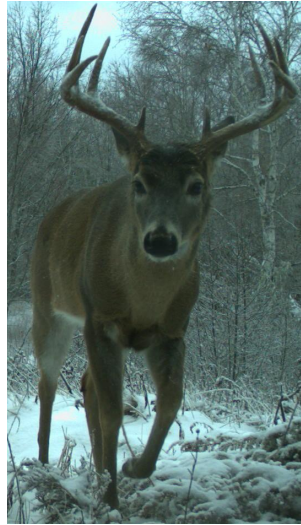


There and back again: characterising SARS-CoV-2 wildlife spillover and spillback

(Pre-Print: 10.1101/2022.02.22.481551v3)

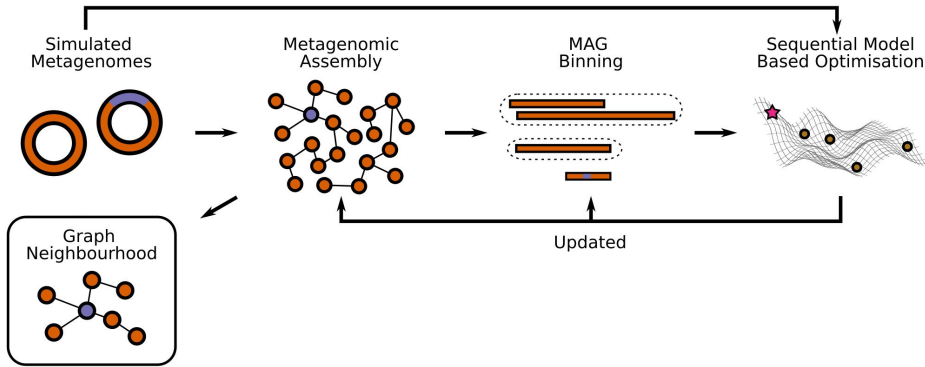


Finlay Maguire

*Computer Science and Community Health & Epidemiology, Dalhousie University, Halifax
Shared Hospital Laboratory, Toronto
Sunnybrook Research Institute, Toronto
Member, Institute of Comparative Genomics, Dalhousie University, Halifax*

Maguire Lab: 2022-

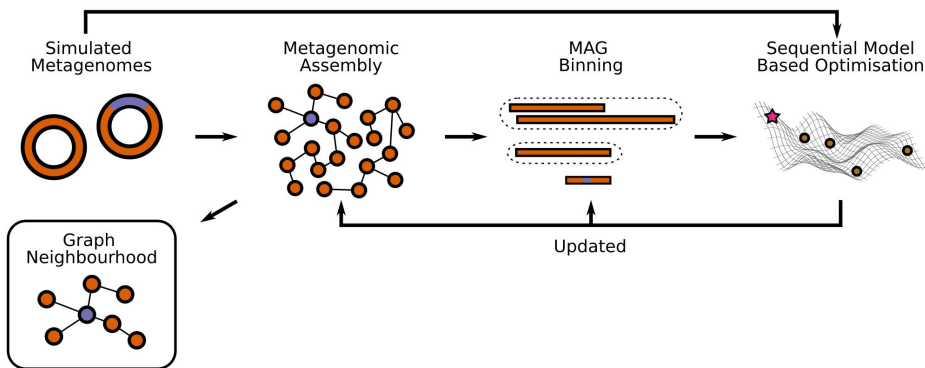
Genomic Epidemiology of Infectious Diseases



- **SARS-CoV-2**
 - CIHR PG: genotype to phenotype
 - NSERC CANMOD: creating genomic SIR models
- **Antimicrobial resistance**
 - NSERC DG: LGT via metagenomic graphs
 - PHA4GE: Emerging threats & public health bioinformatics

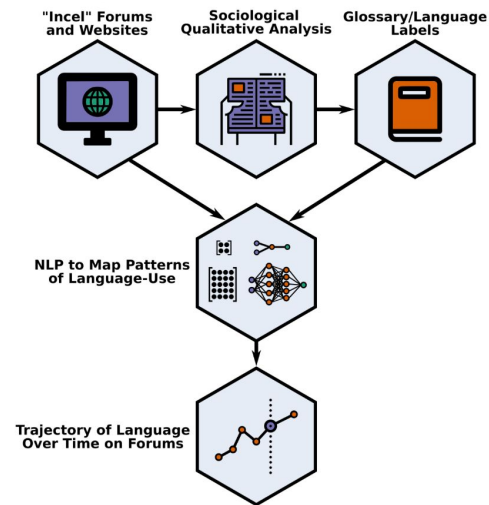
Maguire Lab: 2022-

Genomic Epidemiology of Infectious Diseases



- **SARS-CoV-2**
 - CIHR PG: genotype to phenotype
 - NSERC CANMOD: creating genomic SIR models
- **Antimicrobial resistance**
 - NSERC DG: LGT via metagenomic graphs
 - PHA4GE: Emerging threats & public health bioinformatics

Collaborative Health Data Science



- **Social disconnection**
 - SSHRC IDG: online "Incel" radicalisation
- **Health inequality**
 - SSHRC IDG: modelling interplay of "luck" + frailty/resilience on health outcomes

“WILD-CoV”



Canadian Food
Inspection Agency

National Centre for Foreign Animal Disease

- Bradley Pickering (Iowa State/Manitoba)
- Oliver Lung (Manitoba)
- Peter Kruczkiewicz
- Melissa Goolia, Matthew Suderman, Mathieu Pinette, Greg Smith, Daniel Sullivan, Josip Rudar, Michelle Nebroski, Oksana Vernygora



DALHOUSIE
UNIVERSITY

Computer Science / Epidemiology

- Finlay Maguire

Public
Health
Ontario

- Jennifer Guthrie (Western)
- Alex Marchand-Austin



Sunnybrook
HEALTH SCIENCES CENTRE

Sunnybrook Research Institute

- Samira Mubareka (Toronto)
- Jonathan Kotwa
- Bryan Griffin
- Kuganya Nirmalarajah, Juliette Blais-Savoie, Hsien-Yao Chee, Emily Chien, Winfield Yim, Andra Banete, Lily Yip

Shared Hospital Laboratory

- Patryk Aftanas



Ontario

Ministry of Northern
Development, Mines, Natural
Resources and Forestry

- Jeff Bowman (Trent)
- Tore Buchanan
- Larissa Nituch
- Elizabeth Adey



uOttawa

- Marceline Côté
- Geneviève Laroche
- Ardeshir Ariana, Brett Vahkal



Sinai
Health

- Allison McGeer (Toronto)

Université
de Montréal



CANADIAN
WILDLIFE HEALTH
COOPERATIVE

Forêts, Faune
et Parcs

Québec



Ontario
MINISTRY OF HEALTH

Guillaume Goyette, Andrés Finzi, Claire
Jardine, Ariane Massé Heather McClinchey



CIHR IRSC



Canadian Institutes
of Health Research
Instituts de recherche
en santé du Canada



CoVaRRNet



CANADIAN
WILDLIFE HEALTH
COOPERATIVE

CanCOGeN

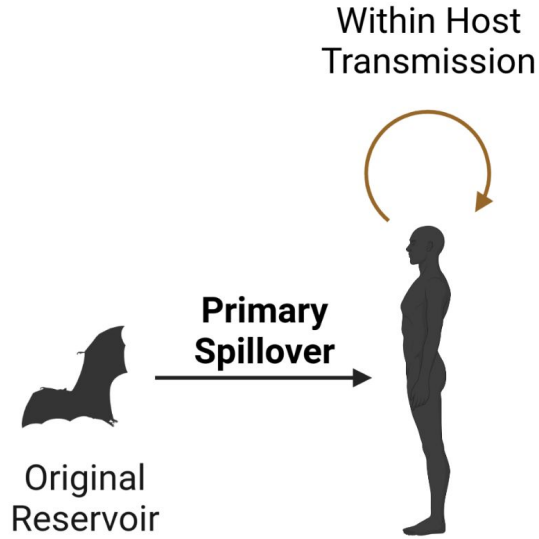
Canadian Safety
and Security Program

Programme canadien
pour la sûreté et la sécurité

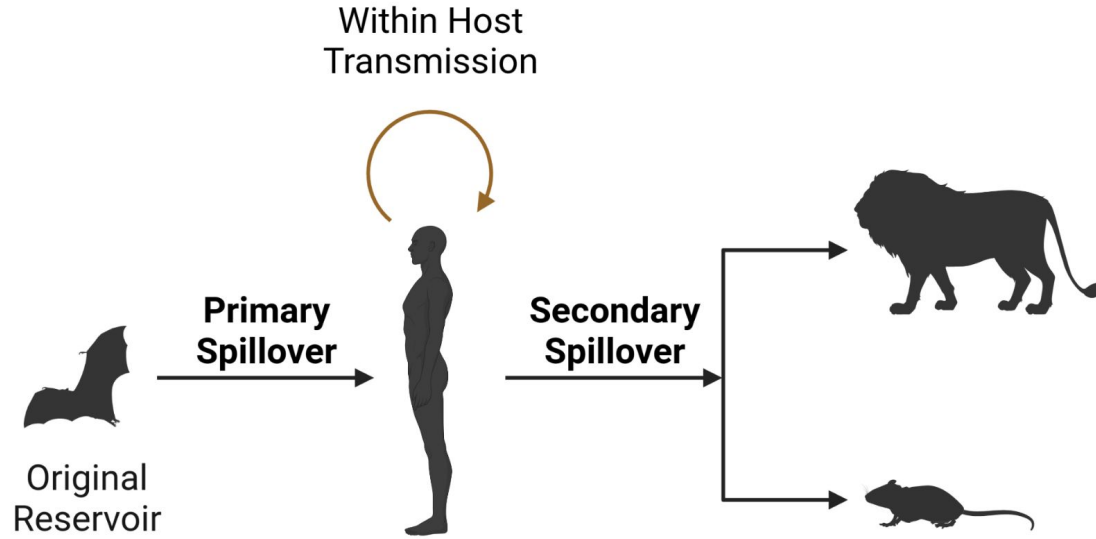


Why monitor non-human mammals?

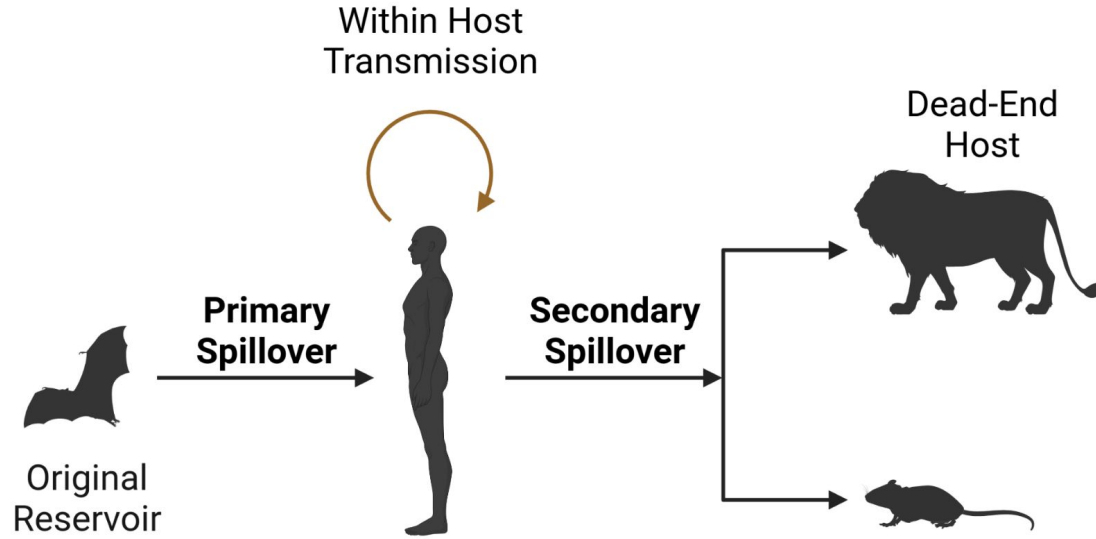
Animal infections can impact evolution of SARS-CoV-2



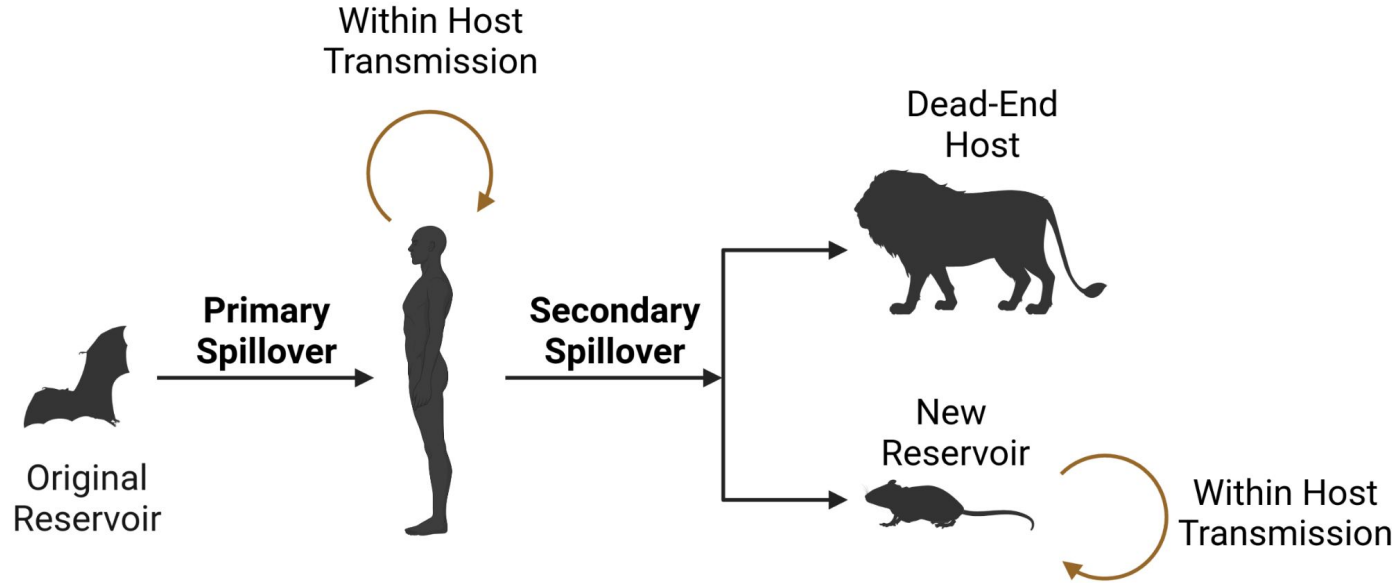
Animal infections can impact evolution of SARS-CoV-2



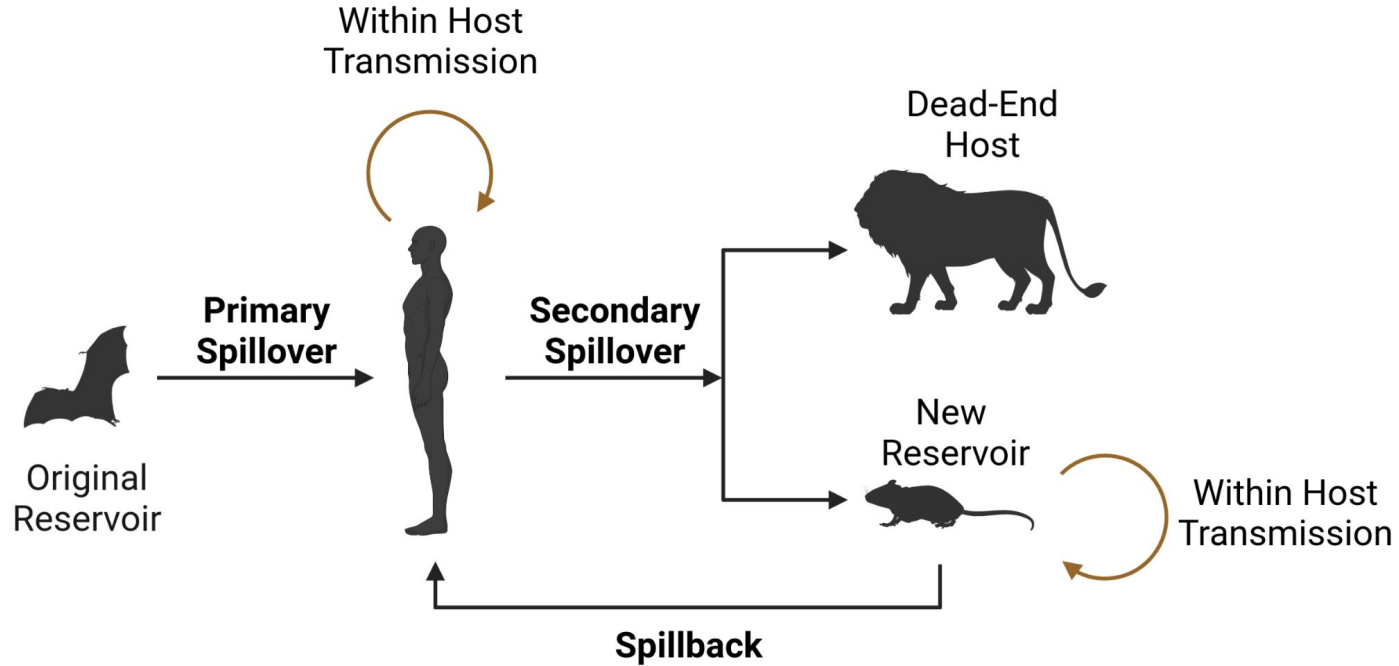
Animal infections can impact evolution of SARS-CoV-2



Animal infections can impact evolution of SARS-CoV-2



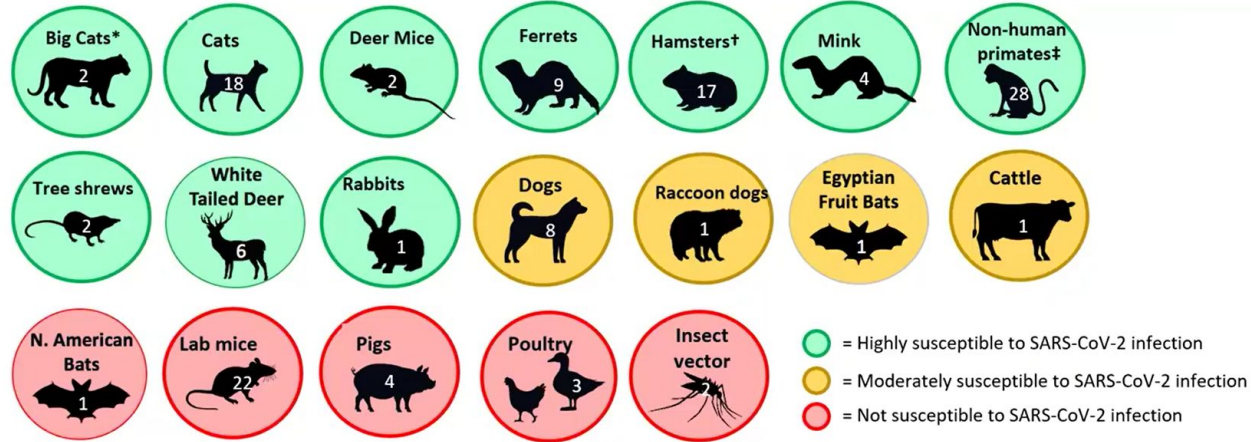
Animal infections can impact evolution of SARS-CoV-2



Are secondary animal reservoirs a problem in
SARS-CoV-2?

