

Viral spillover, cross-species transmission, and emerging tools for outbreak investigation

Louise Moncla

University of Pennsylvania

Department of Pathobiology

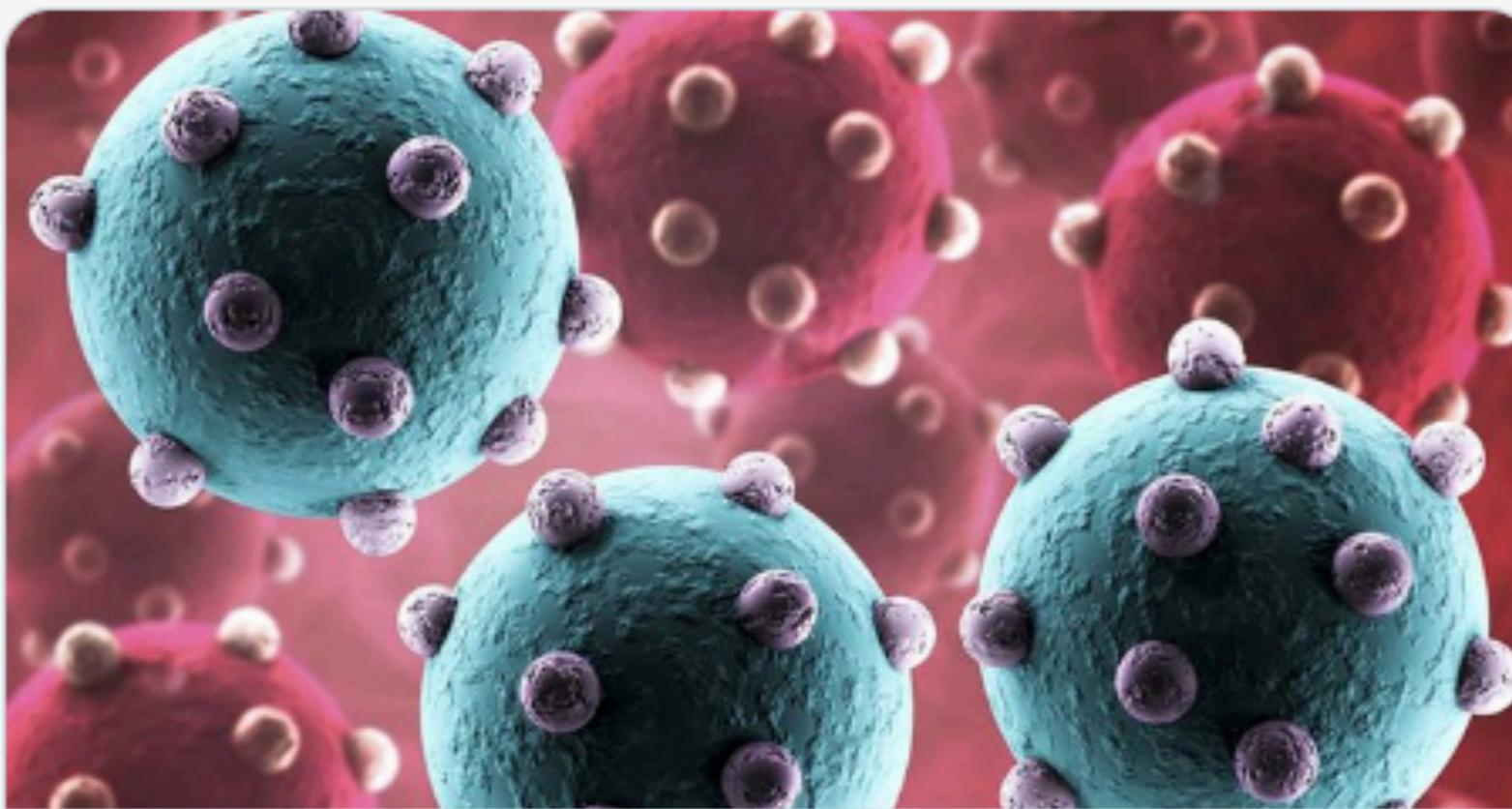
December 4, 2023



Thijs Kuiken @thijskuiken · Feb 23

...

Following the death of an 11-year-old girl from highly pathogenic avian #influenza #H5N1 in Prey Veng province, #Cambodia, Ministry of Health reports 12 more infected people, 4 of whom have begun to show symptoms. Results of diagnosis expected tomorrow.



khmertimeskh.com

After death of girl, 12 more possibly detected with H5N1 bird flu in C...

Ms. Youk Sambath, Secretary of State of the Ministry of Health, has confirmed that the Ministry of Health's emergency response team h...



55



1,023



1,507



625K





National Wildlife Health Center
Madison, Wisconsin
Updated November 27, 2022

Data: USDA, CFIA, WOAH, States
Provisional. All locations are approximations
based on best available information.

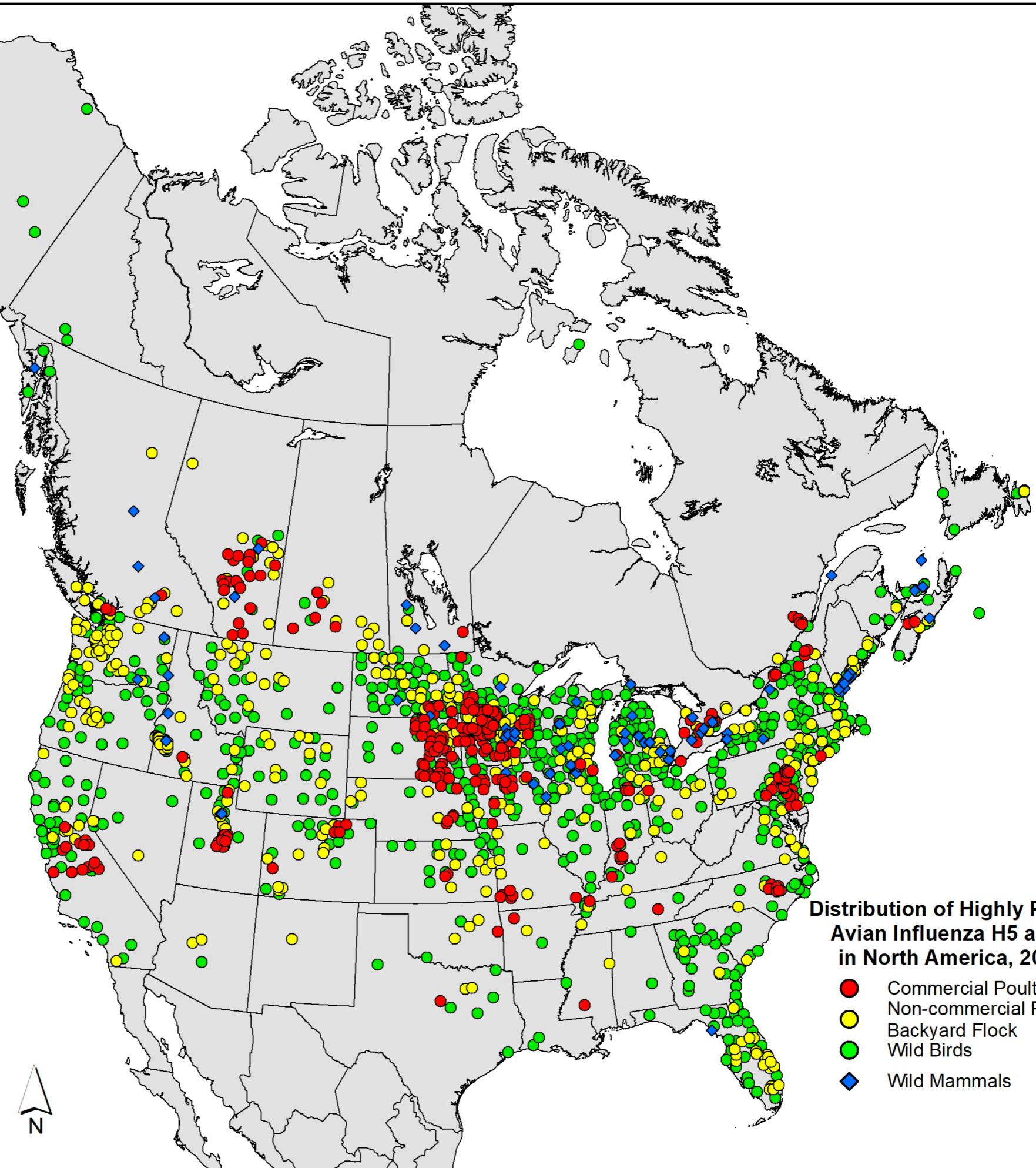
0 250 500 1,000 Miles

Projection: US National Atlas Equal Area



Distribution of Highly Pathogenic Avian Influenza H5 and H5N1 in North America, 2021/2022

- Commercial Poultry
- Non-commercial Poultry,
Backyard Flock
- Wild Birds
- ◆ Wild Mammals





The New York Times

Bird Flu Outbreak Puts Mink Farms Back in the Spotlight

A new variant of avian influenza appears capable of spreading among mammals, highlighting the need for more proactive surveillance, experts said.

February 8,
2023



First new U.S. case of human bird flu confirmed in Colorado prison



An Even Deadlier Pandemic Could Soon Be Here

Feb. 3, 2023 7 MIN READ



A pelican suspected to have died from H5N1 avian influenza on a beach in Peru in December. Ernesto Benavides/Agence France-Presse, via Getty Images



Thijs Kuiken @thijskuiken · Feb 23

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55

1,023

1,507

625K



Jurre Y Siegers, PhD @jurreysi · Feb 23

...

There seems to be a translation error from Khmer to English. The report below (in khmer) states there are 12 contacts to the index case and 4 amongst those 12 report flu like symptoms.

The questions: Is this human case related to the ongoing outbreak in Europe/the Americas? Does this reflect a new epidemiological pattern?



Erik Karlsson
@E_A_Karlsson

...

Happy to announce that the full genome sequence of the Cambodian #H5N1 #avianflu case is now available on @GISAID. This has been an incredible effort by a number of people: (1/)

12:15 AM · Feb 26, 2023 · 532.9K Views

387 Retweets 74 Quotes 1,615 Likes 85 Bookmarks



Phylogeny



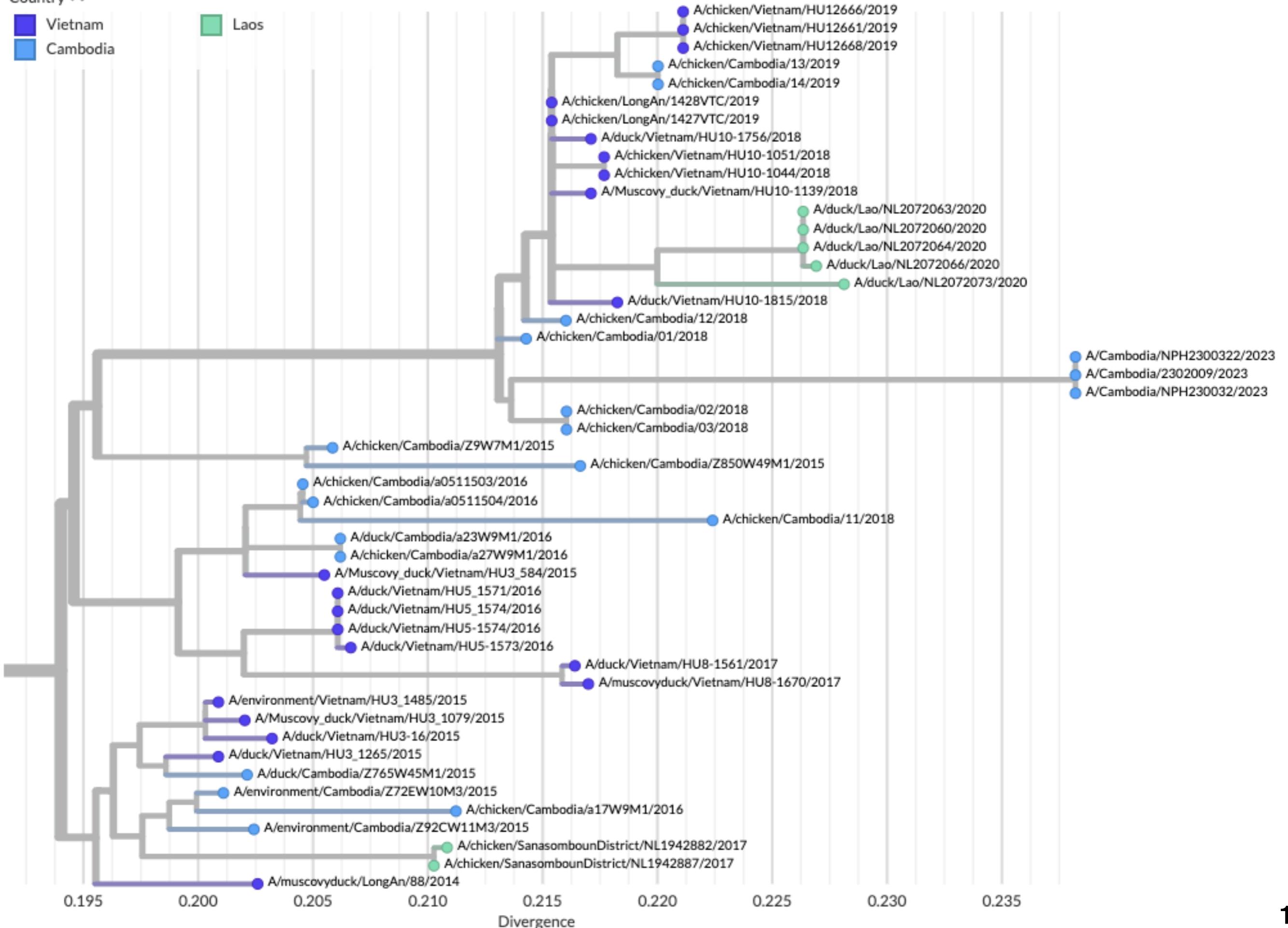
ZOOM TO SELECTED

RESET LAYOUT

Country ▲

- Vietnam
Cambodia

Laos



0.195

0.200

0.205

0.210

0.215
Divergence

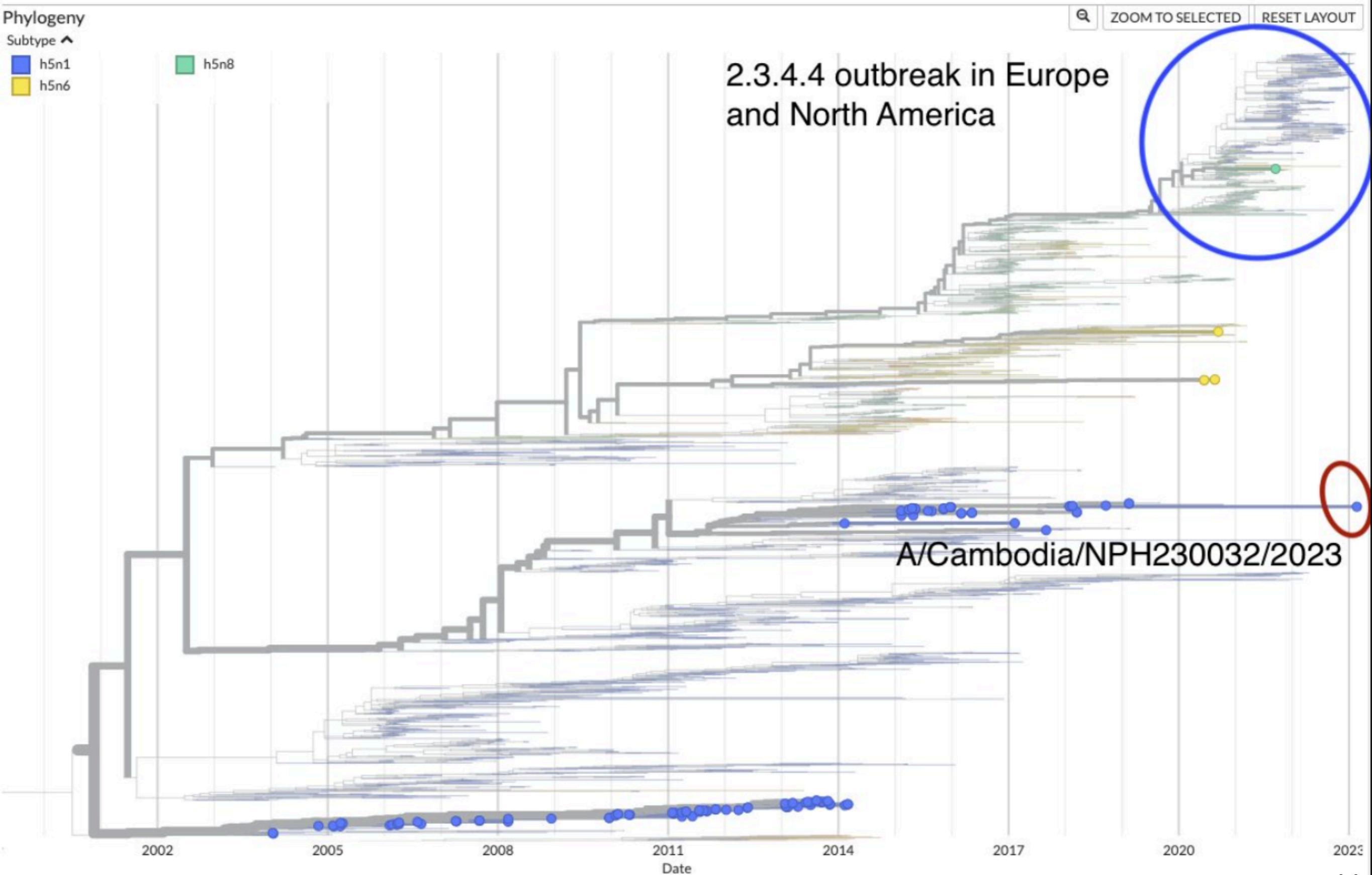
0.220

0.225

0.230

0.235

10



The answers:

- These cases were caused by a lineage endemic to Cambodia, likely by direct interaction with sick, wild birds
- These cases do not represent evolution of human to human transmission of the H5 viruses currently causing the outbreaks in Europe and North America

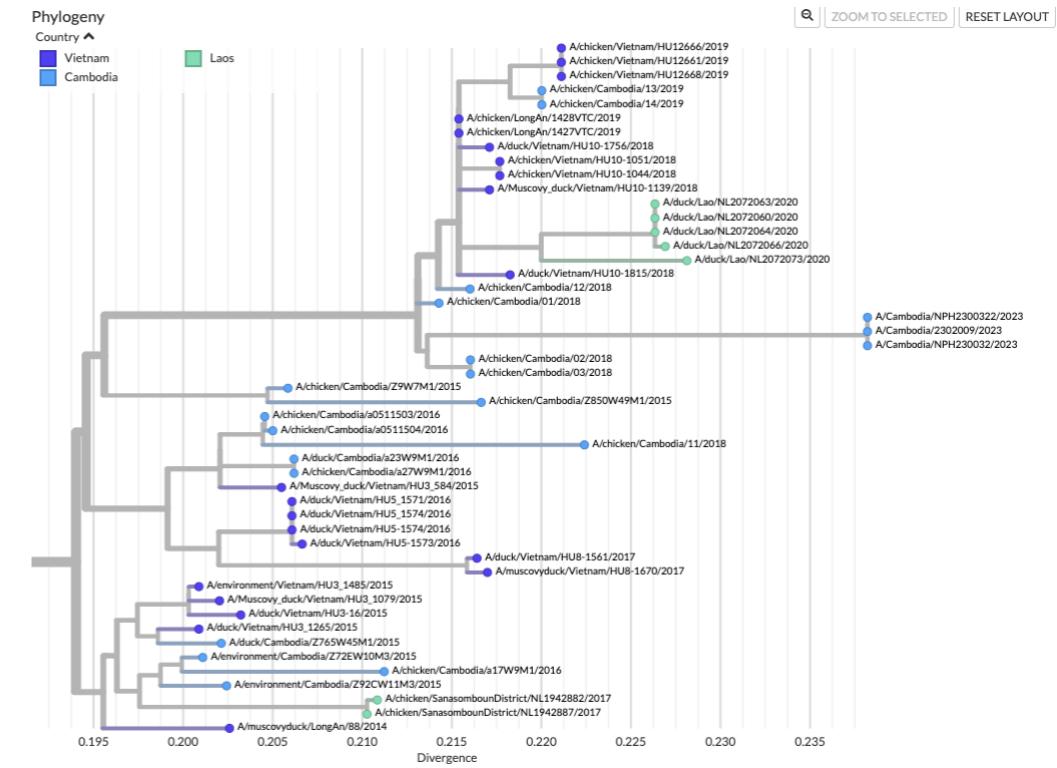
One Health is an approach to public health that recognizes that human, animal, and environmental health are intertwined with each other and cannot be understood in isolation.

ILLUSTRATION

What happened well here?



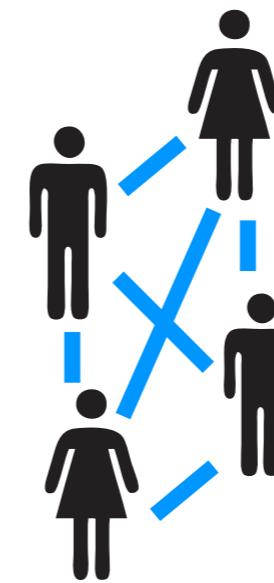
Ongoing, active surveillance
of high-risk animal
populations



Global, rapid data sharing



Effective reporting between
public health agencies



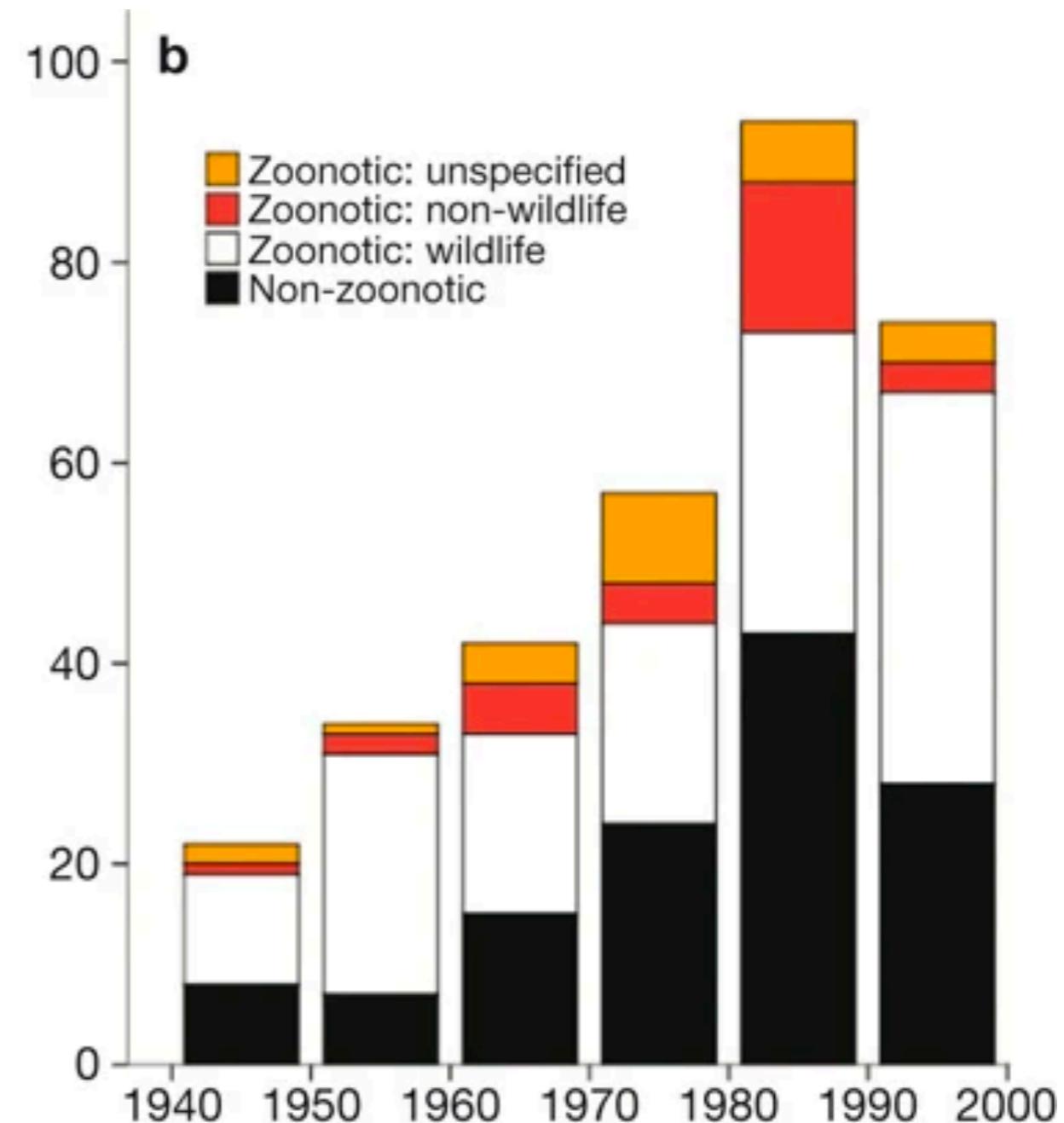
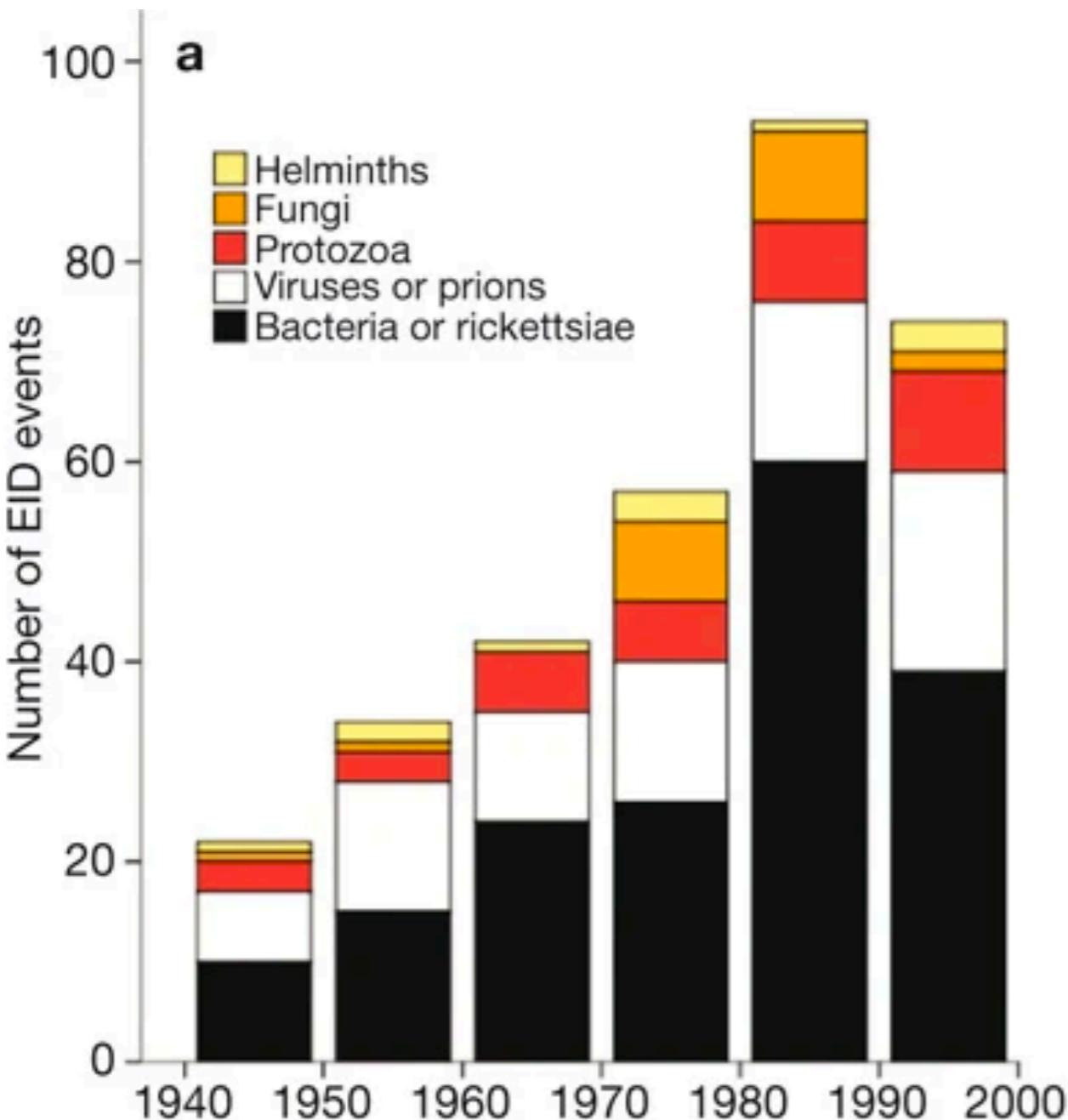
Rapid and effective
contact tracing,
monitoring, and
testing of humans
and animals

Key questions today:

1. Where do new pathogens come from, and how do they end up in humans?
2. What can we learn about viral emergence to reconstruct how outbreaks begin and spread?
3. How can we use this information to improve public health and surveillance practices?

