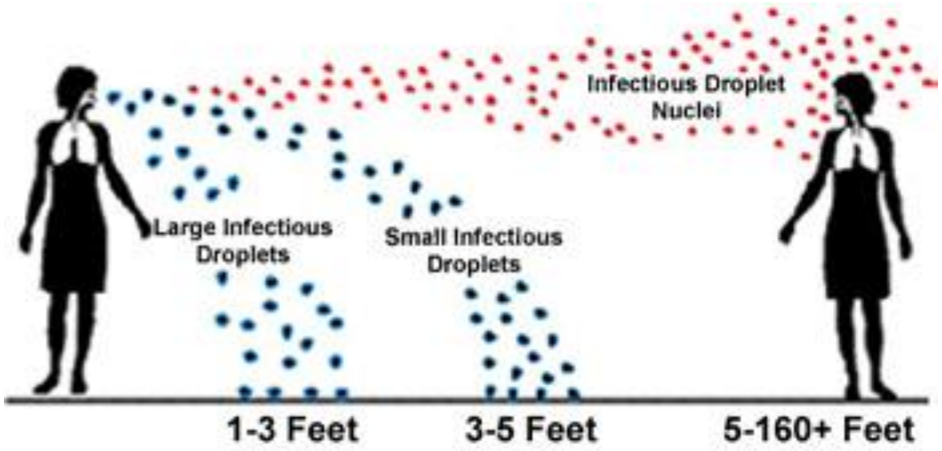
Time Check?

Aerosol Transmission of Infection

- Disease transmission is classified into three routes
  - Airborne
  - Contact
  - Droplet
- Airborne transmission is defined as resulting from the inhalation of small particles, often termed droplet nuclei and considered to have diameters 5-10 μm or less.

Aerosol Transmission of Infection

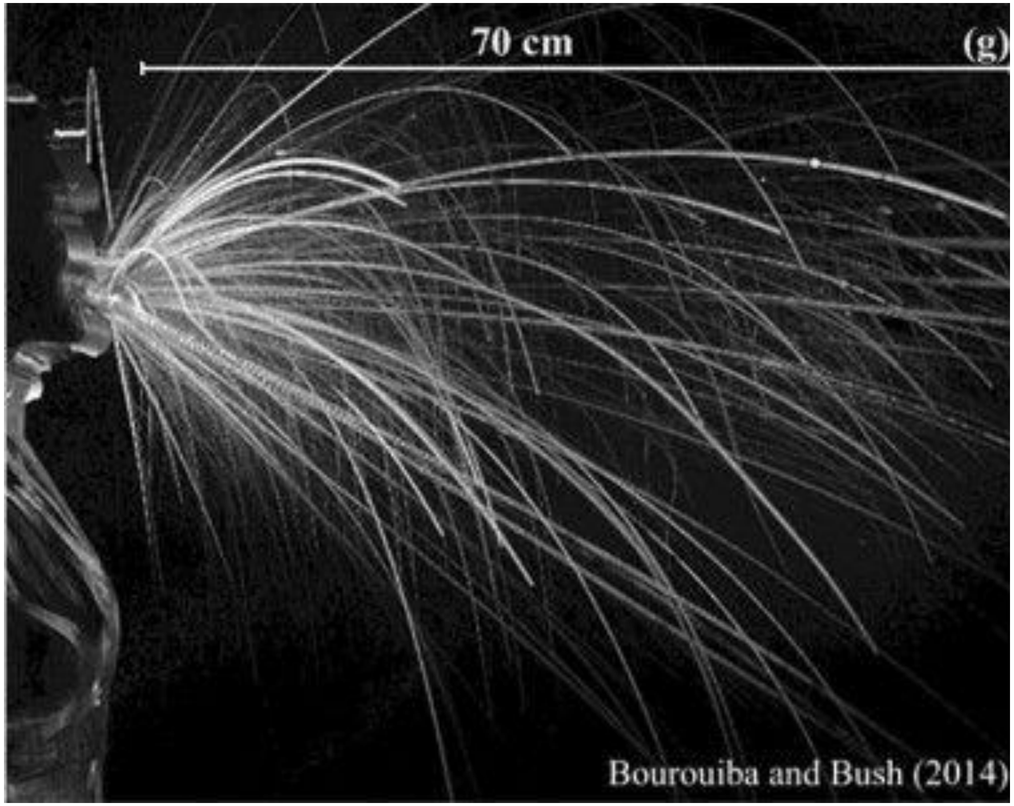
- **Airborne:** aerosols become smaller by evaporation; if inhaled can travel deep into the lung
- **Droplets:** land directly on mucosal lining of nose, mouth, eyes of nearby persons (can be inhaled)
  - **Highest exposure within 3-6 feet**
- **Contact:** Aerosols/secretions contaminate surface. Transmission is through the touch of contaminated surfaces