SARS-CoV-2 can infect ~50 non-human mammals

Published or pre-print articles – SARS-CoV-2 experimental or natural infection in animals



*Big cats include: lions, tigers and puma

†Hamster species include: Chinese hamsters and golden Syrian hamsters

‡Non-human primate species include: African green monkeys, baboons, common marmosets, cynomolgus macaques, pigtail macaques, rhesus macaques, and savanna monkeys



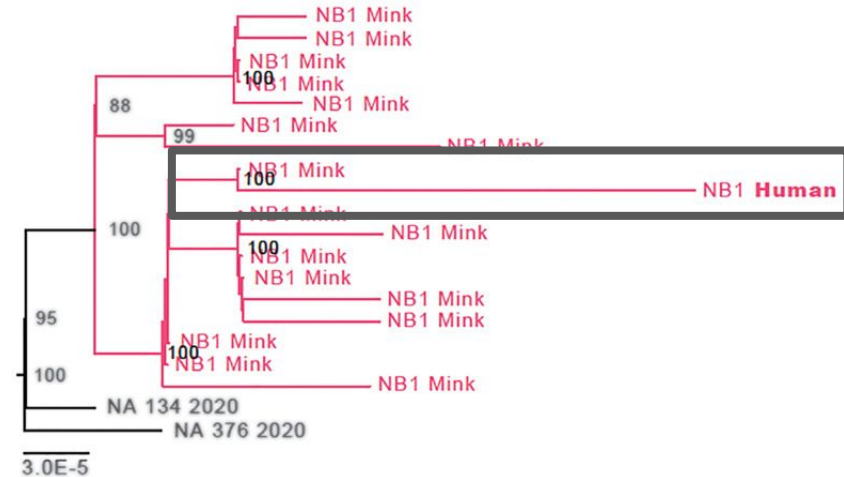
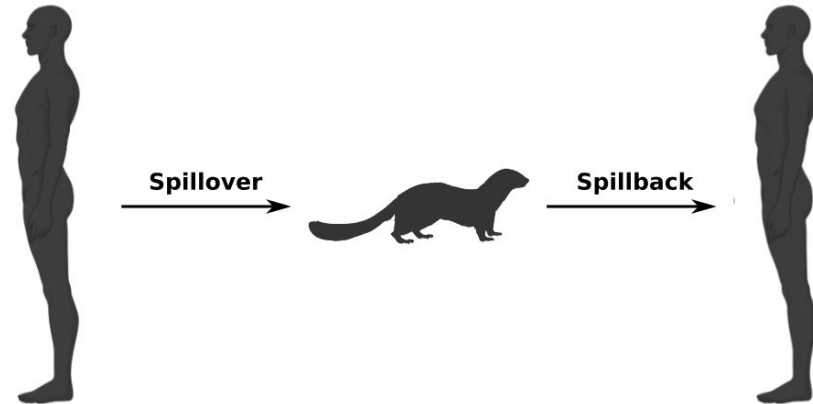
Secondary Spillover into humans has been observed

Transmission of SARS-CoV-2 on mink farms between humans and mink and back to humans

BAS B. OUDE MUNNINK  · REINA S. SIKKEMA · DAVID F. NIEUWENHUIJSE  · ROBERT JAN MOLENAAR  · EMMANUELLE MUNGER  · RICHARD MOLENKAMP 

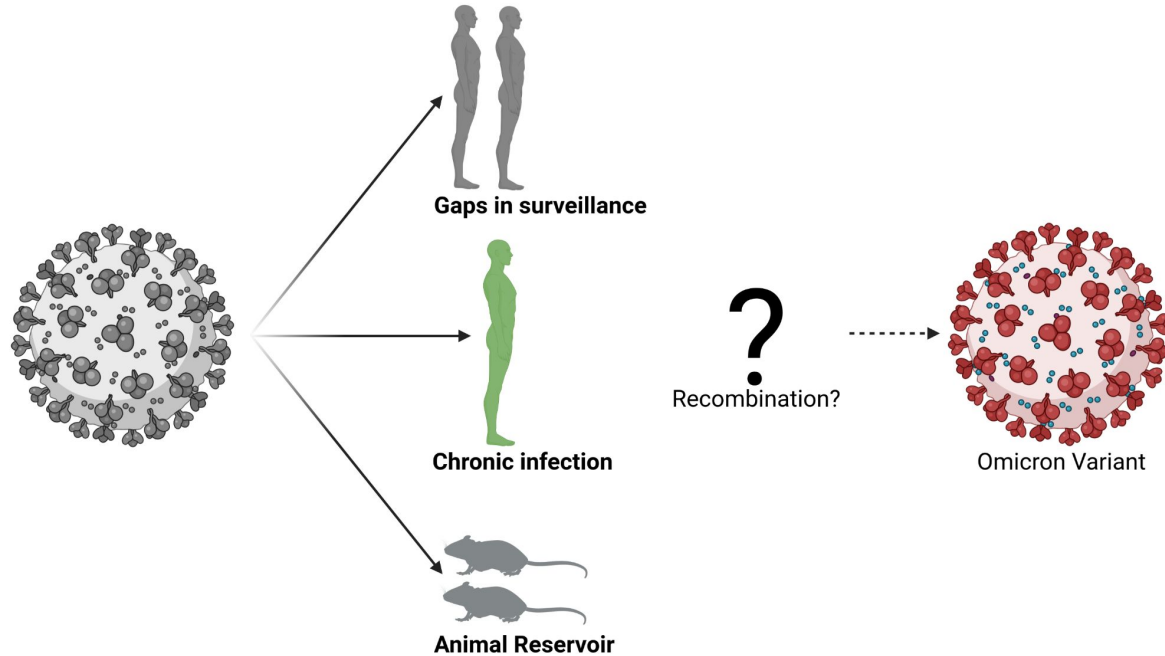
ARCO VAN DER SPEK · PAULIEN TOLSMAN · ARIENE RIETVELD  · MARION P. G. KOOPMANS  · [+13 authors](#) [Authors Info & Affiliations](#)

SCIENCE · 10 Nov 2020 · Vol 371, Issue 6525 · pp. 172-177 · DOI: 10.1126/science.abe5901



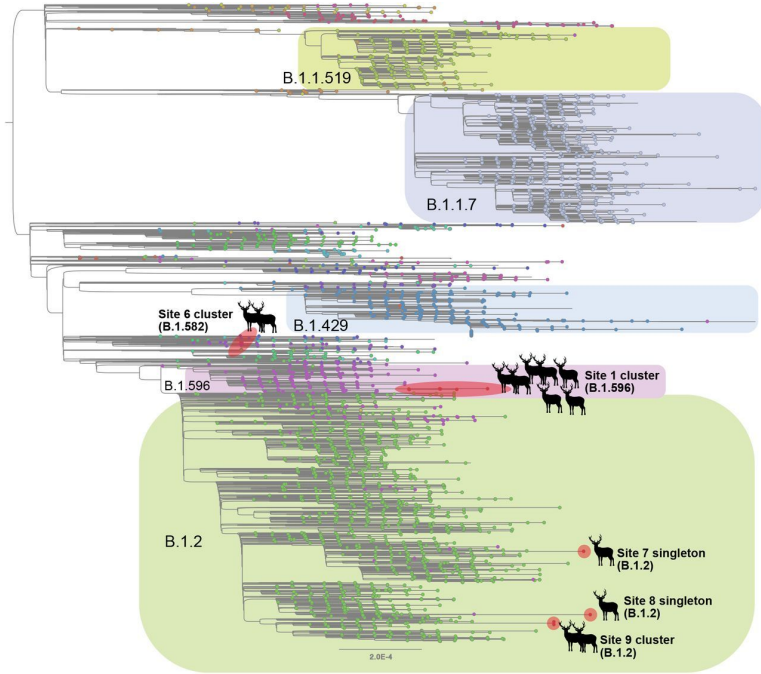
10.1126/science.abe5901

Omicron may have originated in animal reservoirs

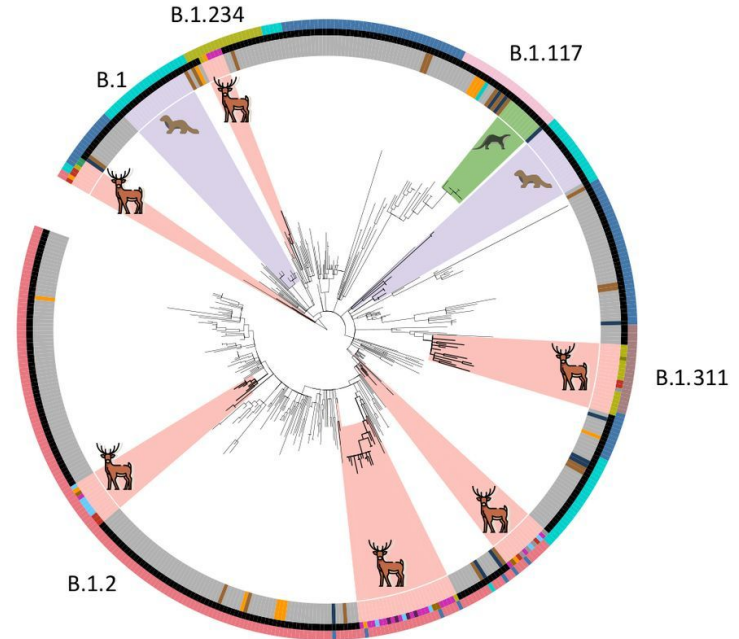


Why are White-Tailed Deer a priority?

White-Tailed Deer are susceptible

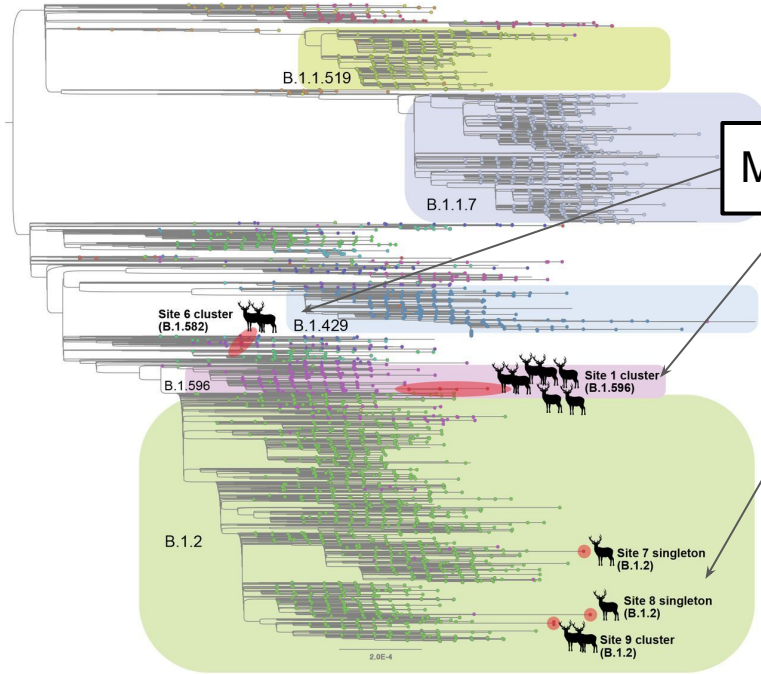


(Hale, 2021) 10.1101/2021.11.04.467308

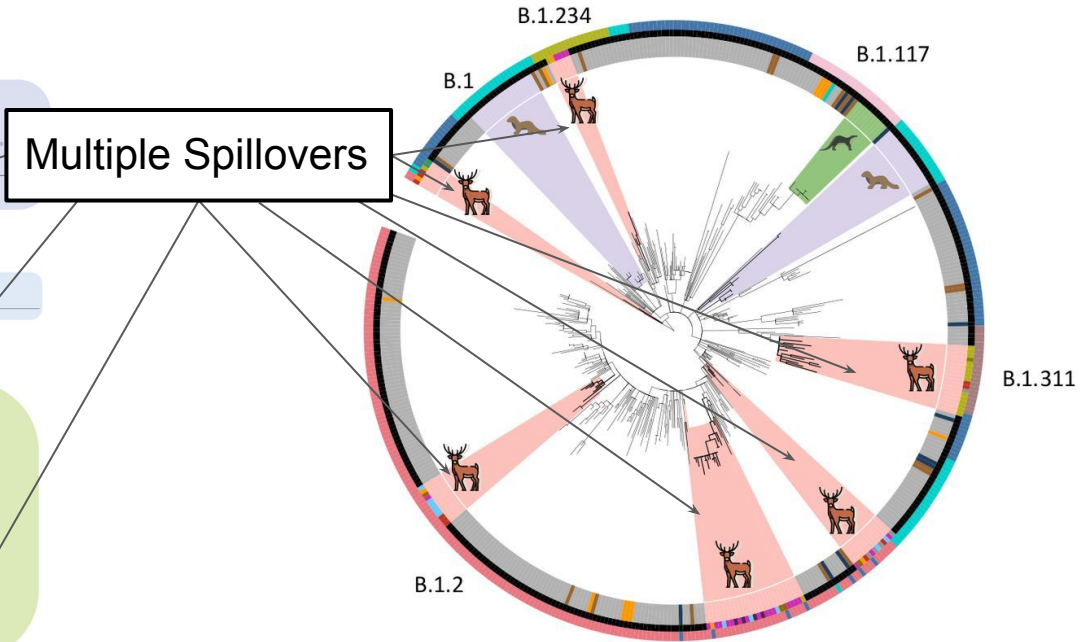


(Kuchipudi, 2021) 10.1101/2021.10.31.466677

White-Tailed Deer are susceptible

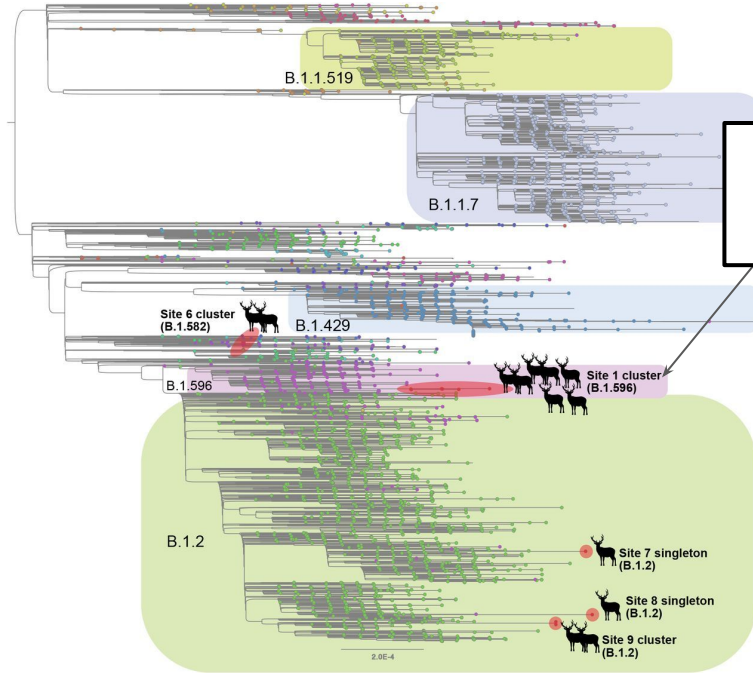


(Hale, 2021) 10.1101/2021.11.04.467308



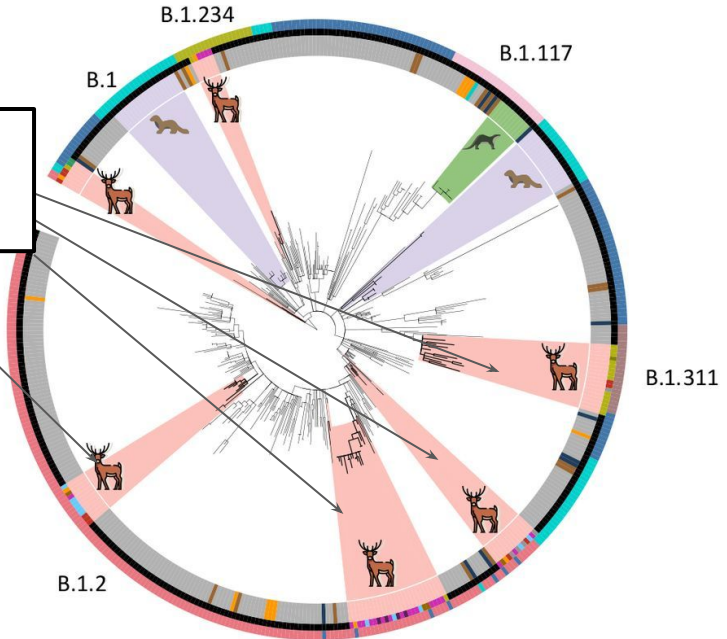
(Kuchipudi, 2021) 10.1101/2021.10.31.466677

White-Tailed Deer are susceptible



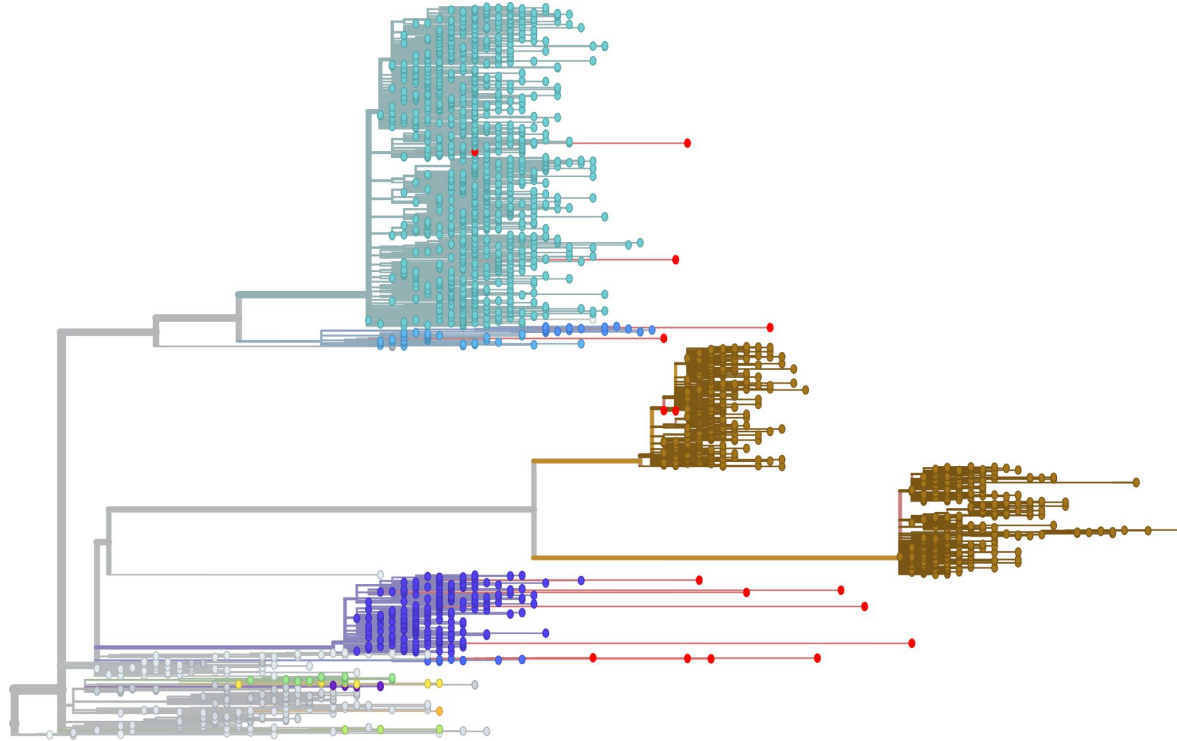
(Hale, 2021) 10.1101/2021.11.04.467308

Deer to Deer
Transmission

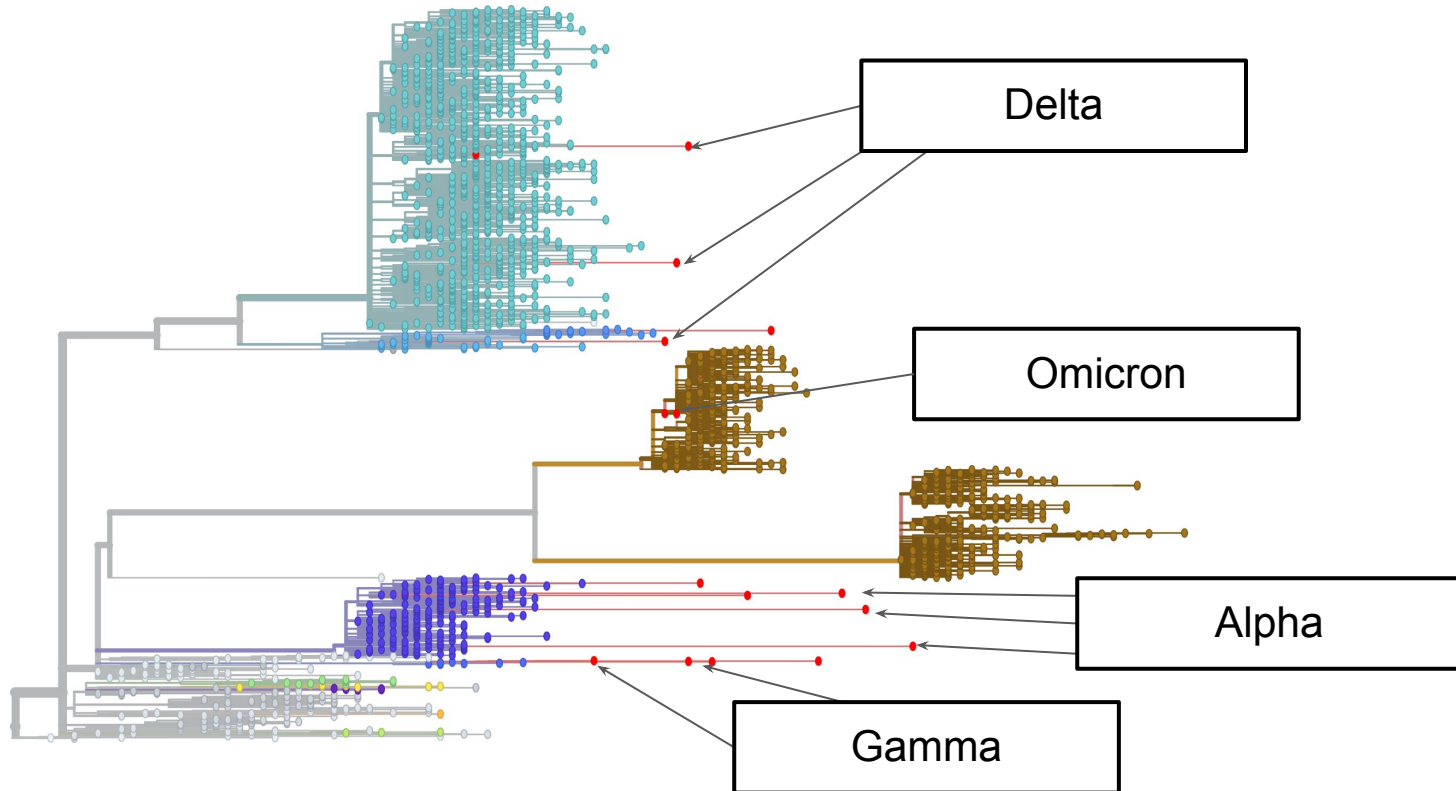


(Kuchipudi, 2021) 10.1101/2021.10.31.466677

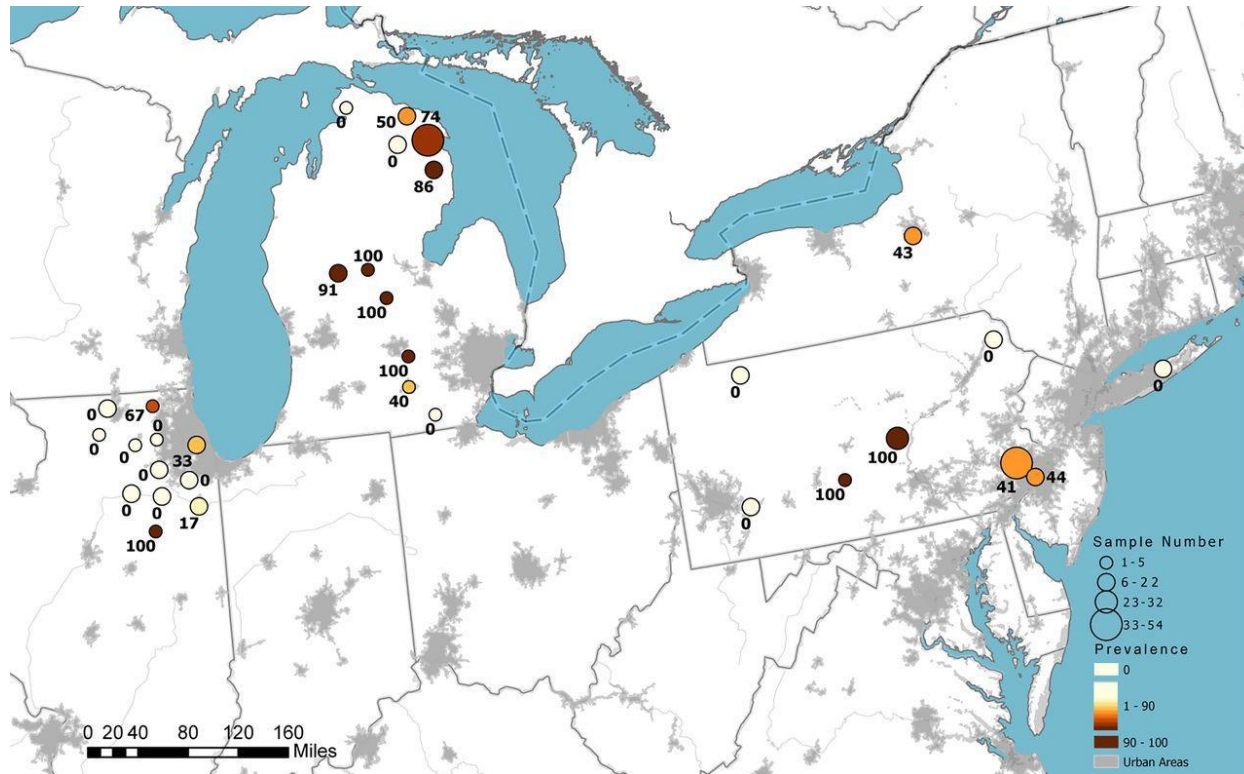
Spillover into White-Tailed Deer includes VOCs