Where do new
viruses come from?

60% of emerging infectious diseases are zoonotic



* Jones et al, Nature, 2008

There are a lot of viruses in the world, and most are uncharacterized

How many viruses on Earth?

17 Comments / By Vincent Racaniello / 6 September 2013



How many different viruses are there on planet Earth? Twenty years ago [Stephen Morse suggested](#) that there were about one million viruses of vertebrates (he arrived at this calculation by assuming ~20 different viruses in each of the 50,000 vertebrates on the planet). The [results of a new study](#) suggest that at least 320,000 different viruses infect mammals.

To estimate unknown viral diversity in mammals, 1,897 samples (urine, throat swabs, feces, roost urine) were collected from the Indian flying fox, *Pteropus giganteus*, and analyzed for viral sequences by consensus polymerase chain reaction. This bat species was selected for the study because it is known to harbor zoonotic pathogens such as Nipah virus. PCR assays were designed to detect viruses from nine viral families. A total of 985 viral sequences from members of 7 viral families were obtained. These included 11 paramyxoviruses (including Nipah virus and 10 new viruses), 14 adenoviruses (13 novel), 8 novel astroviruses, 4 distinct coronaviruses, 3 novel polyomaviruses, 2 bocaviruses, and many new herpesviruses.

When studies look for viruses, they always find new ones

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RESEARCH ARTICLE | VIROME



Cryptic and abundant marine viruses at the evolutionary origins of Earth's RNA virome

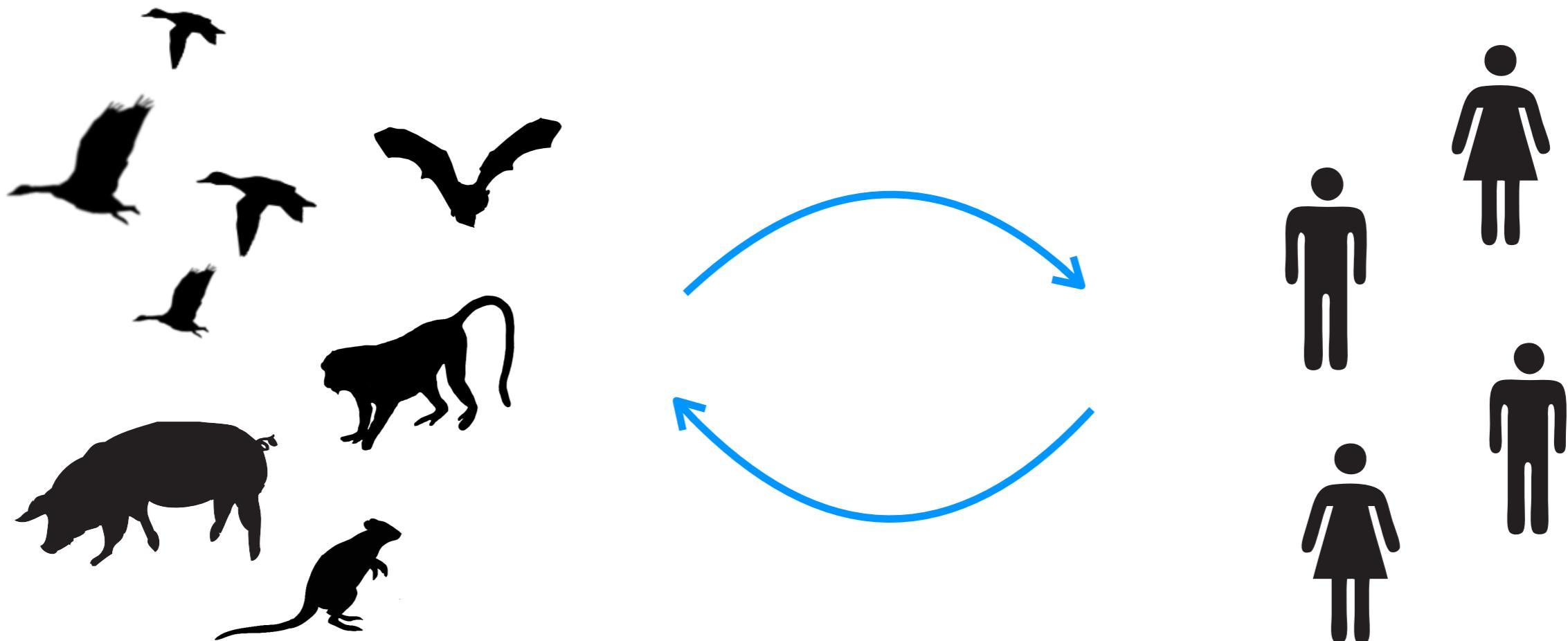
AHMED A. ZAYED , JAMES M. WAINAINA , GUILLERMO DOMINGUEZ-HUERTA , ERIC PELLETIER , JIARONG GUO , MOHAMED MOHSSEN , FUNING TIAN 

, AKBAR ADJIE PRATAMA , BENJAMIN BOLDUC , [...], AND MATTHEW B. SULLIVAN 

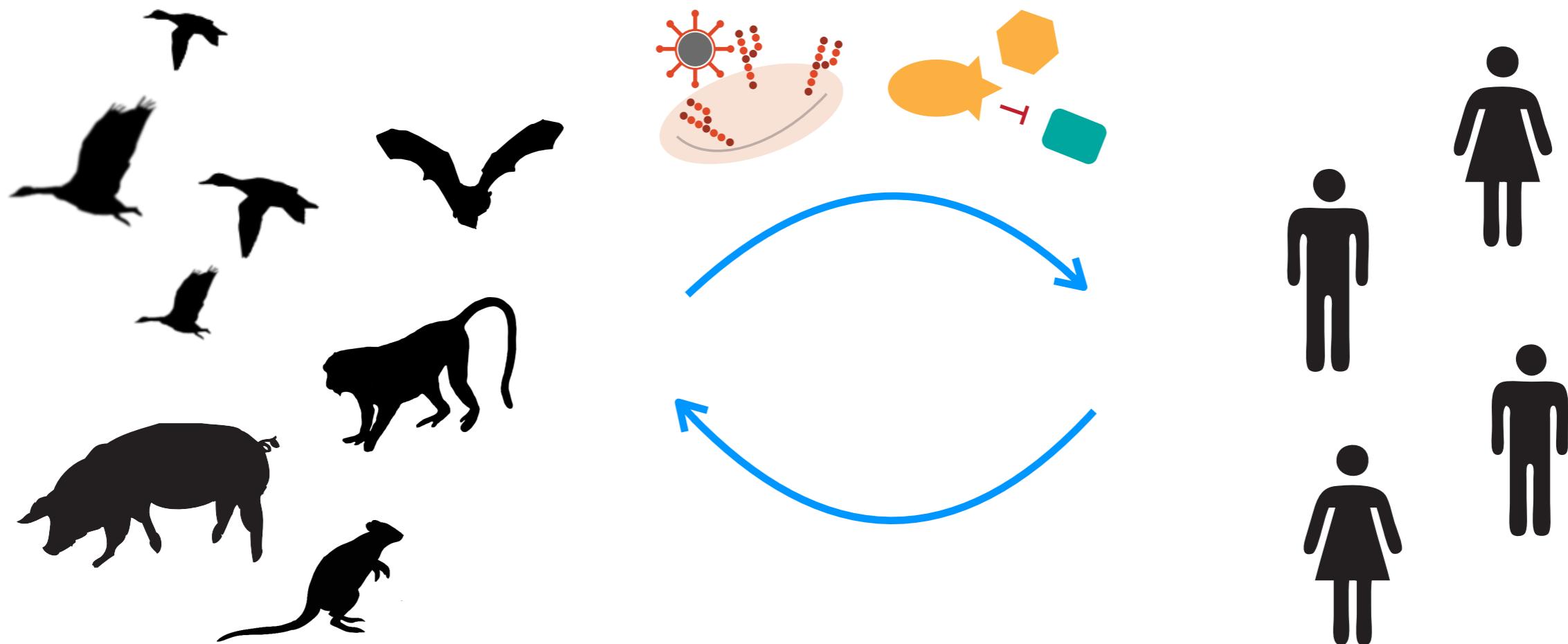
+22 authors

[Authors Info & Affiliations](#)

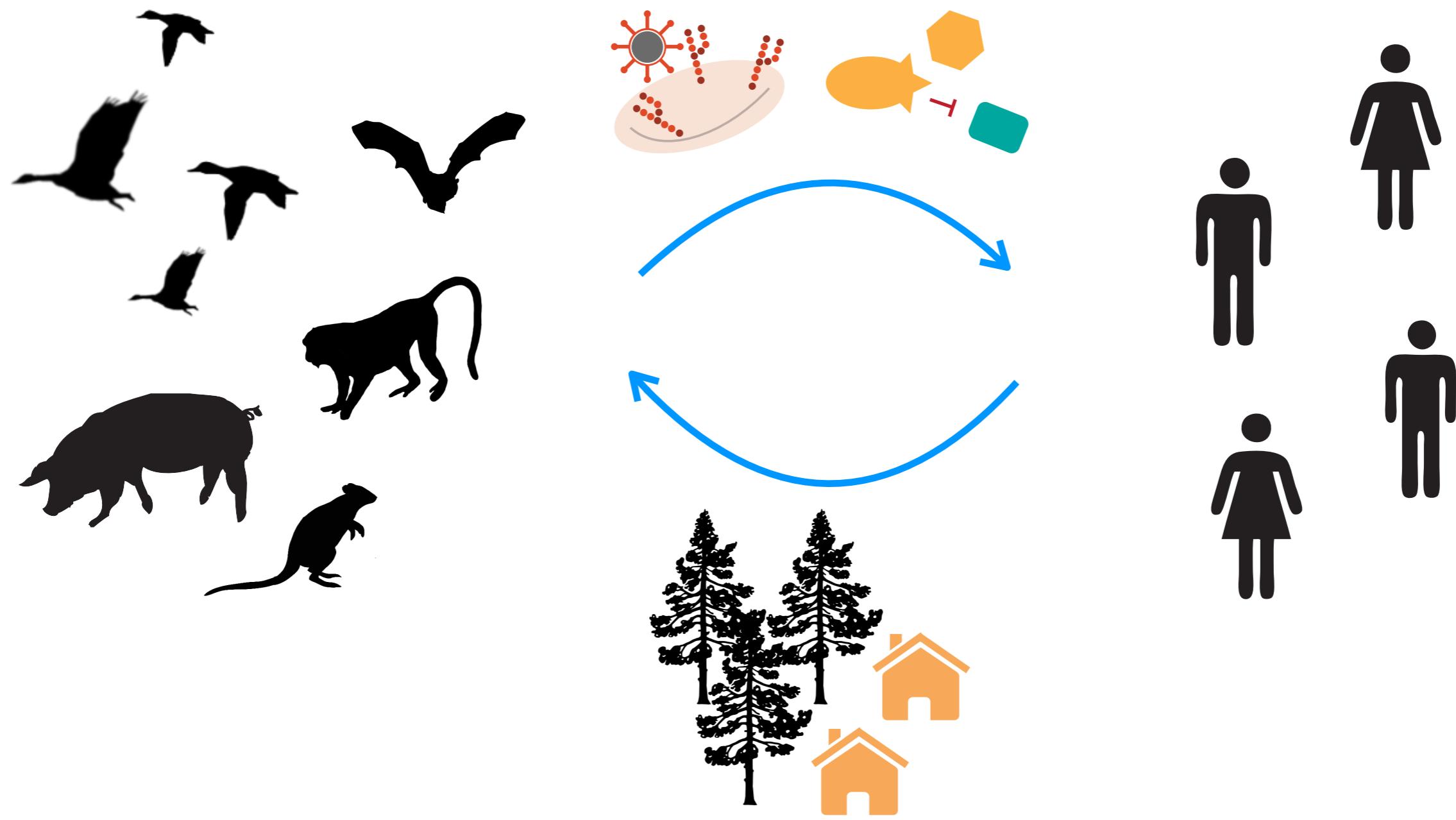
Humans are constantly exposed to viruses from other species



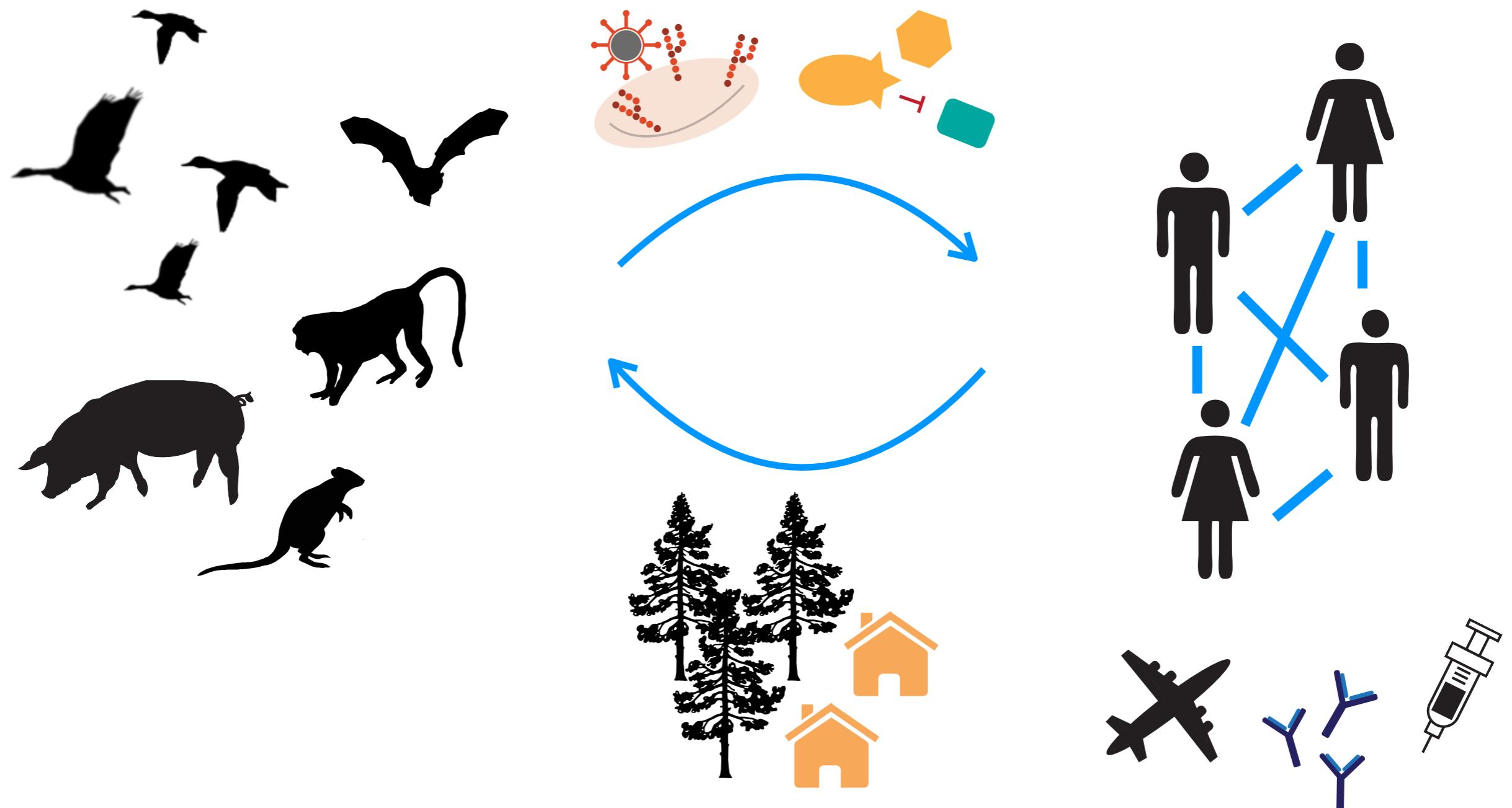
Viral emergence and transmission is complex



Viral emergence and transmission is complex

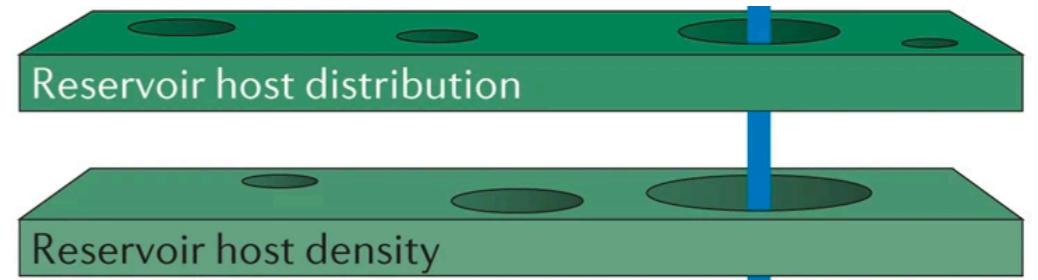


Viral emergence and transmission is complex



Zoonosis is rare because there are a lot of barriers

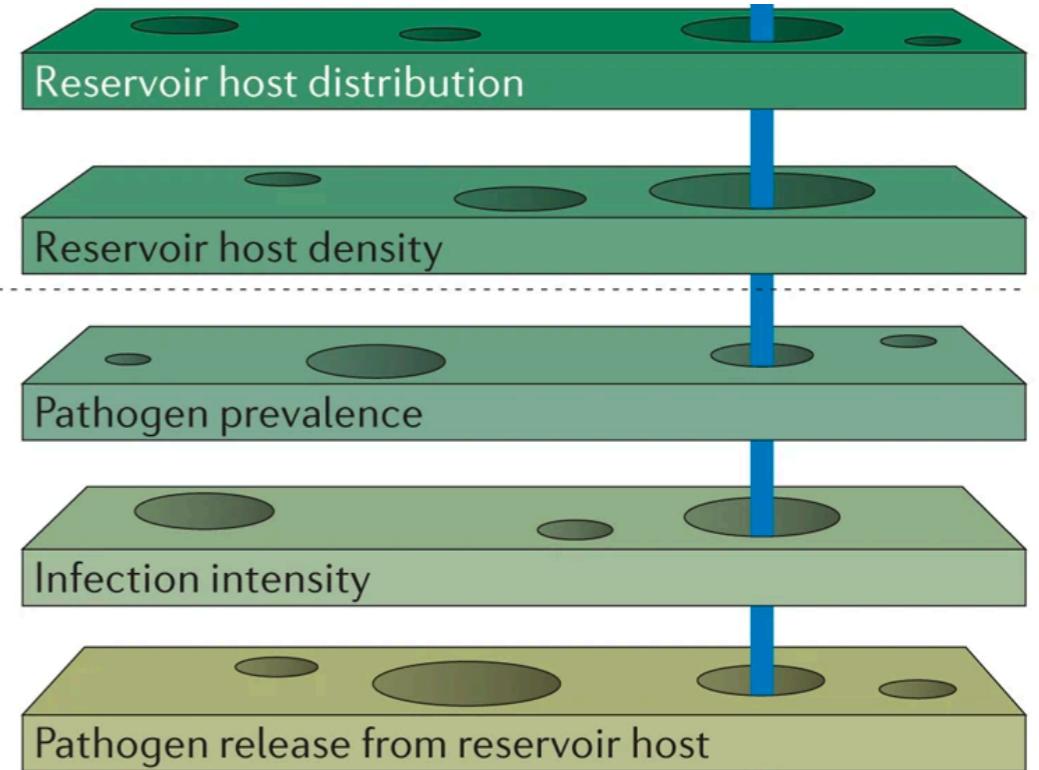
Animal ecology, population biology,
biogeography, behavioural ecology,
landscape ecology, agricultural sciences



Zoonosis is rare because there are a lot of barriers

Animal ecology, population biology,
biogeography, behavioural ecology,
landscape ecology, agricultural sciences

Disease ecology, animal epidemiology,
infectious disease dynamics, immunology,
microbiology, veterinary medicine



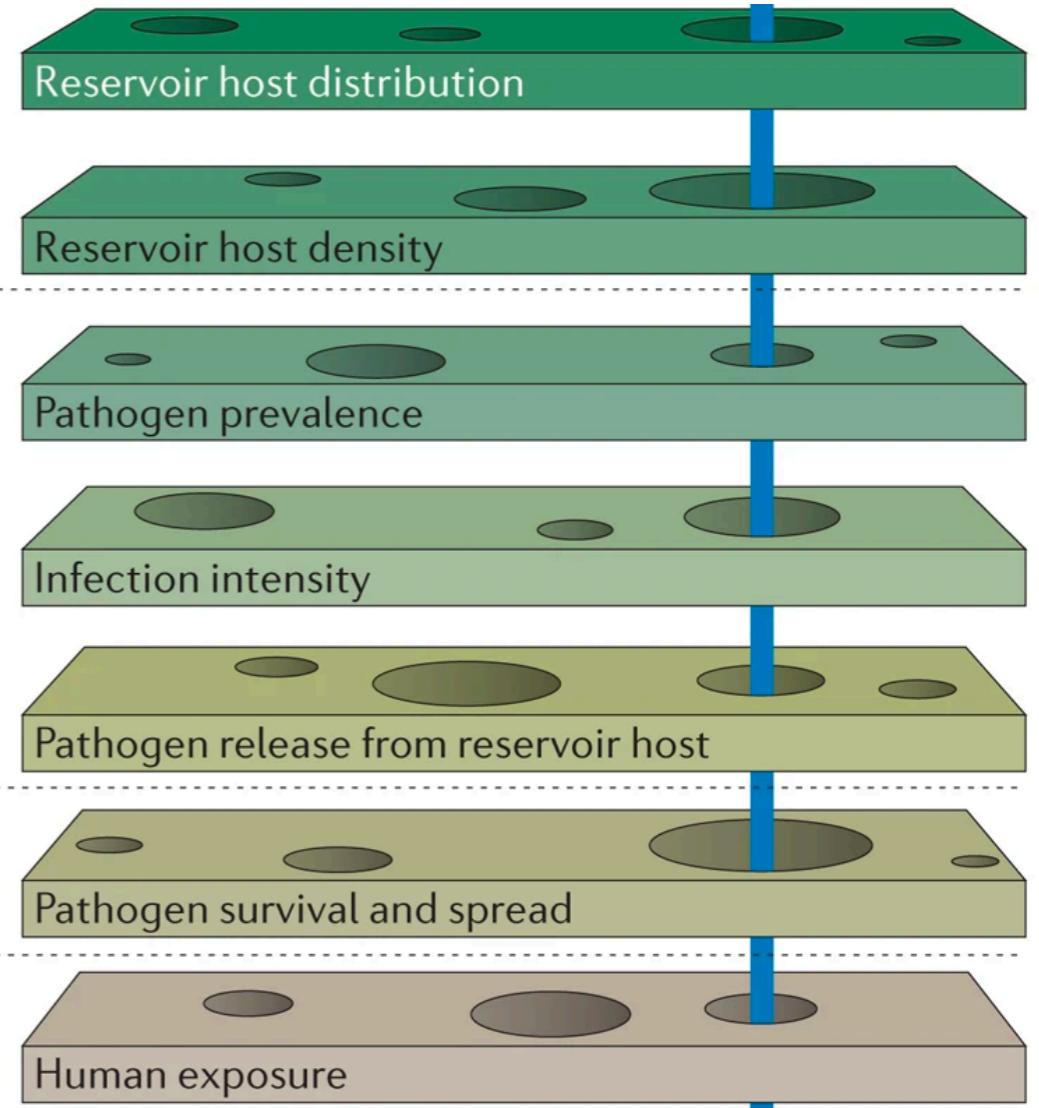
Zoonosis is rare because there are a lot of barriers

Animal ecology, population biology, biogeography, behavioural ecology, landscape ecology, agricultural sciences

Disease ecology, animal epidemiology, infectious disease dynamics, immunology, microbiology, veterinary medicine

Microbiology, disease ecology, vector ecology, epidemiology, spatial ecology, infectious disease dynamics

Human epidemiology, medical anthropology, vector ecology, social sciences, behavioural ecology, infectious disease dynamics