Relative contribution of three routes varies with different agents

Aerosol Transmission of Infection

Aerosol Transmission of Infection

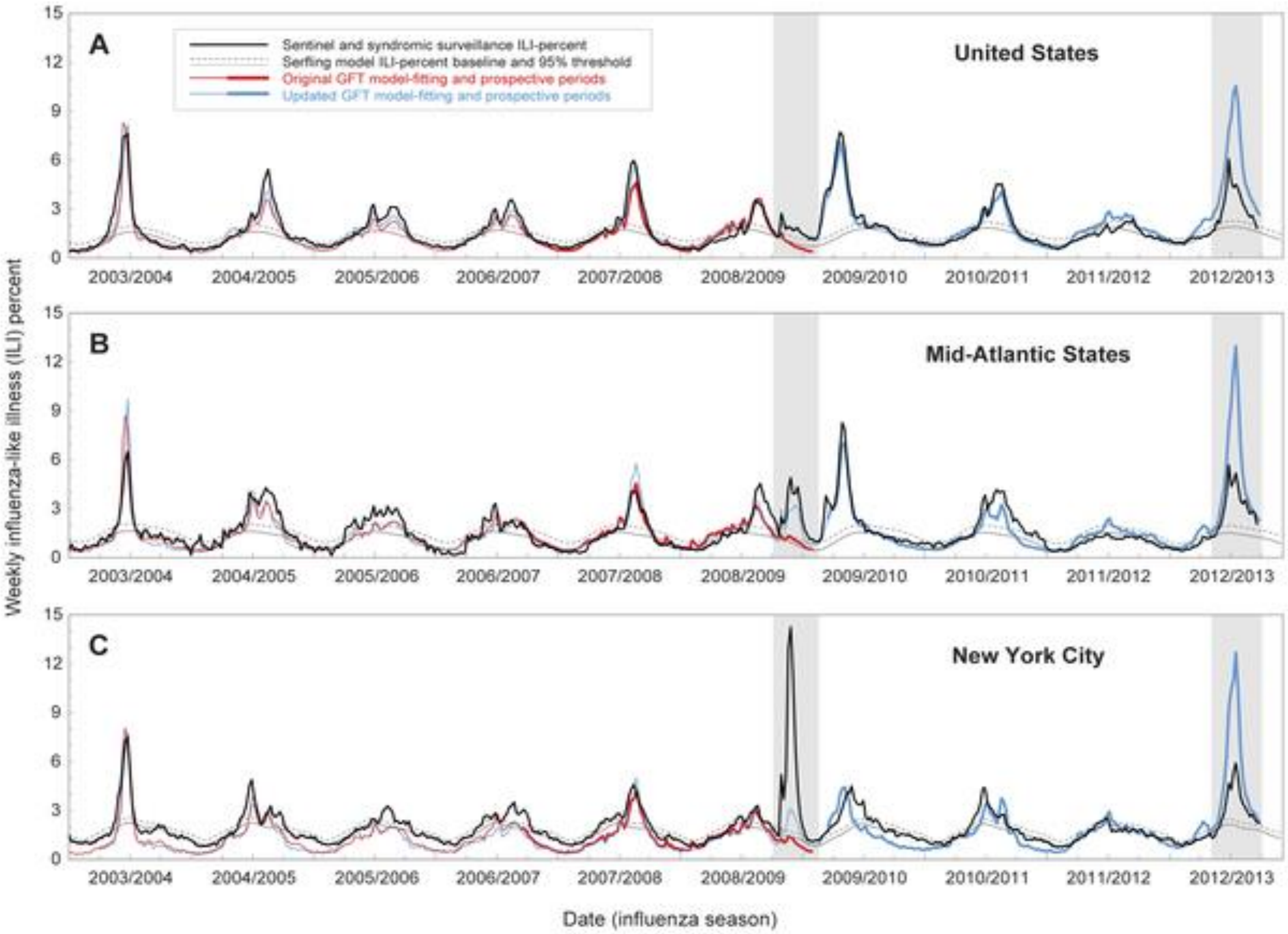


Smaller particles travel farther

Carried on a cloud of turbulence

Can cross a room and get sucked into air vents

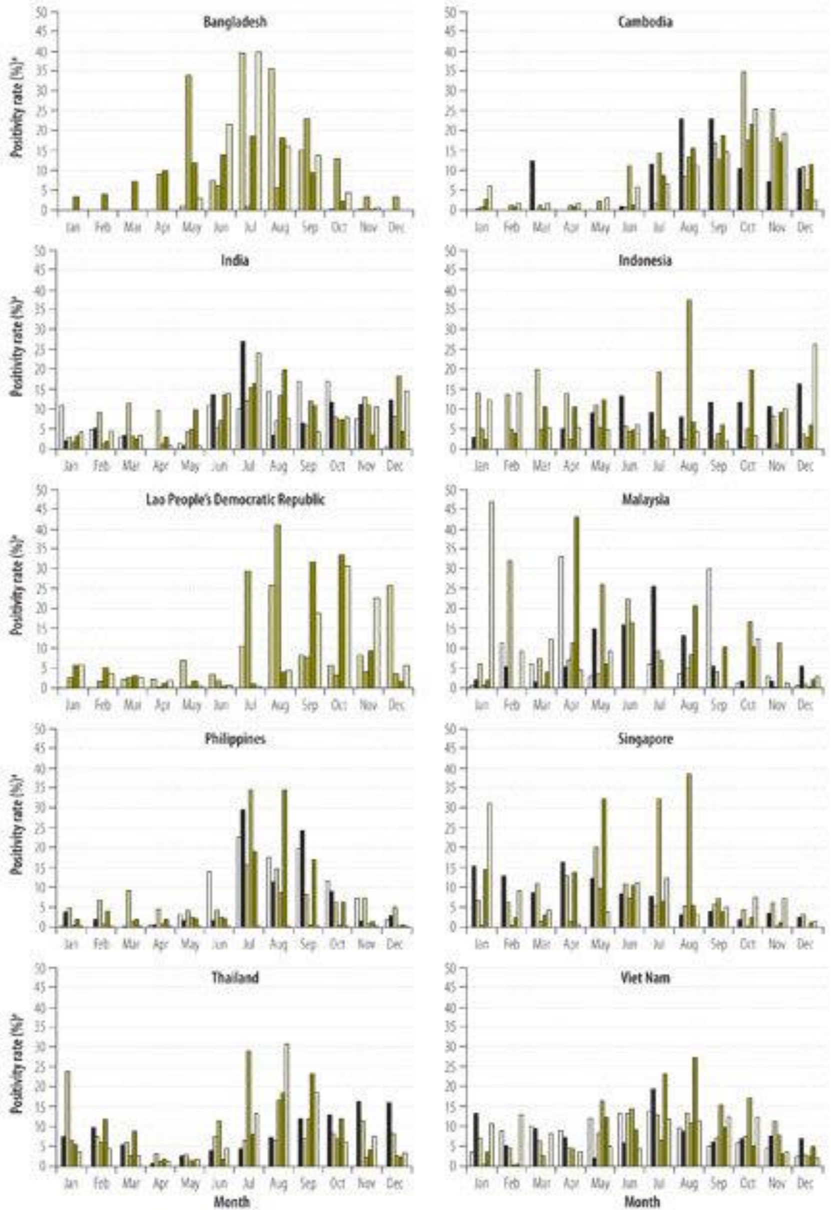
Figure 1. Time-series of weekly influenza-like illness (ILI) surveillance and Google Flu Trends (GFT) search query estimates, June 2003–March 2013.



Olson DR, Konty KJ, Paladini M, Viboud C, Simonsen L (2013) Reassessing Google Flu Trends Data for Detection of Seasonal and Pandemic Influenza: A Comparative Epidemiological Study at Three Geographic Scales. PLoS Comput Biol 9(10): e1003256. doi:10.1371/journal.pcbi.1003256
http://journals.plos.org/ploscompbiol/article?id=info:doi/10.1371/journal.pcbi.1003256