Spillover into White-Tailed Deer includes VOCs

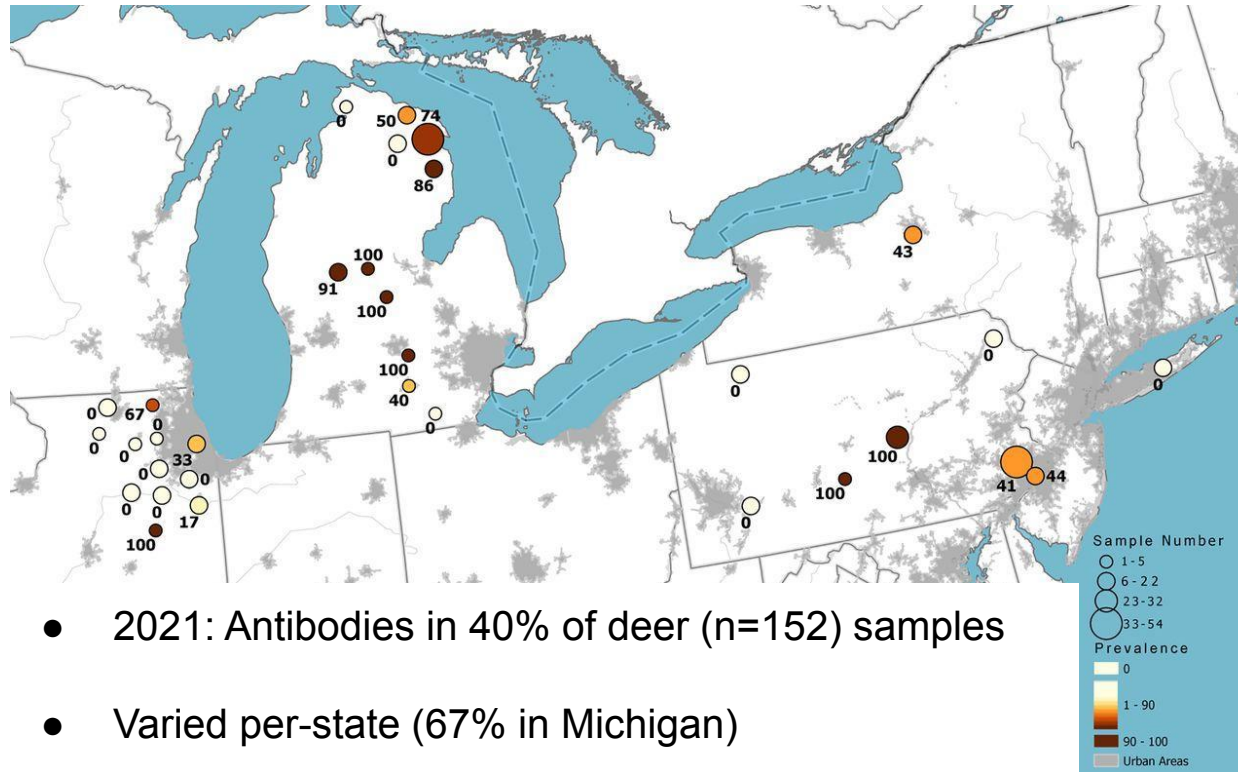


Serology indicates prevalent exposure



(Chandler, 2021) 10.1073/pnas.2114828118

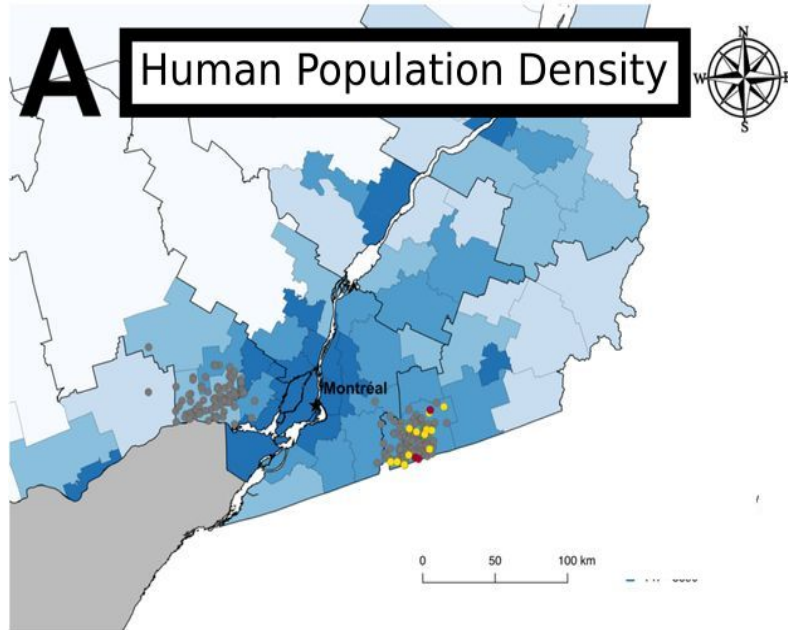
Serology indicates prevalent exposure



- 2021: Antibodies in 40% of deer (n=152) samples
- Varied per-state (67% in Michigan)

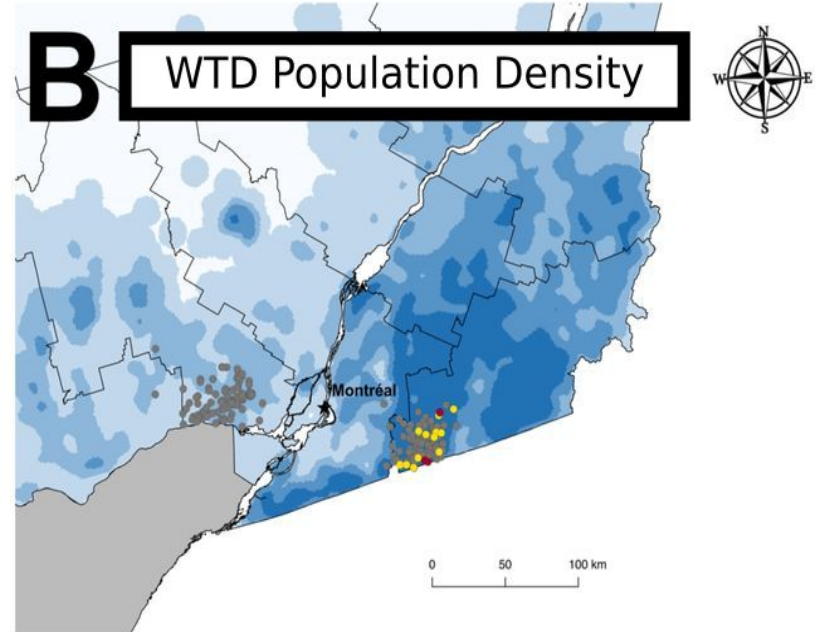
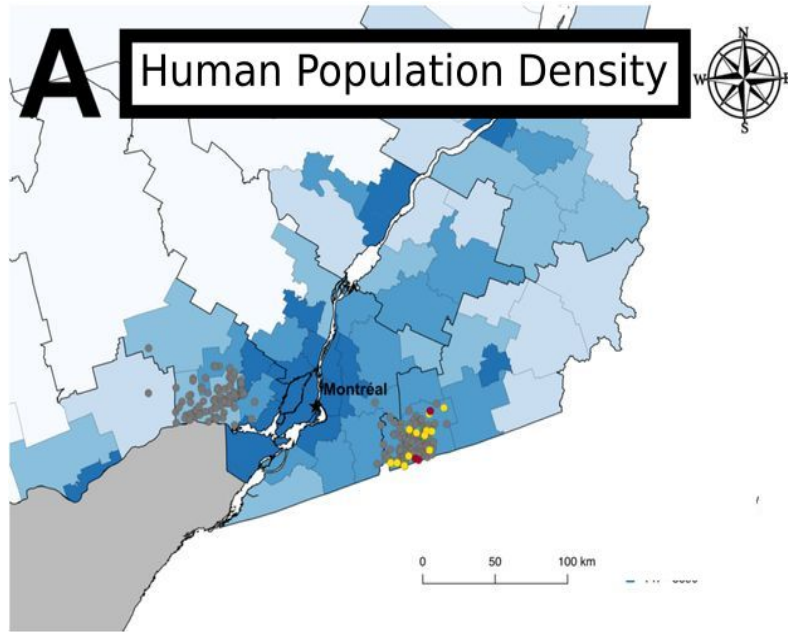
(Chandler, 2021) 10.1073/pnas.2114828118

White-Tailed Deer: large/mobile populations near humans



(Kotwa, 2022) 10.1101/2022.01.20.476458

White-Tailed Deer: large/mobile populations near humans



How can we monitor SARS-CoV-2 evolution
in White-Tailed Deer?

Use pre-existing surveillance programs for WTD

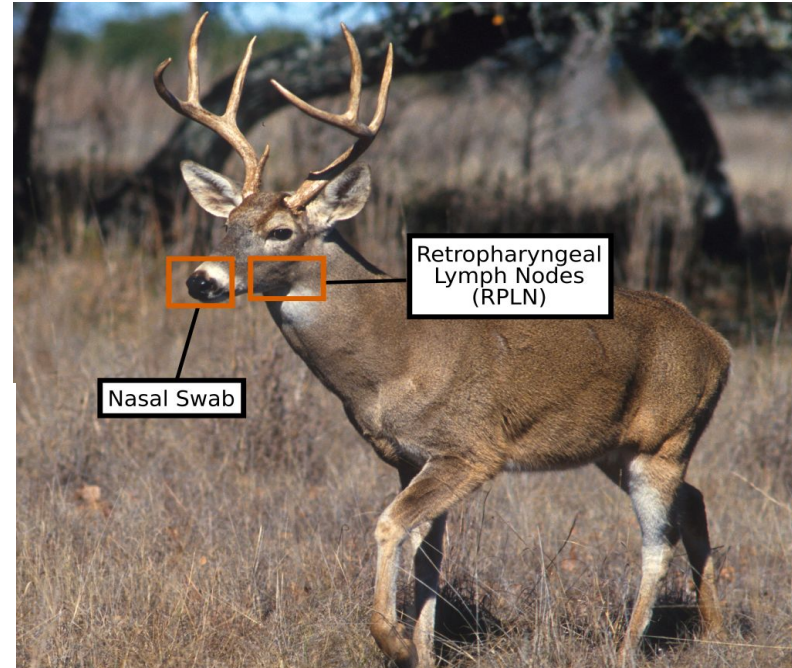
- SARS-CoV-2 sampling added to Chronic Wasting Disease surveillance program (Nov 1st - Dec 31st 2021)



USDA photo by Scott Bauer - Image Number: K5437-3

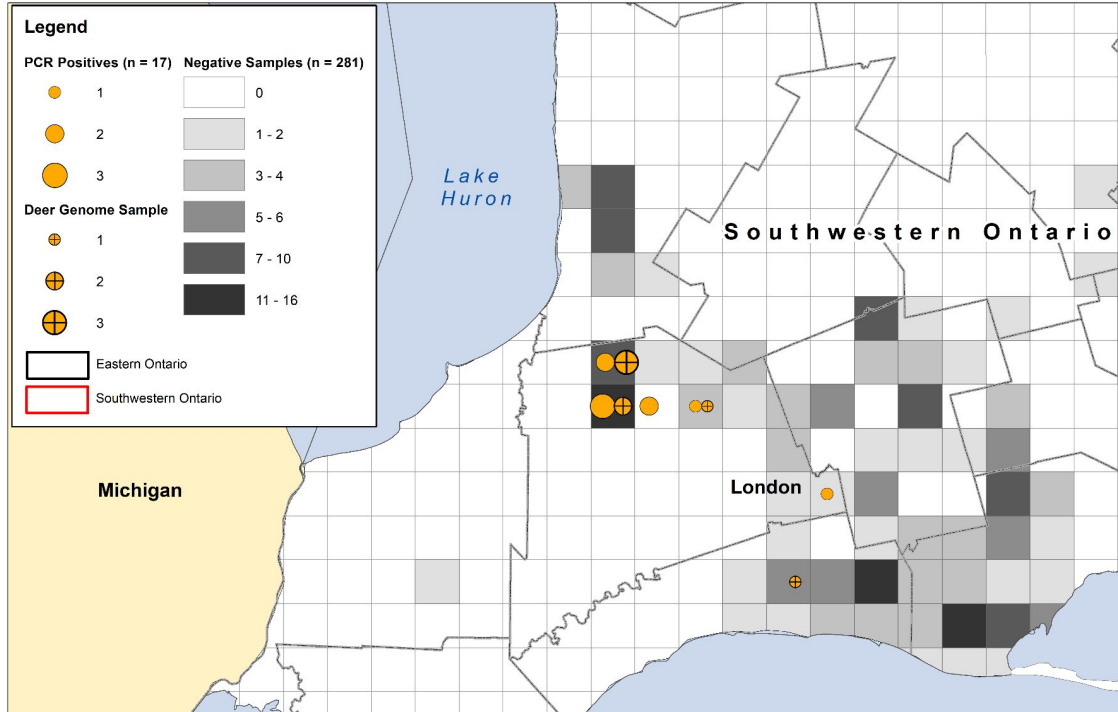
Use pre-existing surveillance programs for WTD

- SARS-CoV-2 sampling added to Chronic Wasting Disease surveillance program (Nov 1st - Dec 31st 2021)
- Nasal swabs and RPLN samples from hunter-harvested carcasses



USDA photo by Scott Bauer - Image Number: K5437-3

17/298 White-Tailed Deer were positive for SARS-CoV-2

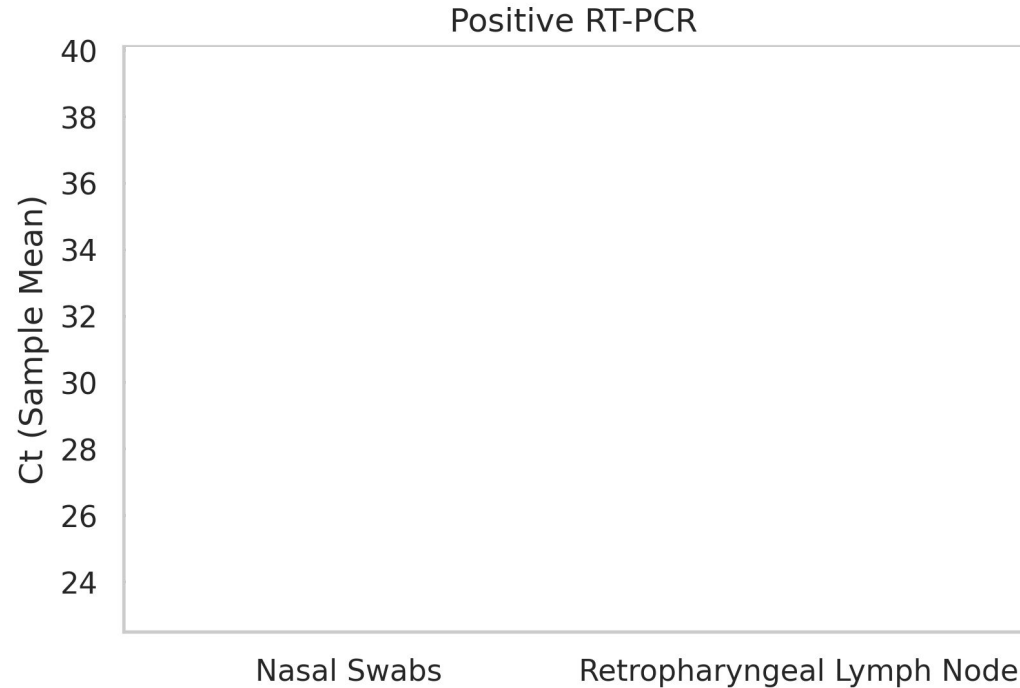


- Independent extraction qRT-PCR (UTR+E, N+E) at separate institutes.
- Viral sequencing attempted for confirmed positive samples.

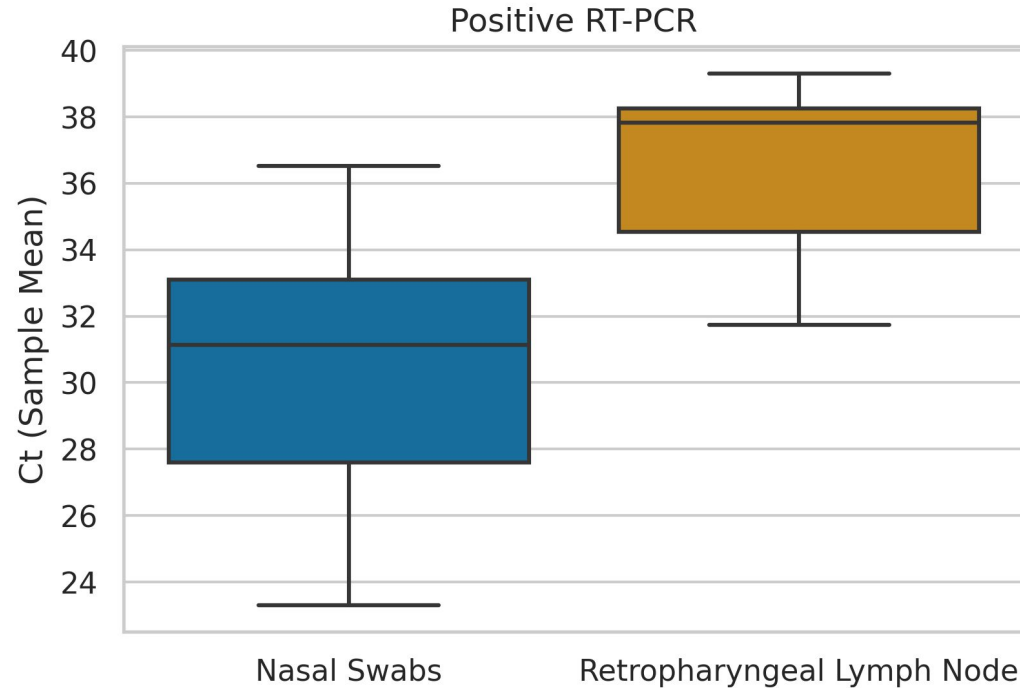
No positive samples from Eastern Ontario

Can we sequence genomes from these
positive samples?

