Phylodynamics & Evolutionary Rates

Lessons from the Biocontrol of Australian Rabbits

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THE UNIVERSITY OF SYDNEY
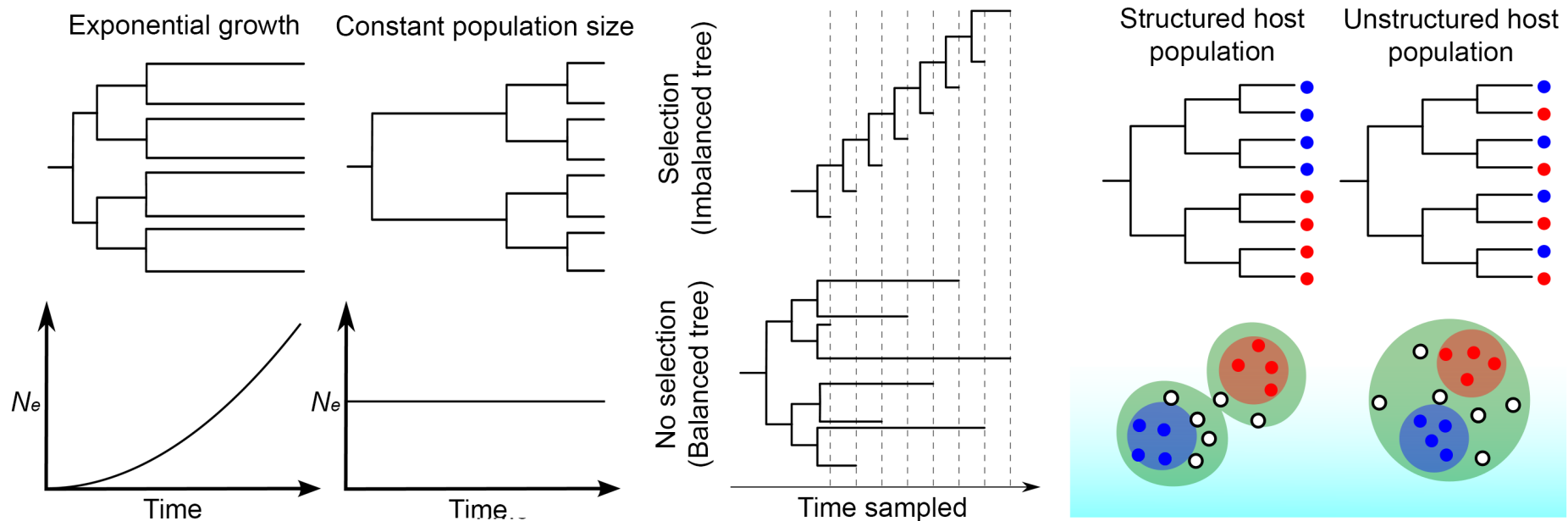
The Westmead Institute FOR MEDICAL RESEARCH

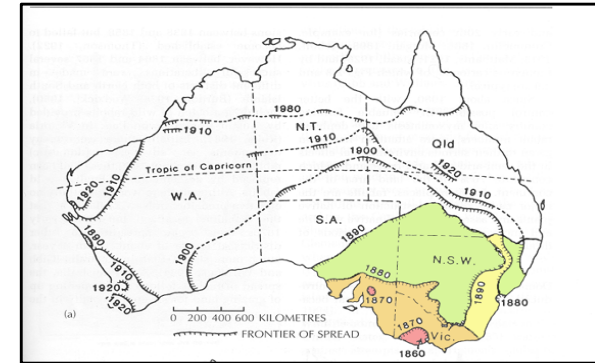# Viral phylodynamics

Fundamental part of Pathogen genomics

Based on the notion that epidemiological, immunological

and evolutionary processes influence the _shape of viral phylogenies_

(Grenfell & Holmes, 2004).

European rabbits in Australia

Spread of the 'Grey Blanket'

- Introduced by early settlers for food and sport in 18th-19th century

- When a few became many… over a billion rabbits by 1950

- Transformed Australian landscape, impacted on native species, and became main vertebrate pest

What do rabbits have to do with viral phylodynamics?