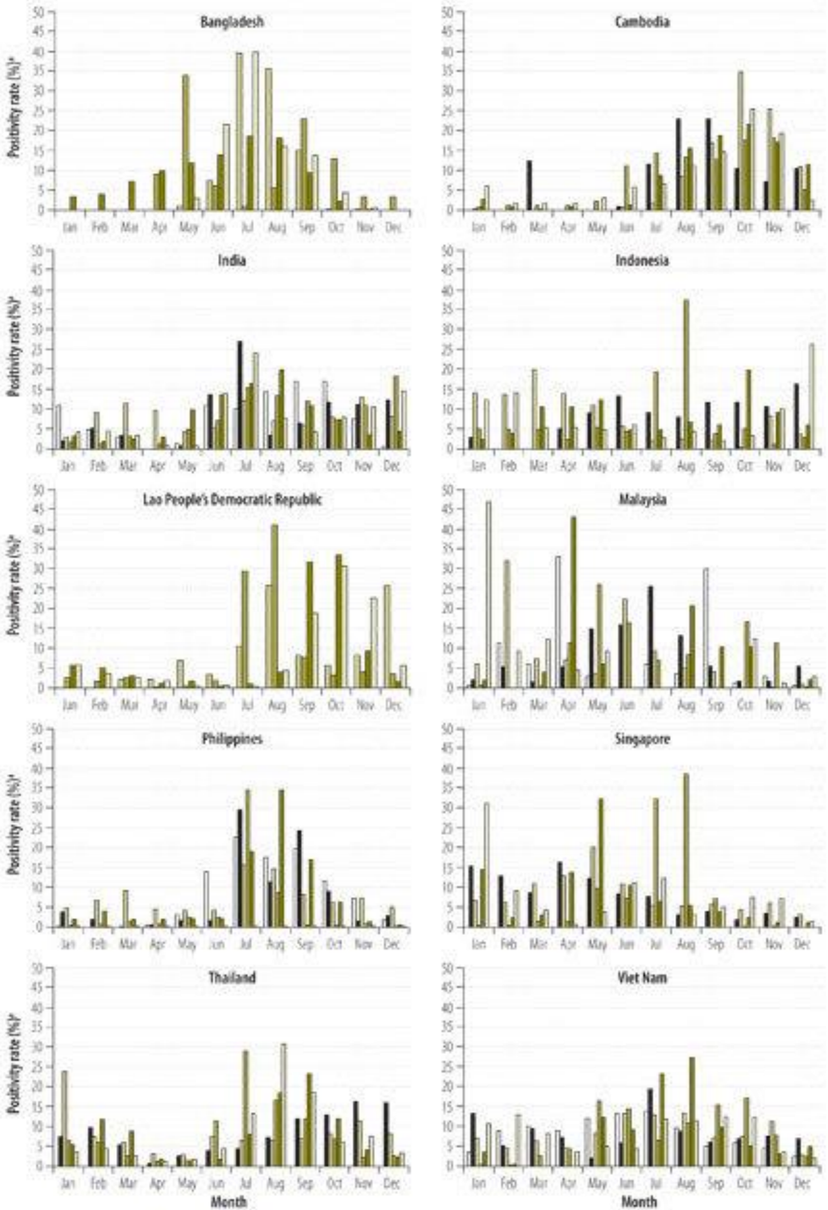
PLOS | COMPUTATIONAL BIOLOGY

What about incidence trends in the tropics?



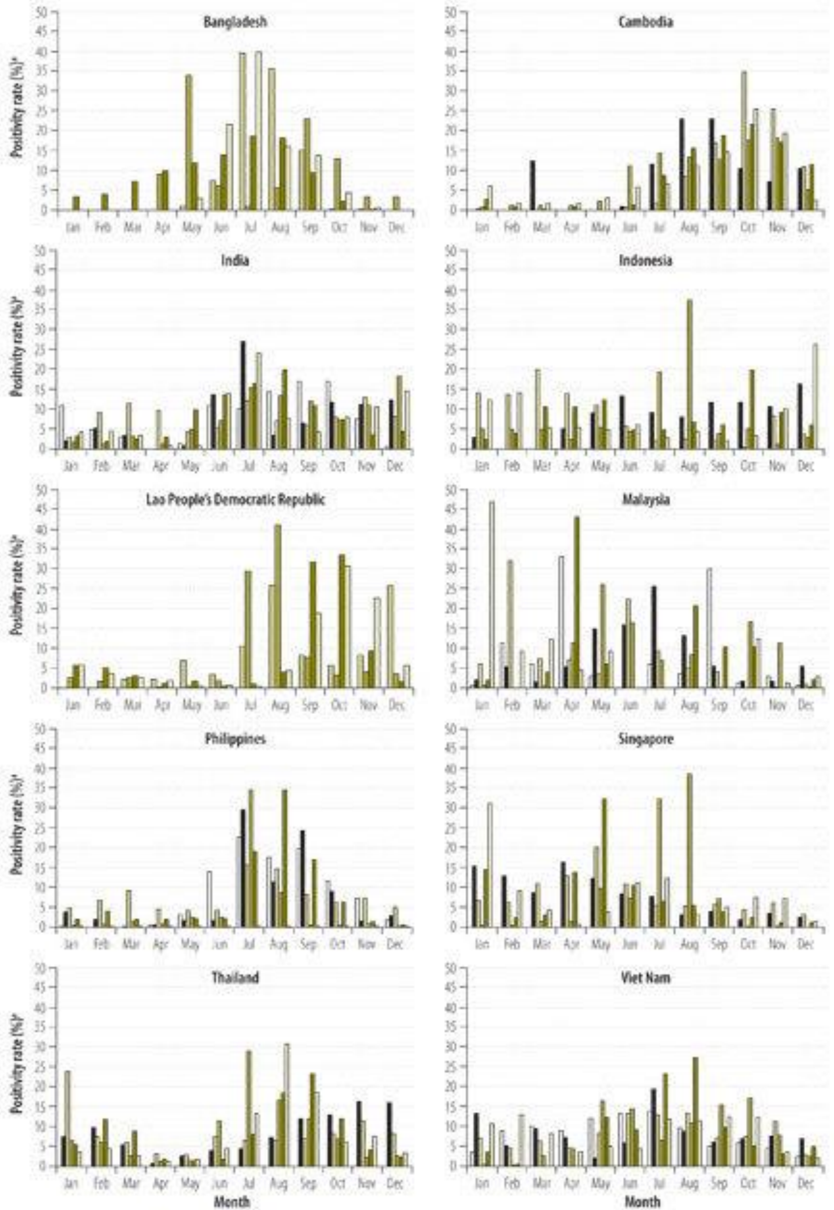
- Variation between countries
- Disease occurs through the year
- No obvious trends – unlike temperate regions

What about incidence trends in the tropics?



- How are these outbreaks linked?
- Do viruses circulate locally continually emerging to cause epidemics, or do they circulate globally moving between populations?

What about incidence trends in the tropics?



- Not obvious from patterns of people getting sick (ili reports)
- Can we infer global transmission dynamics from examination of virus population?
  - Comparative genetics and ecological modeling.
  - Applied Evolutionary Biology