Zoonosis is rare because there are a lot of barriers

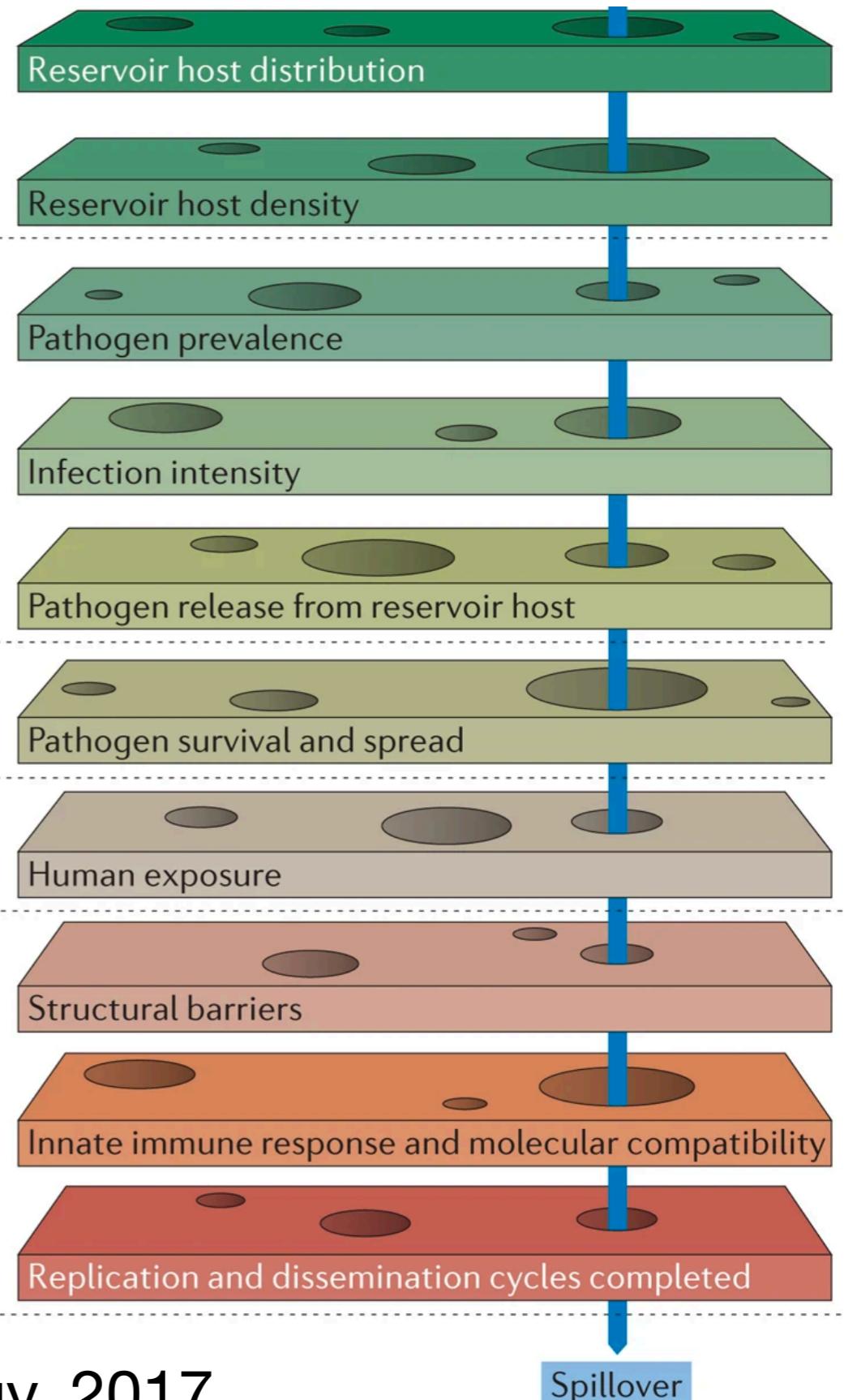
Animal ecology, population biology, biogeography, behavioural ecology, landscape ecology, agricultural sciences

Disease ecology, animal epidemiology, infectious disease dynamics, immunology, microbiology, veterinary medicine

Microbiology, disease ecology, vector ecology, epidemiology, spatial ecology, infectious disease dynamics

Human epidemiology, medical anthropology, vector ecology, social sciences, behavioural ecology, infectious disease dynamics

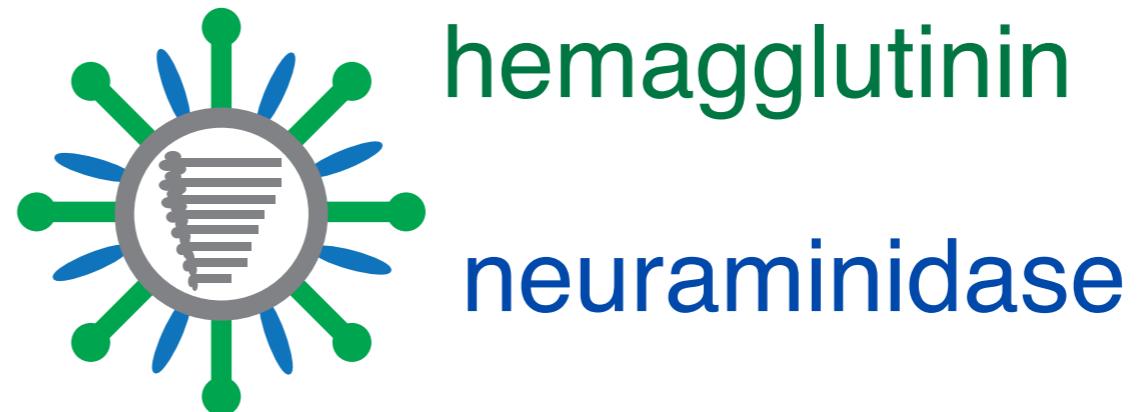
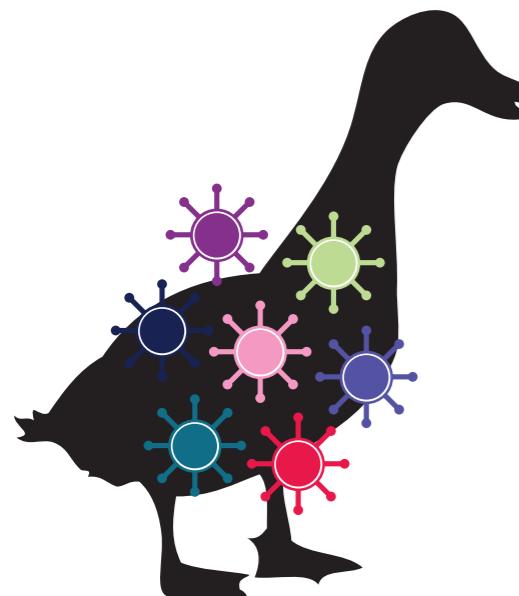
Microbiology, innate and adaptive immunology, cell biology of pathogen–host interactions, pathology, genetics, evolutionary biology



Avian influenza example



Influenza viruses come from wild, aquatic birds



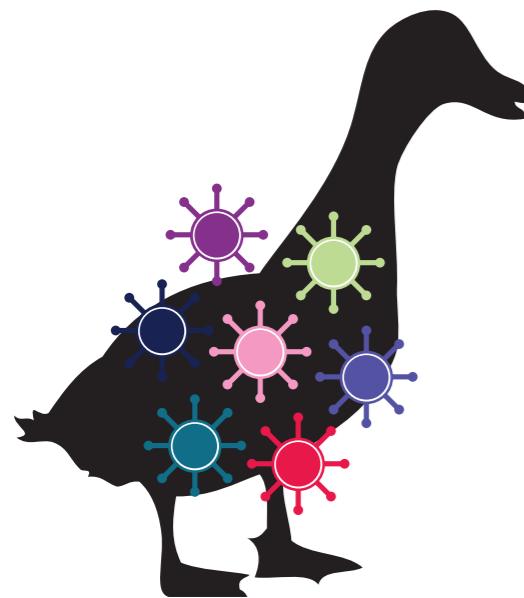
hemagglutinin

neuraminidase

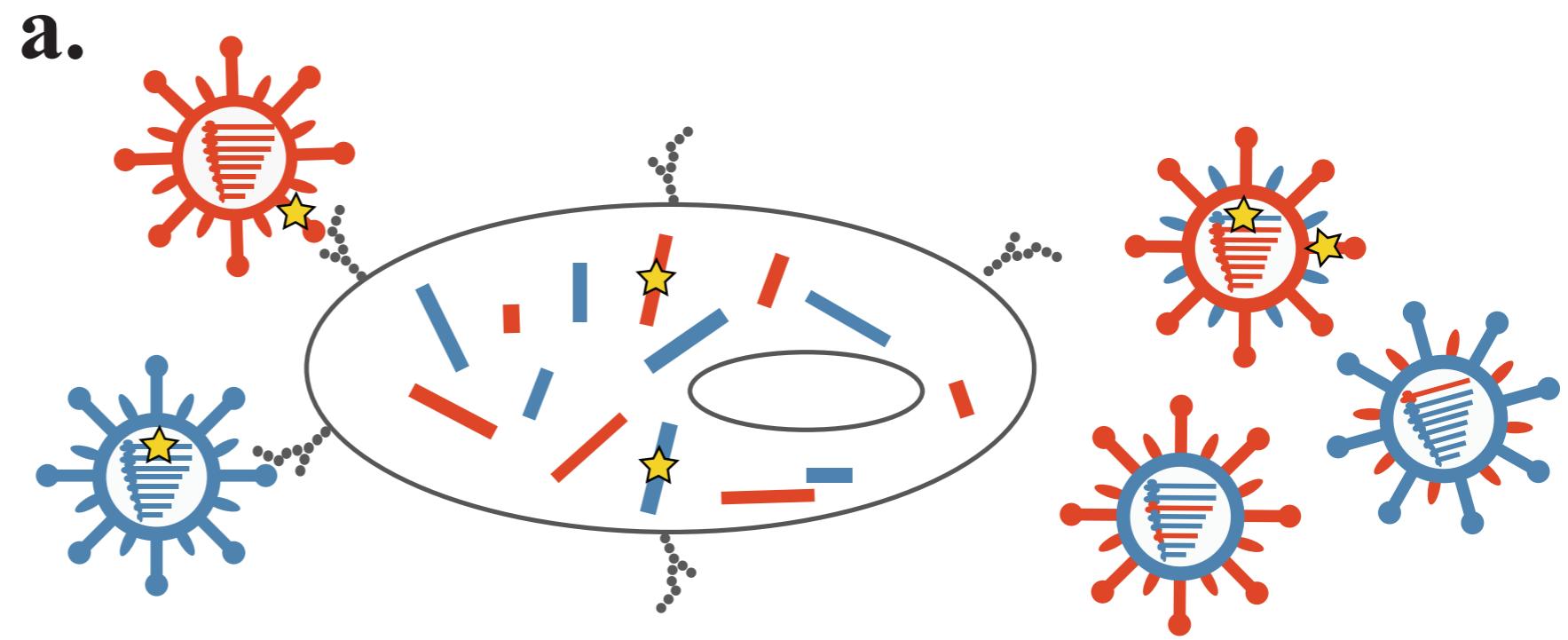
H1-H16
N1-N9

* H17, H18, N10,
N11 are found in
bats

Influenza viruses come from wild, aquatic birds

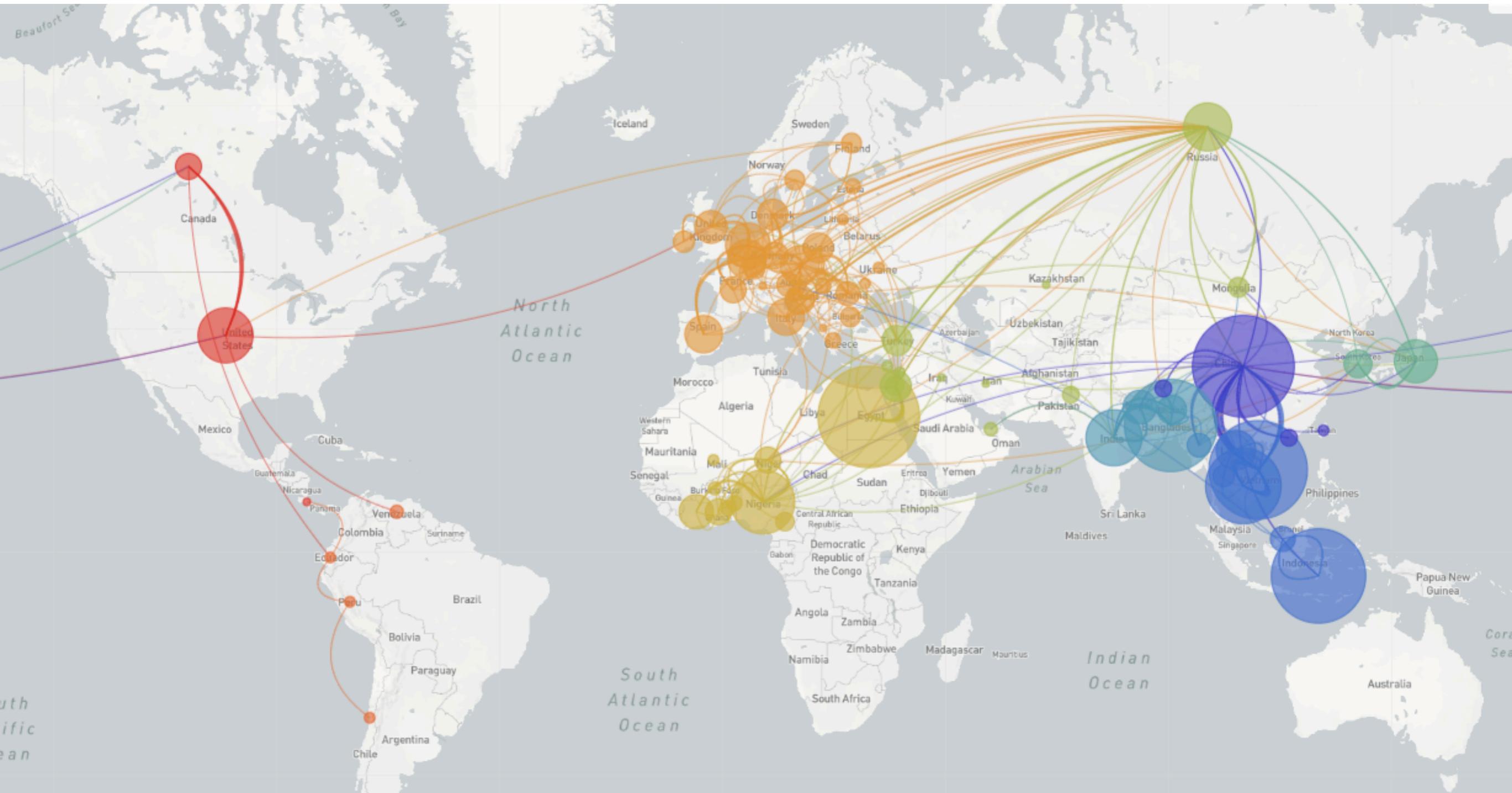


H1-H16
N1-N9



* H17, H18, N10,
N11 are found in
bats

H5 viruses emerged in 1996, and have spread globally

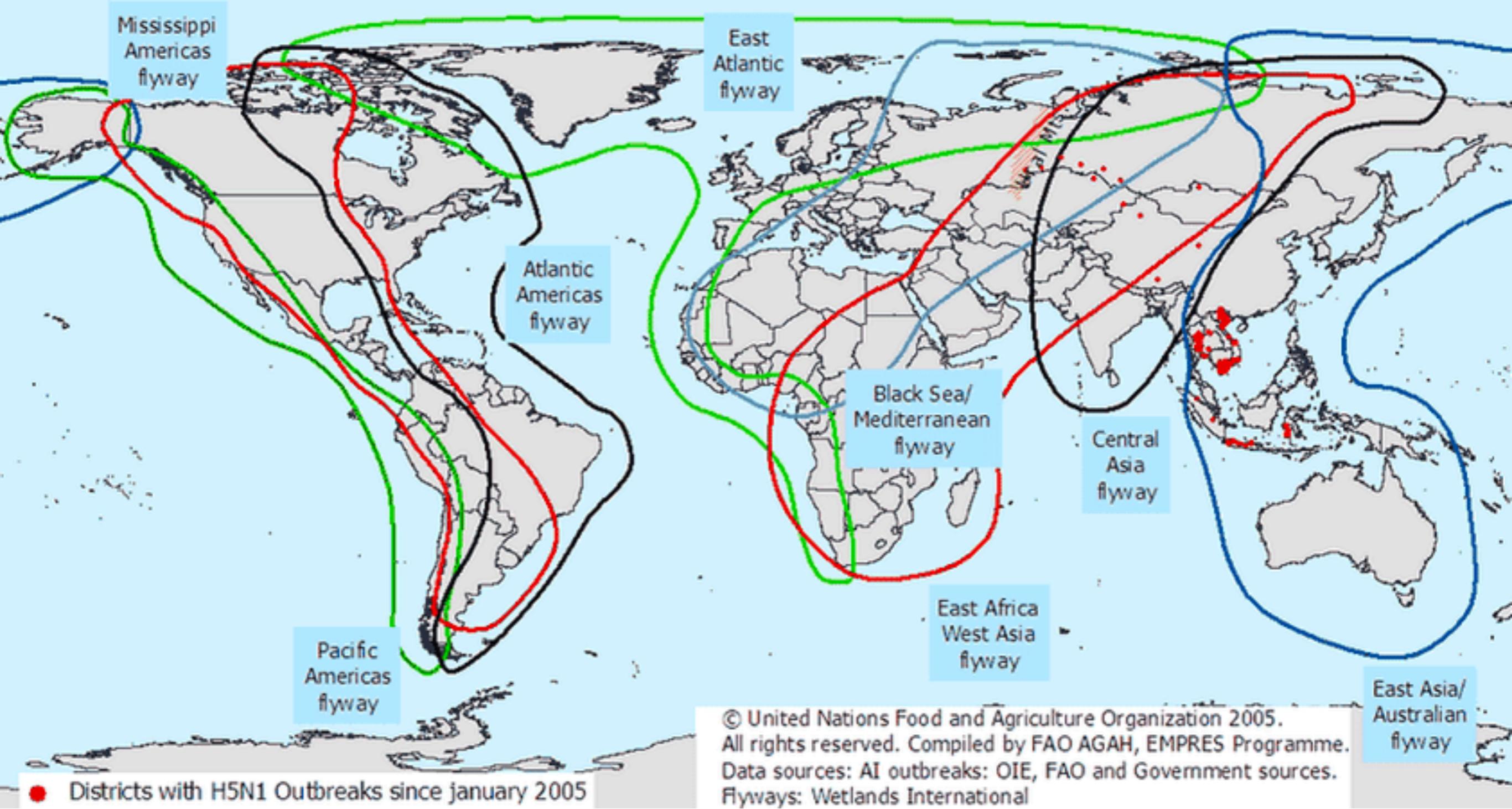


 size = number of genomes from country

nextstrain.org/flu/avian/h5n1/ha

Avian influenza viruses can be spread across continents via bird migration

H5N1 outbreaks in 2005 and major flyways of migratory birds
Situation on 30 August 2005



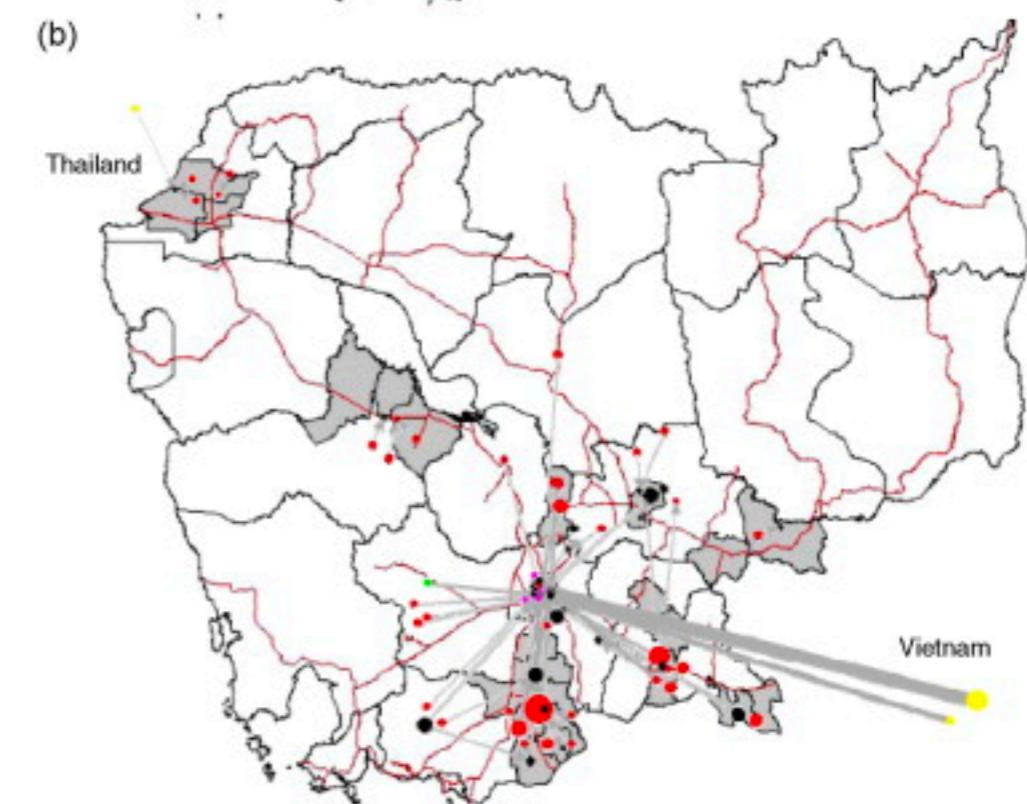
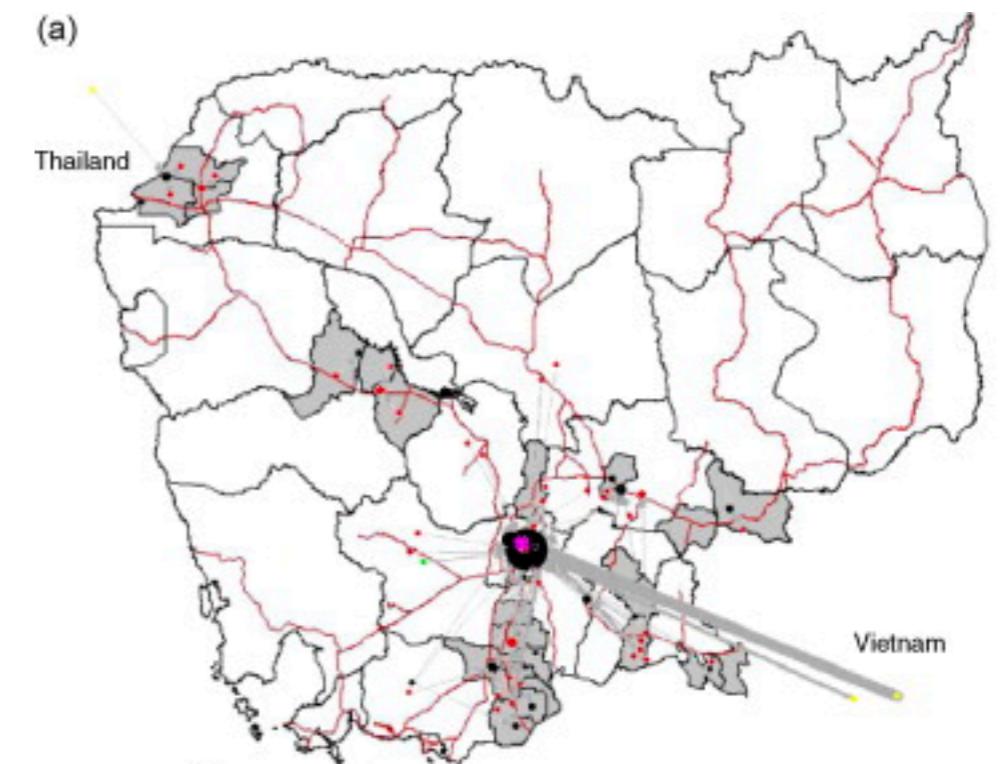
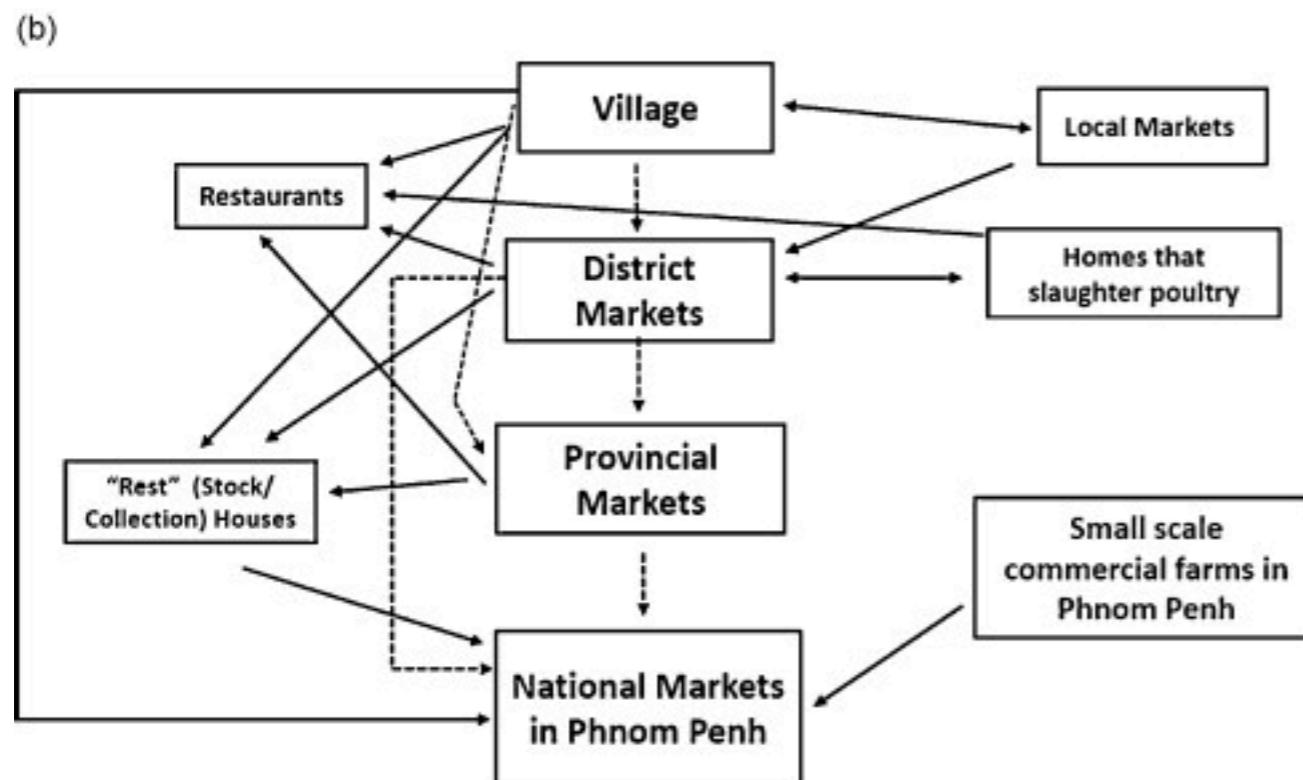
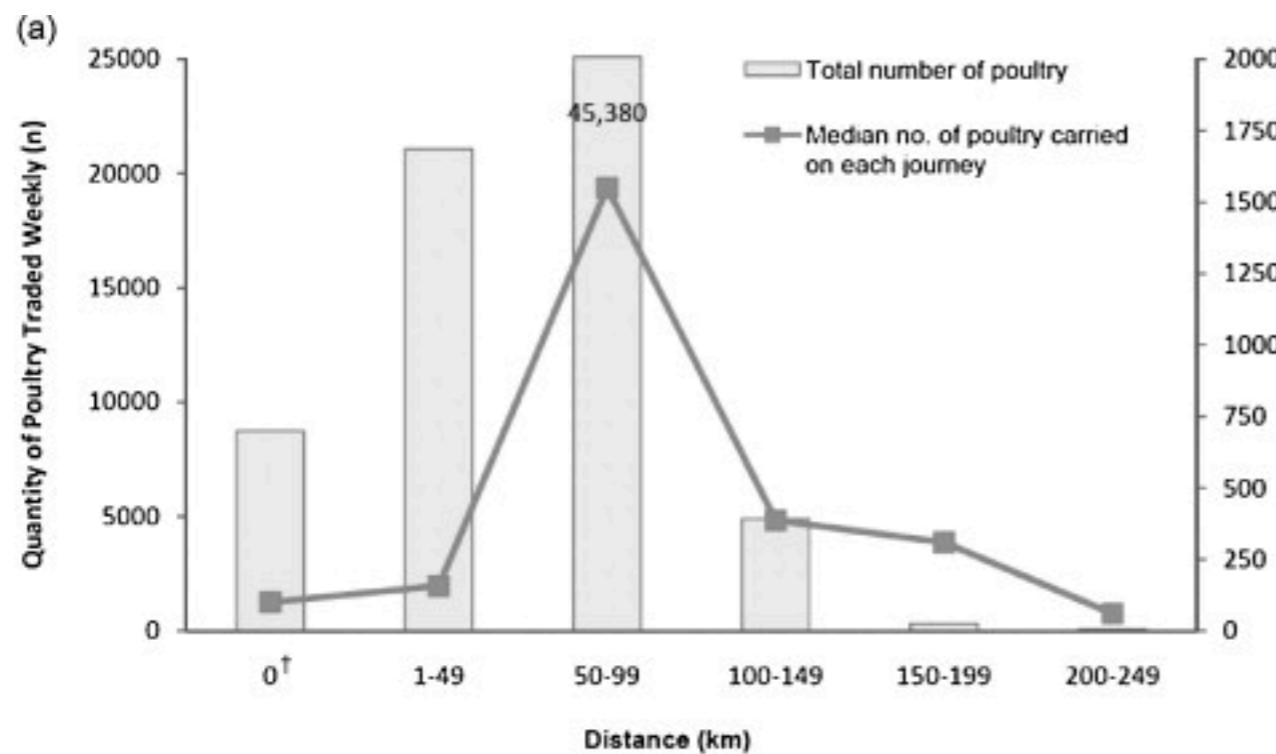
© United Nations Food and Agriculture Organization 2005.
All rights reserved. Compiled by FAO AGAH, EMPRES Programme.
Data sources: AI outbreaks: OIE, FAO and Government sources.
Flyways: Wetlands International