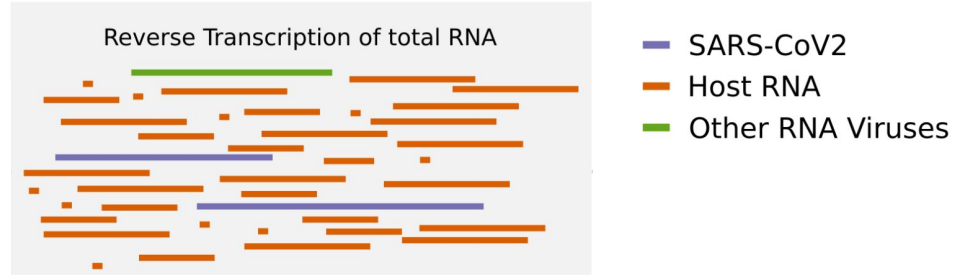
Sequencing challenging due to apparent low viral titre



Sequencing challenging due to apparent low viral titre



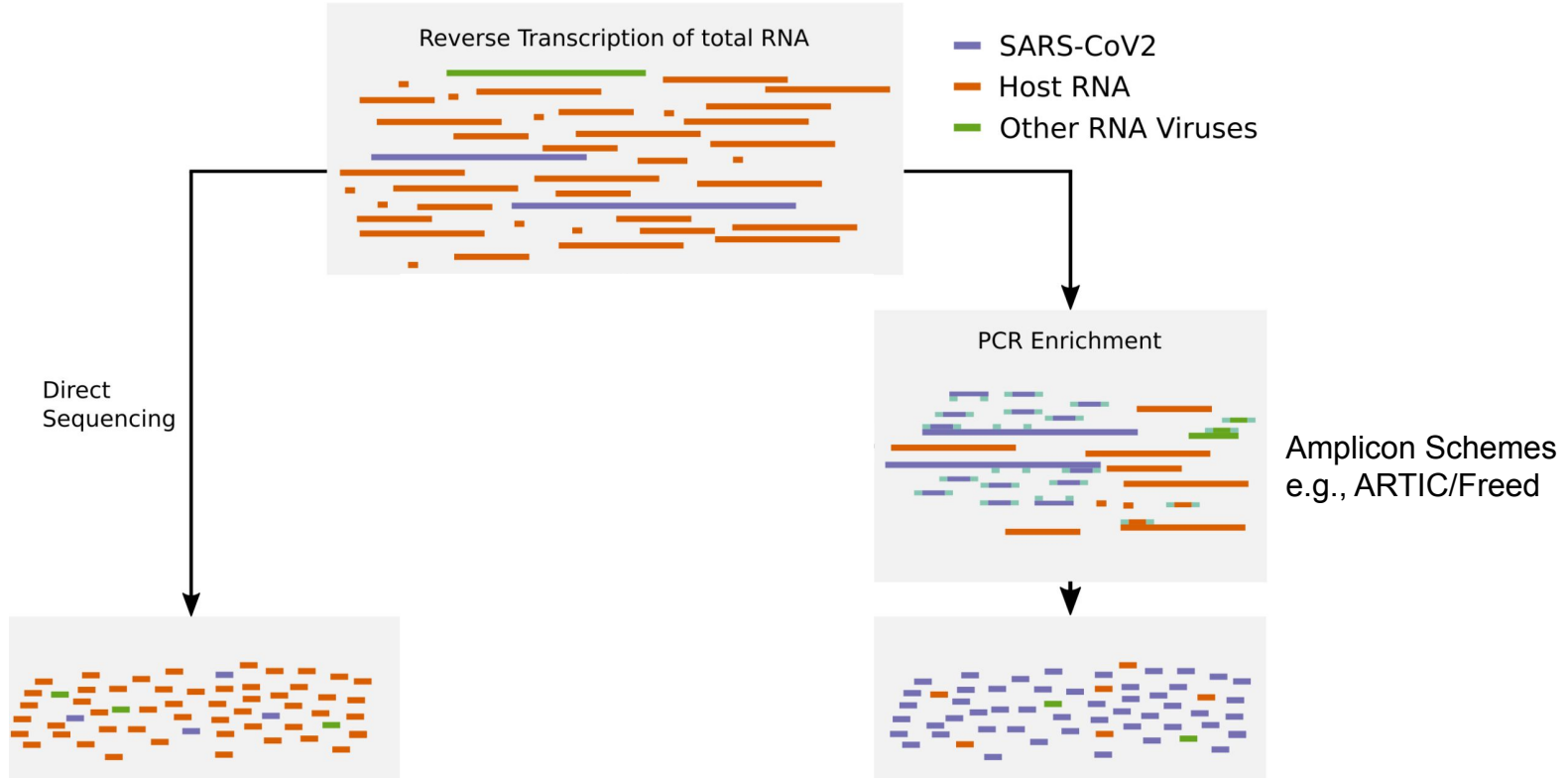
SARS-CoV-2 sequencing requires enrichment



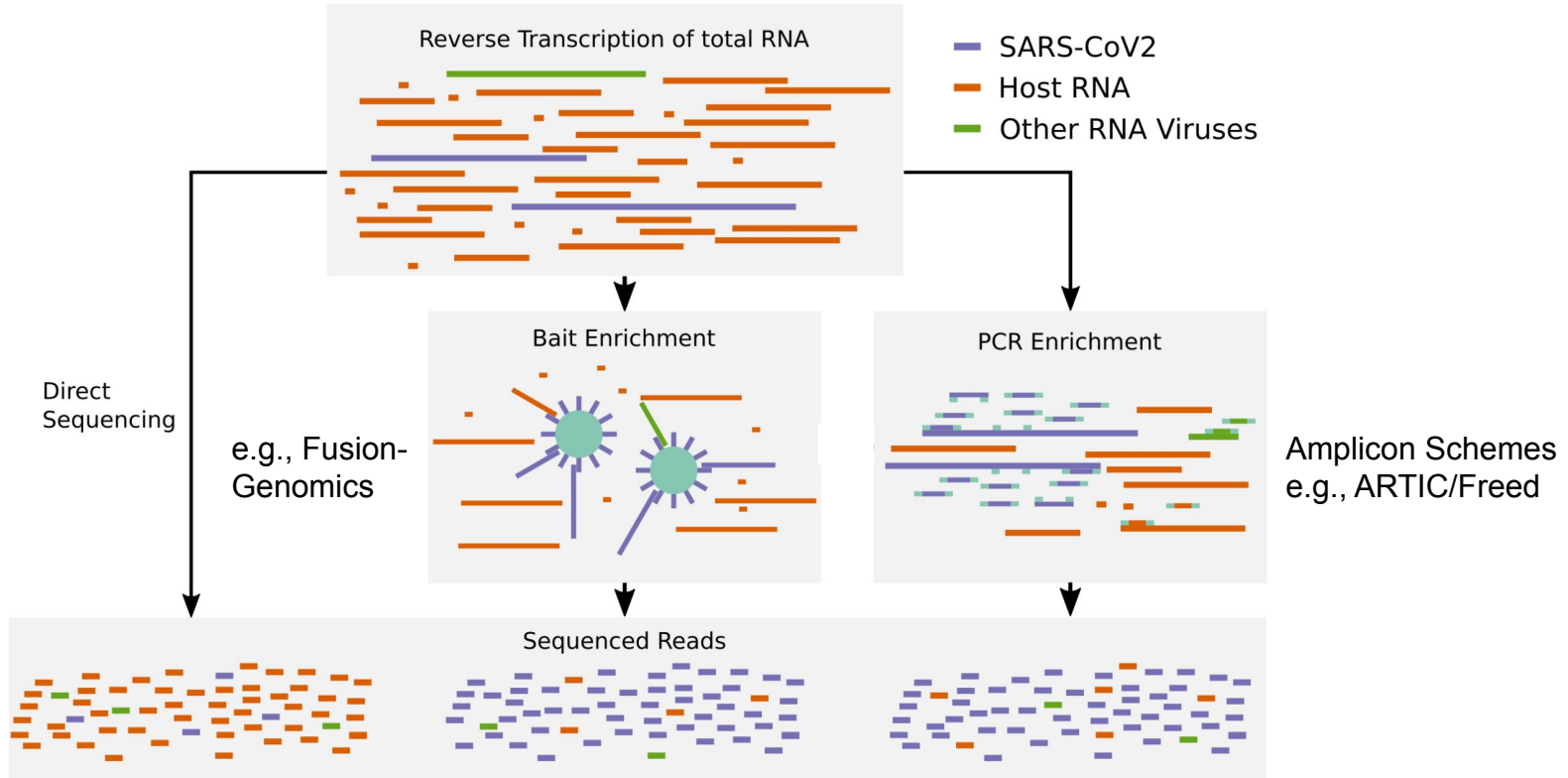
SARS-CoV-2 sequencing requires enrichment



SARS-CoV-2 sequencing requires enrichment



SARS-CoV-2 sequencing requires enrichment



Combining enrichment methods to maximise coverage

ARTICv4 – Illumina (SRI)

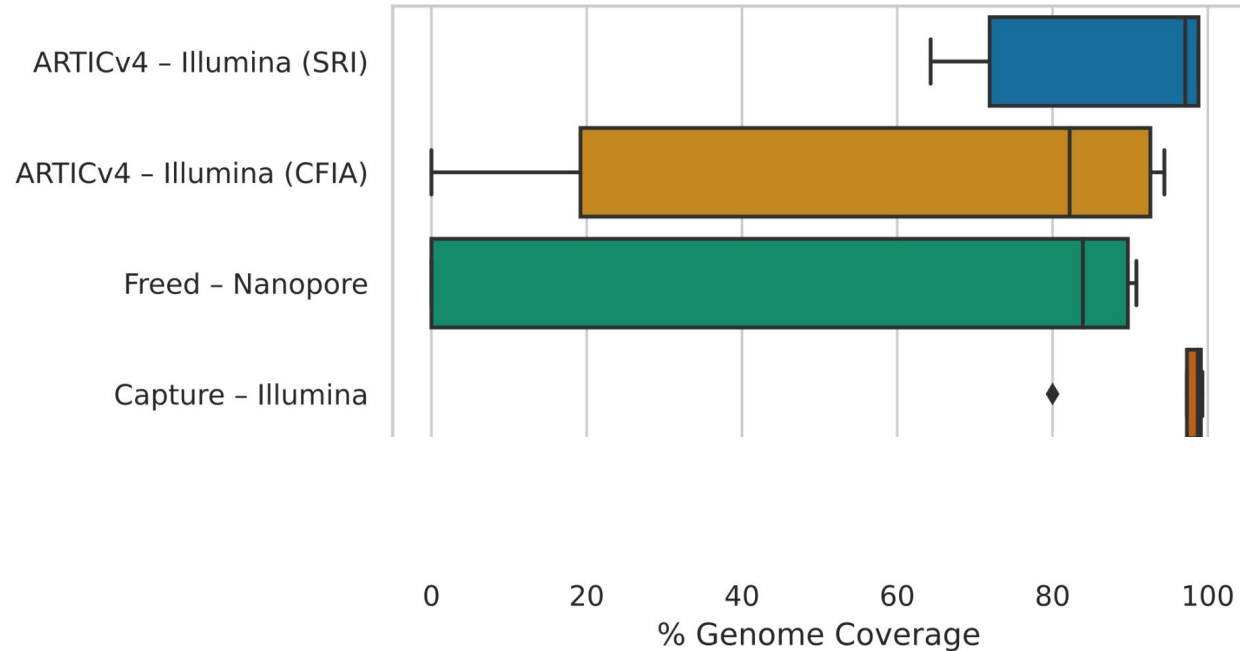
ARTICv4 – Illumina (CFIA)

Freed – Nanopore

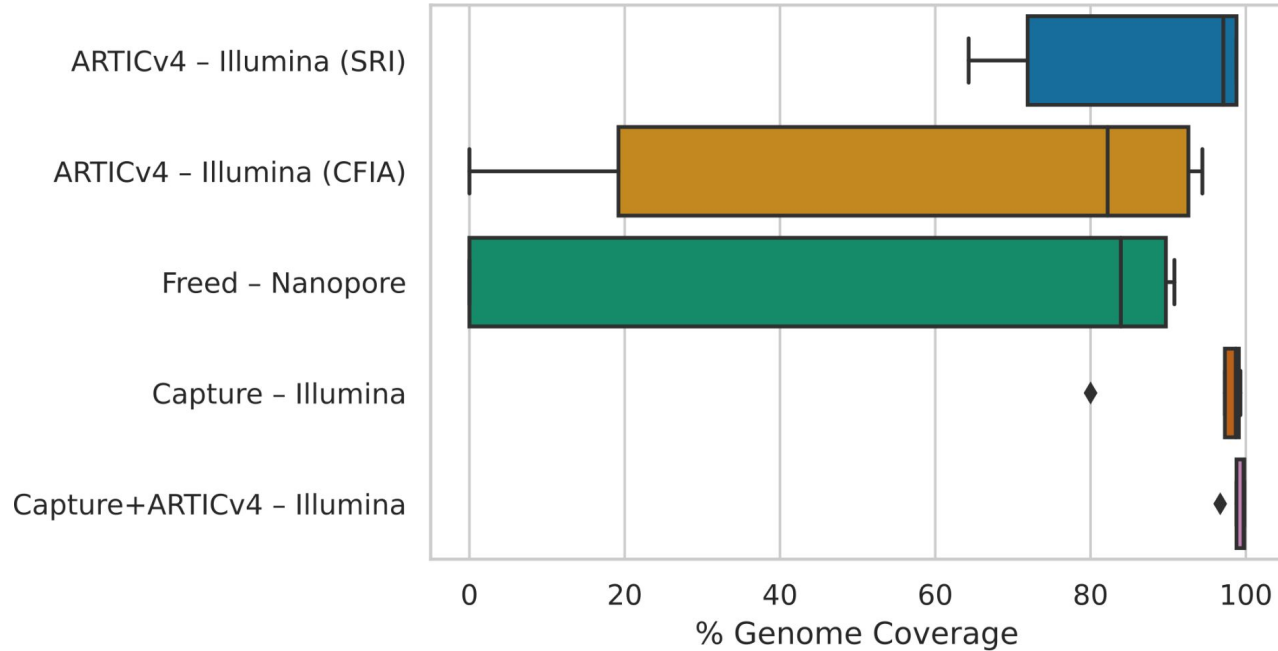
Capture – Illumina

0 20 40 60 80 100
% Genome Coverage

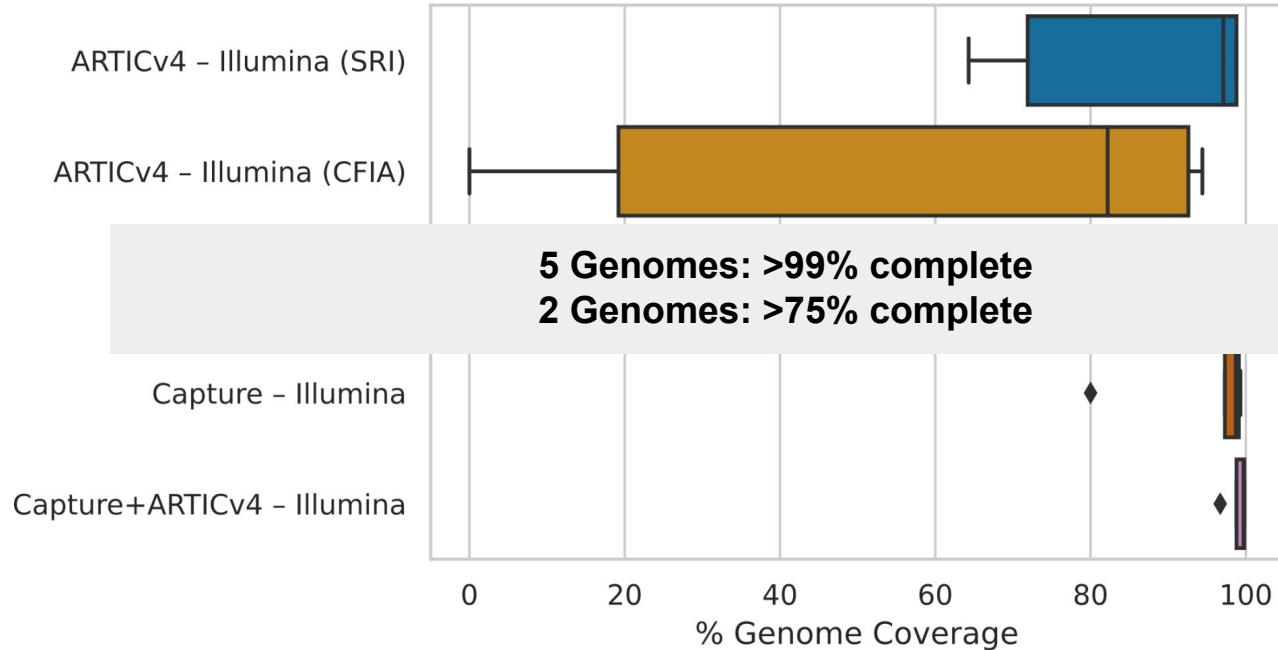
Combining enrichment methods to maximise coverage



Combining enrichment methods to maximise coverage



Combining enrichment methods to maximise coverage



Are these viruses actually from deer or just human contamination?

Ruling out contamination from human SARS-CoV-2

- Independent RNA extractions + qRT-PCR
- Independent sequencing efforts

Ruling out contamination from human SARS-CoV-2

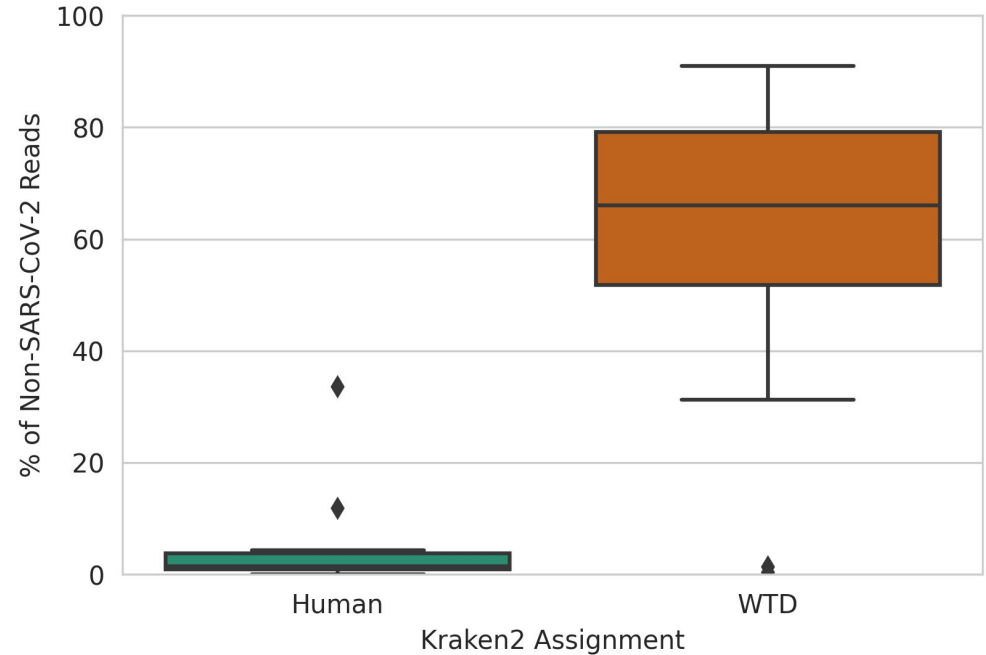
- Independent RNA extractions + qRT-PCR
- Independent sequencing efforts
- Two sample sites: RPLN and Nasal swabs

Ruling out contamination from human SARS-CoV-2

- Independent RNA extractions + qRT-PCR
- Independent sequencing efforts
- Two sample sites: RPLN and Nasal swabs
- Negative Human RNAase PCR

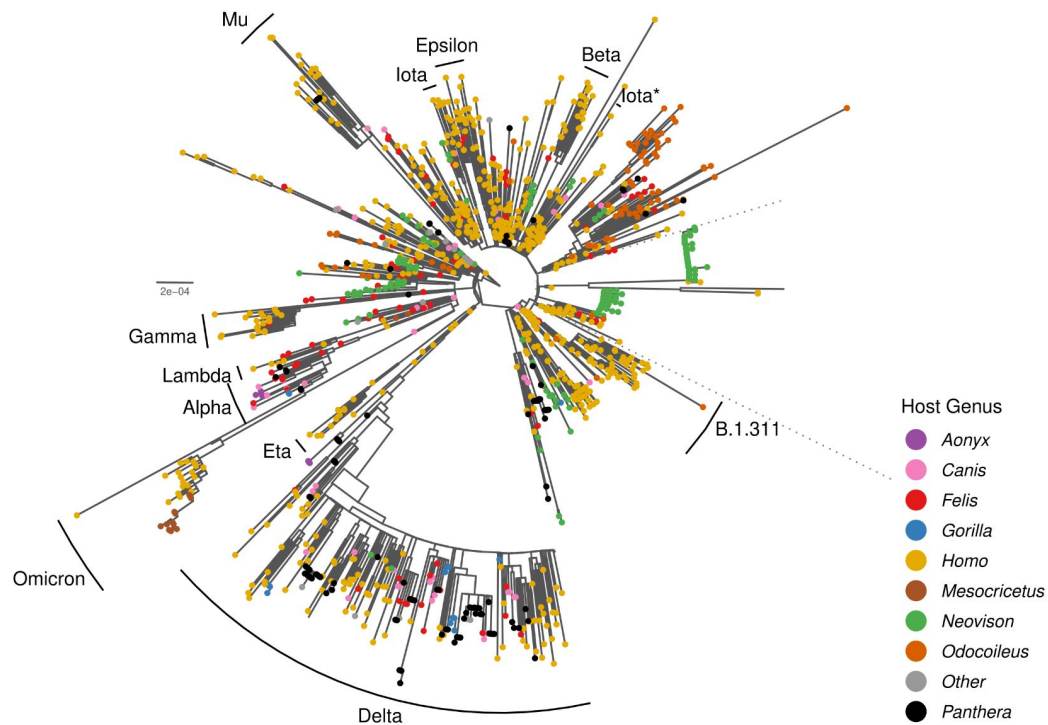
Ruling out contamination from human SARS-CoV-2