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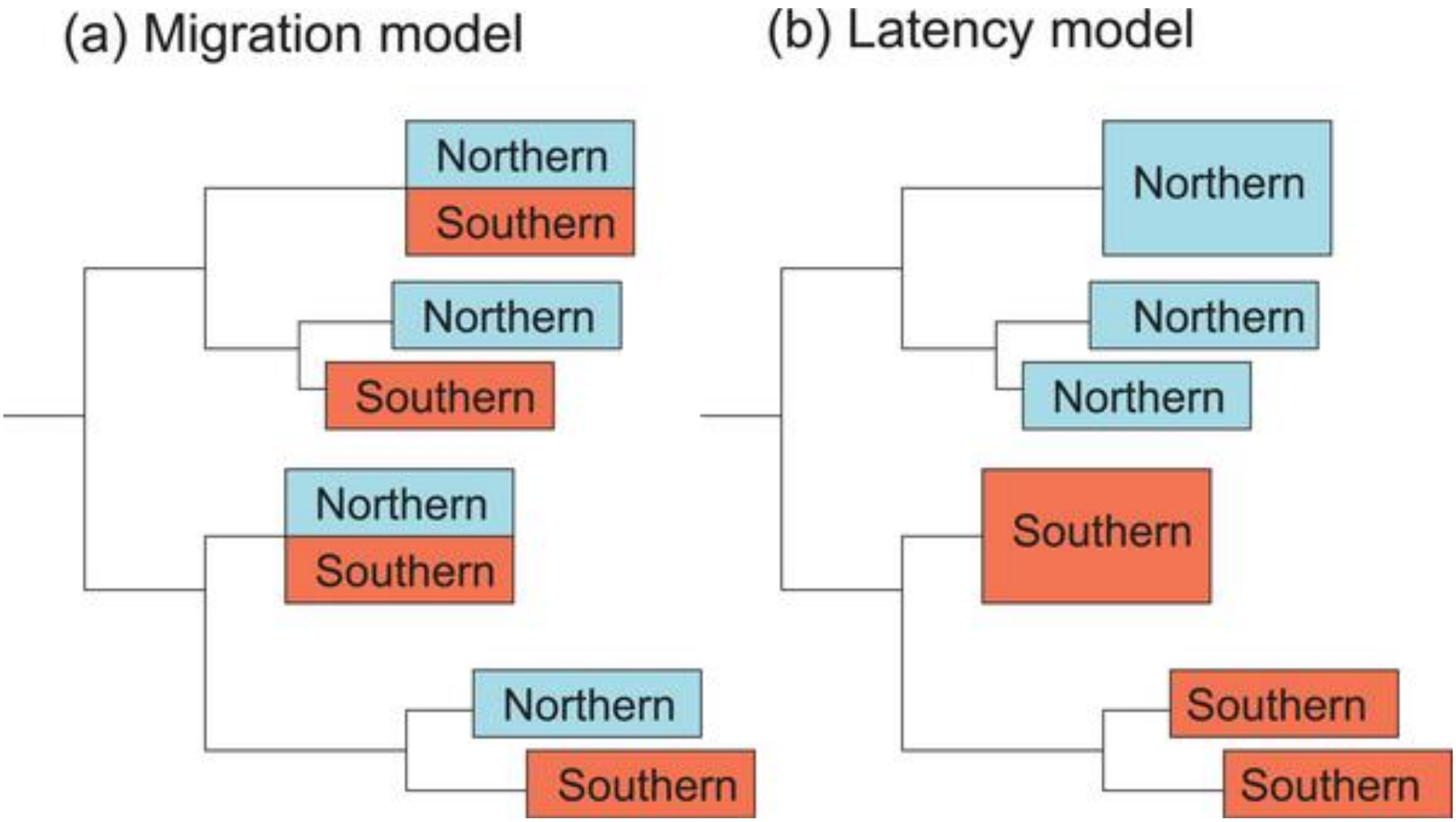
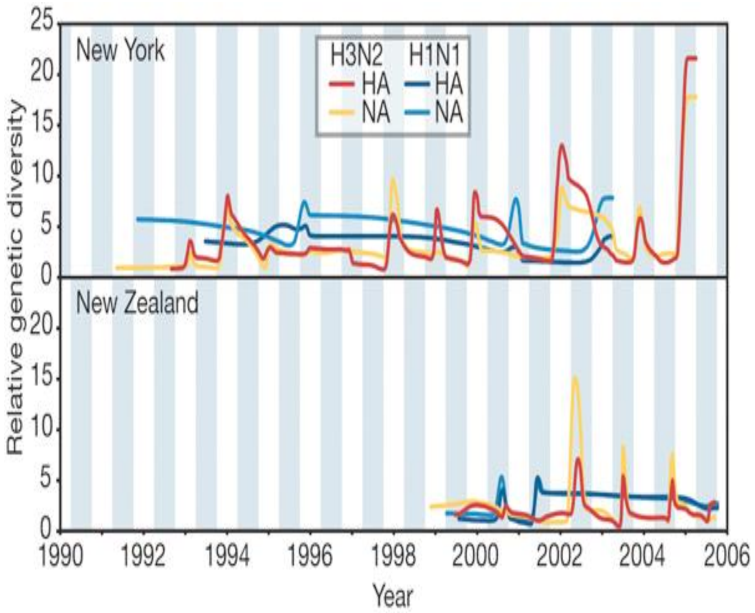
Migration dynamics of Seasonal influenza