● Districts with H5N1 Outbreaks since January 2005



The Food and Land Use Coalition

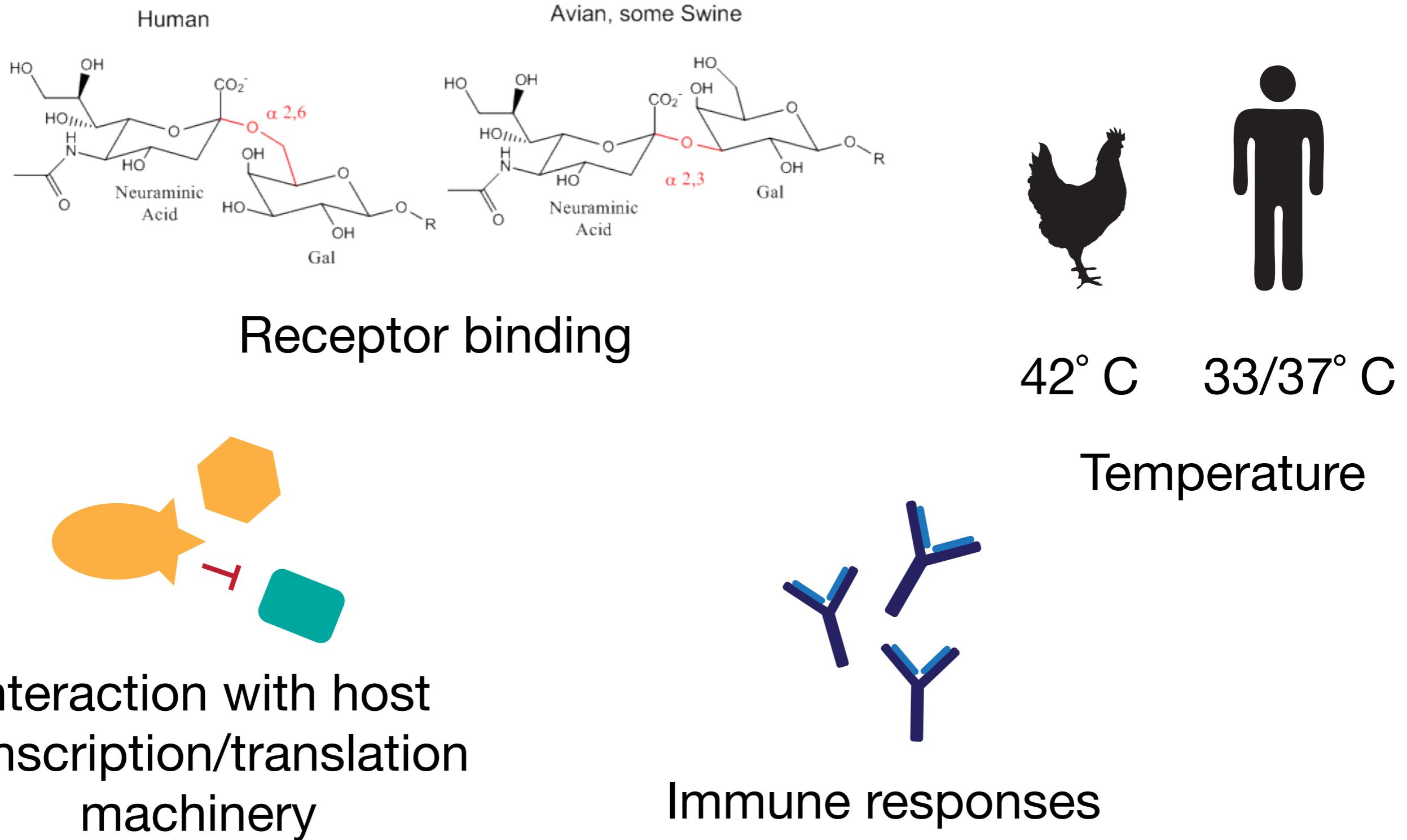
Poultry trade networks aggregate and spread avian influenza





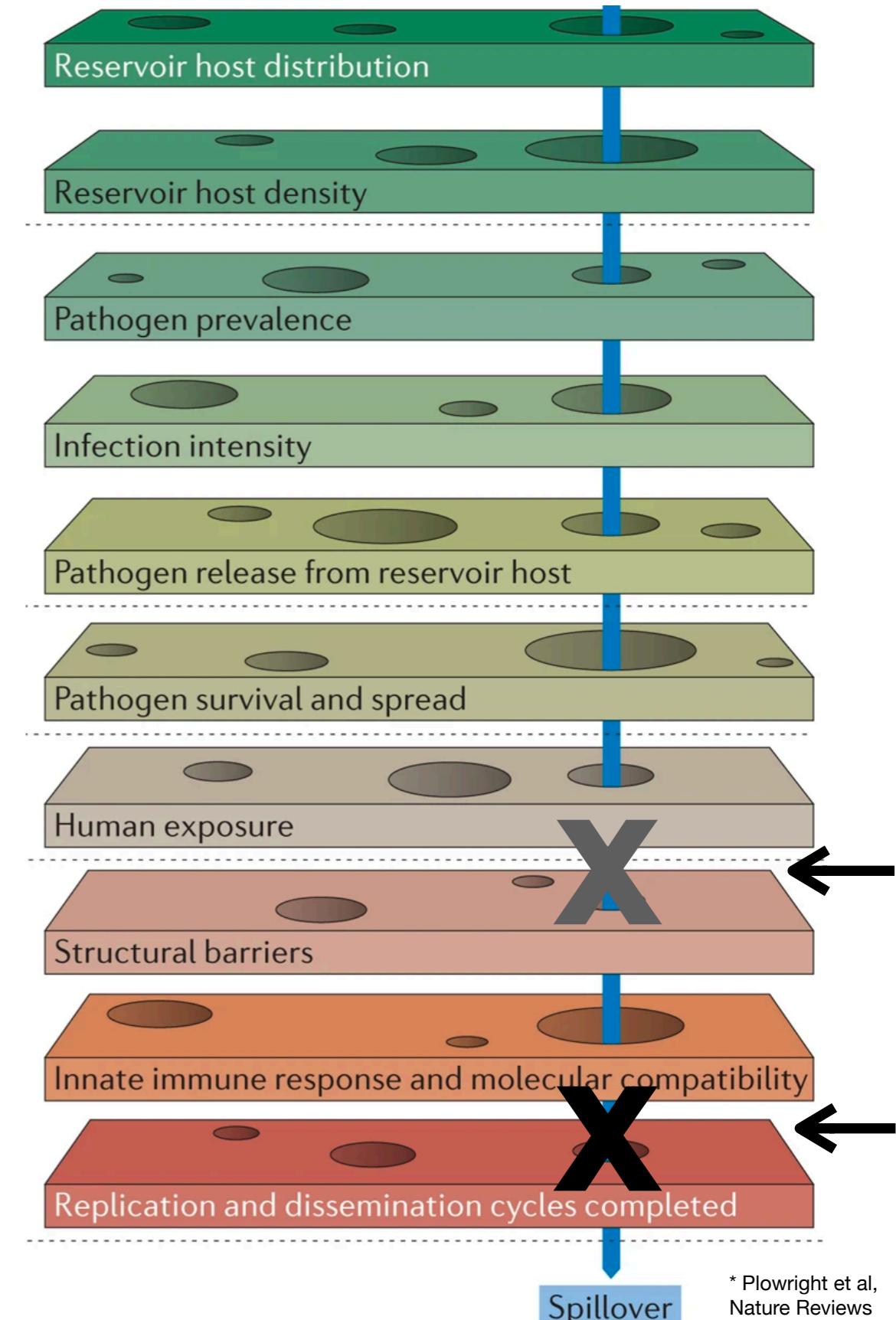
Live bird market in China, 2013

Molecular barriers to avian influenza cross-species transmission



Avian influenza viruses are stuck towards the bottom

- * Wild bird migration spreads H5 viruses long distances
- * Poultry transmission spreads viruses regionally and selects for high pathogenicity
- * More transmission in wild birds leads to more transmission in domestic birds, and vice versa.
- * Establishment of transmission in domestic birds increases human risk.
- * Humans are exposed through direct interaction with birds via wildlife or agriculture
- * Receptor binding, temperature, and intrinsic immune factors prevent human to human transmission



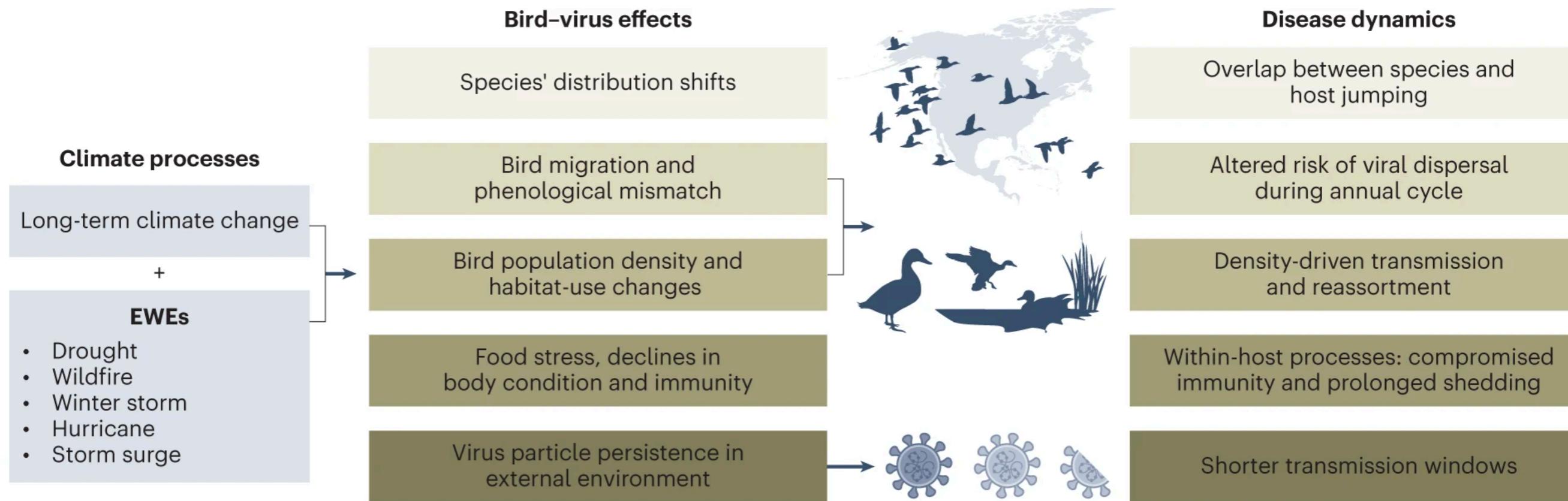
H5 risk and ecology is changing

New host
species
impacted



H5 risk and ecology is changing

Spatiotemporal scale ↓



* Prosser et al, Nature Microbiology, 2023

New host species impacted



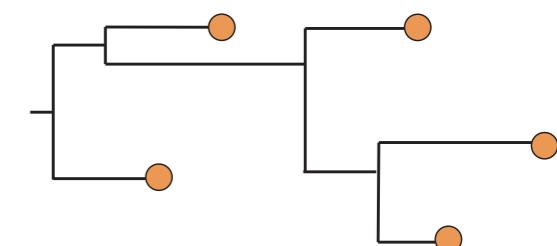
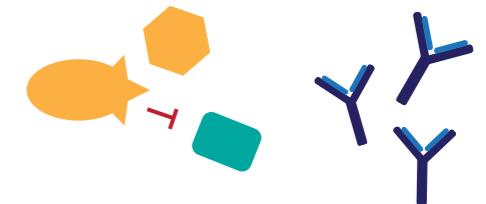
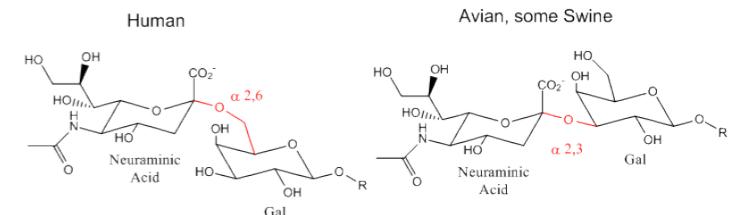
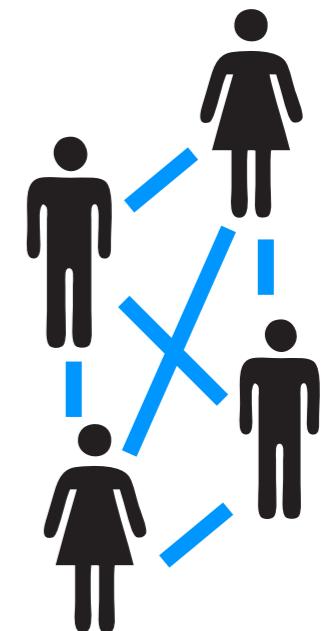
Where do new viruses come from?

- * Most new viruses come from animals
- * Zoonosis requires overcoming a multitude of barriers, and occurs when a series of human, animal, and environmental conditions align that facilitate spillover.
- * One health approaches are meant to integrate across all of these factors.

What can we learn about
viral emergence to
reconstruct how outbreaks
begin and spread?

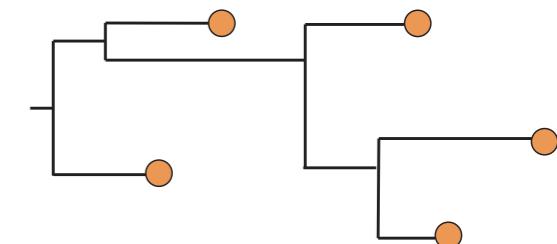
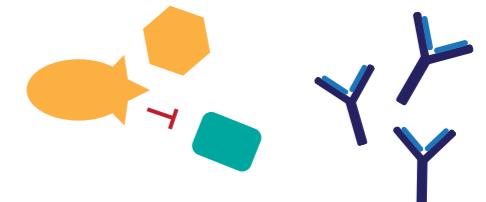
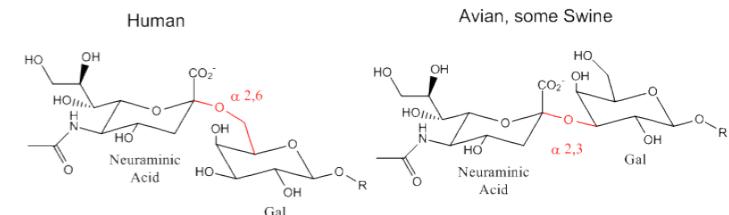
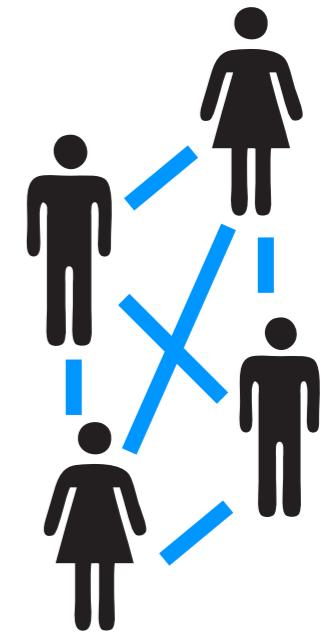
Overview of outbreak tools

1. Ongoing, continuous, active surveillance
2. Epidemiology and epidemiologic modeling
 - Contact tracing, case followup, testing
 - Estimating outbreak growth, transmission rates, differences in rates among population groups
3. Virology/functional studies
4. Genomics
 - New sequencing technologies allow rapid generation of data and data sharing
 - Viral phylodynamics



Overview of outbreak tools

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 - **New sequencing technologies allow rapid generation of data and data sharing**
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Rapid genomic characterization is possible with new, portable sequencing technologies

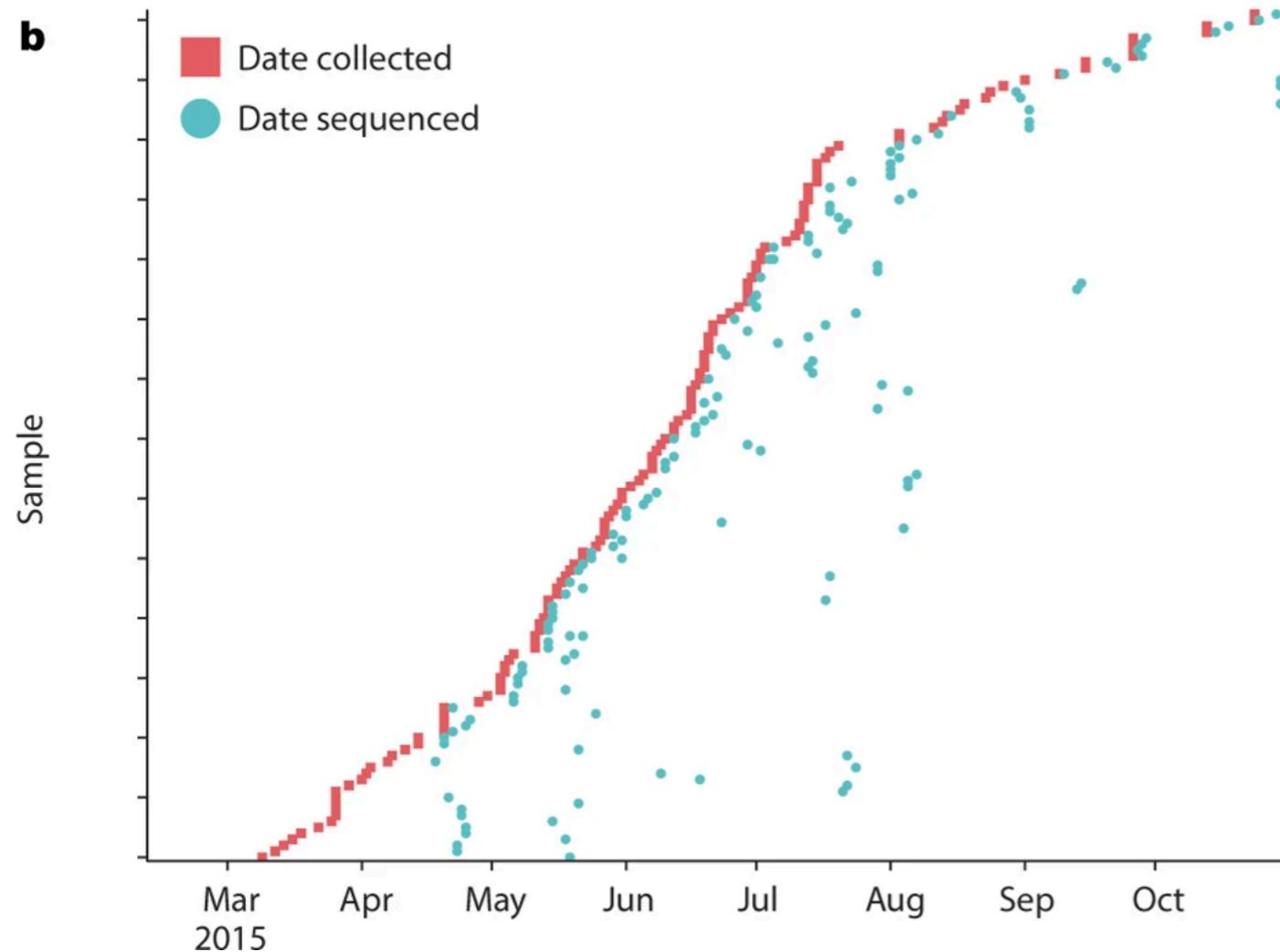
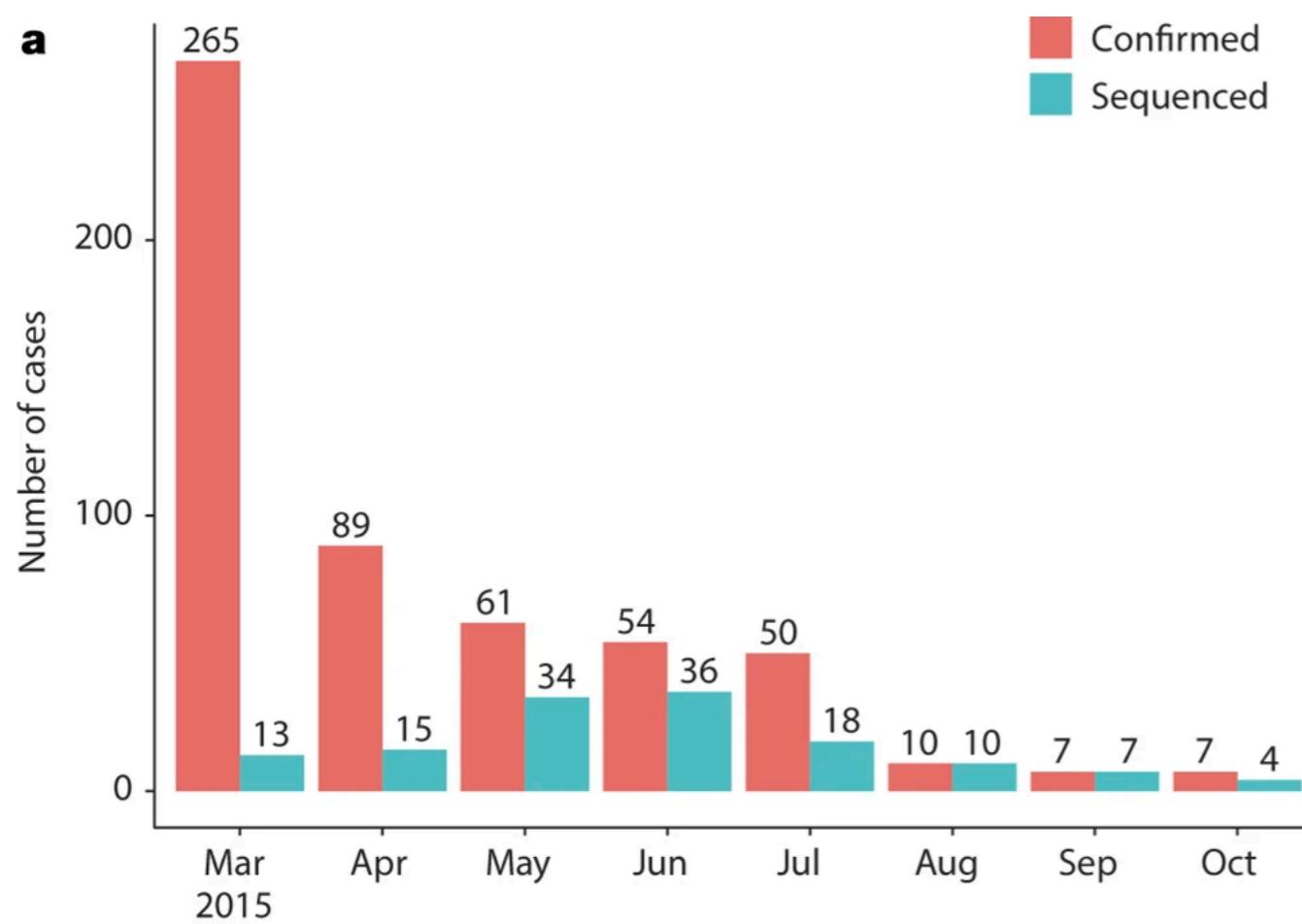
Letter | [Published: 03 February 2016](#)

Real-time, portable genome sequencing for Ebola surveillance

[Joshua Quick](#), [Nicholas J. Loman](#) , [Sophie Duraffour](#), [Jared T. Simpson](#), [Ettore Severi](#),
[Lauren Cowley](#), [Joseph Akoi Bore](#), [Raymond Koundouno](#), [Gytis Dudas](#), [Amy Mikhail](#), [Nobila Ouédraogo](#), [Babak Afrough](#), [Amadou Bah](#), [Jonathan H. J. Baum](#), [Beate Becker-Ziaja](#), [Jan Peter Boettcher](#), [Mar Cabeza-Cabrerozo](#), [Álvaro Camino-Sánchez](#), [Lisa L. Carter](#), [Juliane Doerrbecker](#), [Theresa Enkirch](#), [Isabel García-Dorival](#), [Nicole Hetzelt](#), [Julia Hinzmann](#), ... [Miles W. Carroll](#) + Show authors

[Nature](#) **530**, 228–232 (2016) | [Cite this article](#)





Rapid sequencing allow for quick characterization of emerging outbreaks

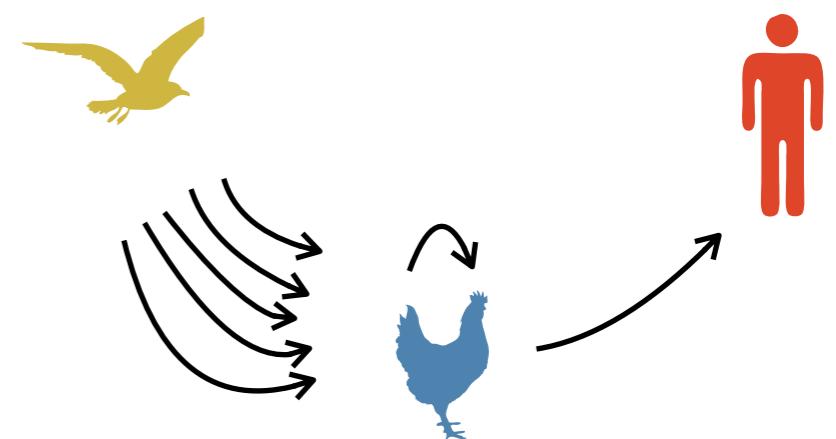
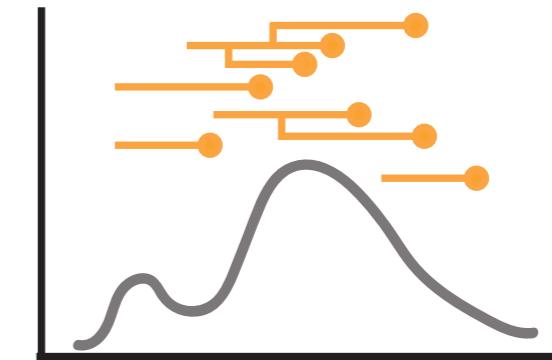


Detection of a SARS-CoV-2 variant of concern in South Africa

[Houriiyah Tegally](#), [Eduan Wilkinson](#), [Marta Giovanetti](#), [Arash Iranzadeh](#), [Vagner Fonseca](#), [Jennifer Giandhari](#), [Deelan Doolabh](#), [Sureshnee Pillay](#), [Emmanuel James San](#), [Nokukhanya Msomi](#), [Koleka Mlisana](#), [Anne von Gottberg](#), [Sibongile Walaza](#), [Mushal Allam](#), [Arshad Ismail](#), [Thabo Mohale](#), [Allison J. Glass](#), [Susan Engelbrecht](#), [Gert Van Zyl](#), [Wolfgang Preiser](#), [Francesco Petruccione](#), [Alex Sigal](#), [Diana Hardie](#), [Gert Marais](#), ... [Tulio de Oliveira](#) + Show authors

Viral genomics can help answer a lot of questions about viral transmission and spread

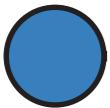
1. When did an outbreak begin, and where? How rapidly is it growing? Who is driving transmission?
2. Who infected whom?
3. How are pathogen populations shaped by natural selection?
4. How are pathogen populations moving across time, space, and hosts?