Viral biocontrol

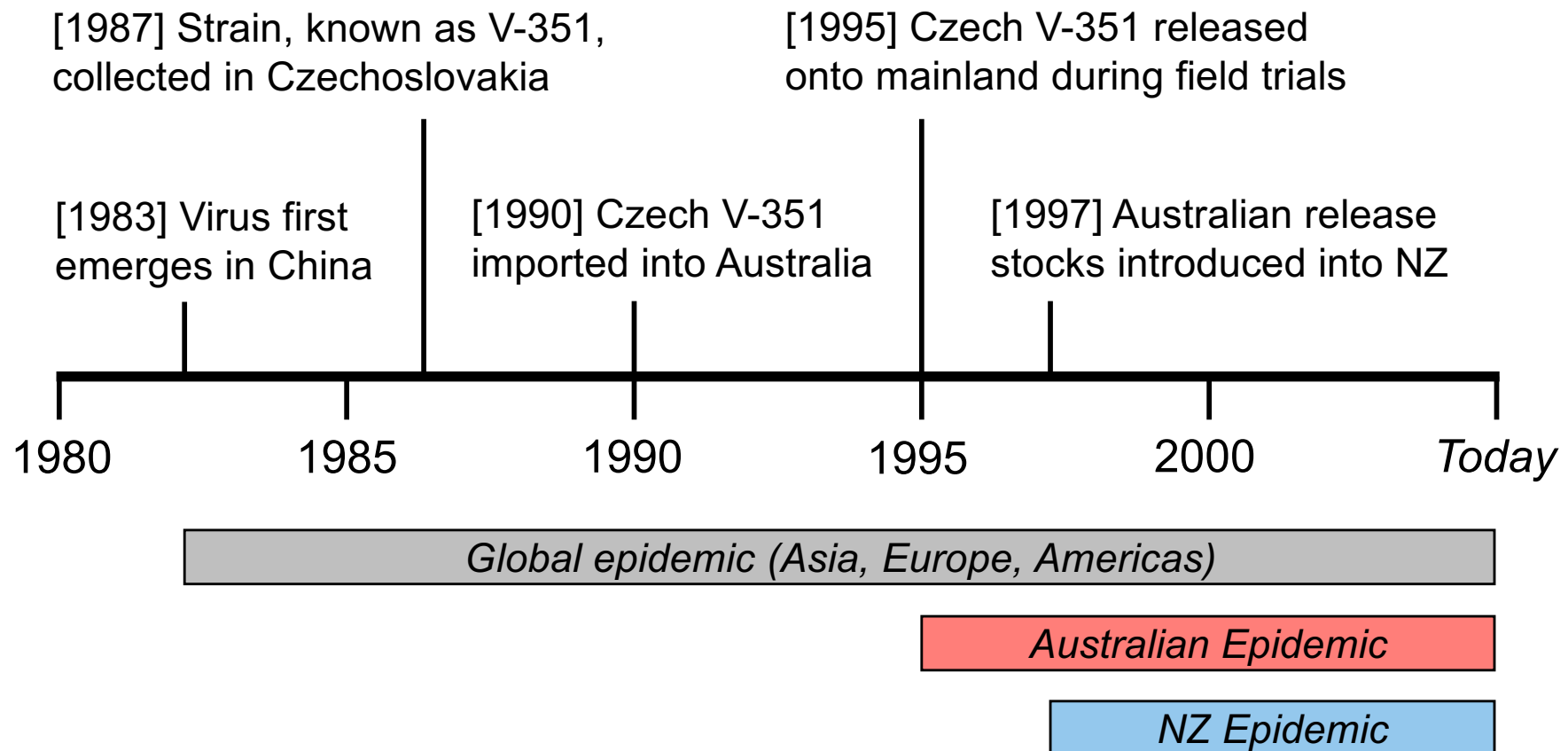**The Australian Perspective…**

"The only good rabbit is a dead one"

- Two viruses have been released into Australia to control rabbit populations

- In 1950, Myxoma Virus released but soon attenuated strains emerged

- Then, in 1995, Rabbit Haemorrhagic Disease Virus was released as alternative

The case study in viral emergence & virulence evolution

RHDV: A known history

Intentional release means you know source and timing of epidemics

[1987] Strain, known as V-351, collected in Czechoslovakia

[1995] Czech V-351 released onto mainland during field trials

[1983] Virus first emerges in China

[1990] Czech V-351 imported into Australia

[1997] Australian release stocks introduced into NZ

1980   1985   1990   1995   2000   *Today*

Global epidemic (Asia, Europe, Americas)

Australian Epidemic

NZ Epidemic

A single release site?