Nelson MI et al (2007) PLoS Pathog 3(9): e131. doi:10.1371/journal.ppat.0030131

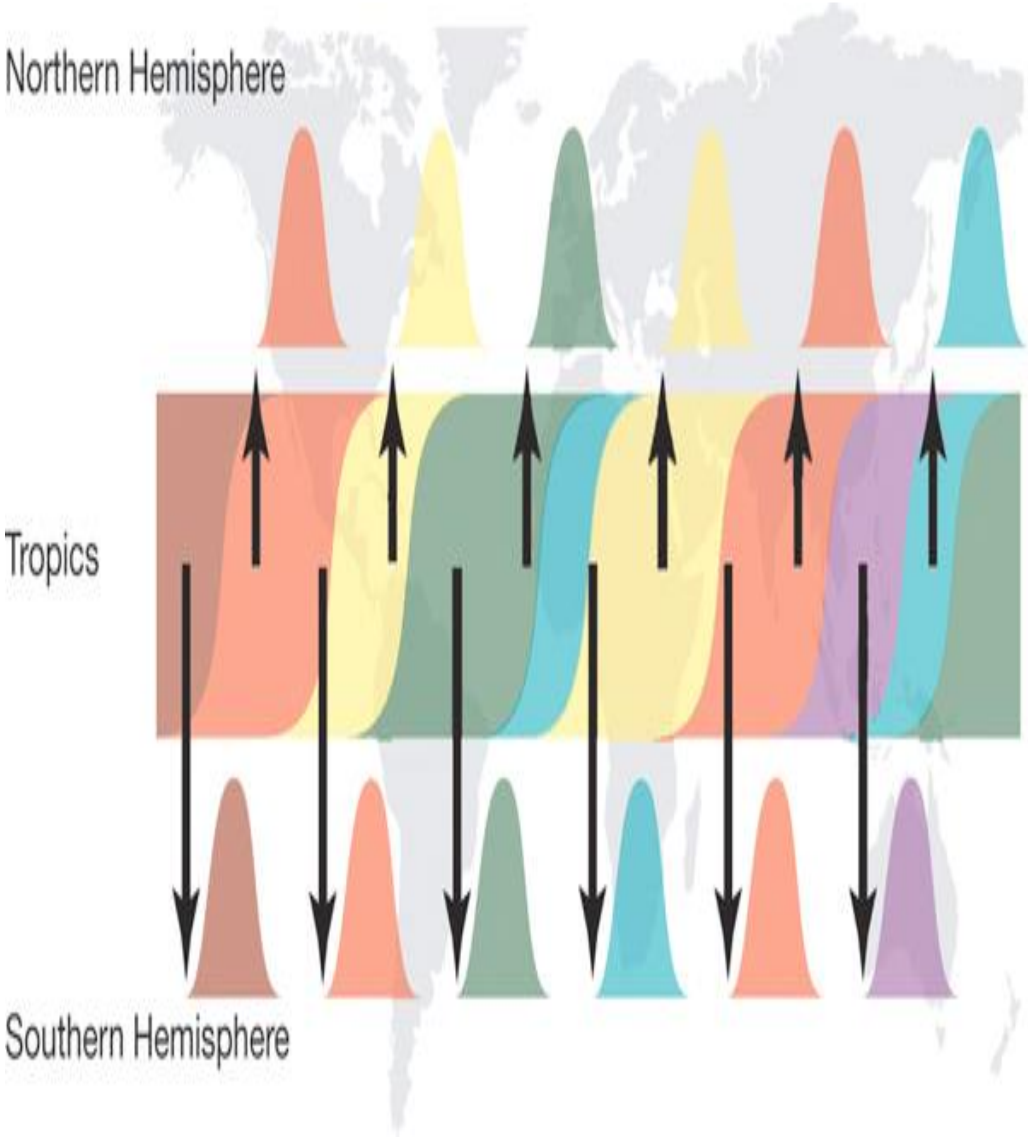
Rambaut et al, Nature 2008



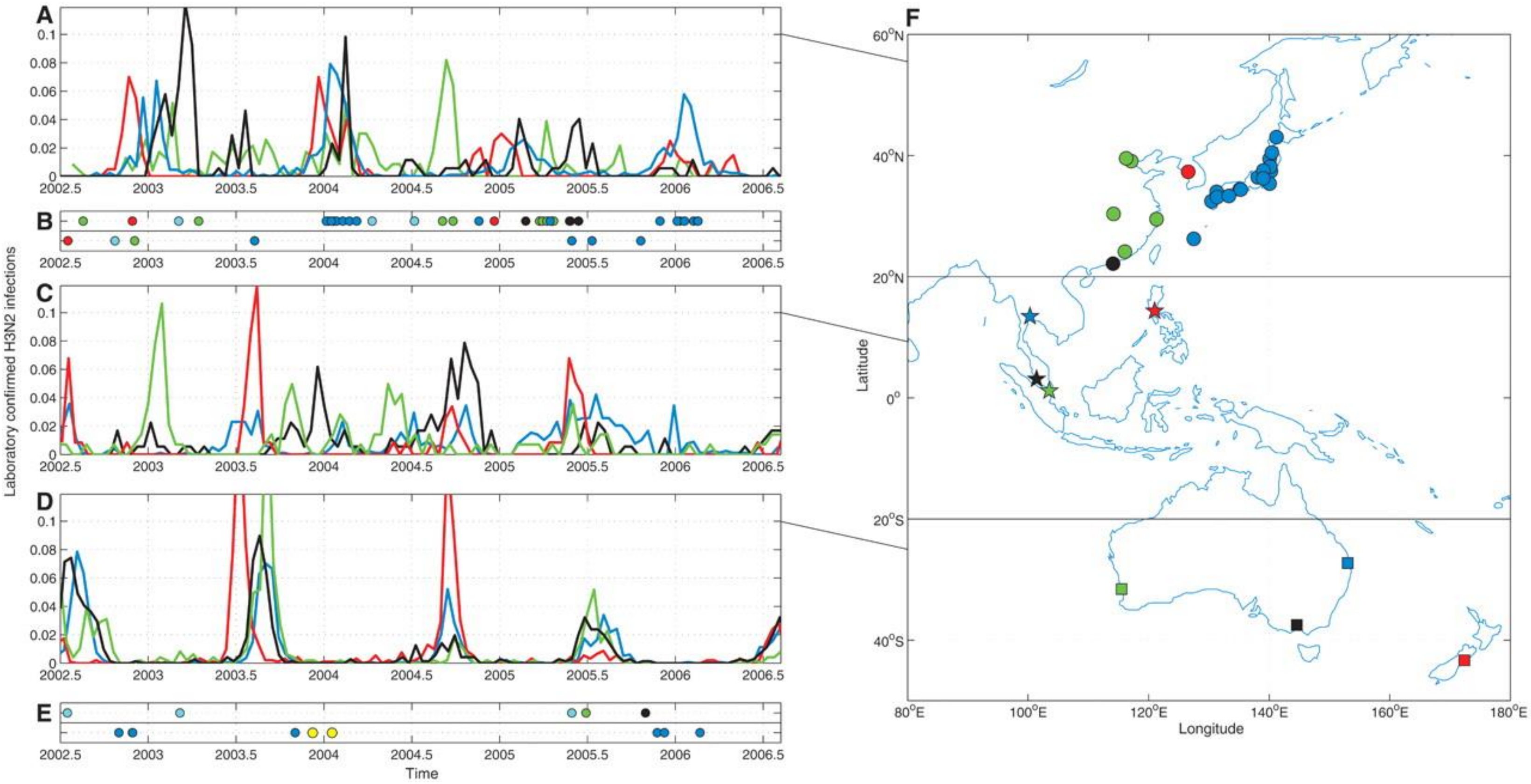
Temporally offset epidemic peaks – strong bottlenecks

- Continual—but largely unidirectional—gene flow from a common source population provides the viruses that ignite each epidemic in populations of the Northern and Southern Hemispheres