Phylogenetics is the study of how epidemiological, immunological, and evolutionary processes act and interact to shape viral phylogenies.

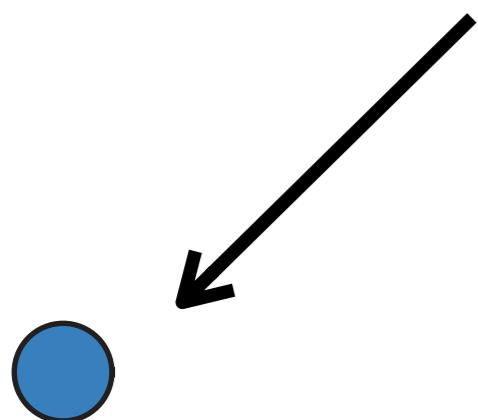
* Volz, Koelle, and Bedford, PLOS Computational Biology, 2013

Viral genomes contain records of a virus's transmission history



Viral genomes contain records of a virus's transmission history

one person
infected with
influenza

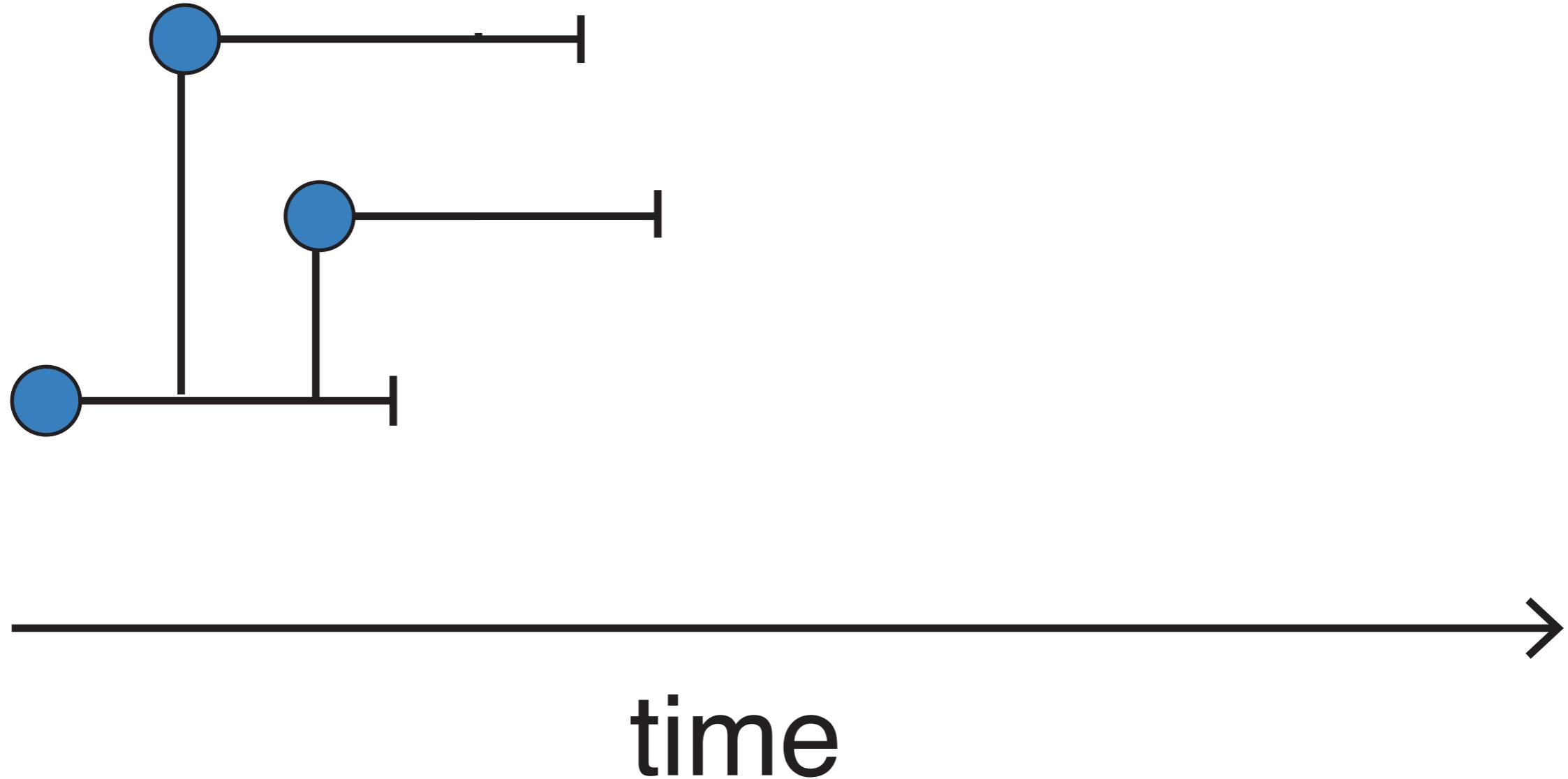


Viral genomes contain records of a virus's transmission history

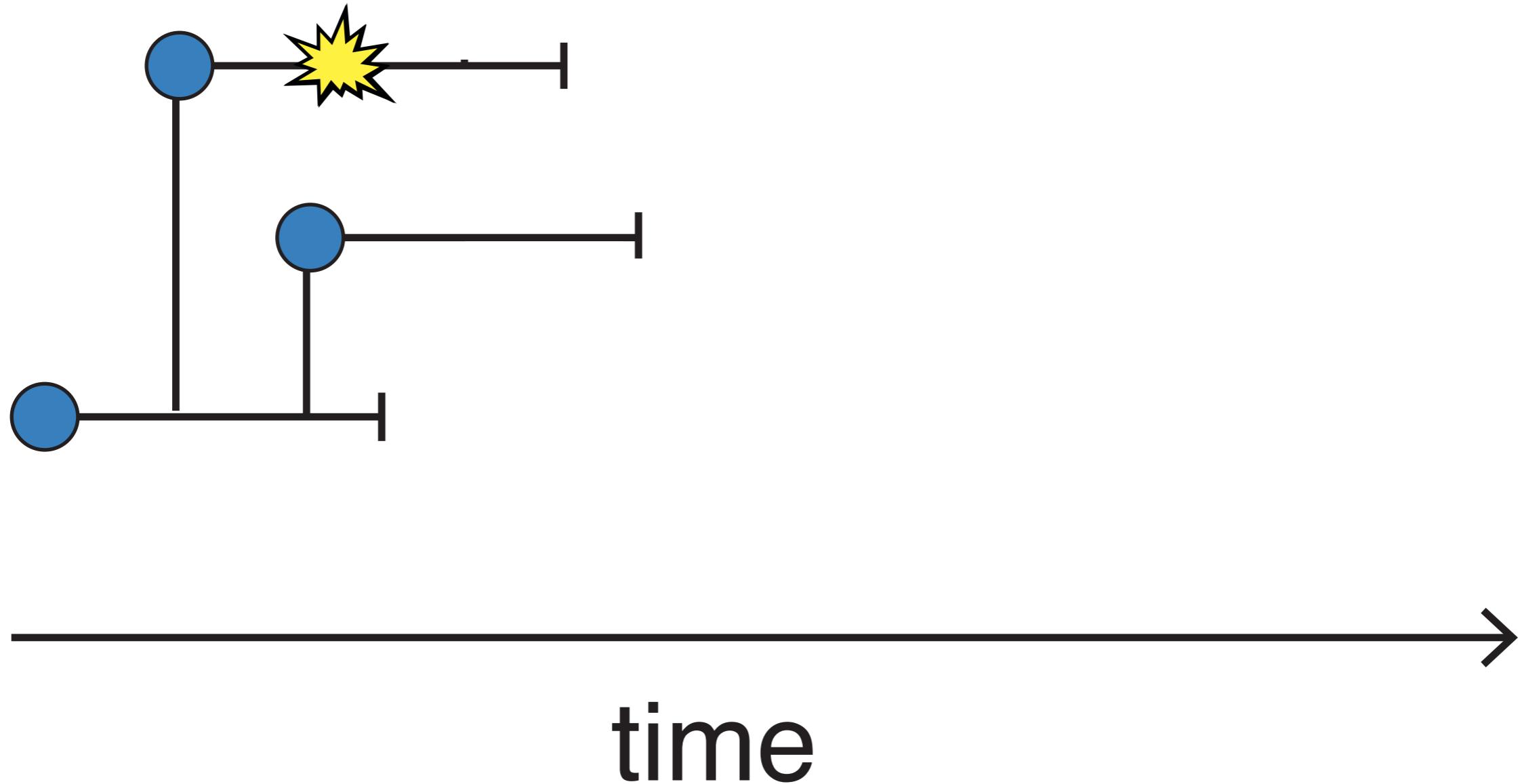
this person's
infectious period



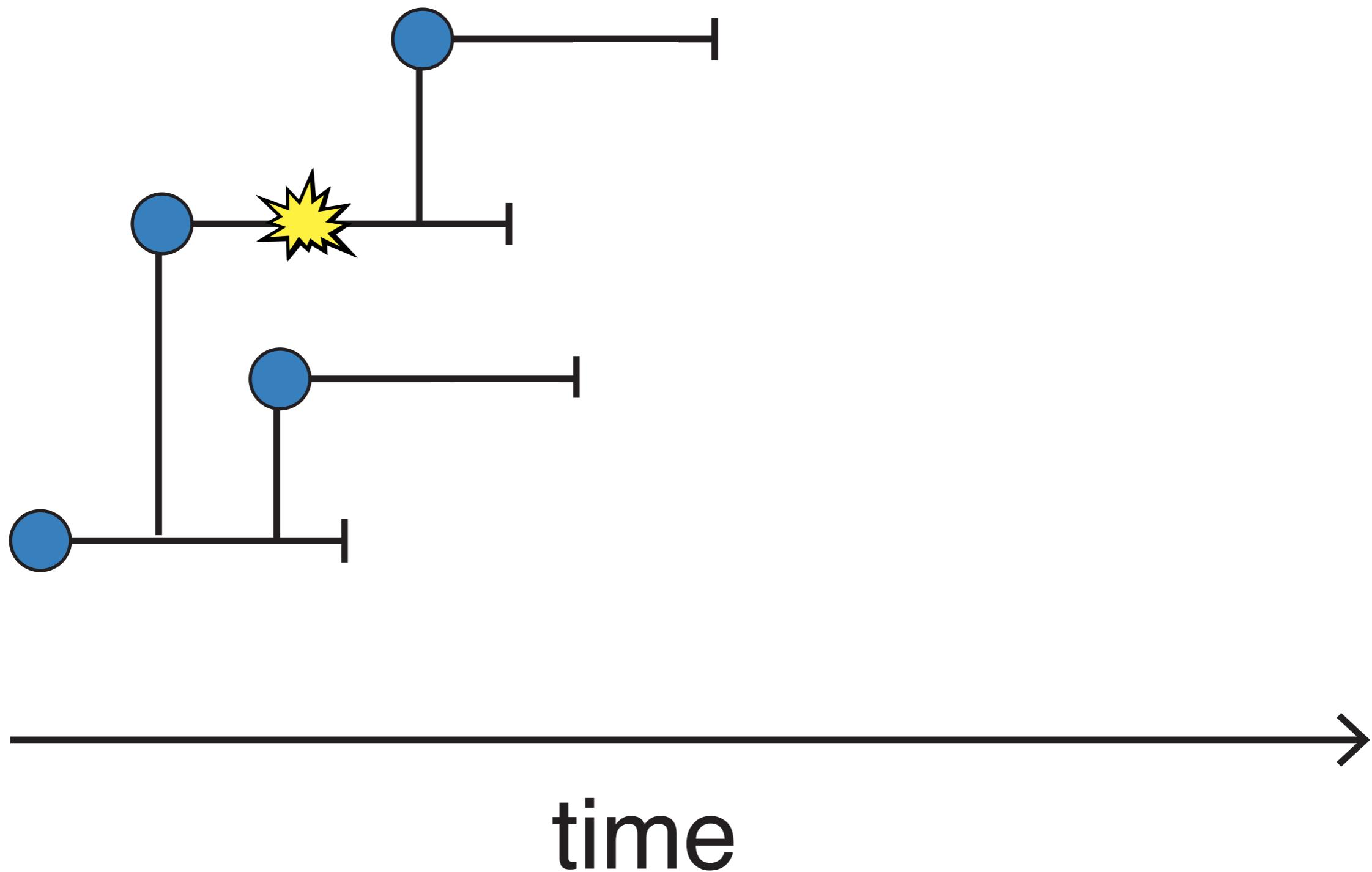
Viral genomes contain records of a virus's transmission history



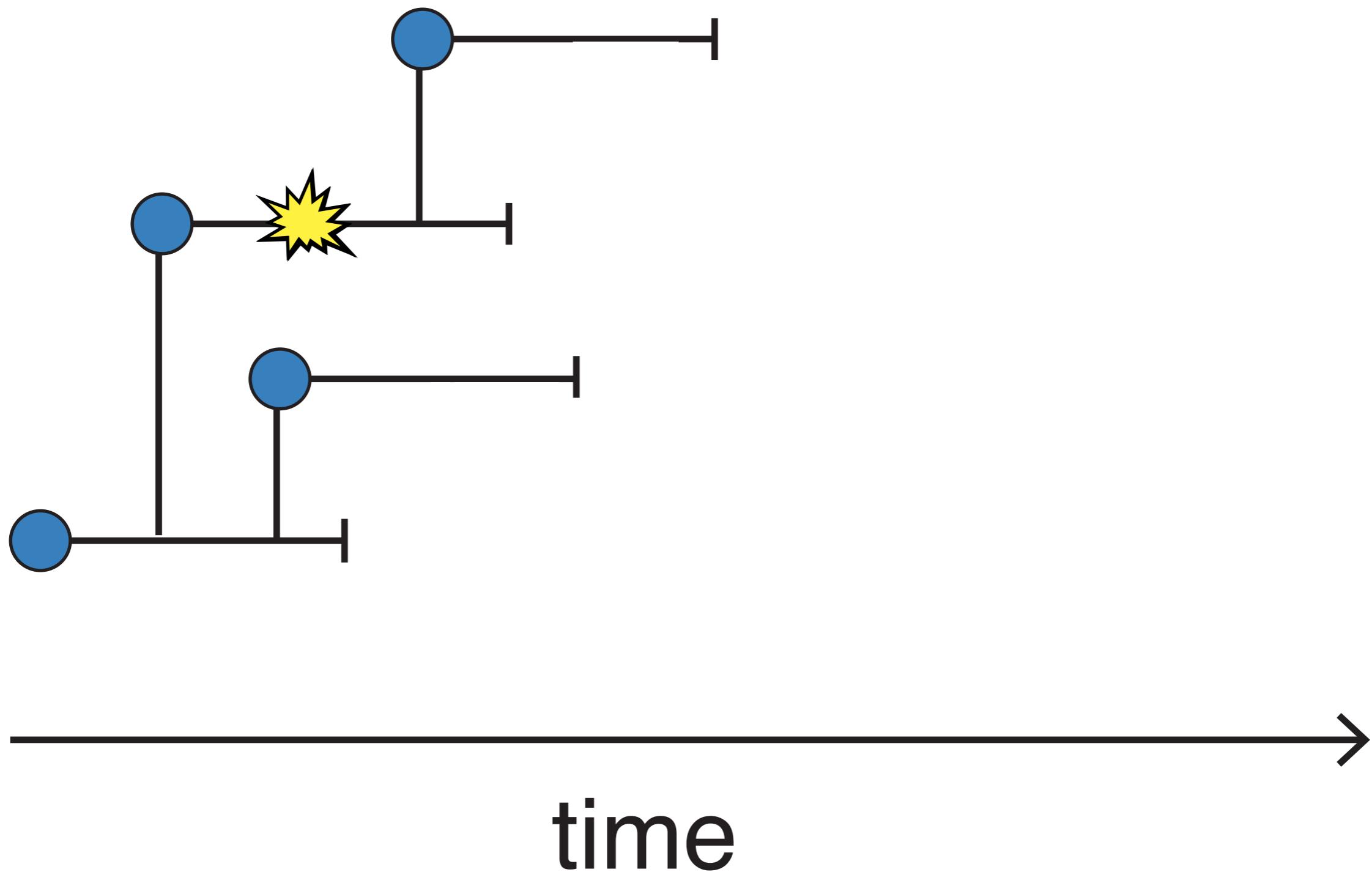
Viral genomes contain records of a virus's transmission history



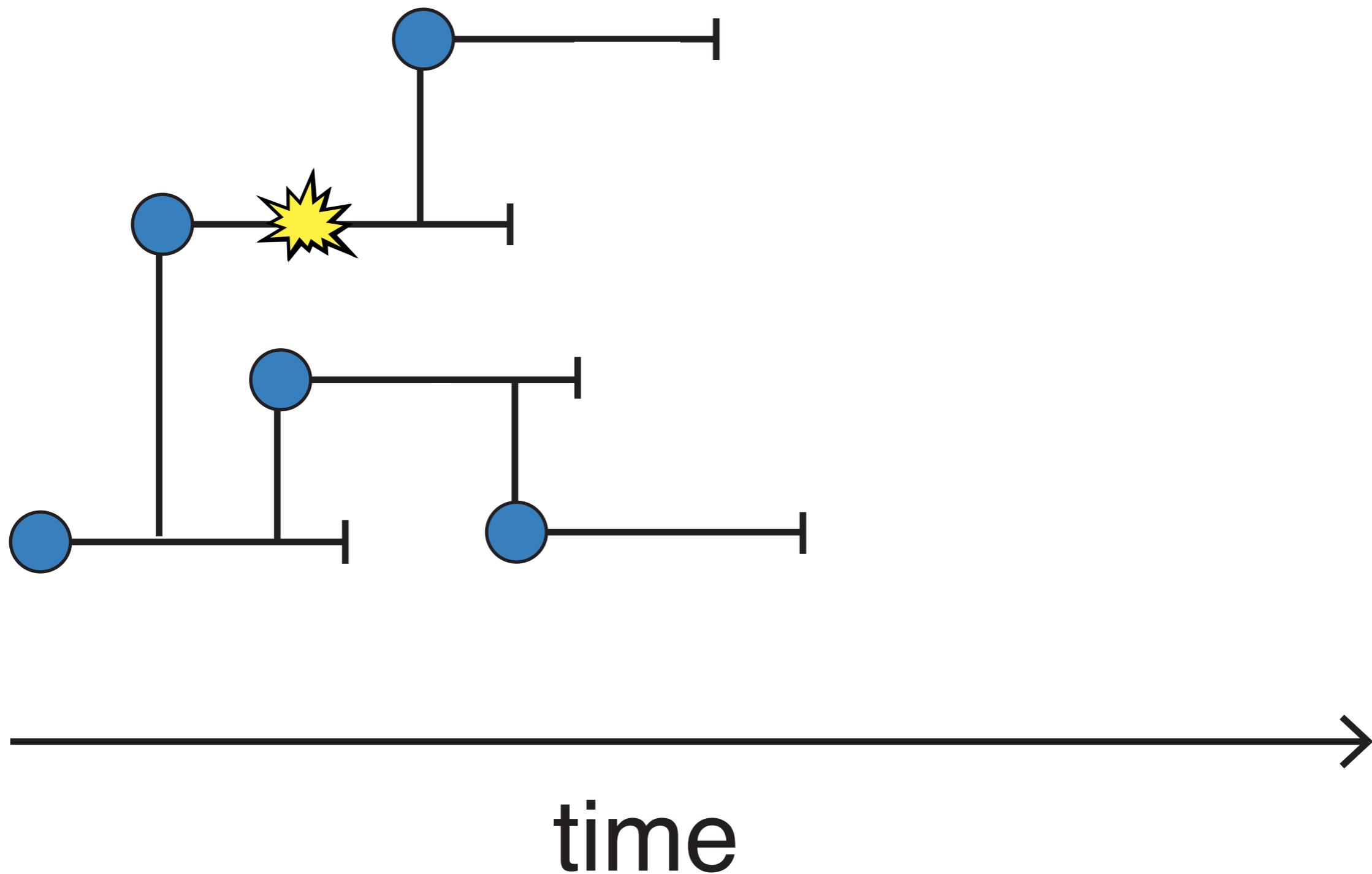
Viral genomes contain records of a virus's transmission history



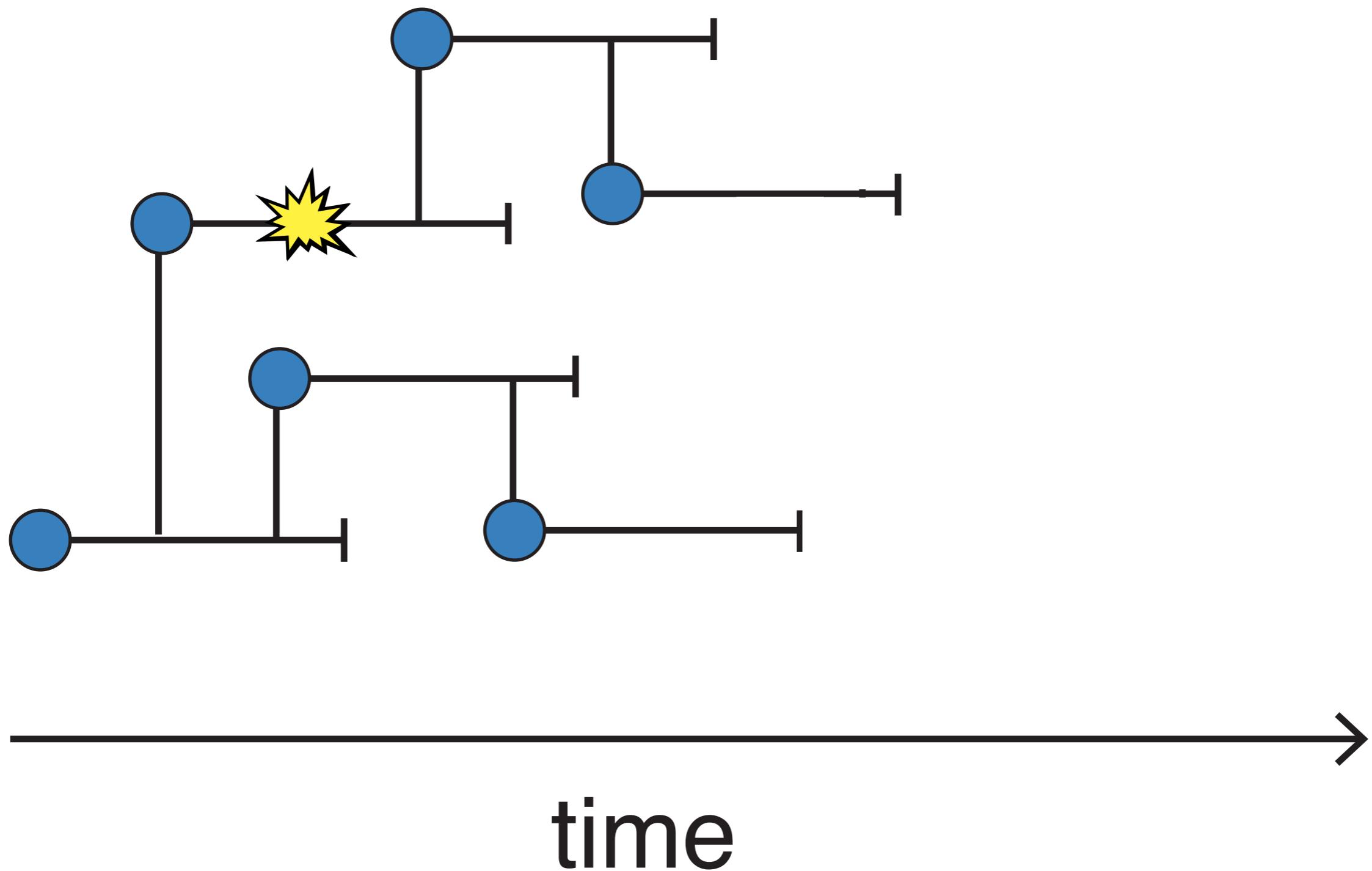
Viral genomes contain records of a virus's transmission history