- Independent RNA extractions + qRT-PCR
- Independent sequencing efforts
- Two sample sites: RPLN and Nasal swabs
- Negative Human RNAase PCR
- Taxonomic read profiling

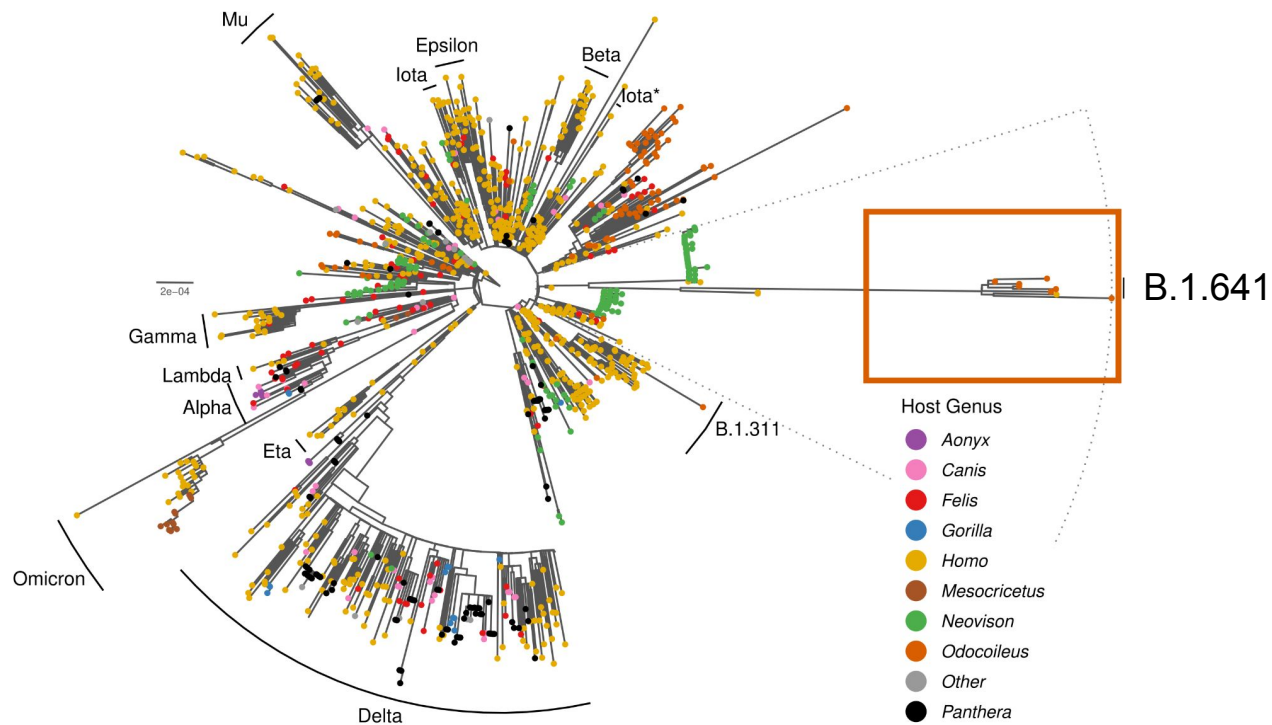


Another recent spillover from local human infections?

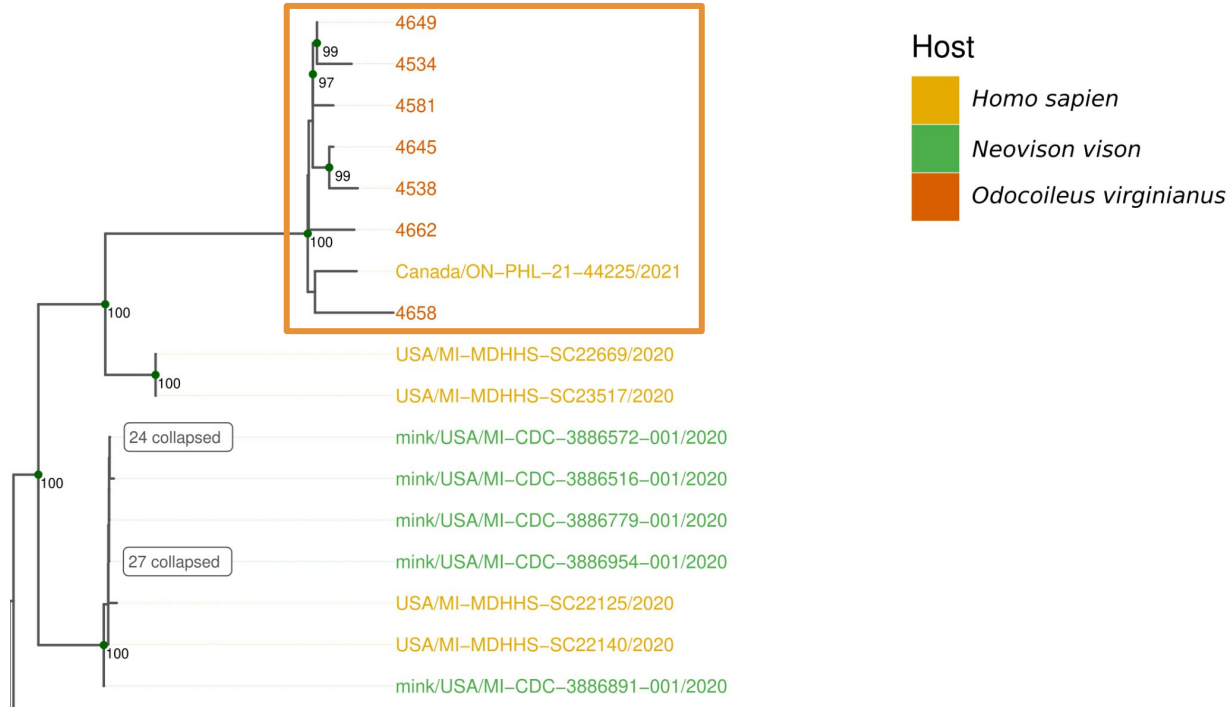
Highly Divergent B.1 Lineage



Highly Divergent B.1 Lineage



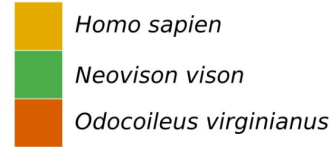
Related to 2020 Michigan Human & Mink



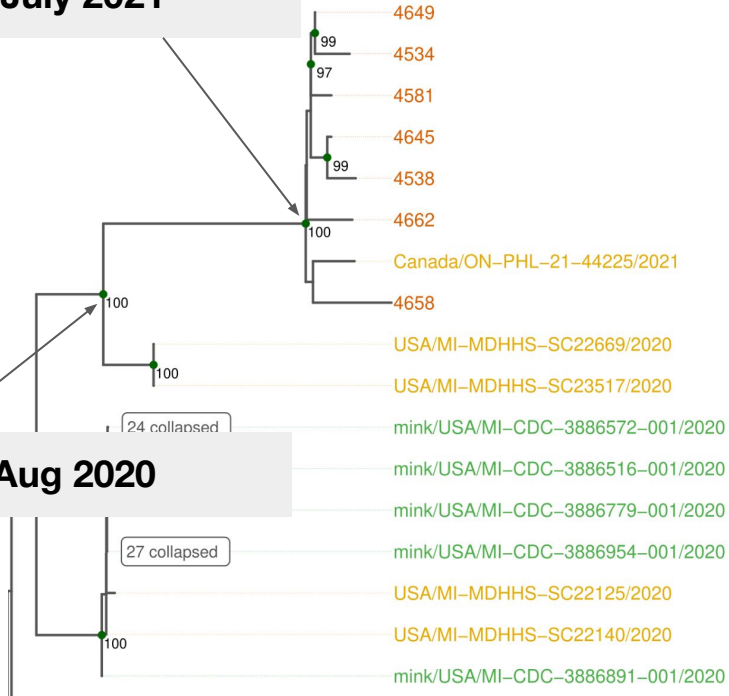
Node dates indicate considerable cryptic transmission

May-July 2021

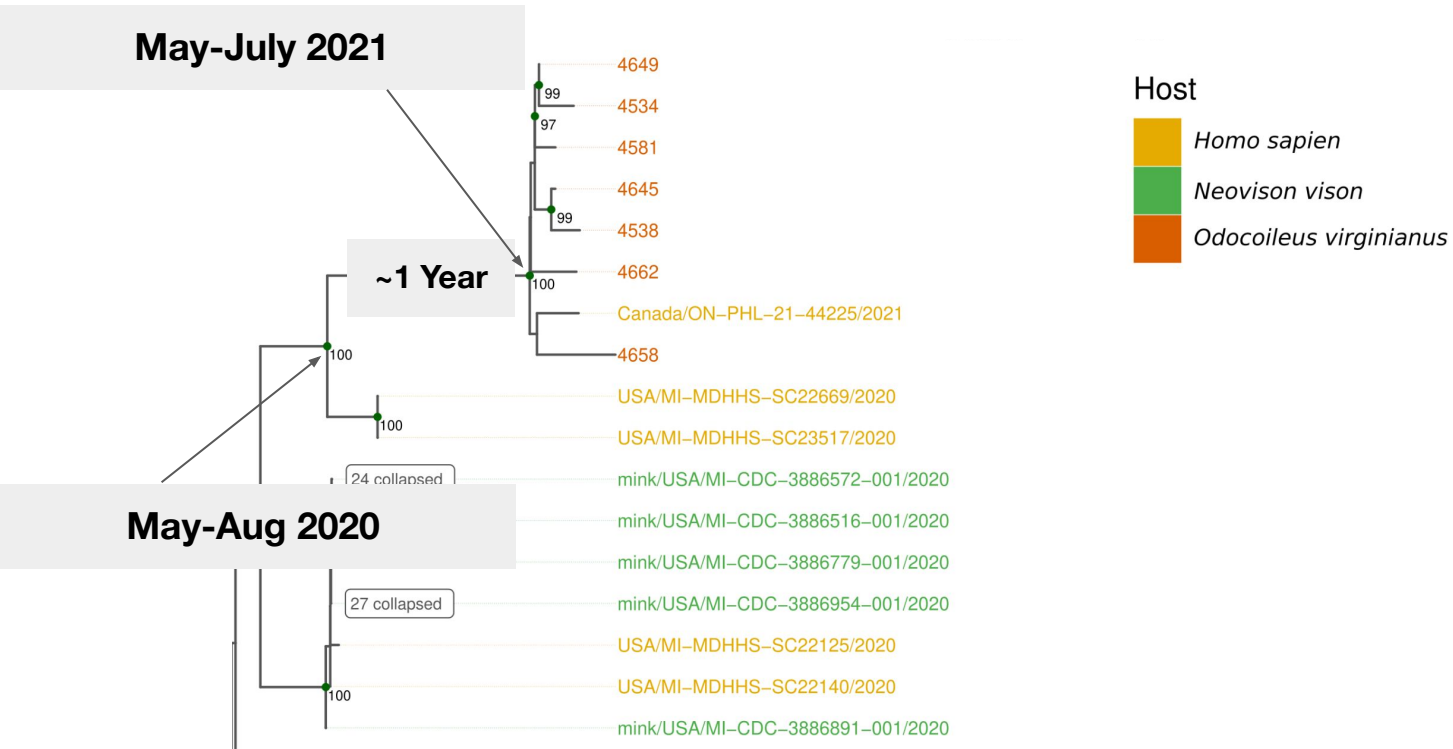
Host



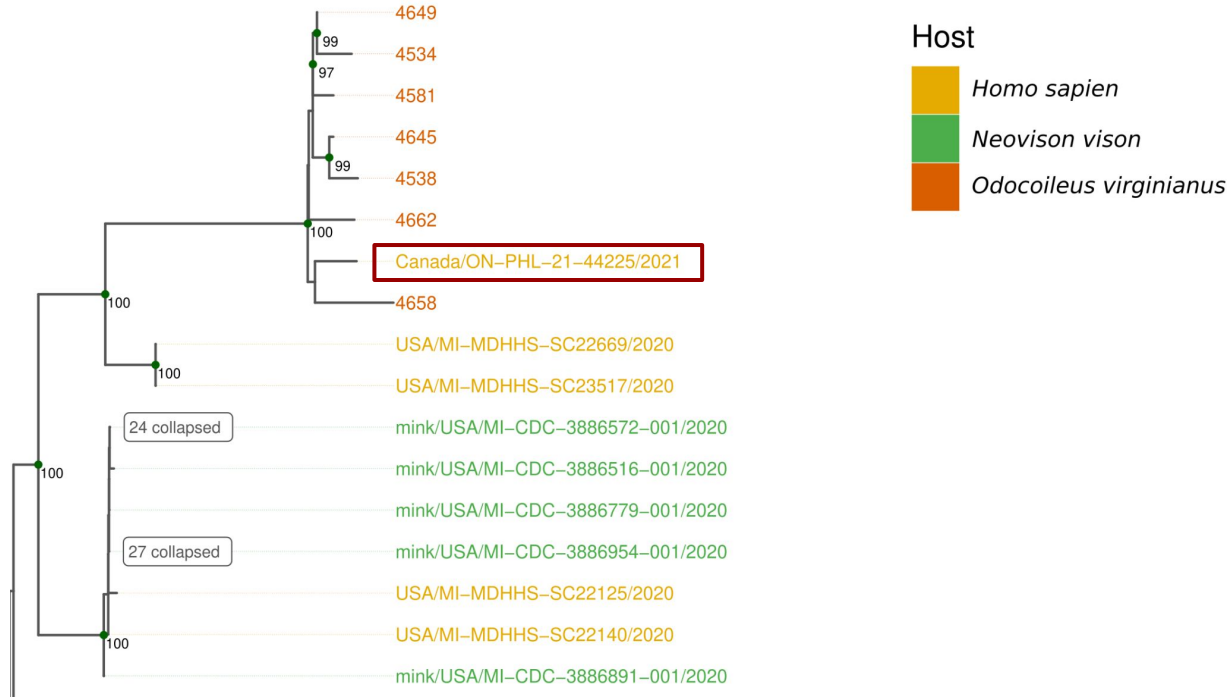
May-Aug 2020



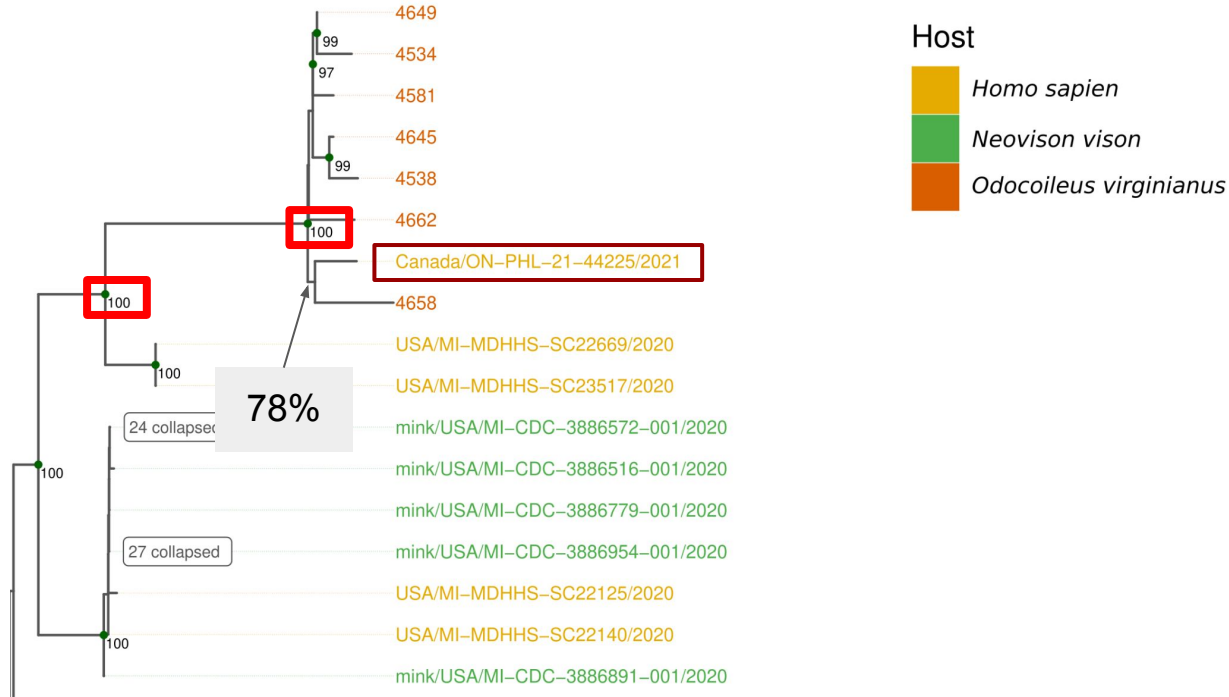
Node dates indicate considerable cryptic transmission



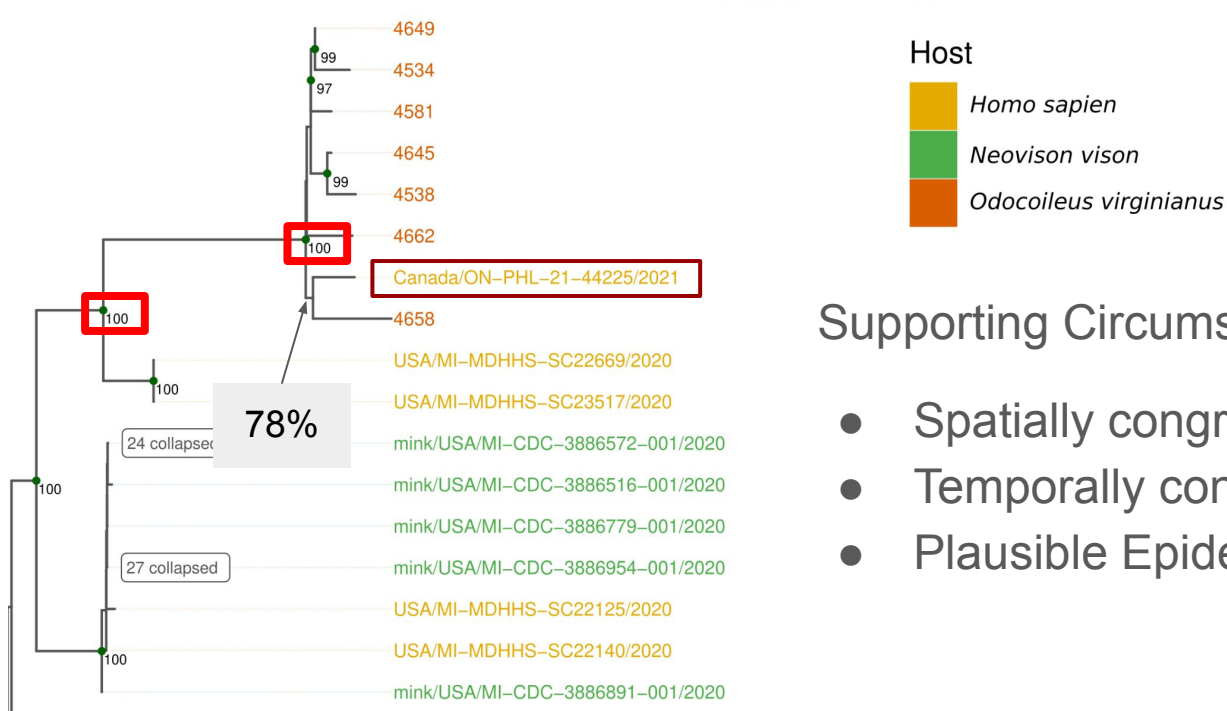
Possible Deer-to-Human transmission



Possible Deer-to-Human transmission



Possible Deer-to-Human transmission



Supporting Circumstantial Evidence:

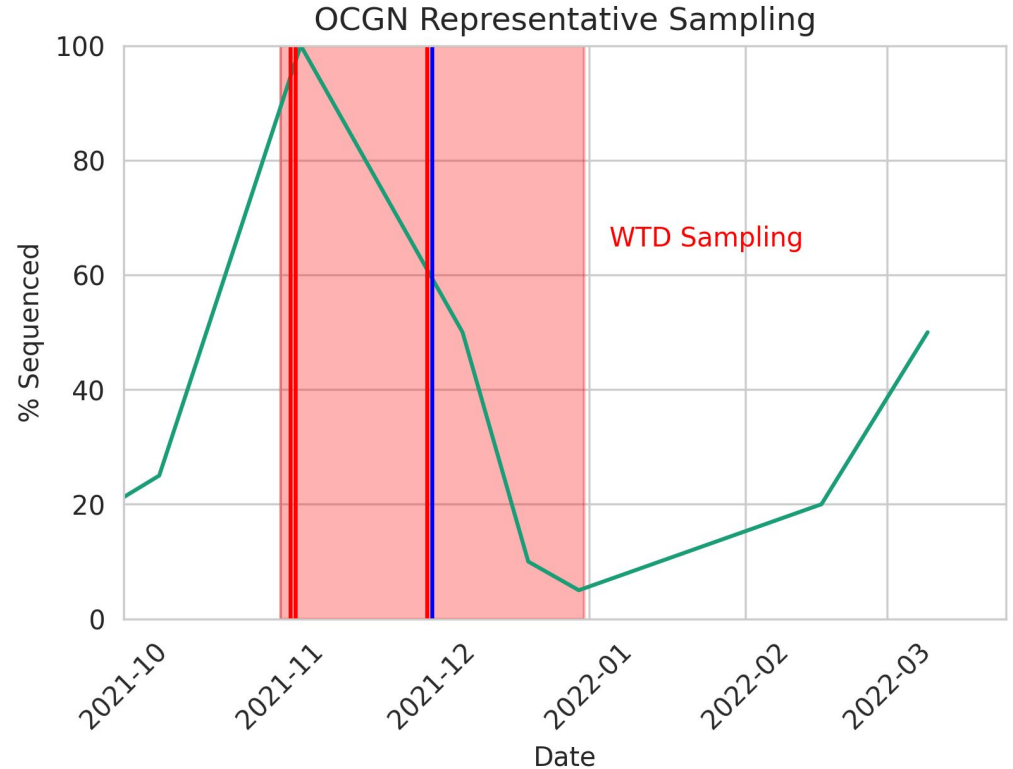
- Spatially congruent
- Temporally congruent
- Plausible Epidemiological Link

No sign of additional human cases

No sign of additional human cases

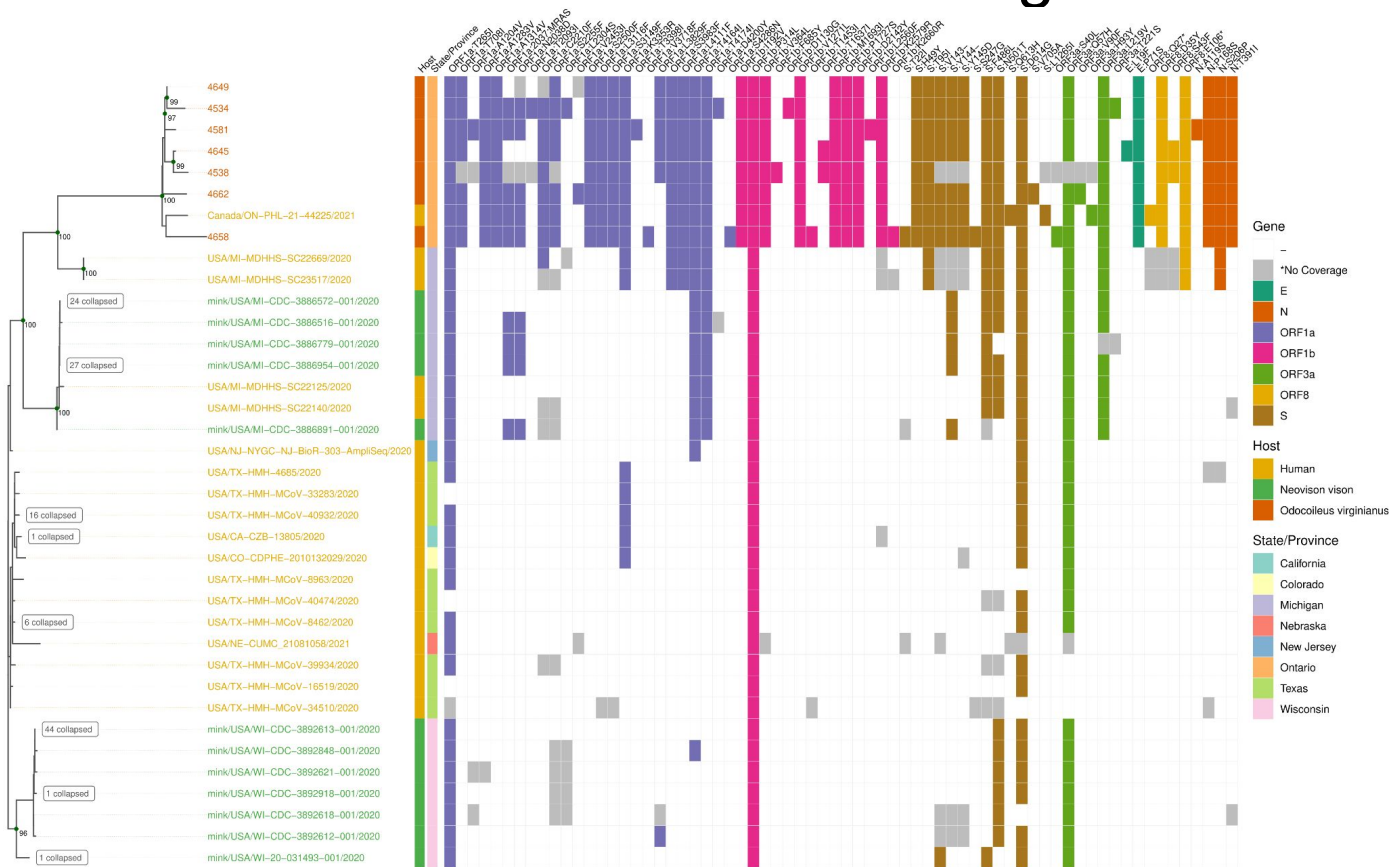
Caveats:

- Omicron induced drop in human surveillance levels
- End of deer hunting season/deer sampling

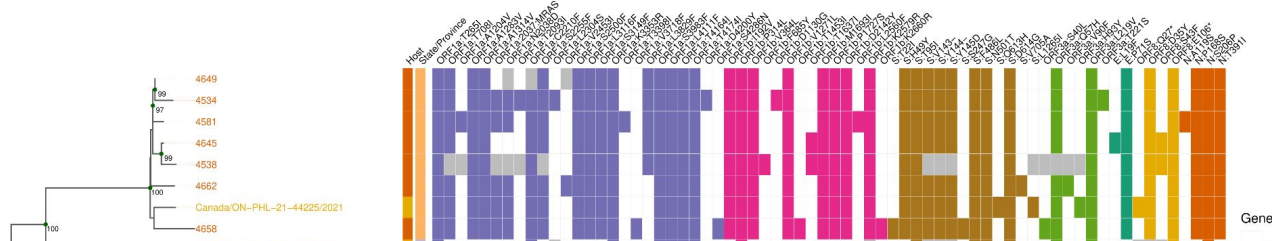


What mutations did we see?

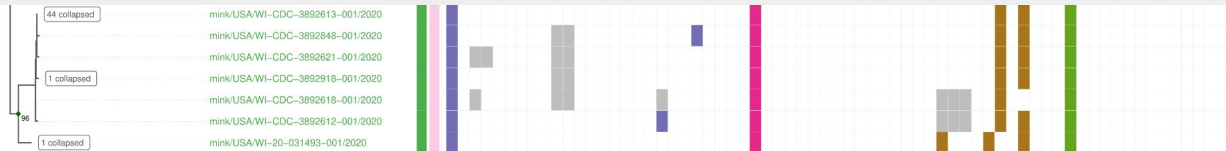
76 Mutations: 49 since MRCA with Michigan Human



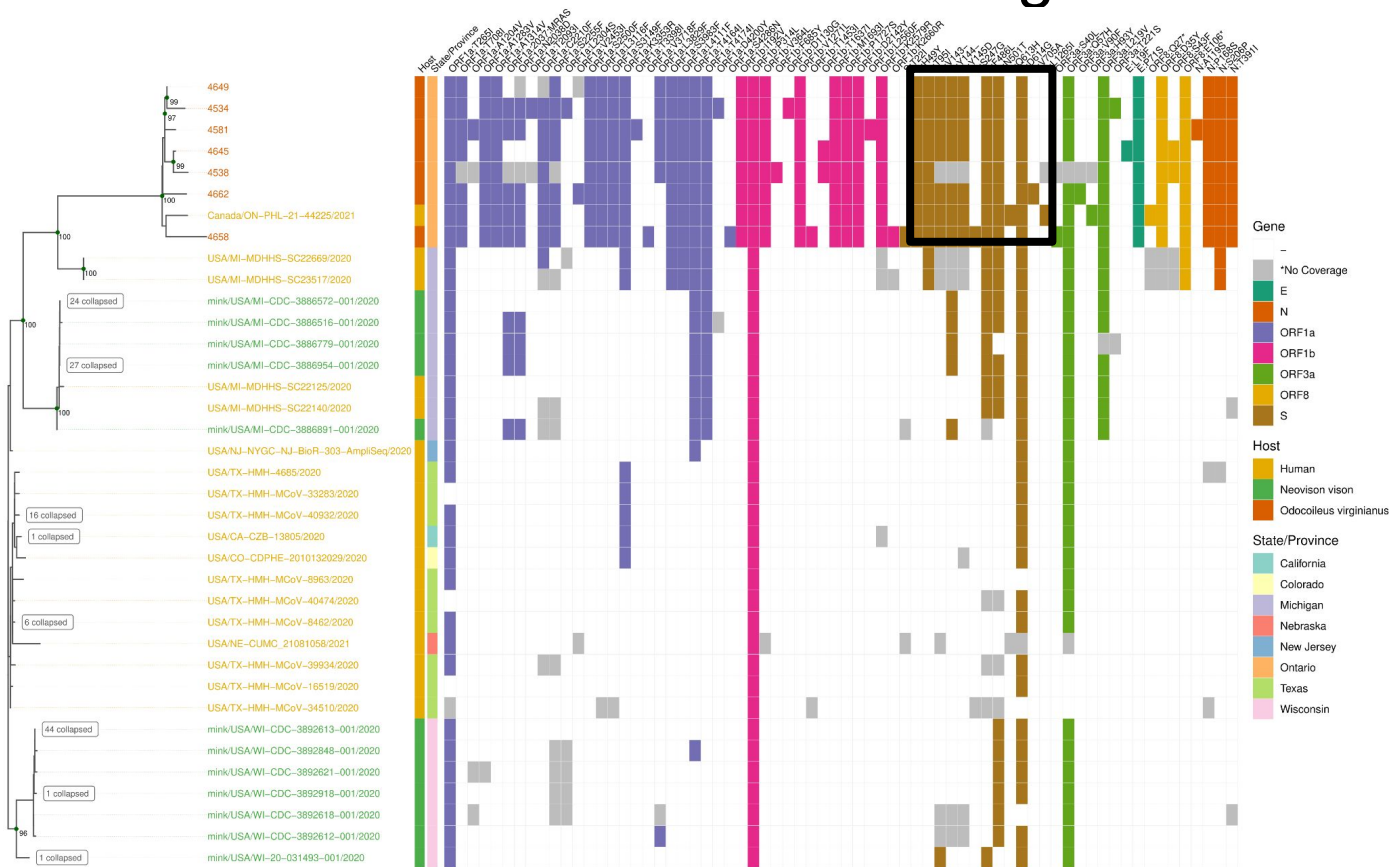
76 Mutations: 49 since MRCA with Michigan Human



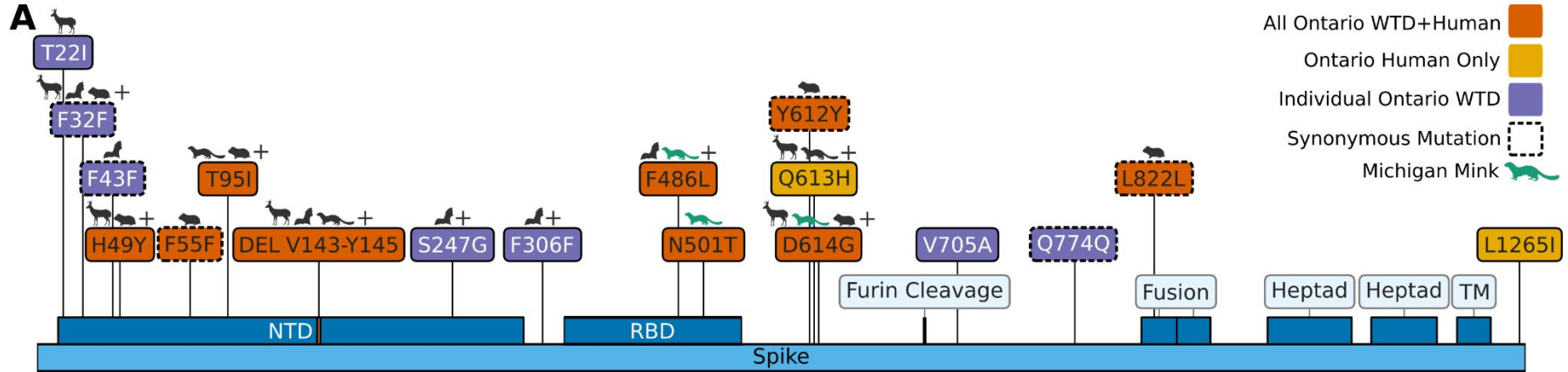
- 38 Synonymous Mutations : 38 Non-synonymous Mutations
- Several rare mutations:
 - ORF1a:insertion2038N/MRASD (n=32, including 31 mink from Michigan, USA)
 - ORF1b:V373L (n=425, all human sequences)
 - S:F486L (n=439)
 - ORF3a:L219V (n=805)
 - ORF10:L37F (n=0)



76 Mutations: 49 since MRCA with Michigan Human



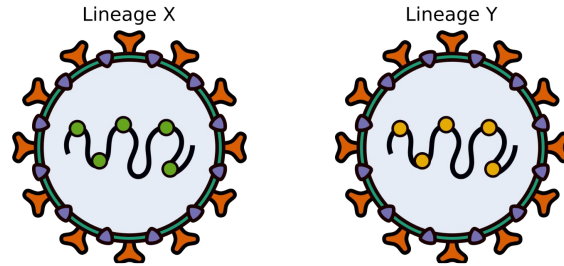
Mutations previously associated with animals



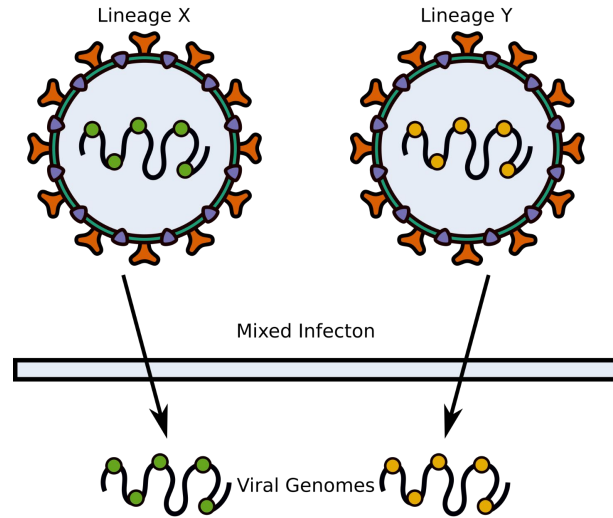
- 37/49 previously associated with non-human animal hosts
- 23 new to WTD

How did all this diversity evolve?

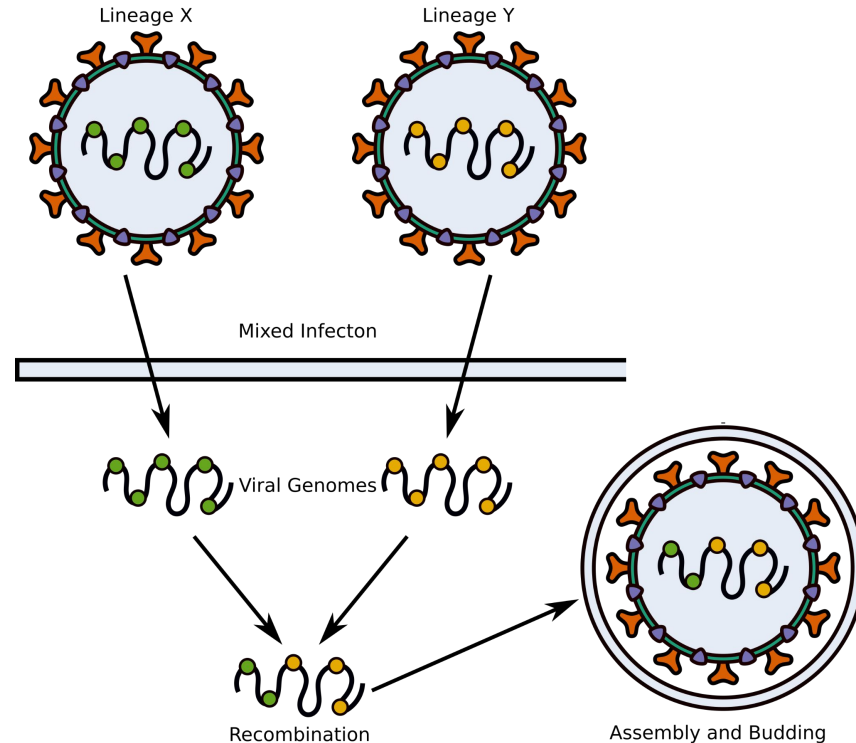
Recombination can lead to sudden novel diversity



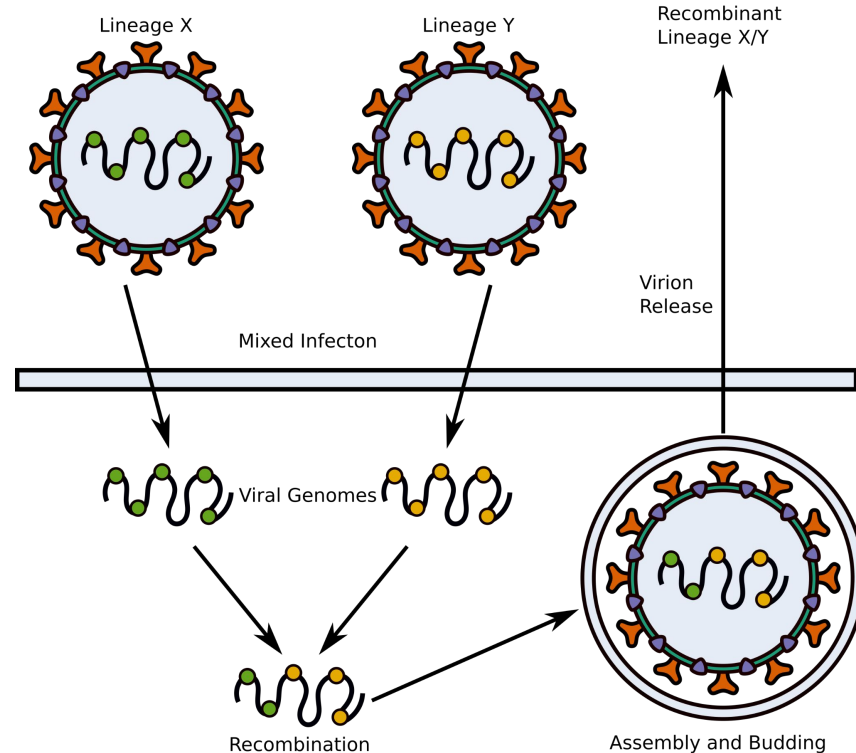
Recombination can lead to sudden novel diversity



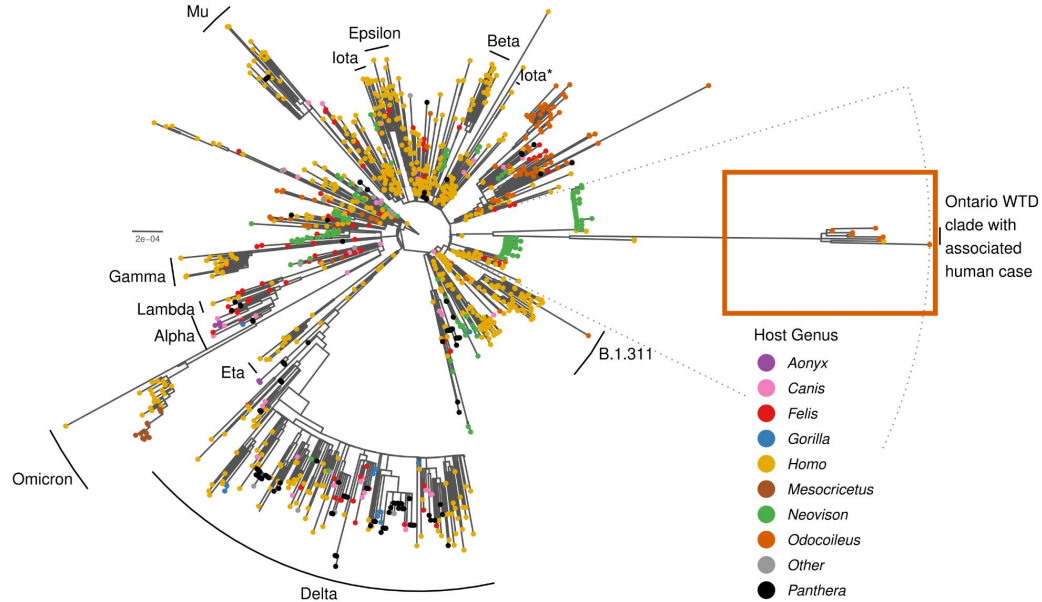
Recombination can lead to sudden novel diversity