Rambaut et al, Nature 2008



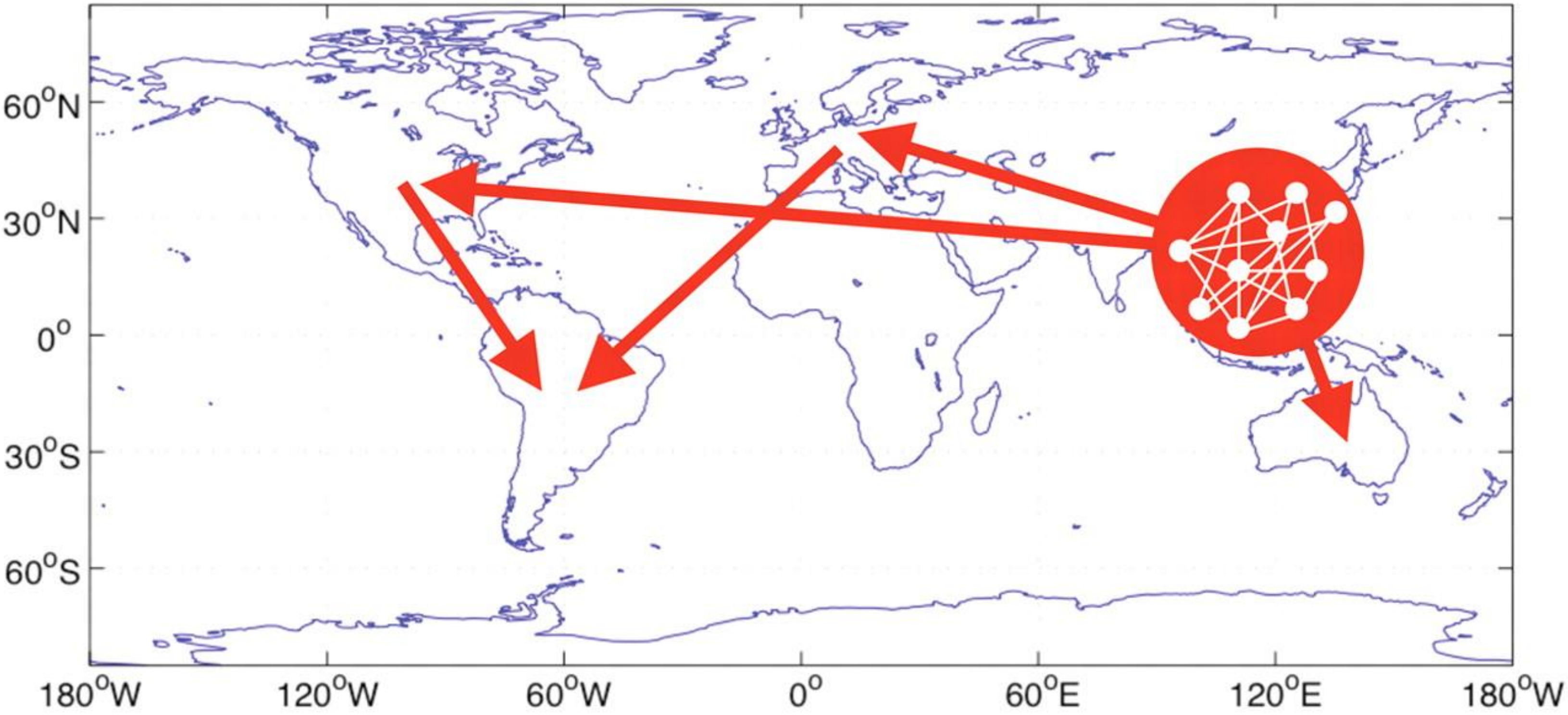
Russell *et al*, Science 2008



C A Russell et al. Science 2008;320:340-346

Published by AAAS

Russell *et al*, Science 2008



C A Russell et al. Science 2008;320:340-346

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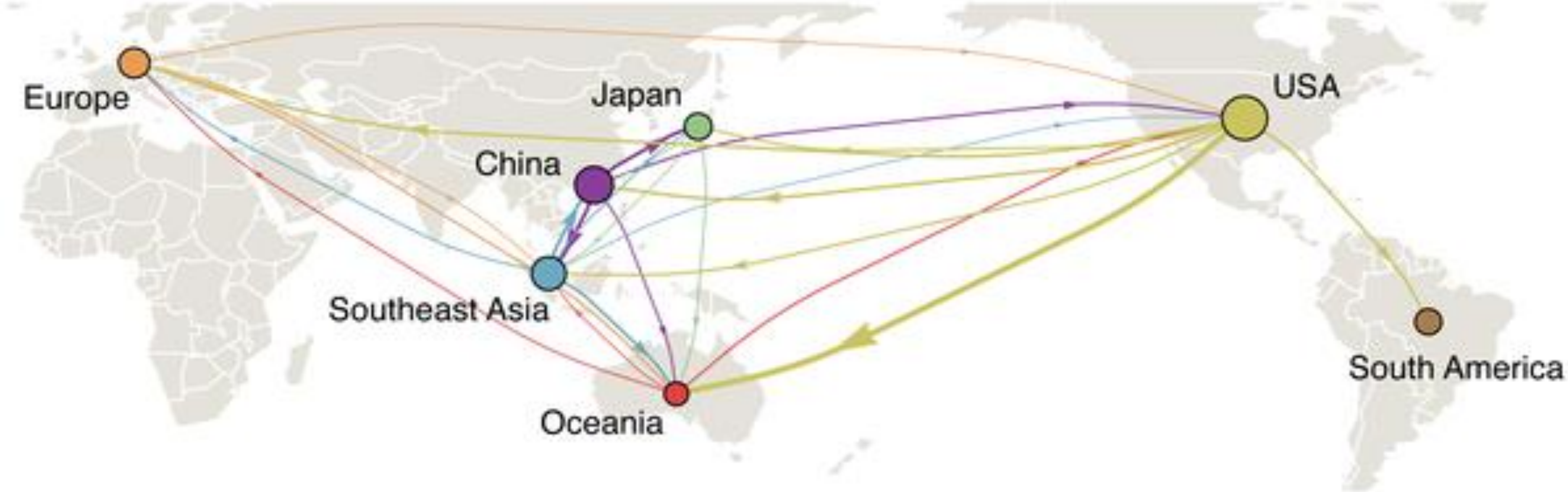
Figure 1. Global migration patterns of influenza A (H3N2) estimated from sequence data between 2002–2008.



Bedford T, Cobey S, Beerli P, Pascual M (2010) Global Migration Dynamics Underlie Evolution and Persistence of Human Influenza A (H3N2). PLoS Pathog 6(5): e1000918. doi:10.1371/journal.ppat.1000918
http://www.plospathogens.org/article/info:doi/10.1371/journal.ppat.1000918

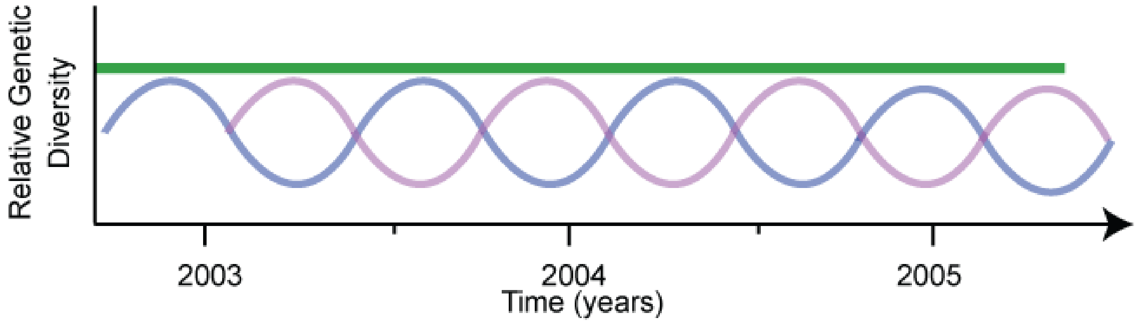
Evolutionary dynamics of H3N2 seasonal influenza
What tools do we have?