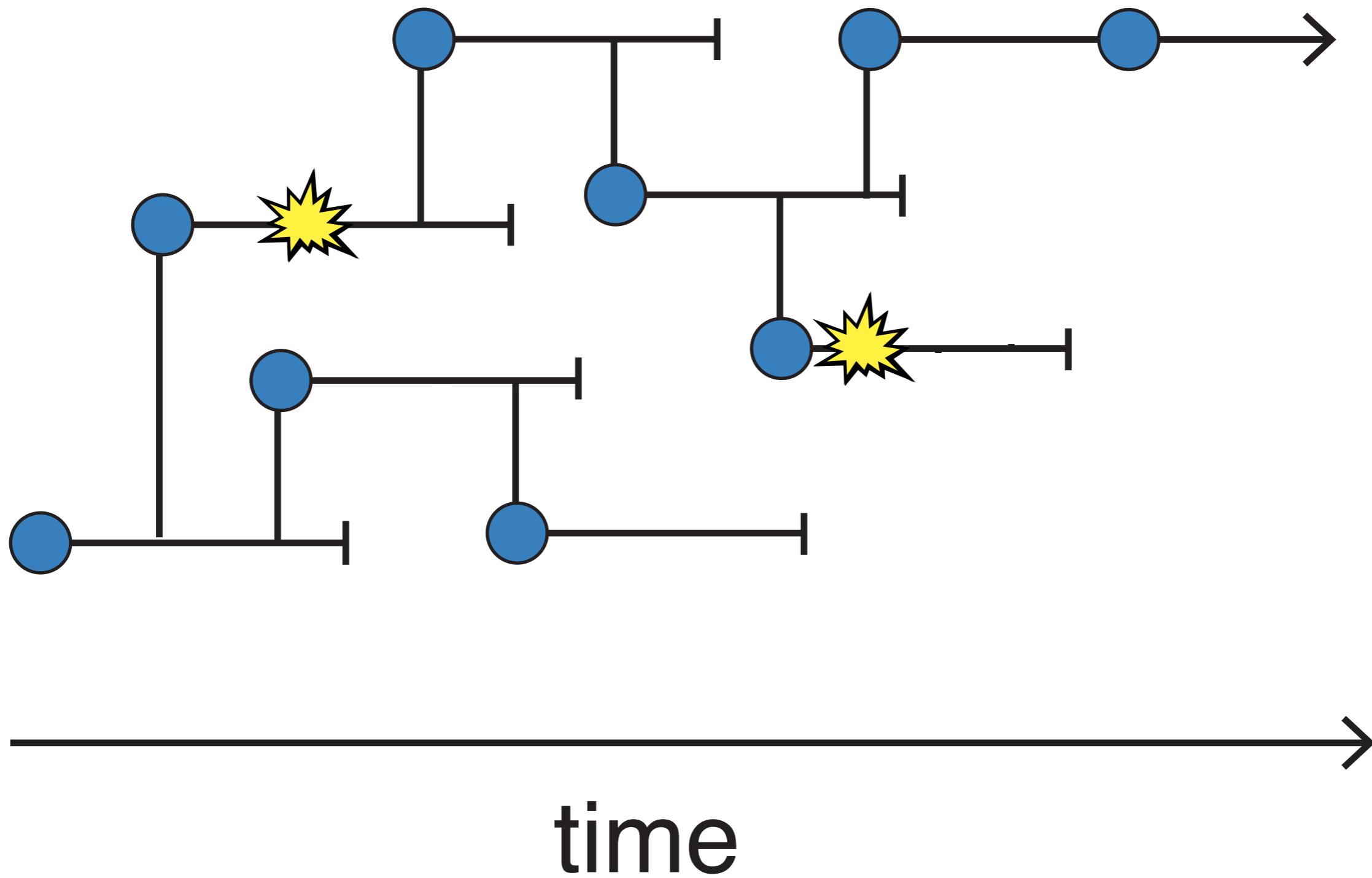
Viral genomes contain records of a virus's transmission history



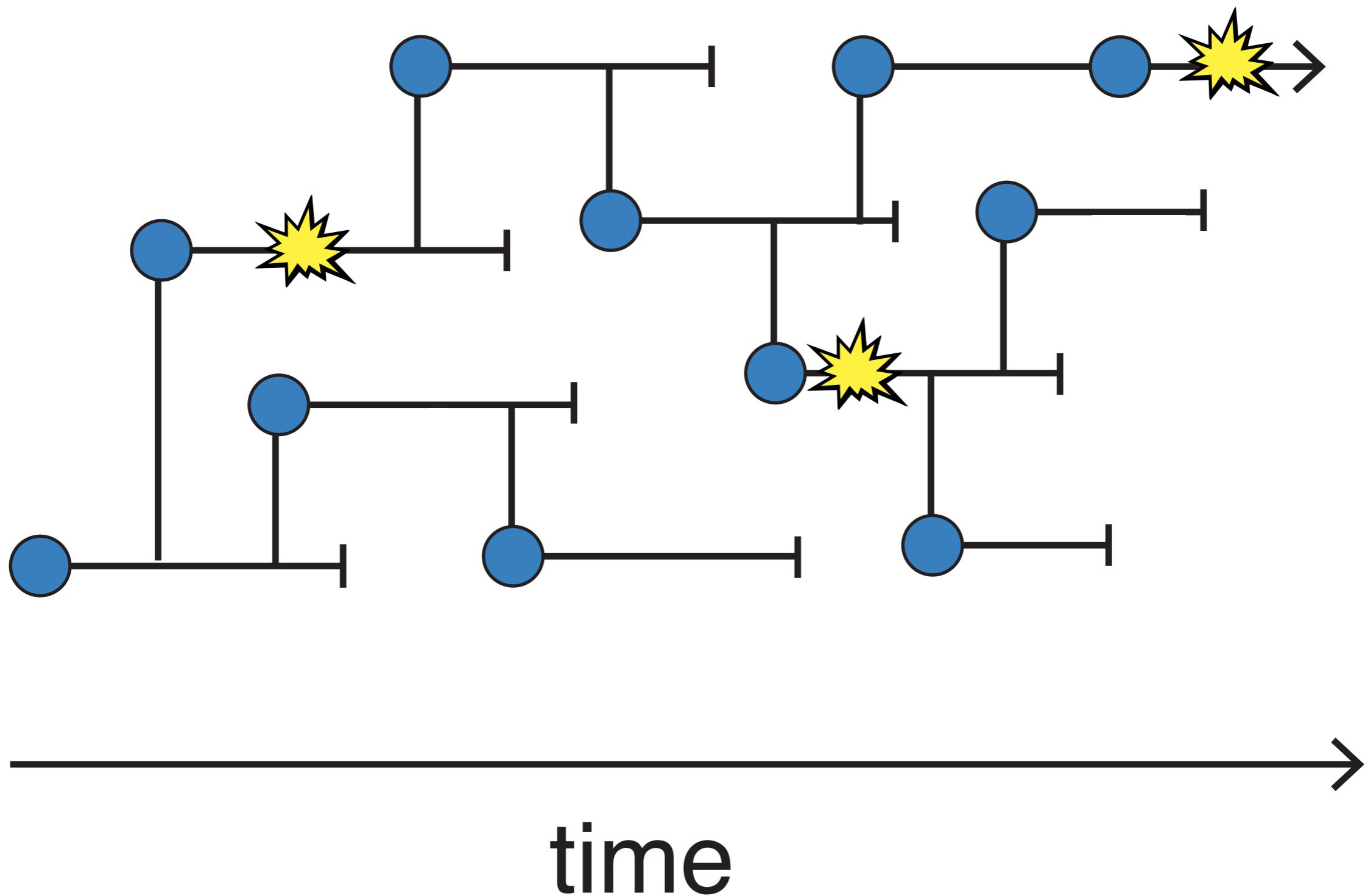
Viral genomes contain records of a virus's transmission history



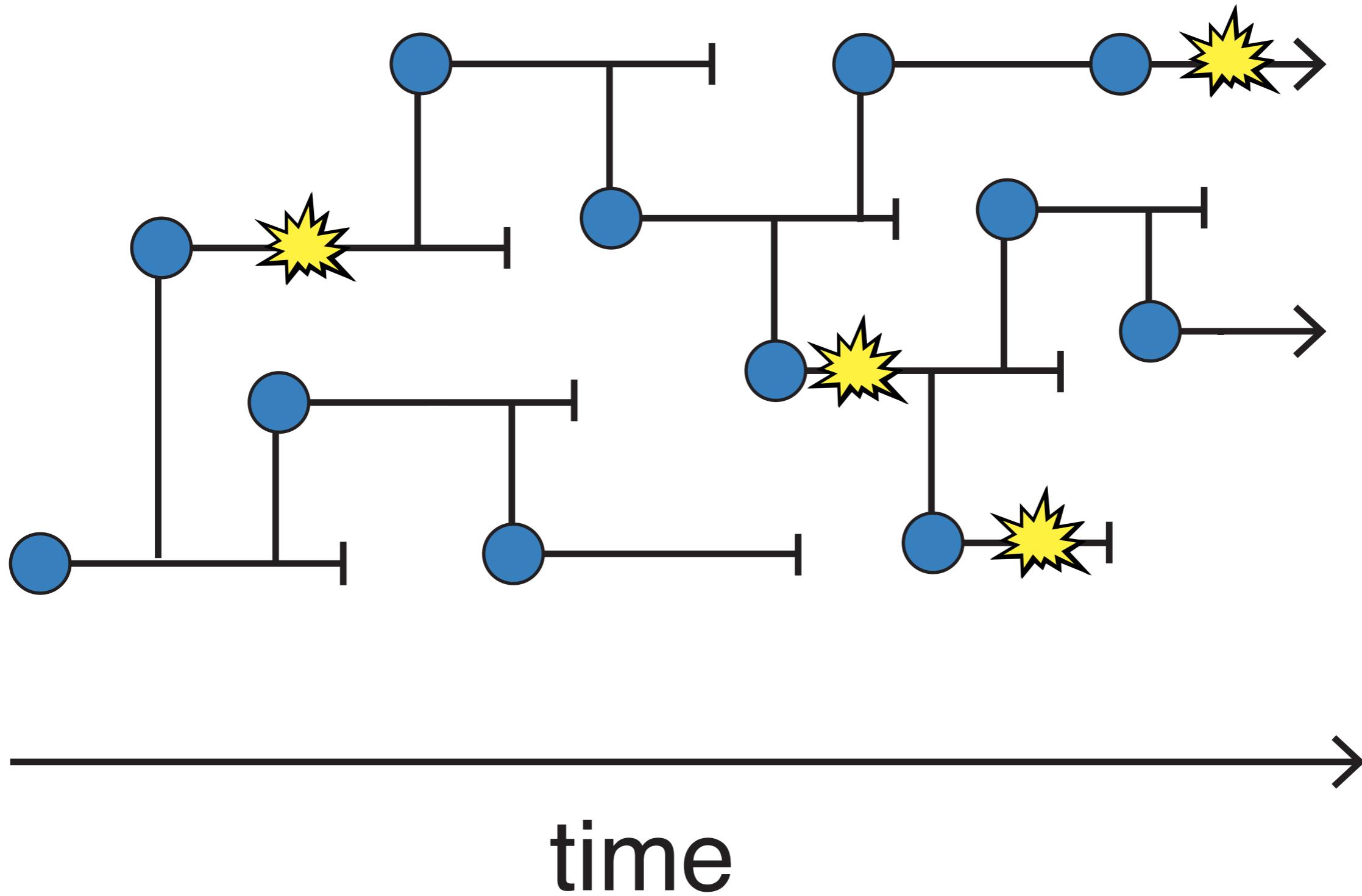
Viral genomes contain records of a virus's transmission history



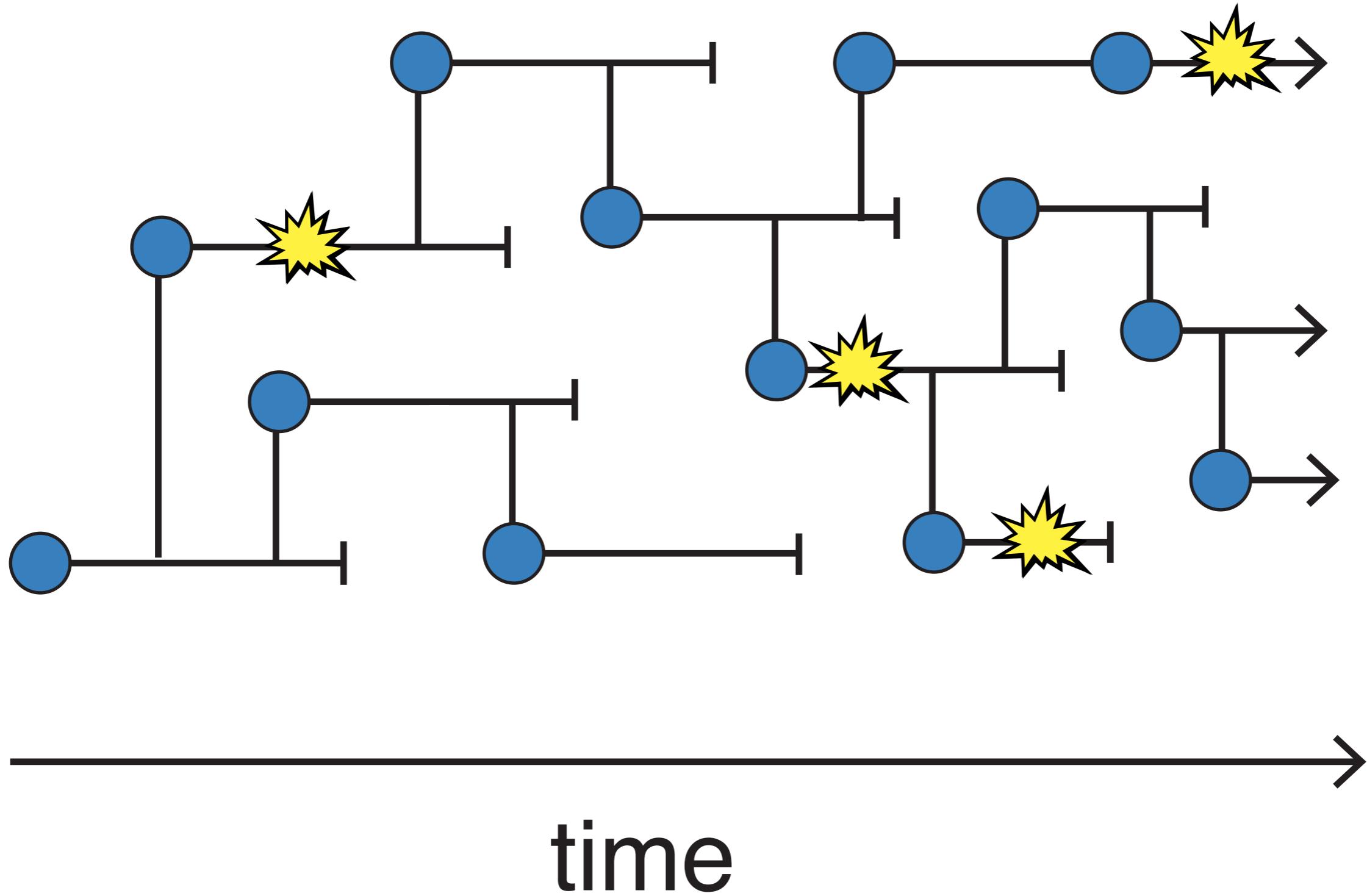
Viral genomes contain records of a virus's transmission history



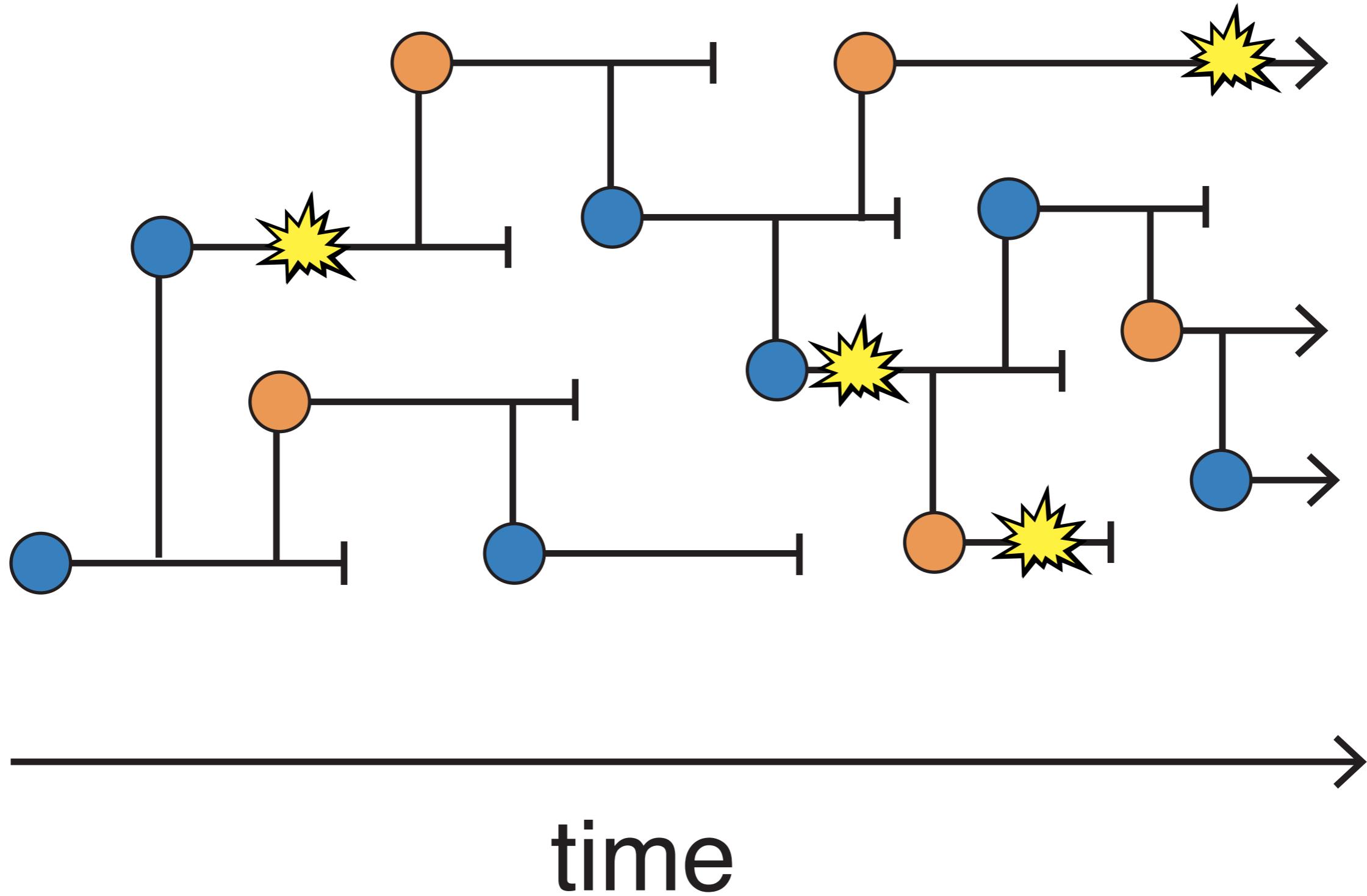
Viral genomes contain records of a virus's transmission history



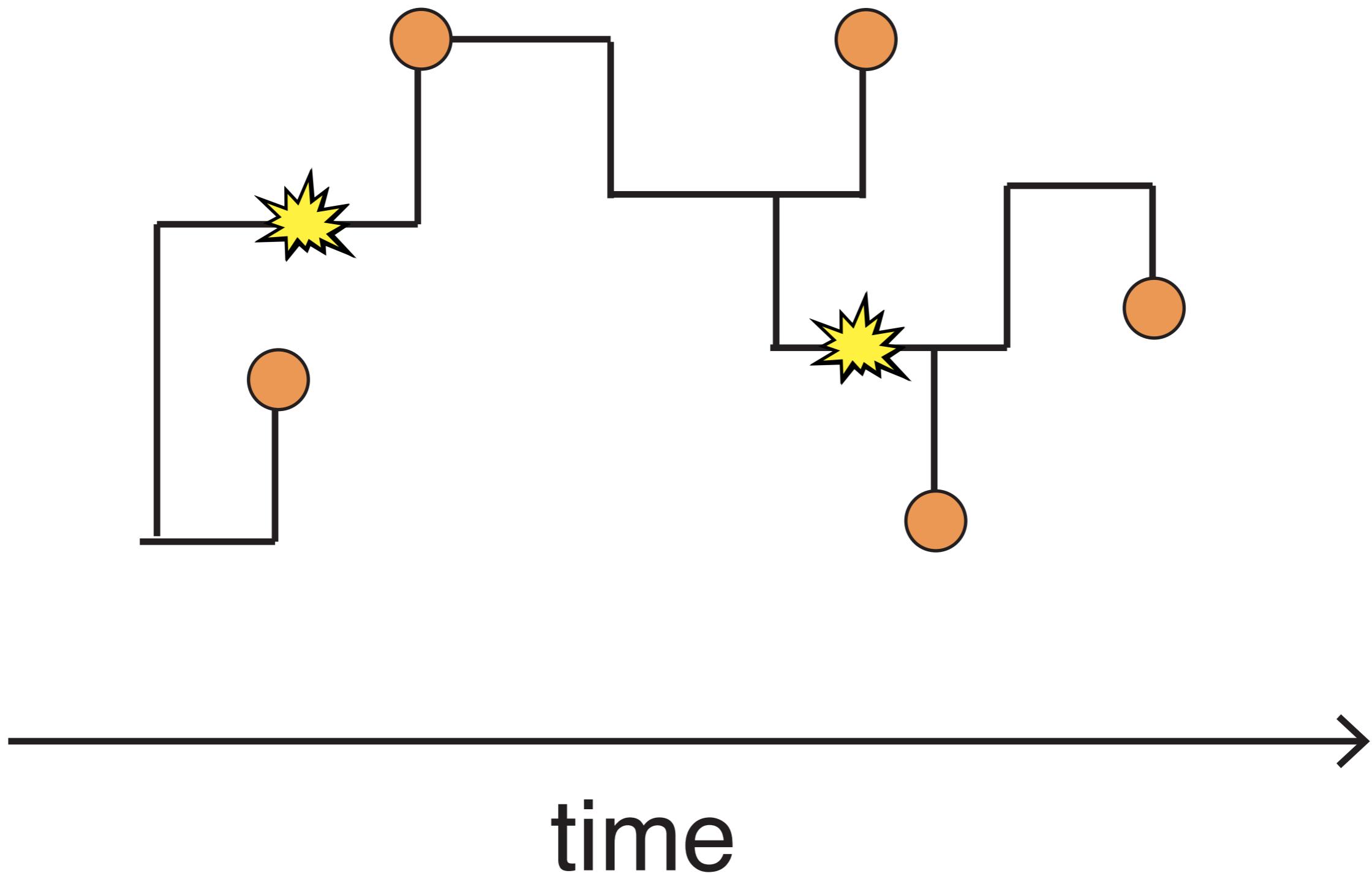
Viral genomes contain records of a virus's transmission history



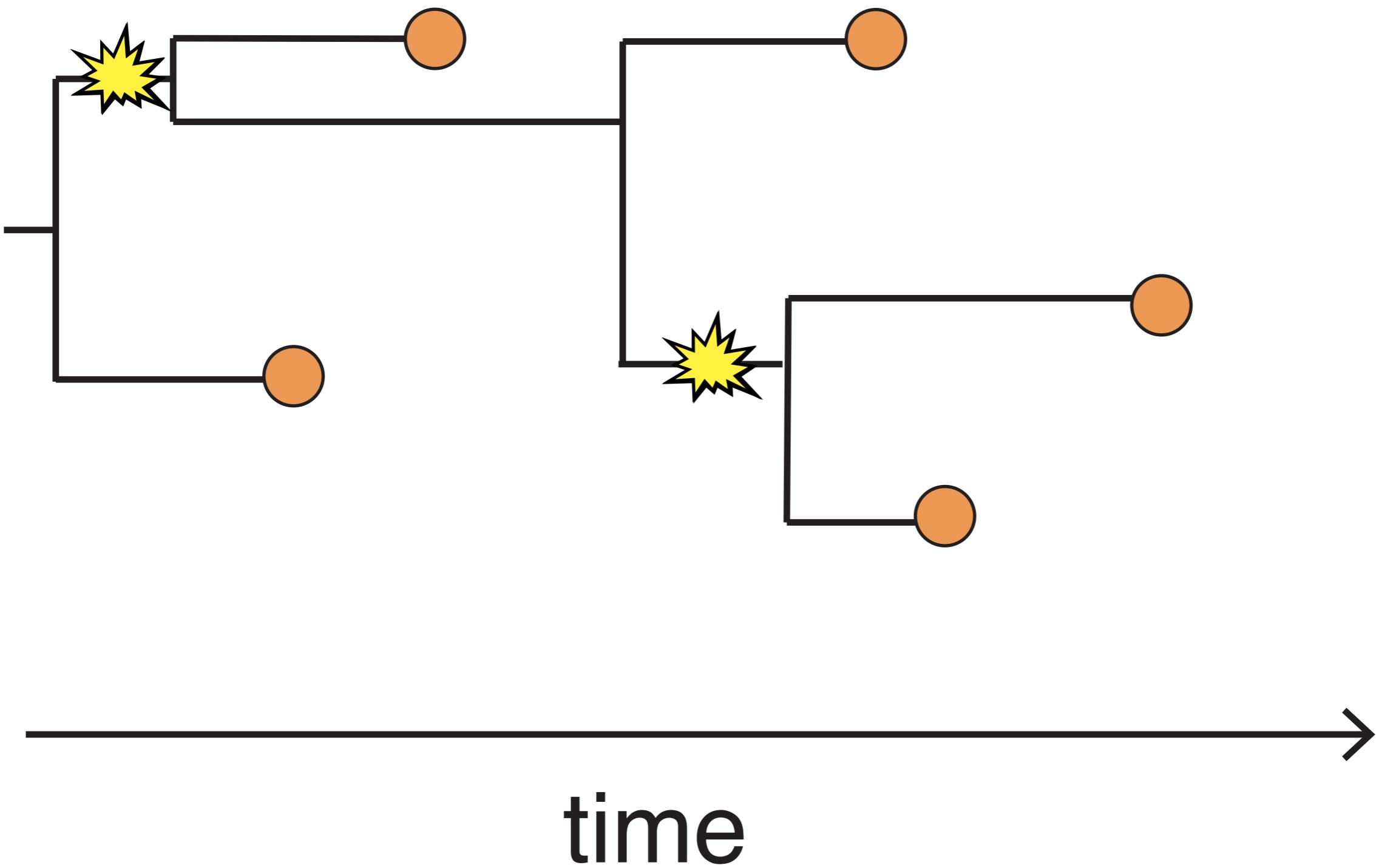
Viral genomes contain records of a virus's transmission history



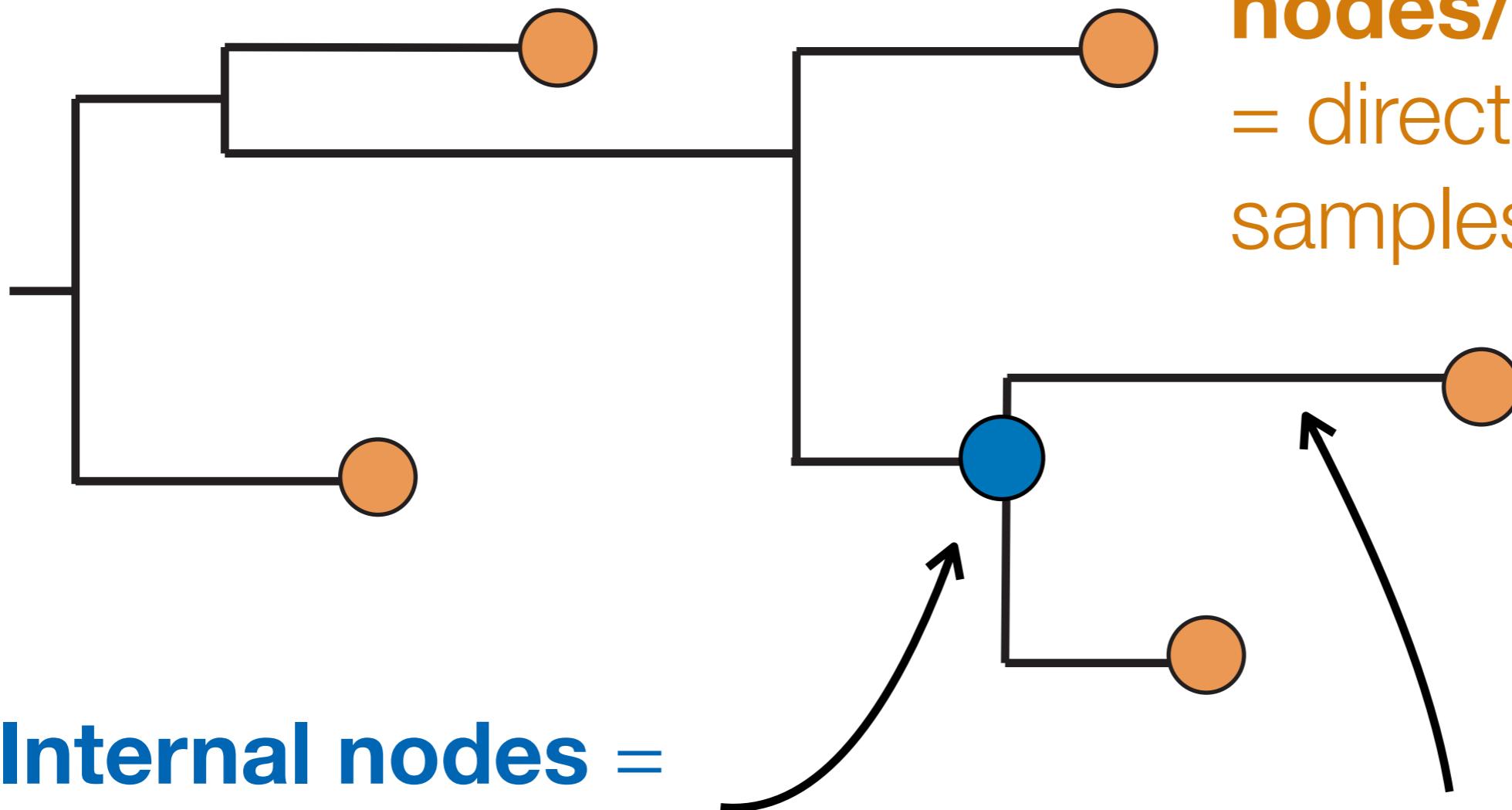
Viral genomes contain records of a virus's transmission history