Recombination can lead to sudden novel diversity

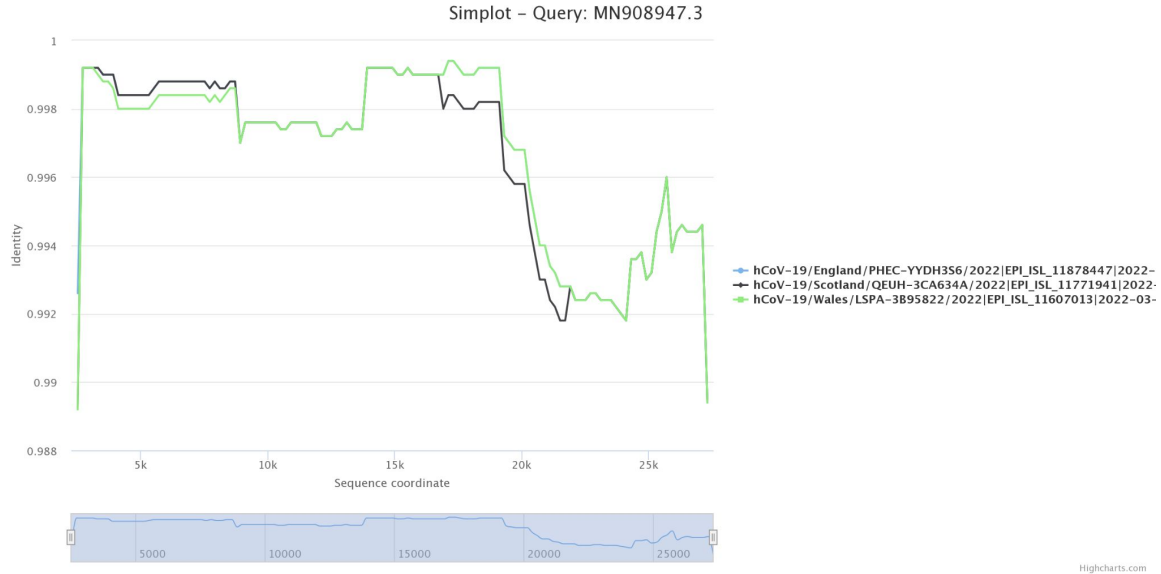


No indication of recombination



- SNP tables (**sc2rf**)

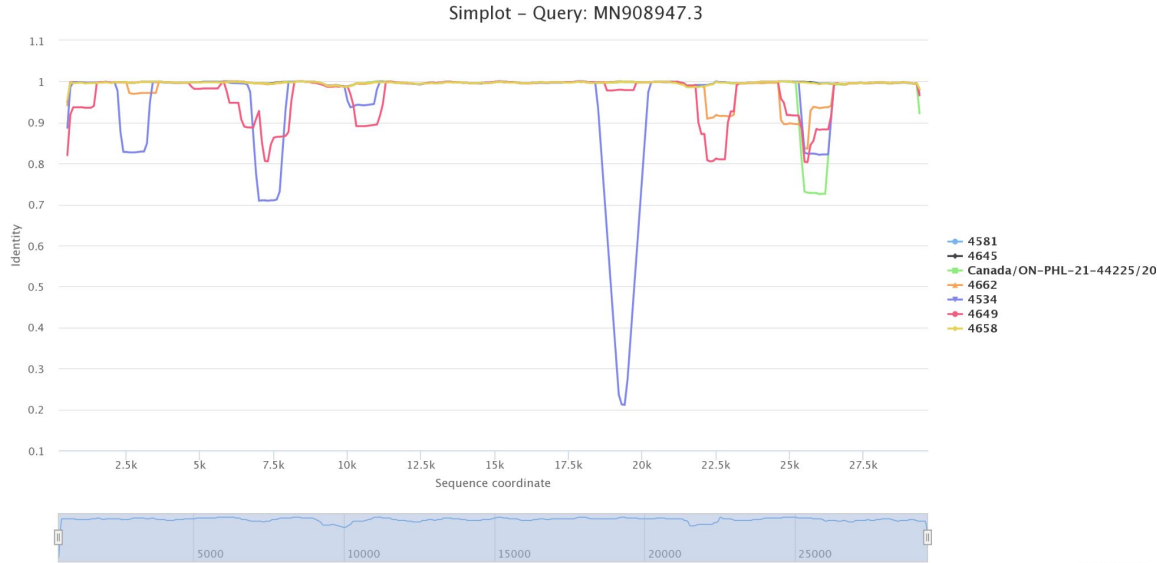
No indication of recombination



- SNP tables (**sc2rf**)

- Mosaicism (**3Seq**)

No indication of recombination

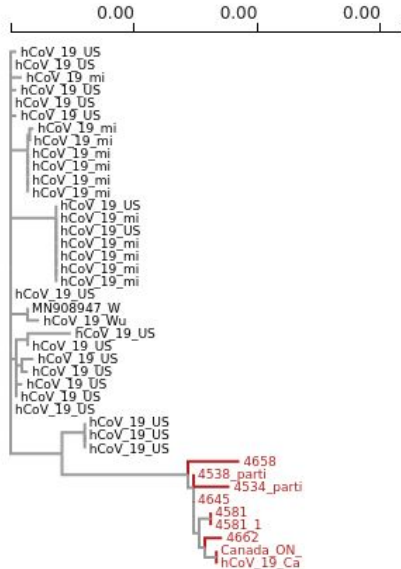


- SNP tables (**sc2rf**)

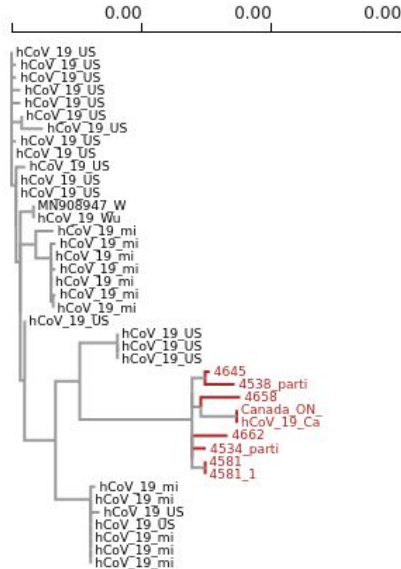
- Mosaicism (**3Seq**)

No indication of recombination

Tree 1, coordinate range 1-12731

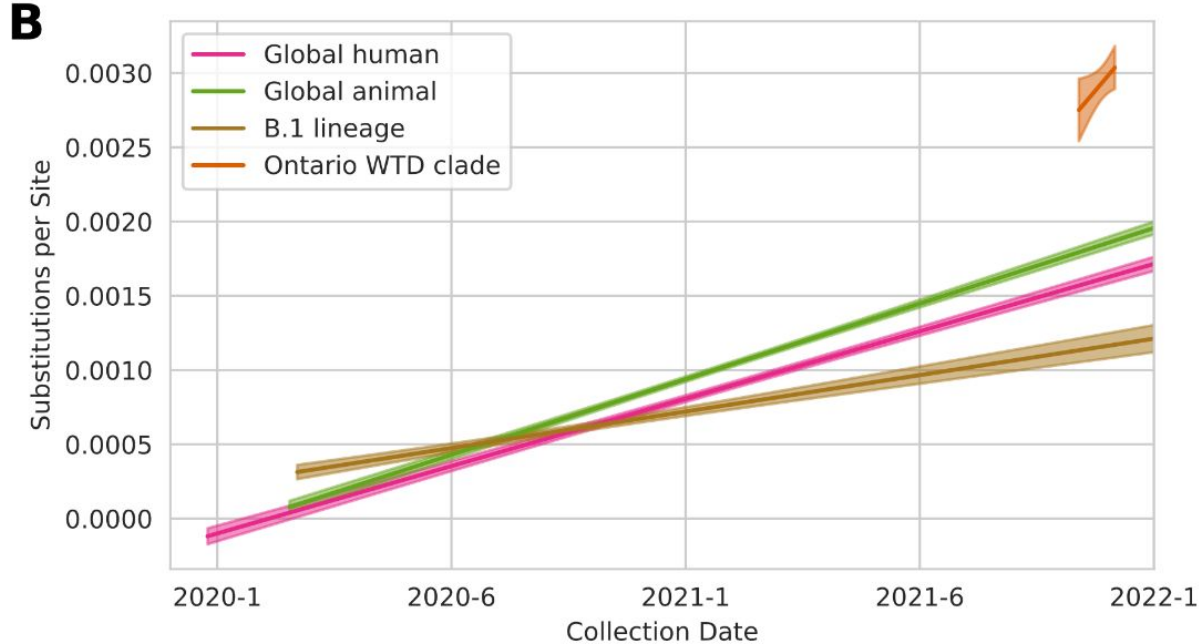


Tree 2, coordinate range 12732-29903



- SNP tables (**sc2rf**)
- Mosaicism (**3Seq**)
- Phylogenetic incongruence (**Hyphy-GARD**)

Possibly elevated mutation rate



Global human: 0.9×10^{-3} to 1.0×10^{-3}

Global animal: 1.0×10^{-3} to 1.1×10^{-3}

B.1 lineage: 0.4×10^{-3} to 0.6×10^{-3}

Ontario WTD: 0 to 8×10^{-3}

Possibly elevated mutation rate

Evolutionary rate of SARS-CoV-2 increases during zoonotic infection of farmed mink

SARS-CoV-2 coronavirus SARS-CoV-2 Molecular Evolution



ash.porter

3d

Evolutionary rate of SARS-CoV-2 increases during zoonotic infection of farmed mink

Authors:

Ashleigh F. Porter (1*), Damian F.J. Purcell (1), Benjamin P. Howden (1,2), Sebastian Duchene (1*).

Affiliations:

1. Department of Microbiology and Immunology, The University of Melbourne at The Peter Doherty Institute for Infection and Immunity, Melbourne, VIC, Australia.
2. Microbiological Diagnostic Unit Public Health Laboratory, The University of Melbourne at The Peter Doherty Institute for Infection and Immunity, Melbourne, VIC, Australia.

*Corresponding author. Email: ashleigh.porter@unimelb.edu.au,
sebastian.duchene@unimelb.edu.au

Global human: 0.9×10^{-3} to 1.0×10^{-3}

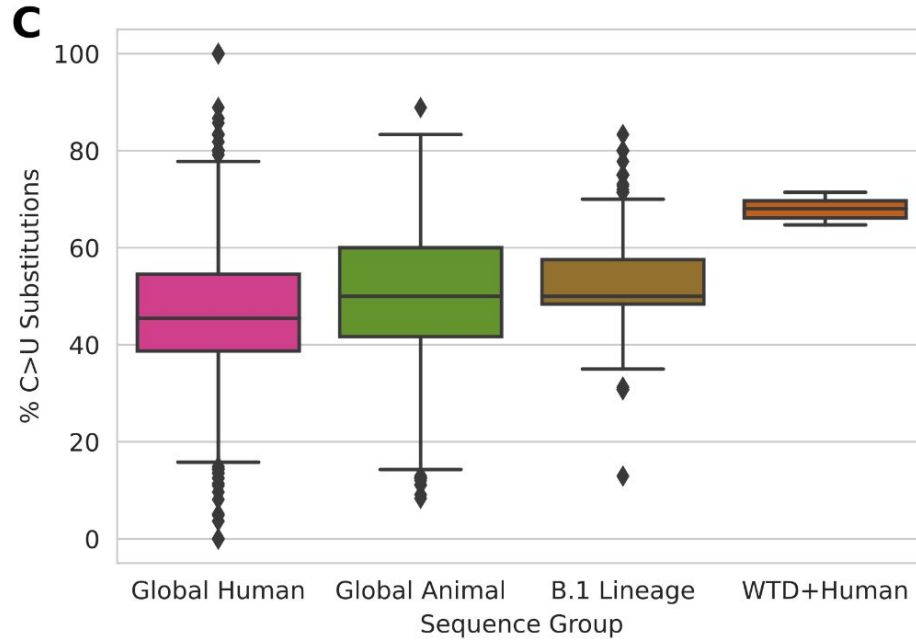
Global animal: 1.0×10^{-3} to 1.1×10^{-3}

B.1 lineage: 0.4×10^{-3} to 0.6×10^{-3}

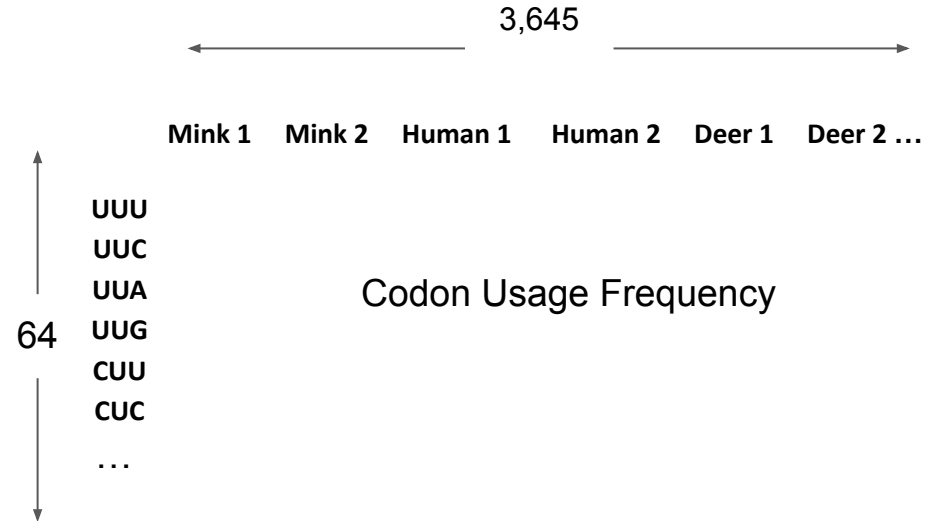
Ontario WTD: 0 to 8×10^{-3}

Mink: 3.18×10^{-3} to 10.6×10^{-3}

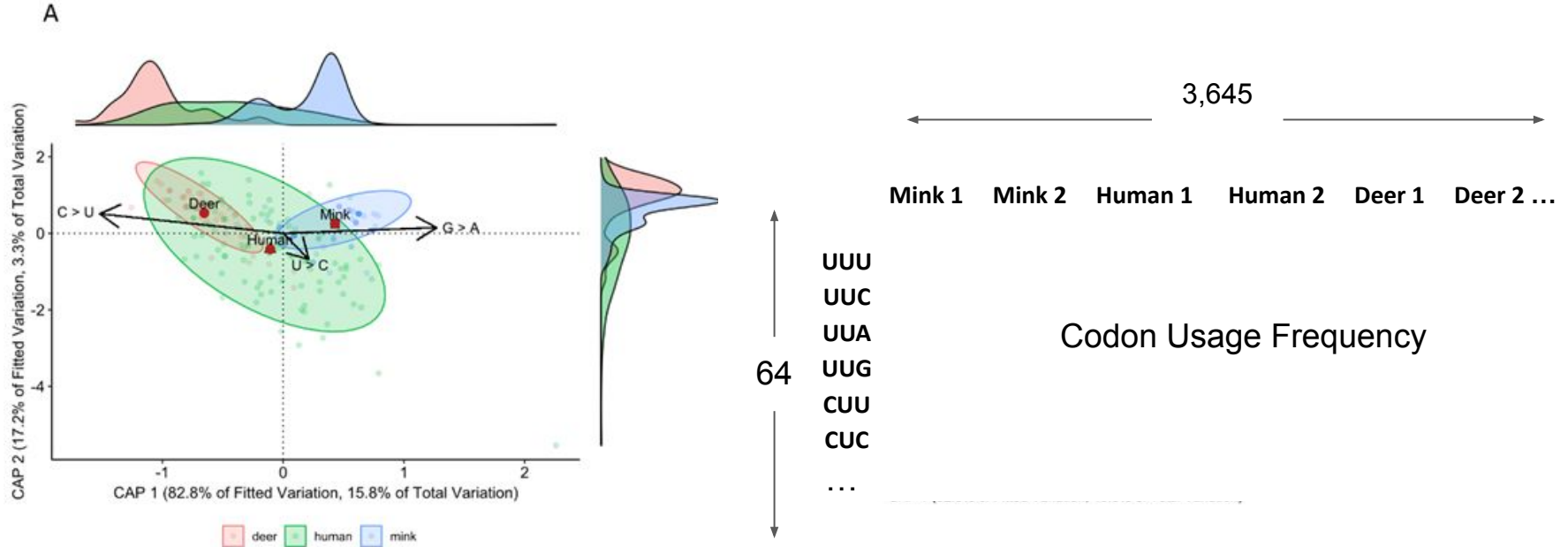
Elevated C>U indicative of host adaptation



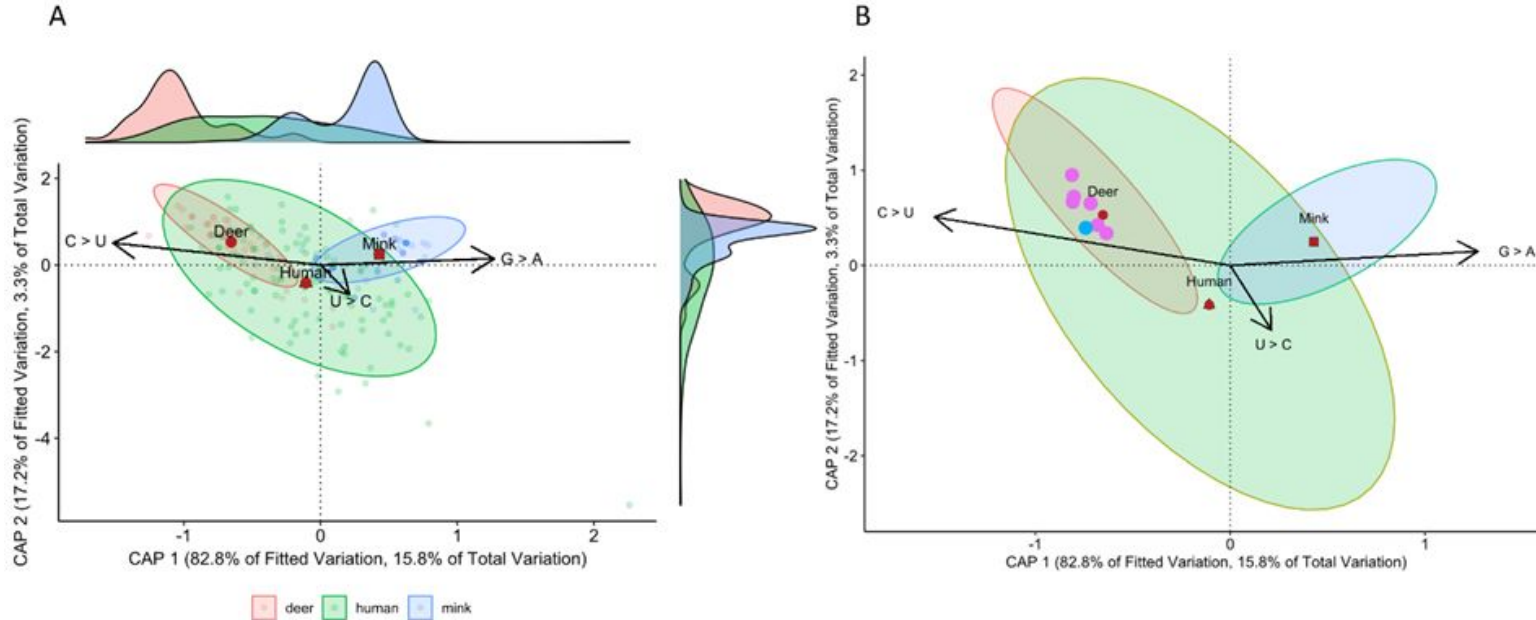
Codon usage patterns also support host adaptation



Codon usage patterns also support host adaptation



Codon usage patterns also support host adaptation



But, no sign of diversifying selection!

dN/dS:

- >1 positive/diversifying selection
- ~1 neutral evolution
- <1 negative/purifying selection

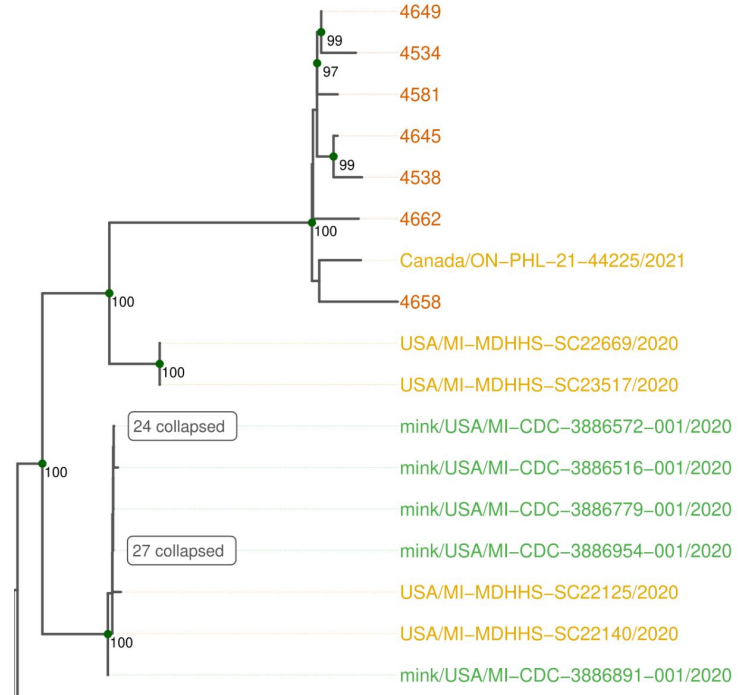
But, no sign of diversifying selection!

dN/dS:

- >1 positive/diversifying selection
- ~1 neutral evolution
- <1 negative/purifying selection