Source-Sink Model

Northern sink population

Southeast asian source population

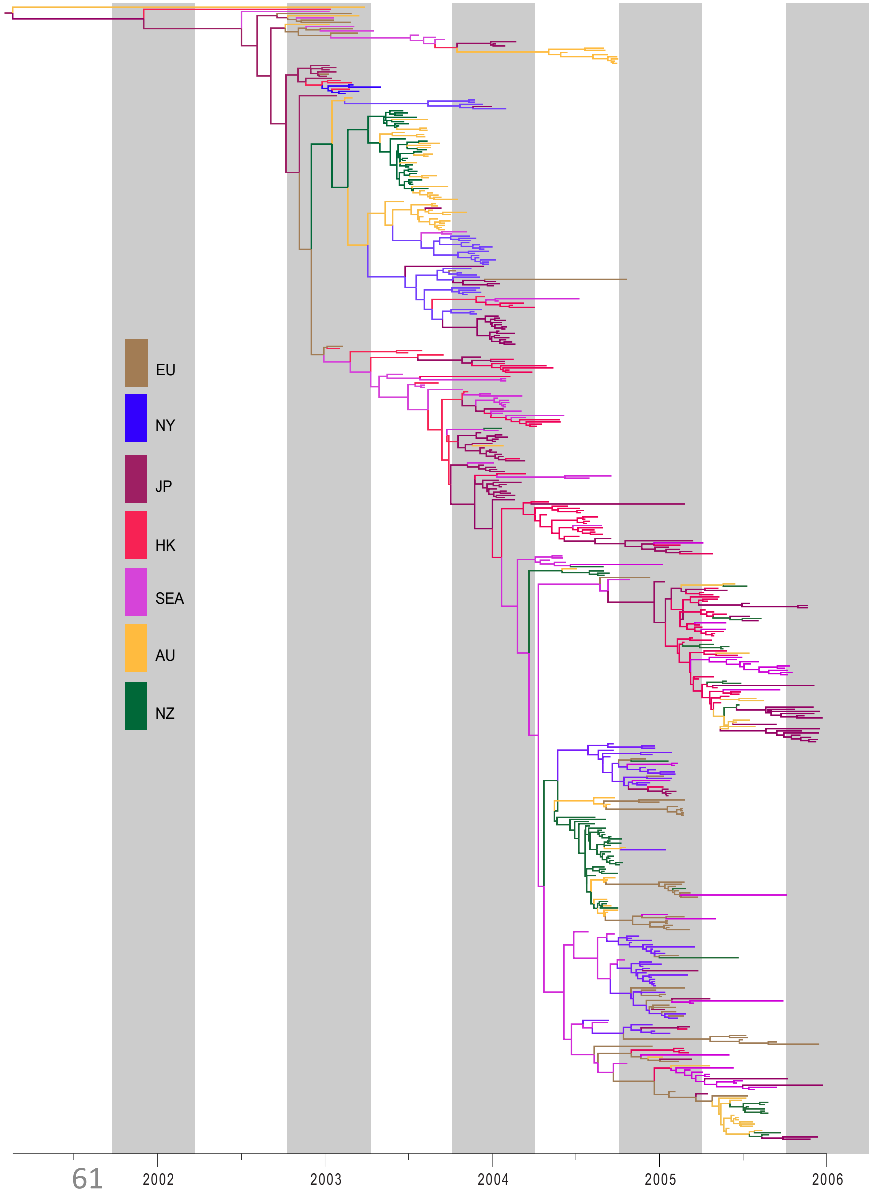
Southern sink population



Source population maintains high levels of diversity without seasonal fluctuations. Seeds outbreaks in northern and southern temperate regions

Phylogeography – transition between discrete states

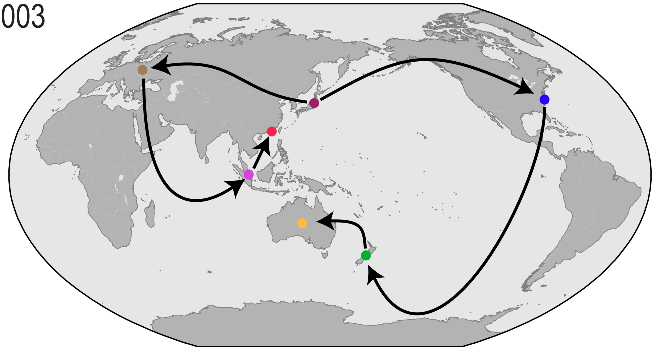
Supplementary Figure 8



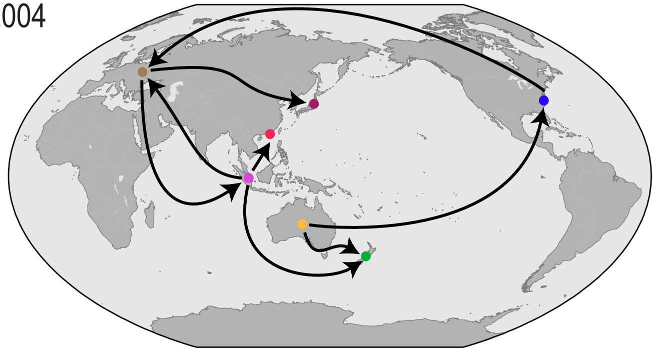
- Tree trunk alternates between Japan, SEA, HK, Europe
- Within a single year, all locations occupy part of the tree backbone