Viral genomes contain records of a virus's transmission history



A phylogenetic tree is a hypothesis of shared descent

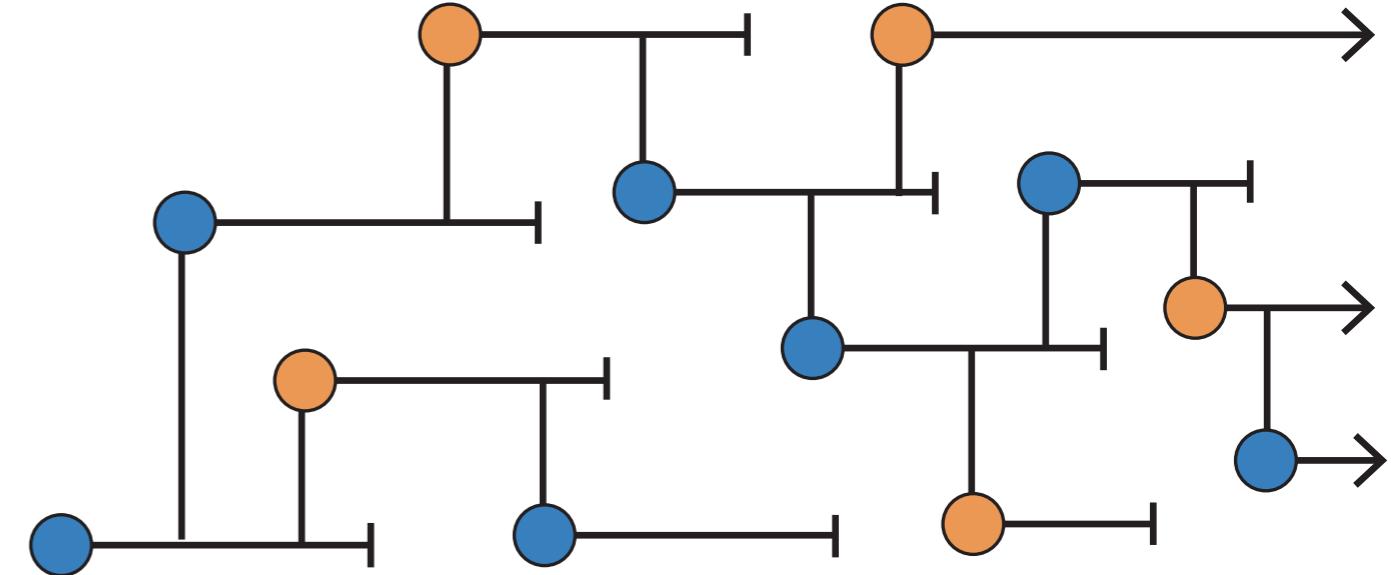
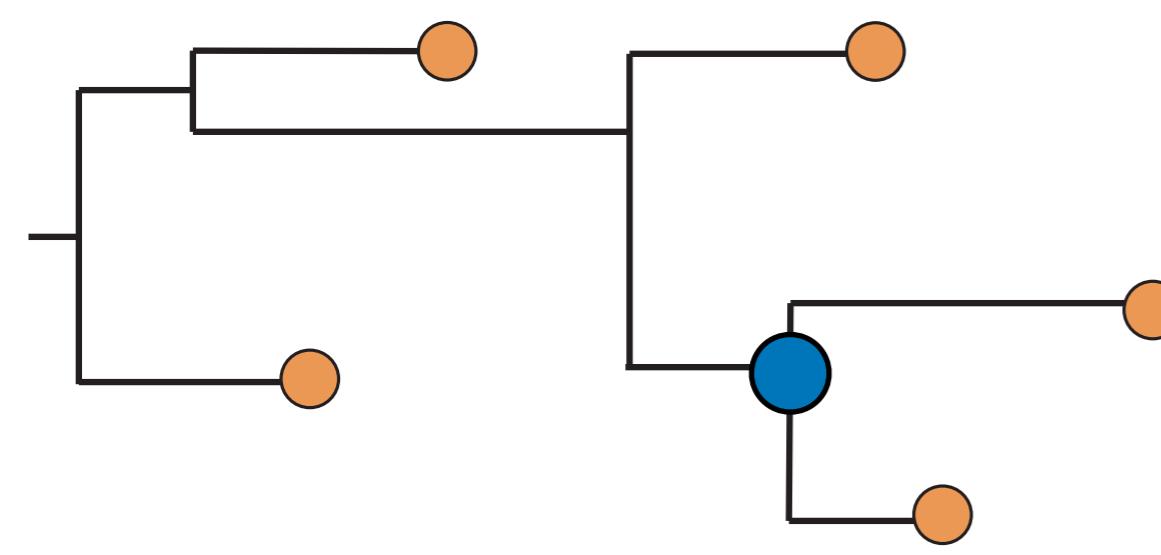


Internal nodes =
hypothetical,
inferred ancestors

**Tips/terminal
nodes/leaves/taxa**
= directly observed
samples

branches = a way to
connect tips and nodes; units
can be mutations or time

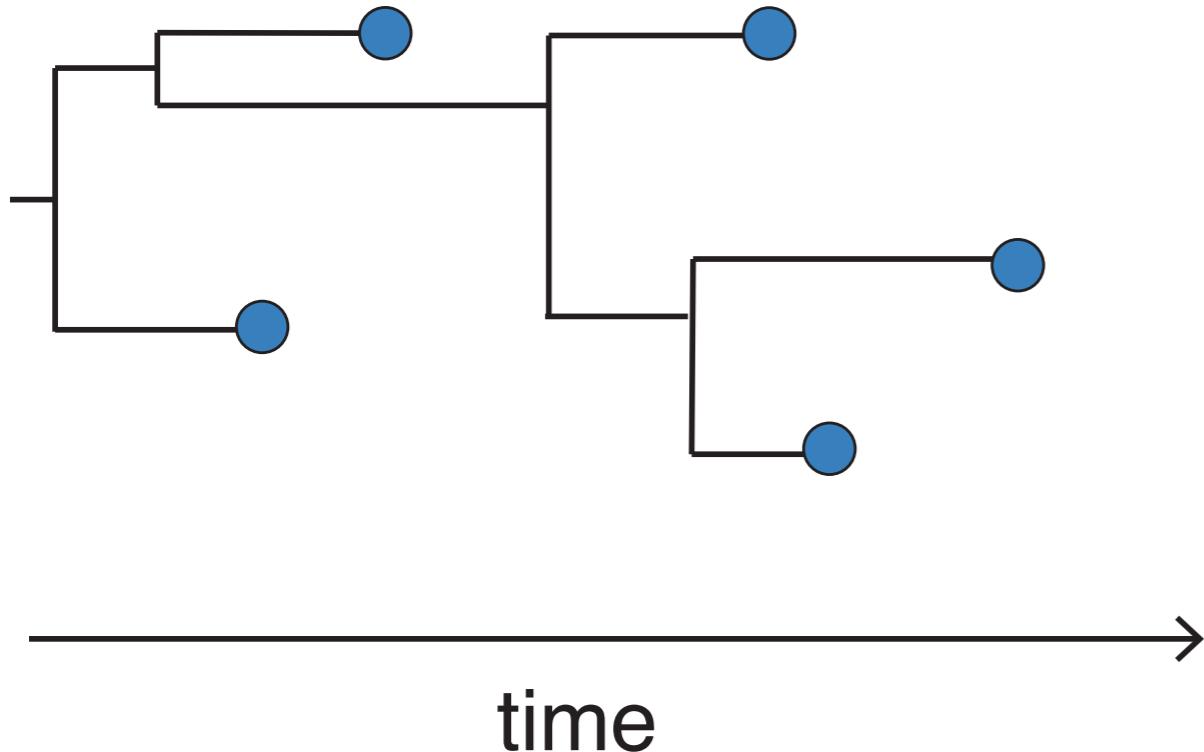
Phylogenetic trees are distinct from transmission trees



Phylogenetic tree:
hypothesis of shared
ancestry

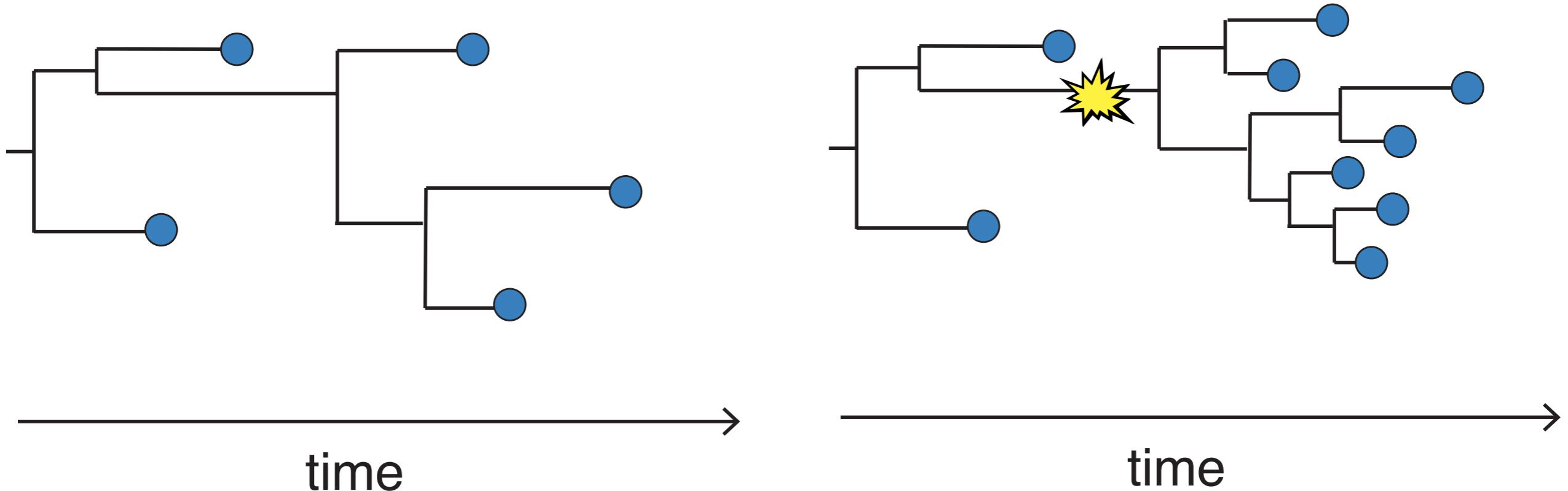
Transmission tree: true
transmission history

The shape of phylogenetic trees changes under different evolutionary, ecological, and epidemiologic scenarios



Original tree

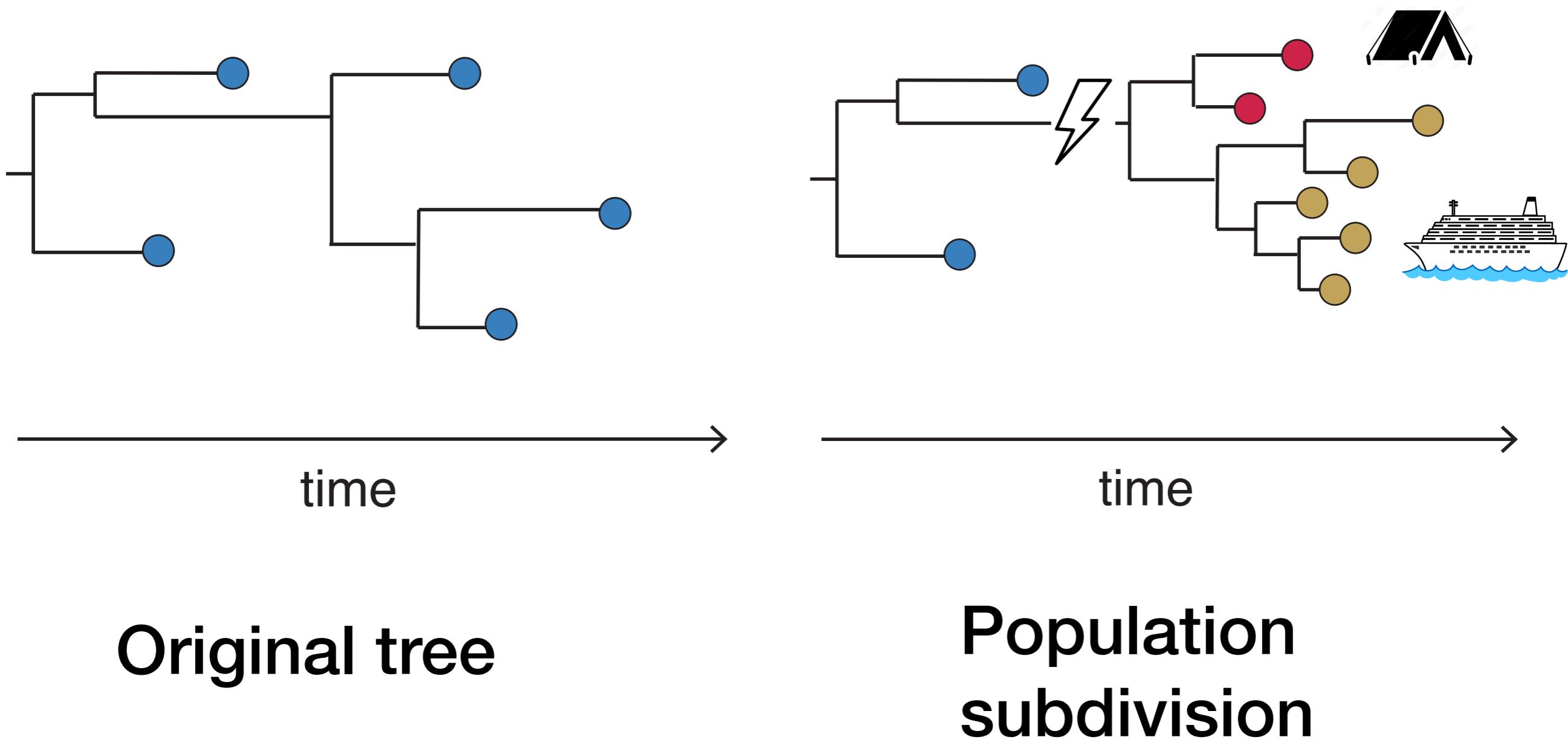
The shape of phylogenetic trees changes under different evolutionary, ecological, and epidemiologic scenarios



Original tree

Increased
transmissibility

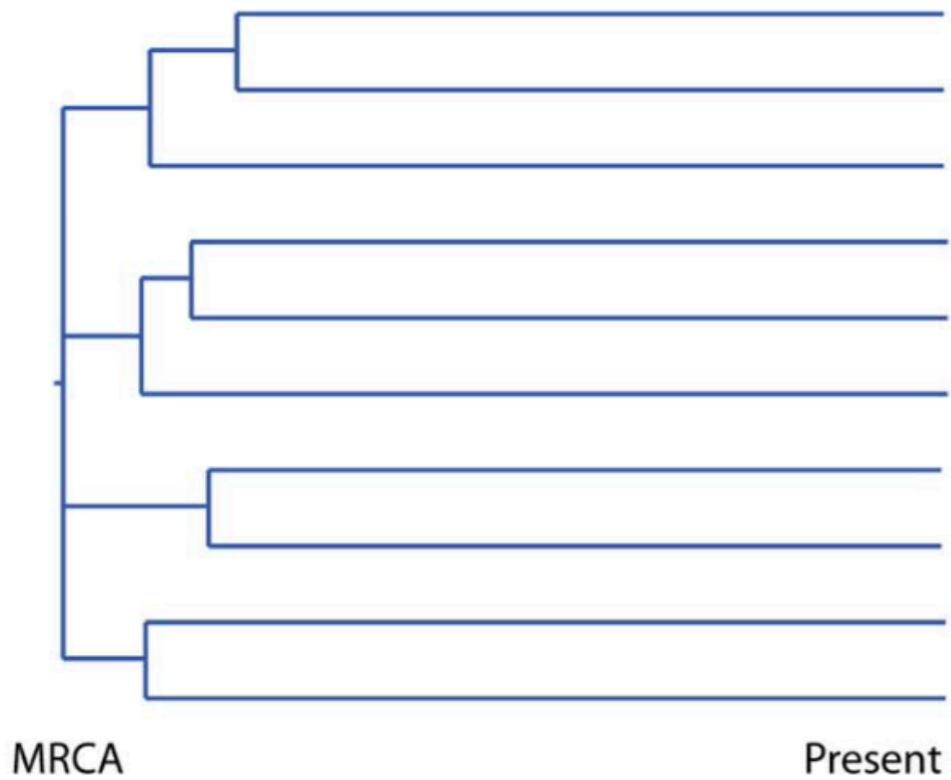
The shape of phylogenetic trees changes under different evolutionary, ecological, and epidemiologic scenarios



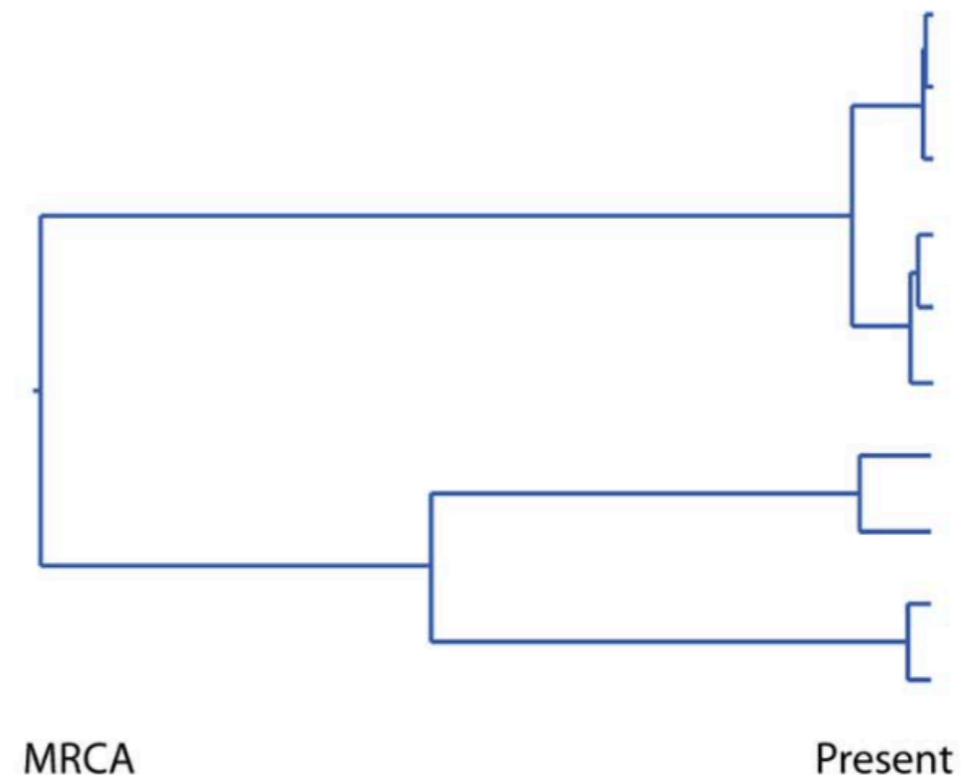
Original tree

Population
subdivision

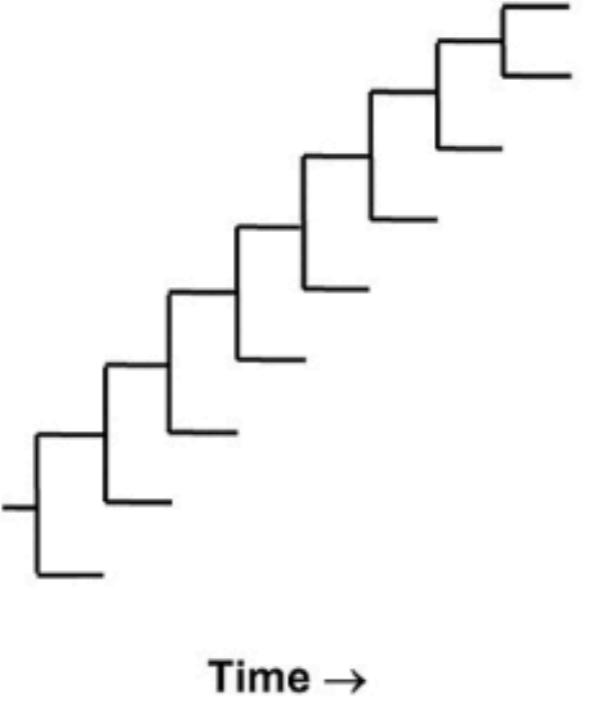
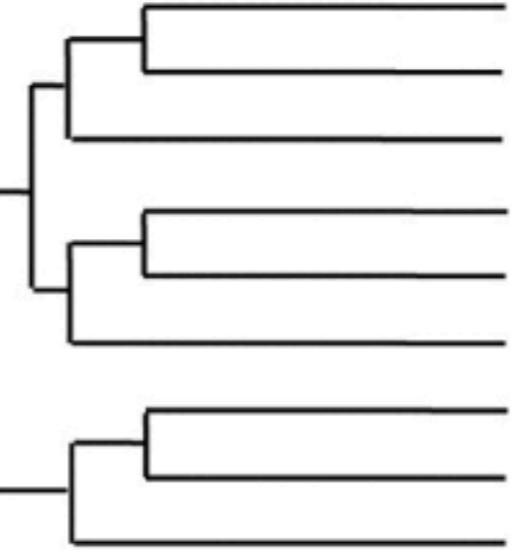
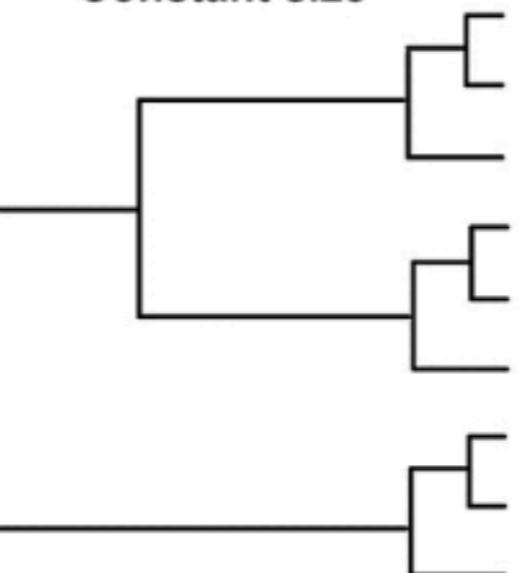
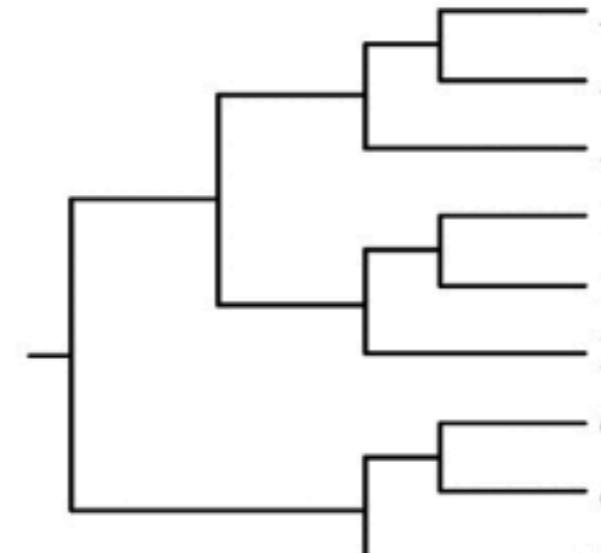
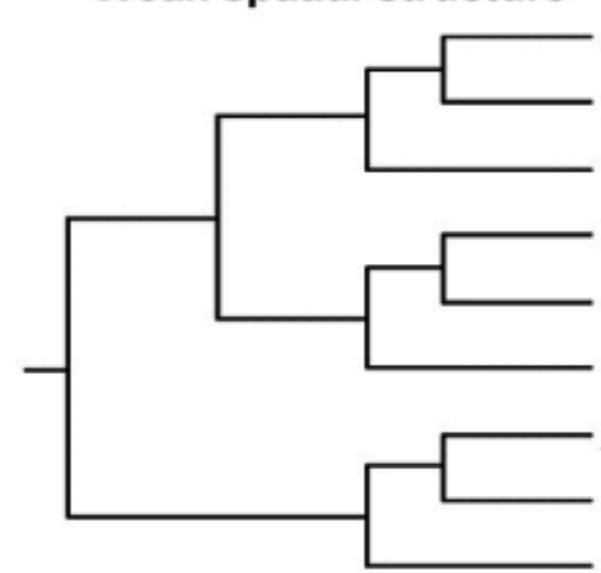
Trees look different in large and small populations