Challenge:

- Mutation rates vary over time/branches



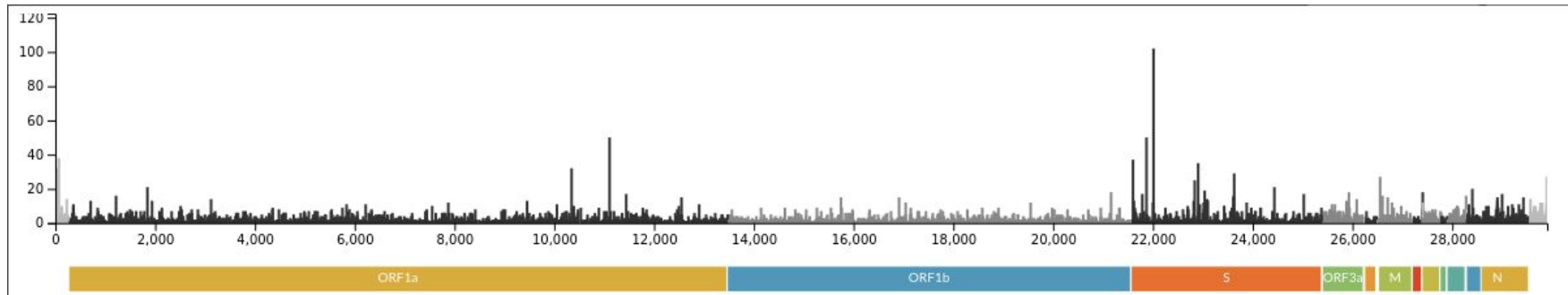
But, no sign of diversifying selection!

dN/dS:

- >1 positive/diversifying selection
- ~1 neutral evolution
- <1 negative/purifying selection

Challenge:

- Mutation rates vary over time/branches
- Mutation rates vary across the genome



But, no sign of diversifying selection!

dN/dS:

- >1 positive/diversifying selection
- ~1 neutral evolution
- <1 negative/purifying selection

Challenge:

- Mutation rates vary over time/branches
- Mutation rates vary across the genome

Codon-alignment gene-based phylogenies

Hyphy:

- **abSREL**: positive selection in proportion of branches
- **BUSTED**: gene-wide positive selection
- **RELAX**: selection changing in set of branches

But, no sign of diversifying selection!

dN/dS:

- >1 positive/diversifying selection
- ~1 neutral evolution
- <1 negative/purifying selection

Challenge:

- Mutation rates vary over time/branches
- Mutation rates vary across the genome

Codon-alignment gene-based phylogenies

Hyphy:

- **abSREL**: positive selection in proportion of branches
- **BUSTED**: gene-wide positive selection
- **RELAX**: selection changing in set of branches



But, no sign of diversifying selection!

dN/dS:

- >1 positive/diversifying selection
- ~1 neutral evolution
- <1 negative/purifying selection

Challenge:

- Mutation rates vary over time/branches
- Mutation rates vary across the genome

Codon-alignment gene-based phylogenies

Hyphy:

- **abSREL**: positive selection in proportion of branches
- **BUSTED**: gene-wide positive selection
- **RELAX**: selection changing in set of branches

No significant positive selection or intensification of selection detected

Relaxation of selection on ORF1ab amongst WTD clade $p=0.0032$)



But, no sign of diversifying selection!

76 total consensus mutations in WTD

ORF1ab (71% of genome):

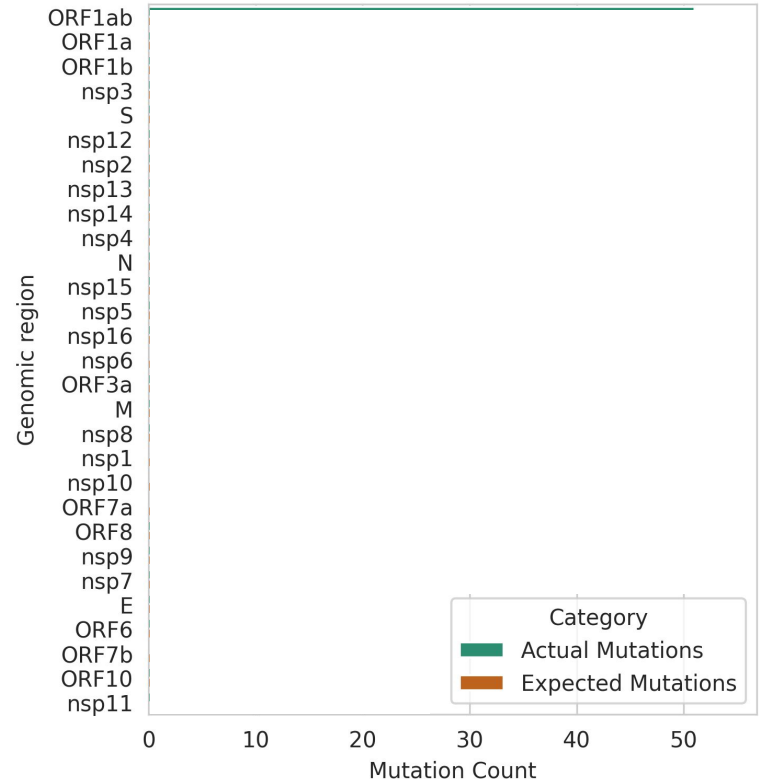
Expected mutations: **54.1**

Actual mutations: ?

S (12.7% of genome):

Expected mutations: **9.7**

Actual mutations: ?



But, no sign of diversifying selection!

76 total consensus mutations in WTD

ORF1ab (71% of genome):

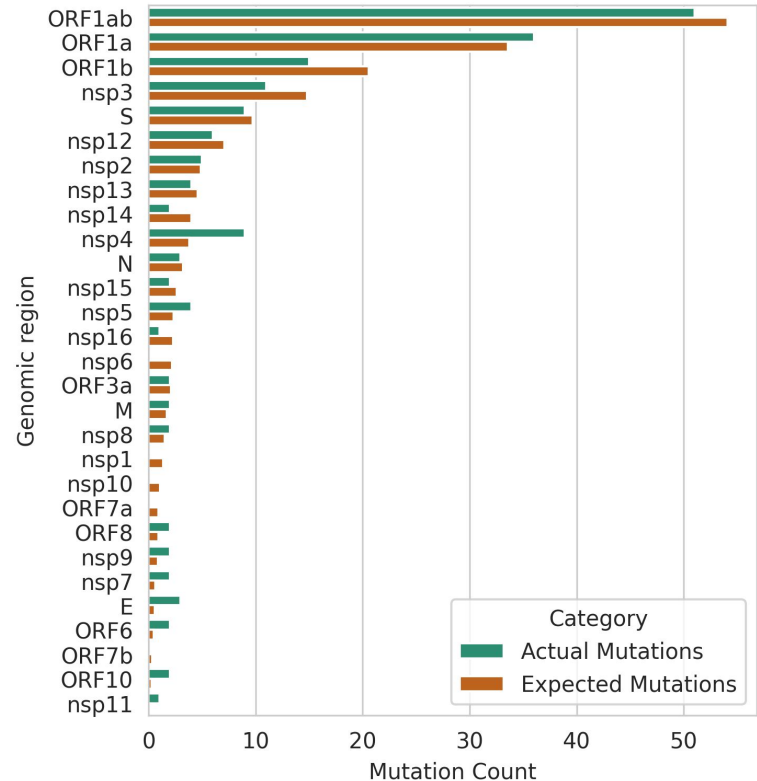
Expected mutations: **54.1**

Actual mutations: **51**

S (12.7% of genome):

Expected mutations: **9.7**

Actual mutations: **9**



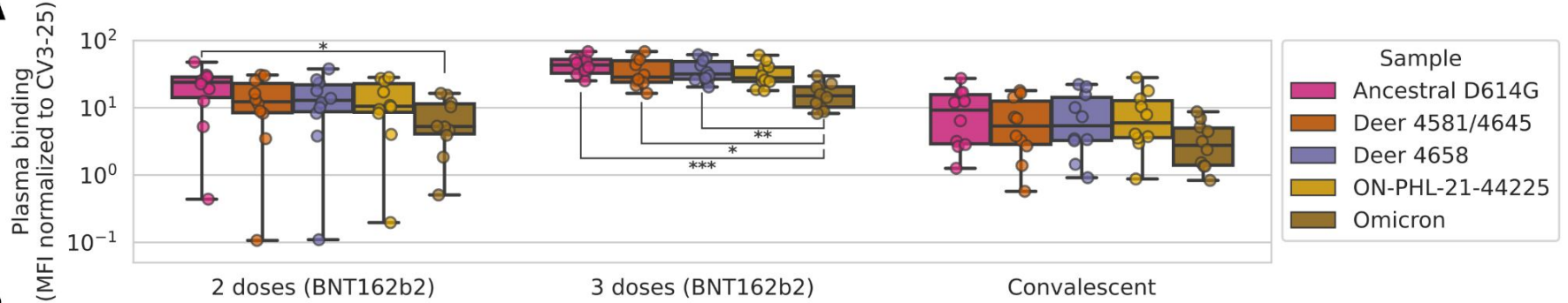
So, what does all this mean in terms of
phenotype?

No change of spike antigenicity



No change of spike antigenicity

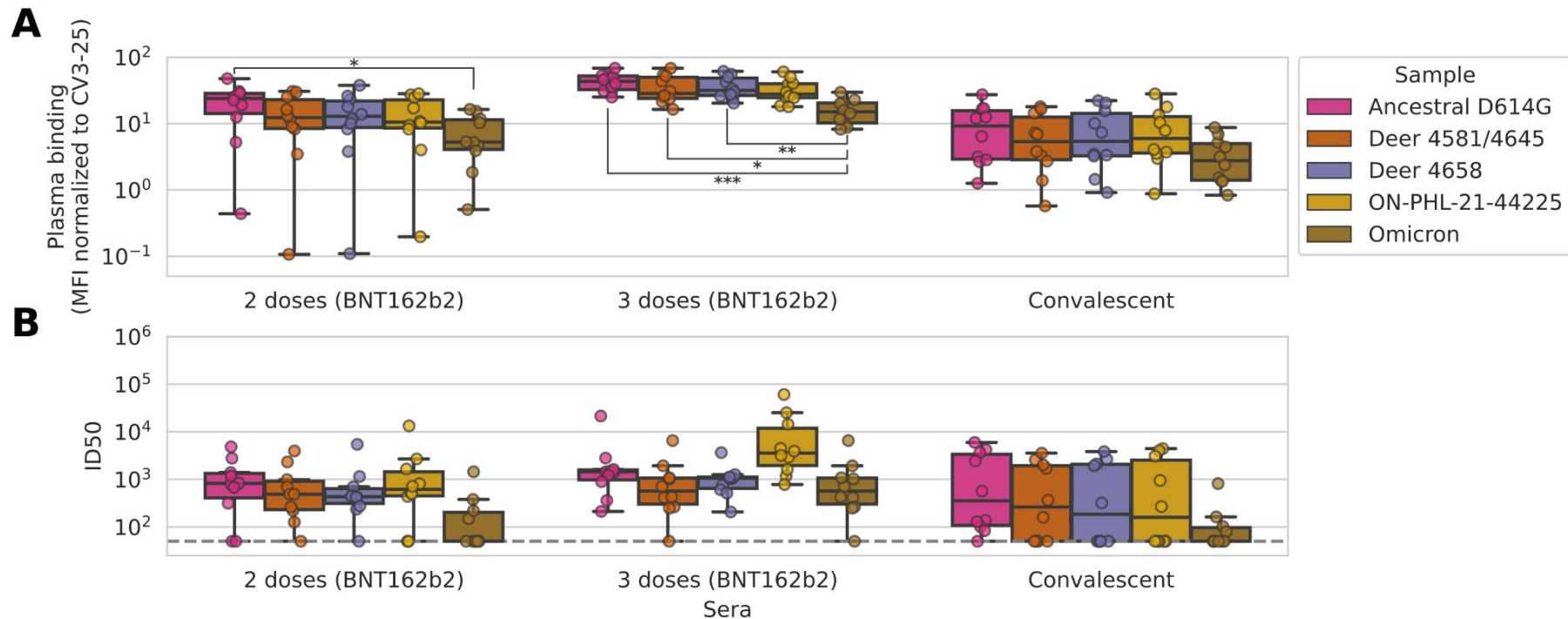
A



B

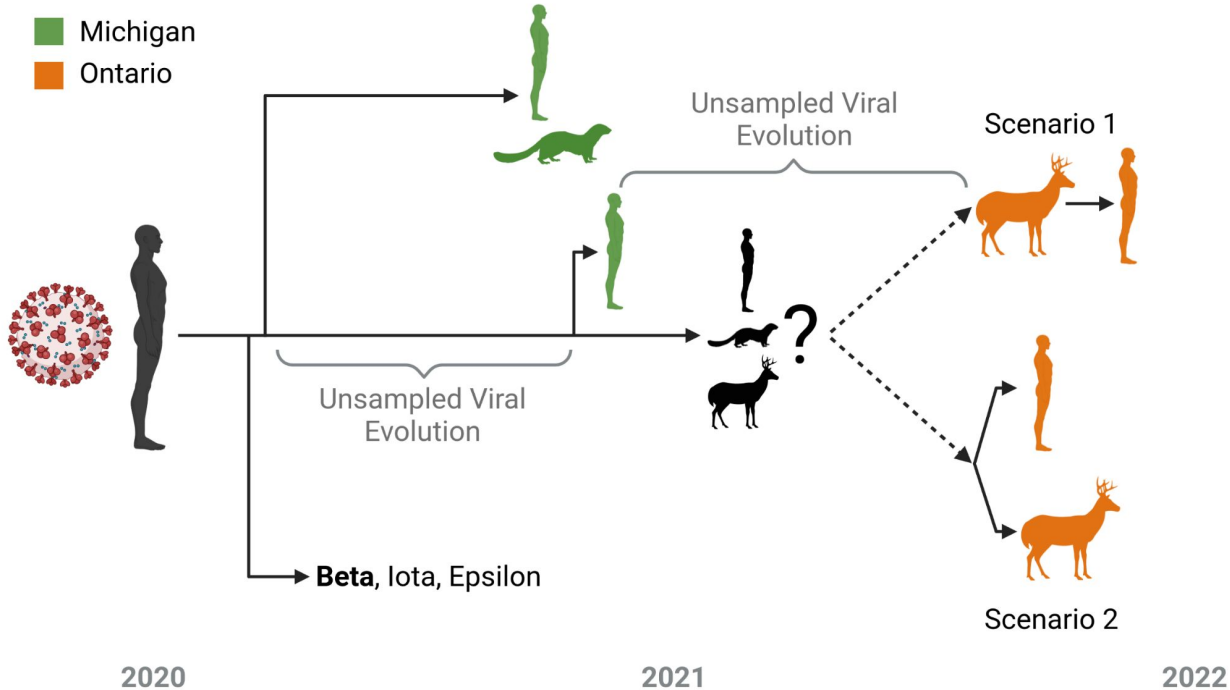


No change of spike antigenicity



So, are WTD a secondary reservoir?

Limited “One Health” surveillance means lots of uncertainty



Take-aways

- Highly divergent SARS-CoV-2 in WTD with likely deer-to-human transmission
- Signs of genomic adaptation to animal host (animal-associated mutations, C>U, codon usage, elevated mutation rate)
- No indication of strong selection or change of antigenicity (*c.f.* Omicron)

Take-aways

- Highly divergent SARS-CoV-2 in WTD with likely deer-to-human transmission
 - Signs of genomic adaptation to animal host (animal-associated mutations, C>U, codon usage, elevated mutation rate)
 - No indication of strong selection or change of antigenicity (*c.f.* Omicron)
-
- Need to better understand the interplay of virus, disease, and immune selection in non-human animals (and impact on pathogen evolution)
 - Similar scenarios could generate future variants of concern
 - Therefore, clear need for broader “One-Health” surveillance

“WILD-CoV”



Canadian Food
Inspection Agency

National Centre for Foreign Animal Disease

- Bradley Pickering (Iowa State/Manitoba)
- Oliver Lung (Manitoba)
- Peter Kruczkiewicz
- Melissa Goolia, Matthew Suderman, Mathieu Pinette, Greg Smith, Daniel Sullivan, Josip Rudar, Michelle Nebroski, Oksana Vernygora



DALHOUSIE
UNIVERSITY

Computer Science / Epidemiology

- Finlay Maguire

Public
Health
Ontario

- Jennifer Guthrie (Western)
- Alex Marchand-Austin



Sunnybrook

HEALTH SCIENCES CENTRE

Sunnybrook Research Institute

- Samira Mubareka (Toronto)
- Jonathan Kotwa
- Bryan Griffin
- Kuganya Nirmalarajah, Juliette Blais-Savoie, Hsien-Yao Chee, Emily Chien, Winfield Yim, Andra Banete, Lily Yip

Shared Hospital Laboratory

- Patryk Aftanas



Ontario

Ministry of Northern
Development, Mines, Natural
Resources and Forestry

- Jeff Bowman (Trent)
- Tore Buchanan
- Larissa Nituch
- Elizabeth Adey



uOttawa

- Marceline Côté
- Geneviève Laroche
- Ardeshir Ariana, Brett Vahkal



Sinai
Health

- Allison McGeer (Toronto)

Université
de Montréal



CANADIAN
WILDLIFE HEALTH
COOPERATIVE

Forêts, Faune
et Parcs

Québec



Ontario
MINISTRY OF HEALTH

Guillaume Goyette, Andrés Finzi, Claire
Jardine, Ariane Massé Heather McClinchey



CIHR IRSC



Canadian Institutes
of Health Research

Instituts de recherche
en santé du Canada



CoVaRR+Net



CANADIAN
WILDLIFE HEALTH
COOPERATIVE

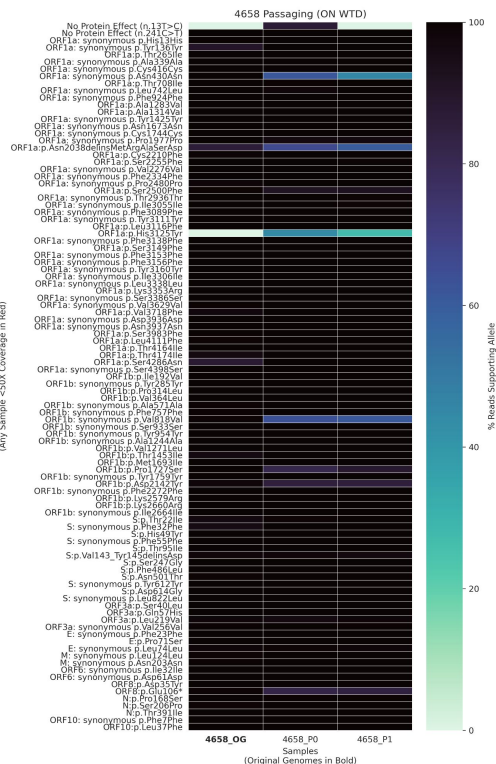
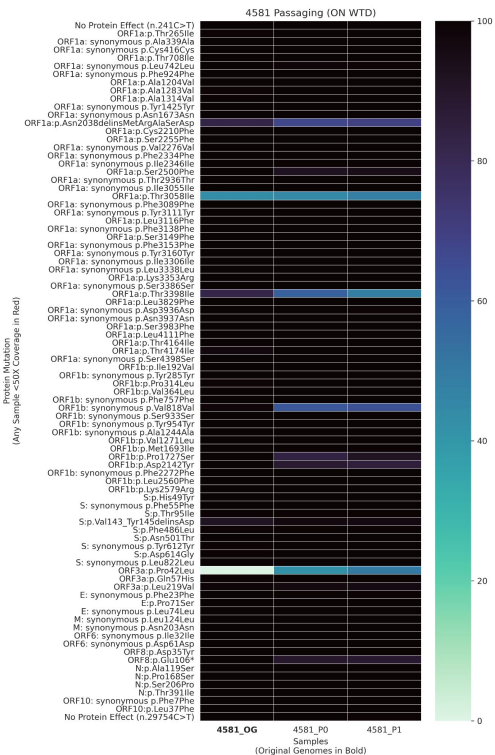
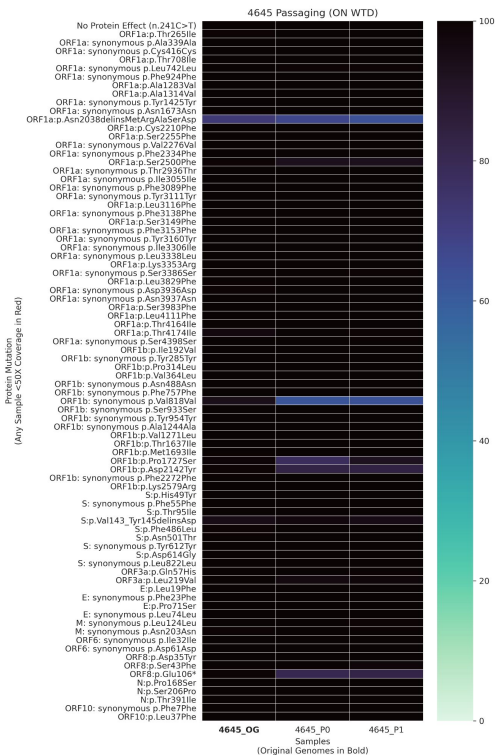
CanCOGeN

Canadian Safety
and Security Program

Programme canadien
pour la sûreté et la sécurité



Viral cultures



Viral cultures