Phylogeography – transition between discrete states

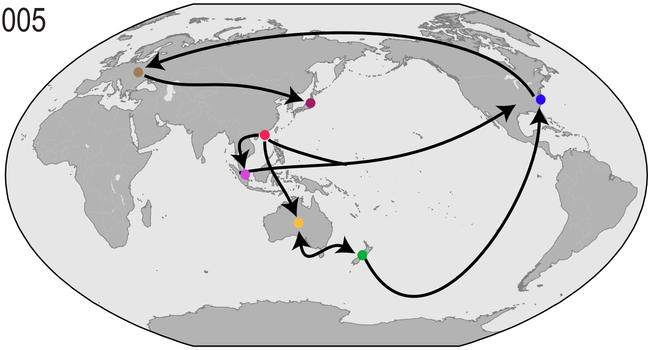
2003



2004



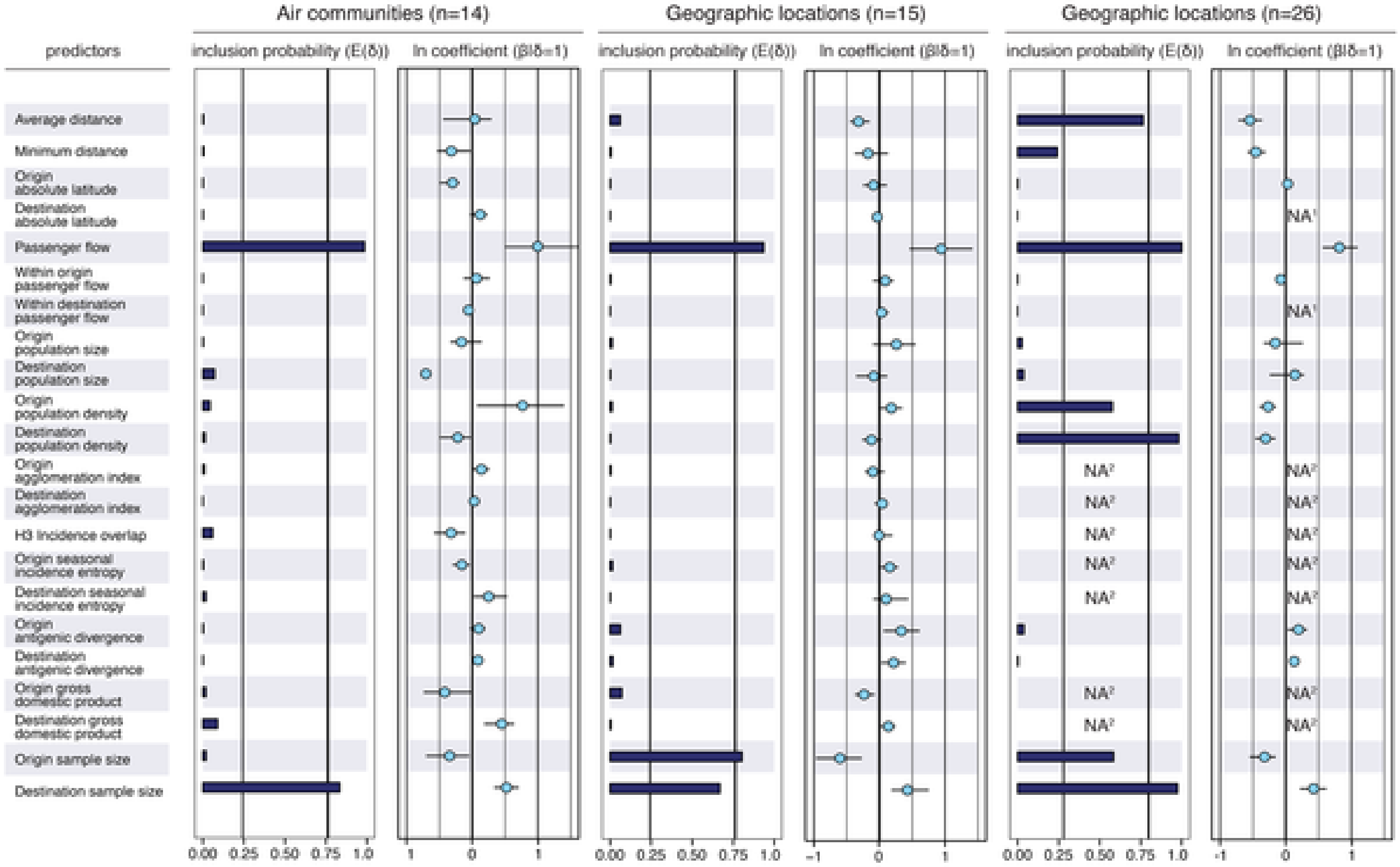
2005



- No model supports persistence in SEA or HK

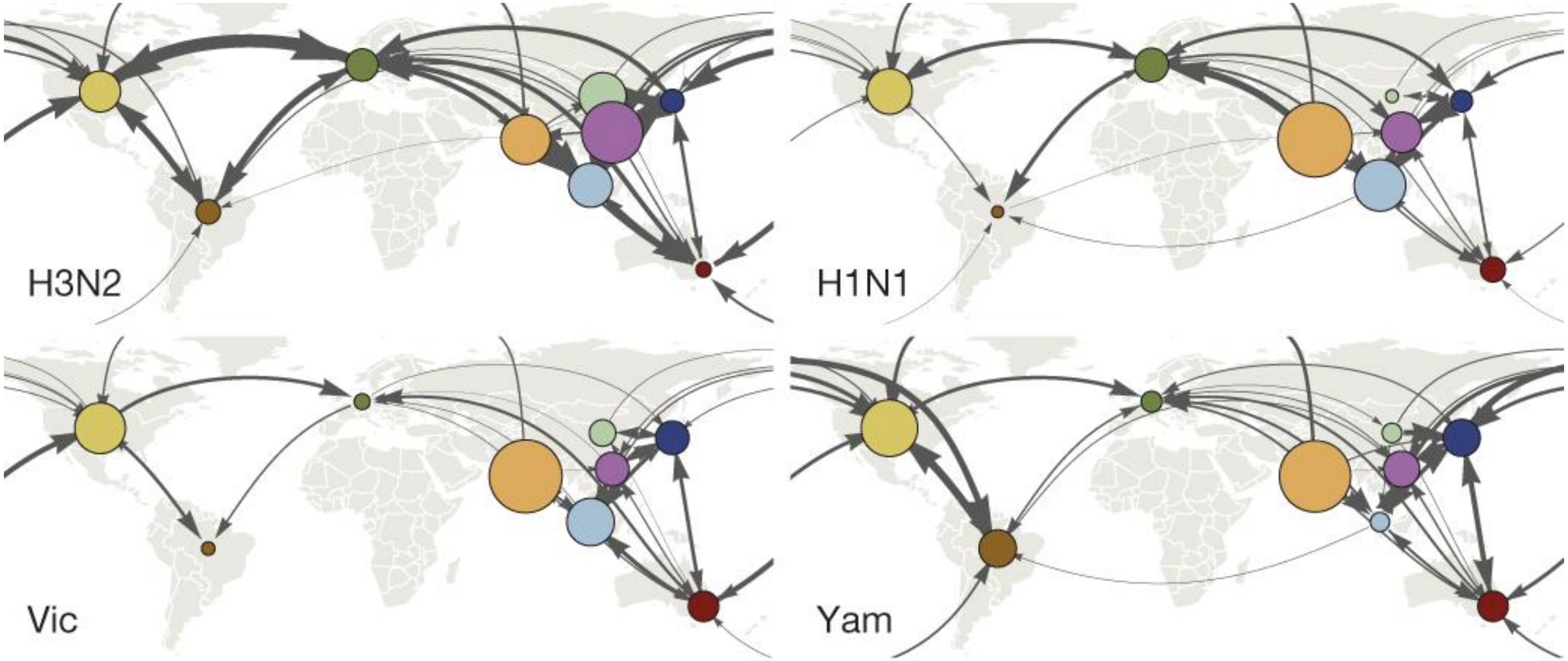
Source-Sink Model for seasonal influenza is not supported

Figure 2. Predictors of global H3N2 diffusion among the 14 air communities and the 15 & 26 geographic locations.



Lemey P, Rambaut A, Bedford T, Faria N, Bielejec F, et al. (2014) Unifying Viral Genetics and Human Transportation Data to Predict the Global Transmission Dynamics of Human Influenza H3N2. PLoS Pathog 10(2): e1003932. doi:10.1371/journal.ppat.1003932
http://journals.plos.org/plospathogens/article?id=info:doi/10.1371/journal.ppat.1003932

Estimates of mean pairwise virus migration rate.



T Bedford *et al. Nature* **000**, 1-4 (2015) doi:10.1038/nature14460

nature

Summary

- If each population is considered to be somewhat isolated, this produces a network of flight-connected population centers with multiple epidemic peaks globally distributed at any given time.
- New York is an extremely well connected global centre. It may be that outbreaks in New York are transmitting globally during seasonal epidemics, but, only establish in other centers where suitable (i.e. other northern temperate regions). Transmission into SEA may be crucial for transmission to the southern temperate regions.