Big population

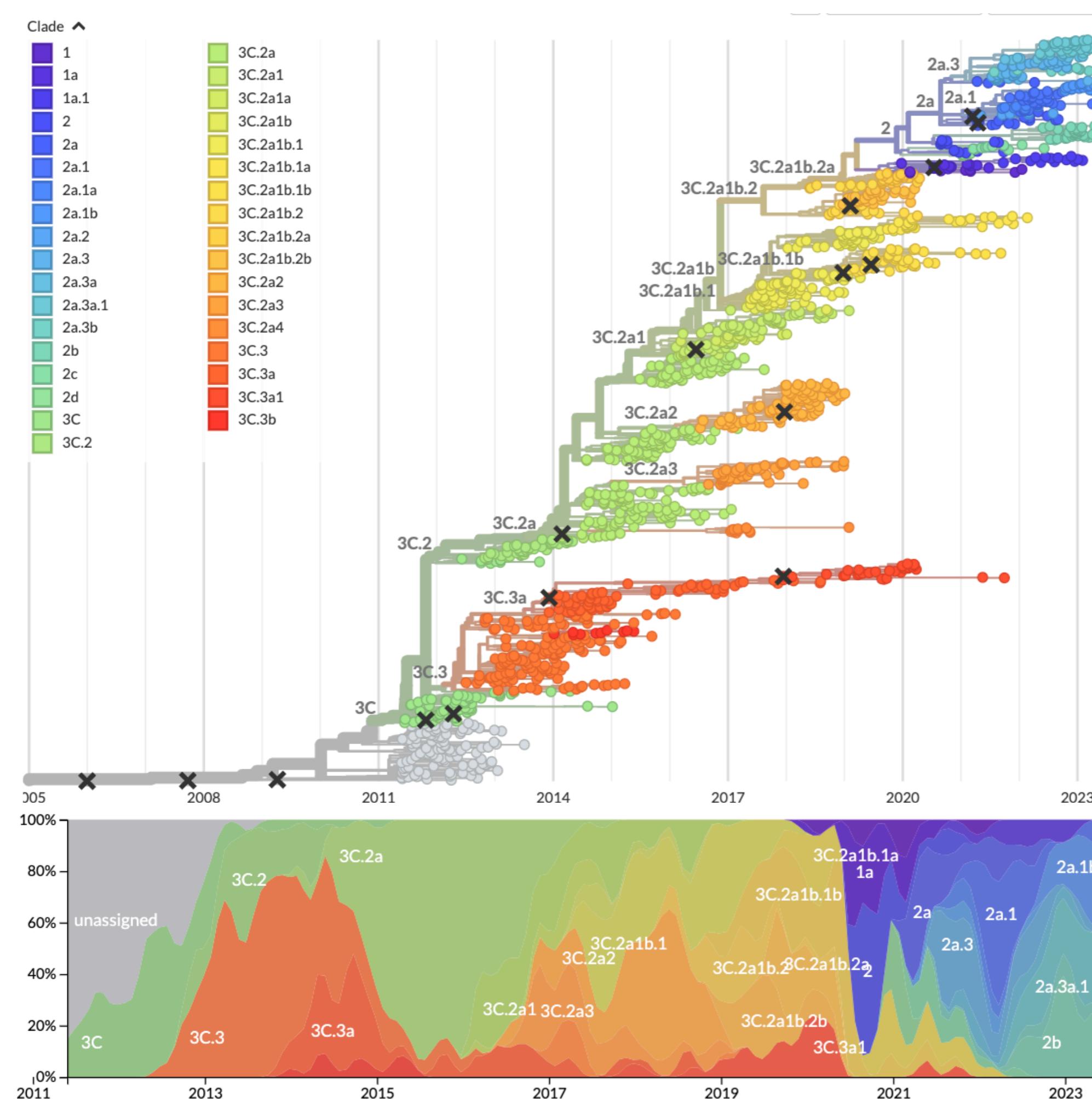


Small population

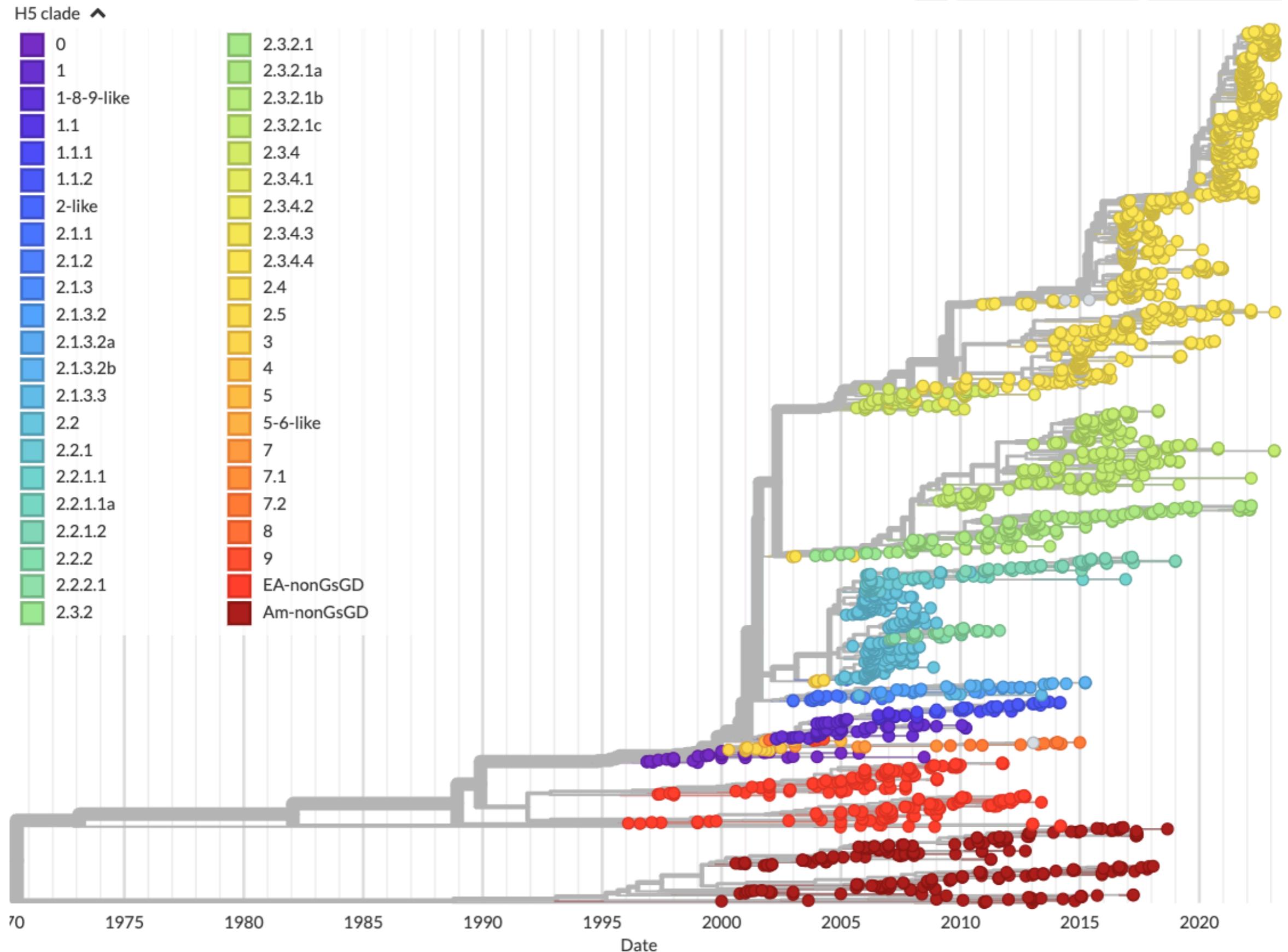
| Continual Immune Selection | | Weak or Absent Immune Selection | |
|-----------------------------------|---|--|--|
| | | Tree shape controlled by non-selective population dynamic processes | |
| Idealized Phylogeny Shapes |  | Population size dynamics | Spatial dynamics |
| | Exponential growth  Constant size  | Strong 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 |

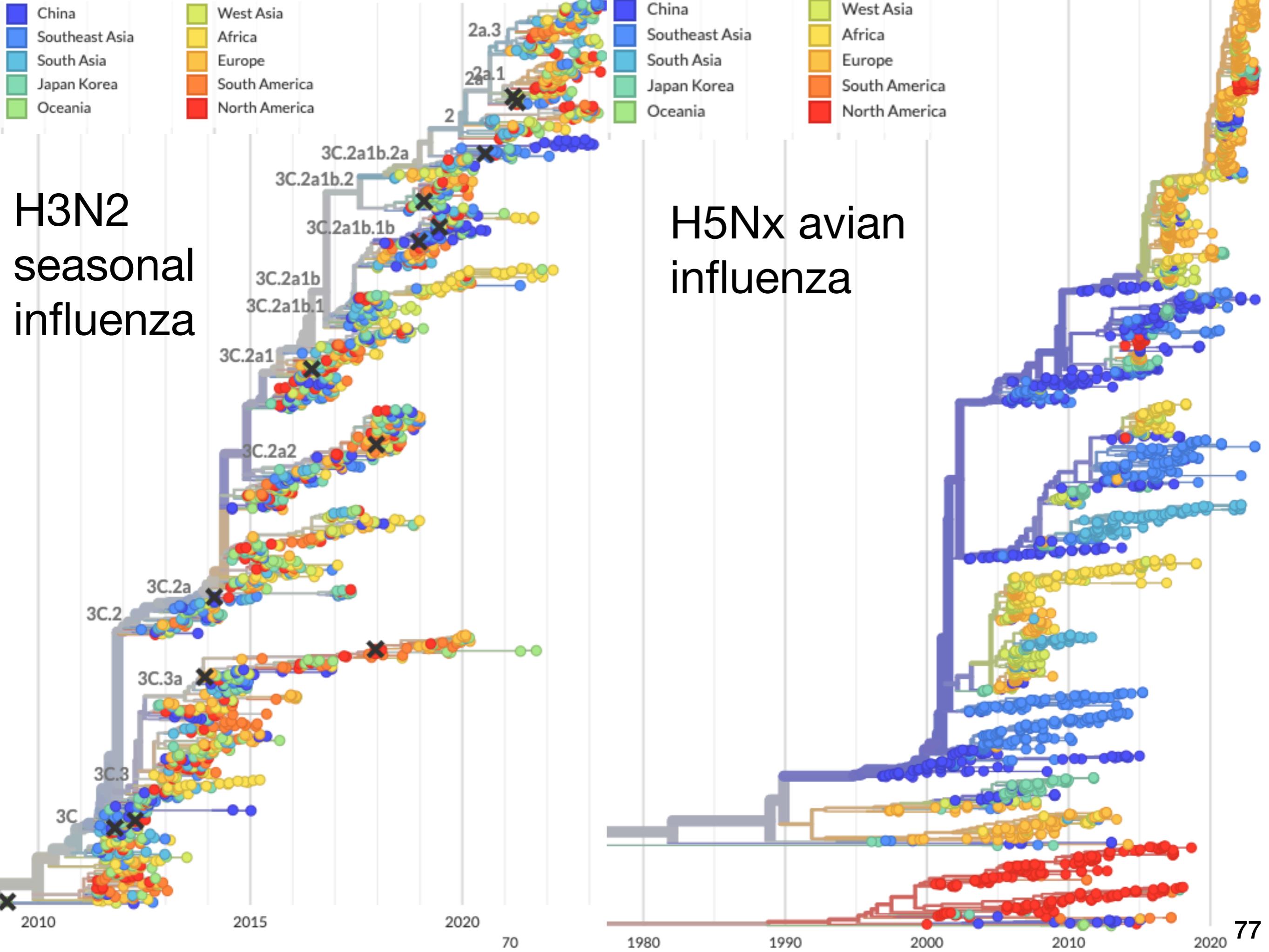
Some real life examples

Seasonal influenza evolves yearly, necessitating vaccine updates

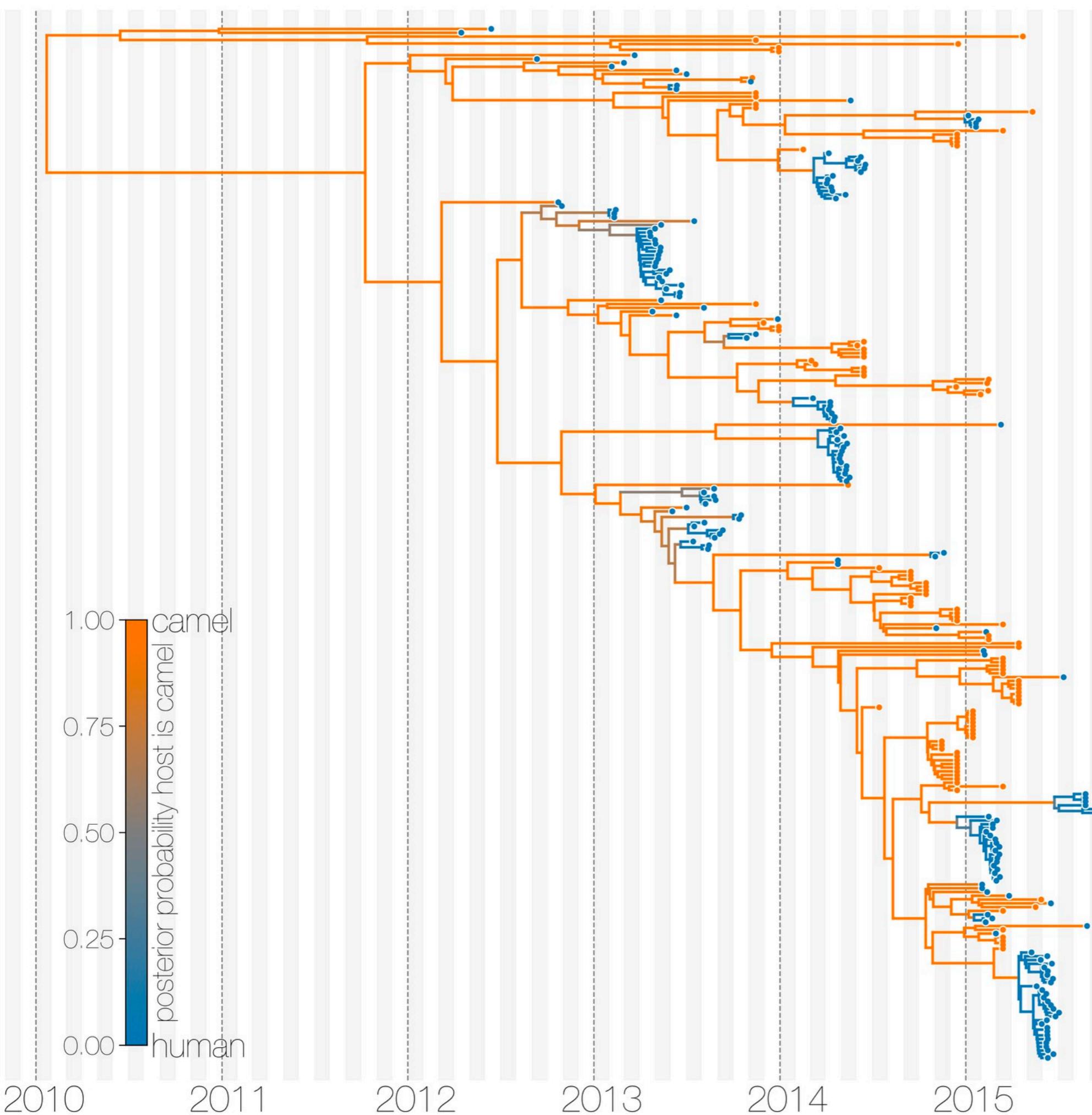


Avian influenza evolves with less selection, and more geographic structure





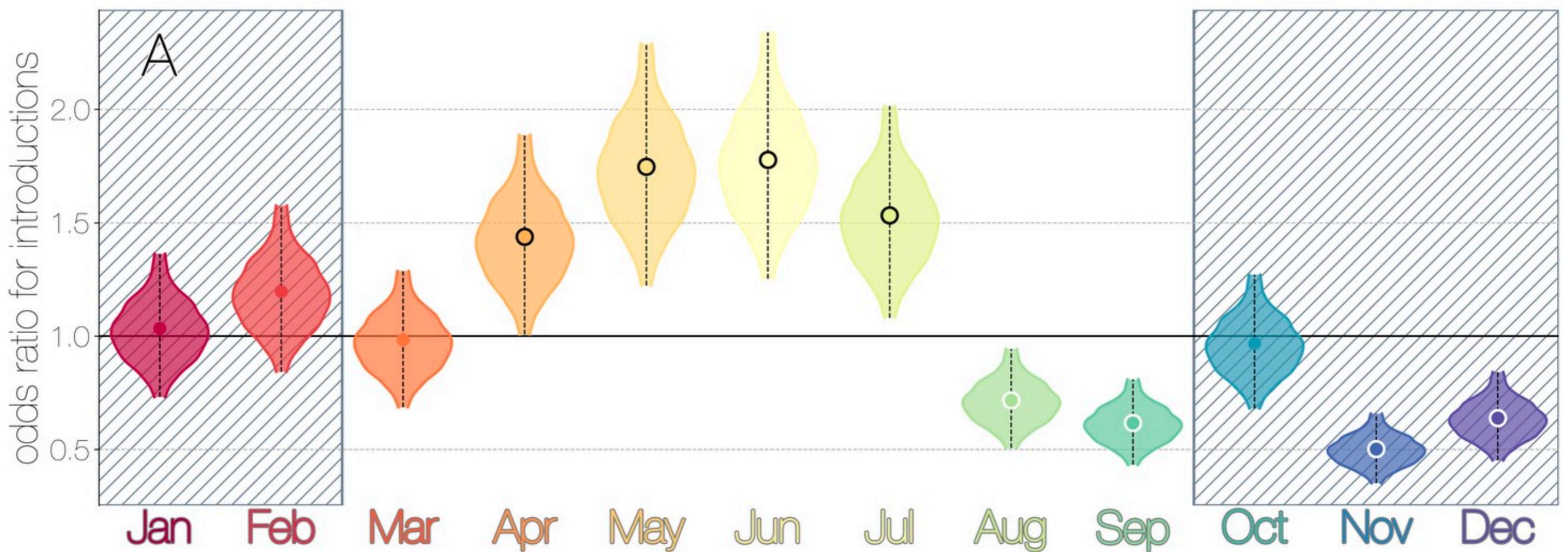
Inferring host switching for MERS



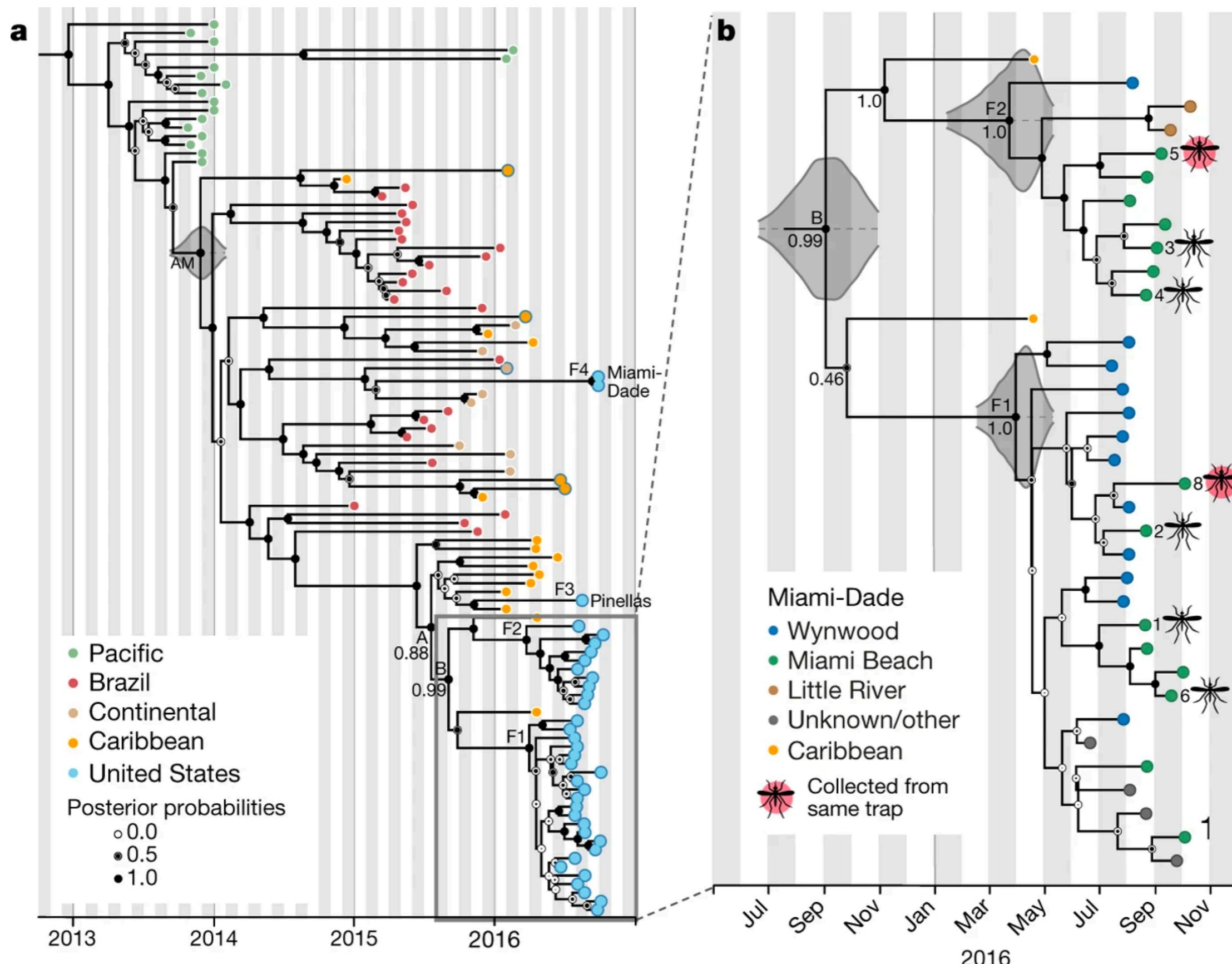
NeTau estimates:
Humans:
0.24 years
Camels: 3.5 years

Dudas et al, eLife
2020

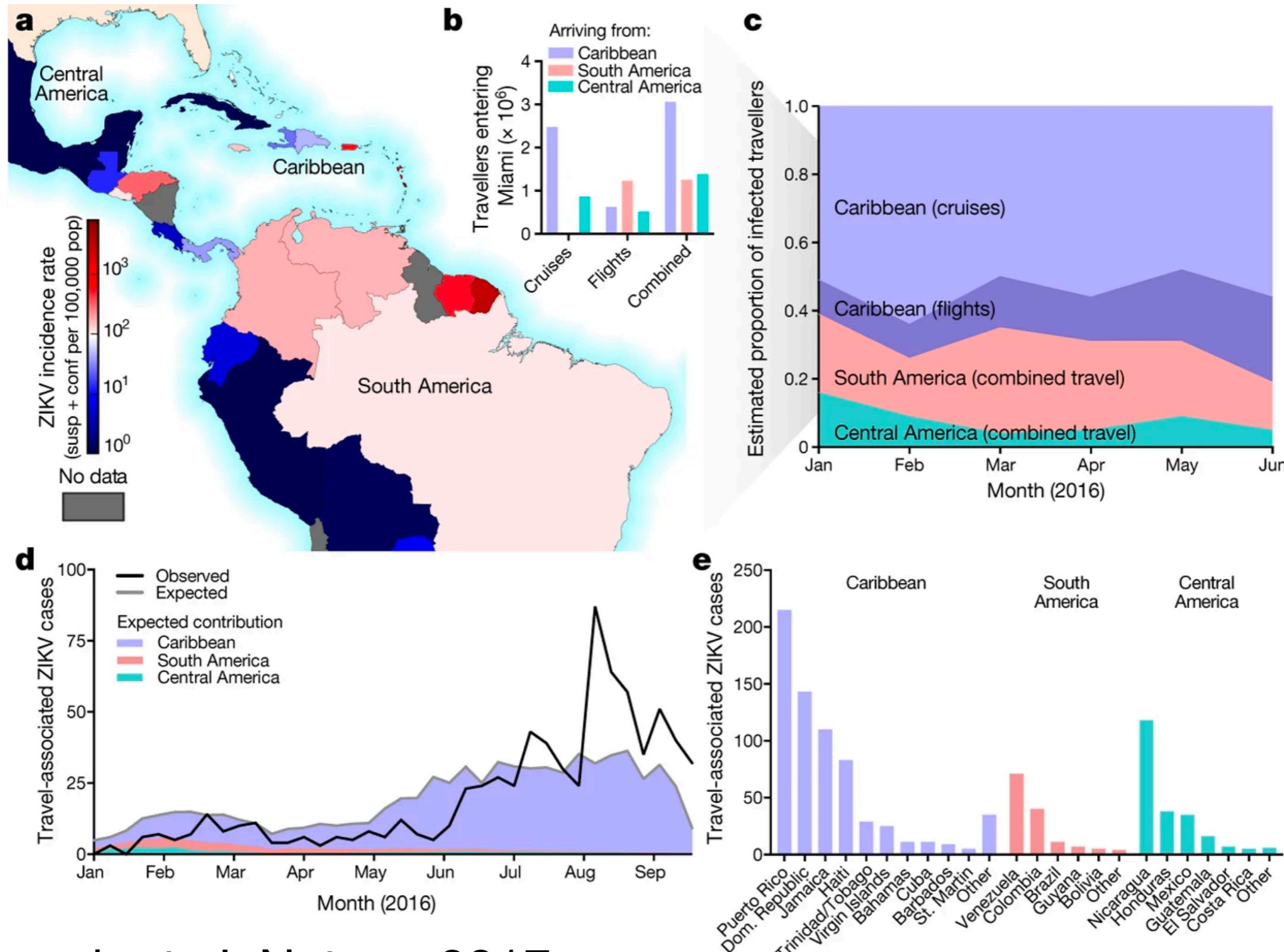
Spring calving season correlates with an increase in the number of cross-species transmission events



Inferring Zika introductions into Florida



Mosquito abundance and cruise ship traffic made Miami uniquely at risk for Zika



What can we learn about viral emergence to reconstruct how outbreaks begin and spread?

- * Emerging sequencing technologies allow for rapid outbreak response and characterization in resource-limited settings.
- * Phylogenetics is an emerging field that uses viral genomes to reconstruct how viruses emerge and spread.

Future work focuses on integrating this information to better formulate risk assessments, interventions, and surveillance practices

- Vaccine strain selection
- Risk assessment pipelines that integrate genomic and functional data
- Inferences about outbreak spread and drivers that inform public health
 - targeted vaccination
 - Efficacy of border control measures and vector control

The key take-home messages:

1. Spillover and cross-species transmission occurs when changes in human, animal, and environmental conditions align that facilitate spillover.
2. Human, animal, and environmental health are intertwined, and interact. One Health approaches attempt to understand health within this context.
3. Zoonosis is rare, and requires overcoming a multitude of barriers.
4. Emerging technologies allow for rapid outbreak reconstruction, response, and mitigation.
5. Phylodynamic approaches allow us to better understand how viruses emerge and spread.
6. Future development is focused on integrating data across multiple disciplines to better formulate surveillance and outbreak mitigation strategies, and on using computational approaches to forecast outbreak dynamics and risk.

We are going to use Nextstrain to explore an example of cross-species transmission and phylodynamic inference



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Nextstrain

Real-time tracking of pathogen evolution

Nextstrain is an open-source project to harness the scientific and public health potential of pathogen genome data. We provide a continually-updated view of publicly available data alongside powerful analytic and visualization tools for use by the community. Our goal is to aid epidemiological understanding and improve outbreak response. If you have any questions, or simply want to say hi, please give us a shout at hello@nextstrain.org.

[READ MORE](#)

H3Nx influenza viruses have repeatedly spilled into new species

- Form groups of 2-3 people
- Navigate to: <https://github.com/lmoncla/teaching-lectures-and-labs/>

Extra resources and cool examples:

- West Nile virus in the Americas: <https://nextstrain.org/narratives/twenty-years-of-WNV> and <https://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1008042>
- Ebola in West Africa: <https://www.nature.com/articles/nature22040>
- HIV in humans: <https://www.science.org/doi/full/10.1126/science.1256739>
- Cryptic transmission of SARS-CoV-2 in Washington state: <https://www.science.org/doi/full/10.1126/science.abc0523>
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