"Unintentional" release

- Virus 'escaped' off Wardang Island, South Australia during field trials

- Phylodynamics would predict these events are written in the genomic history of the viruses
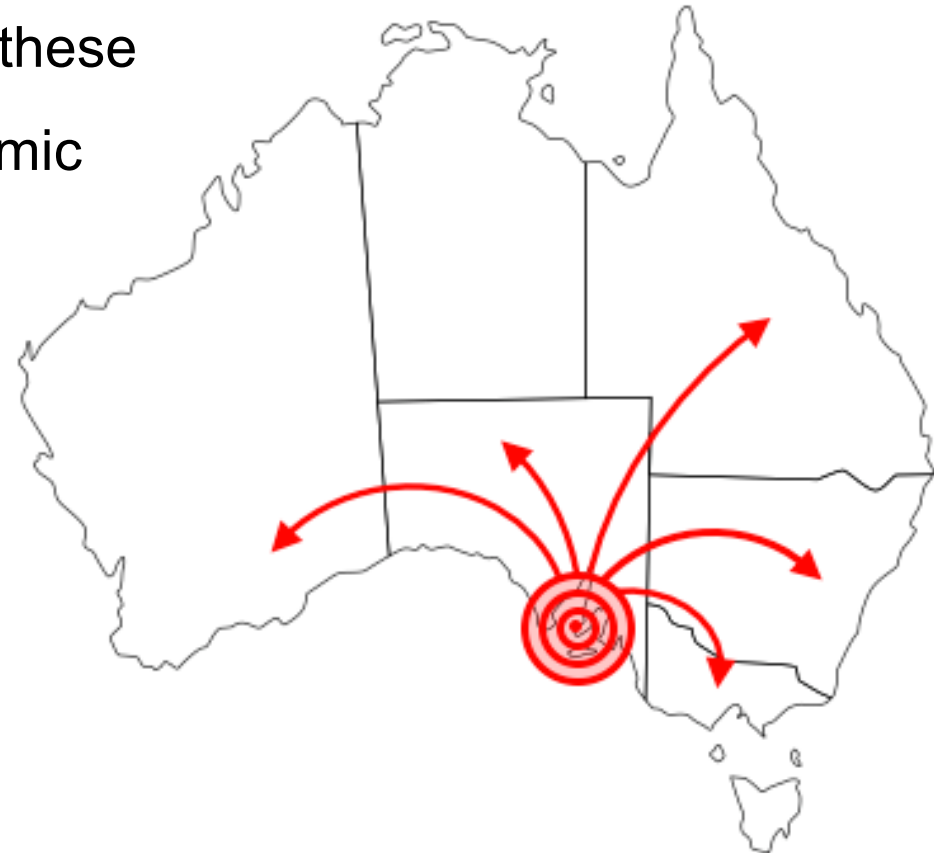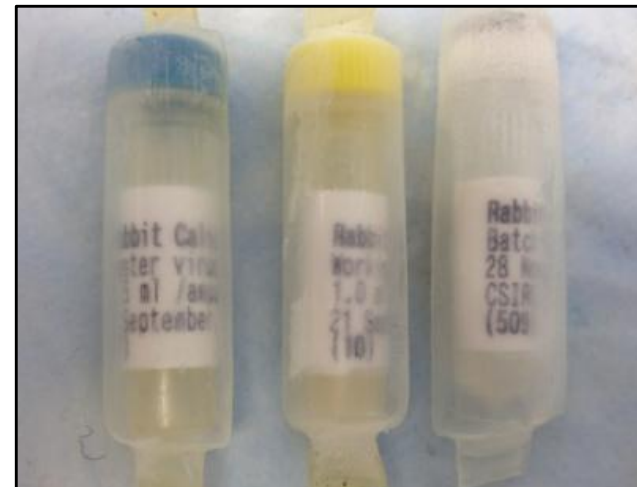
A single founder virus?

Sequencing original RHDV stocks

- Original Australian CSIRO/AAHL stocks

- Independent sources Australian 'release' stocks

- Early Australian source used in NZ

Master

↓

Working

↓

Release
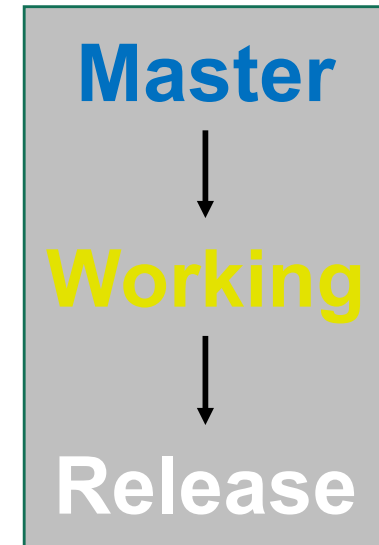
All (Australian) viruses should share a single common ancestor

A robust phylogeny

Required for any phylodynamic inference
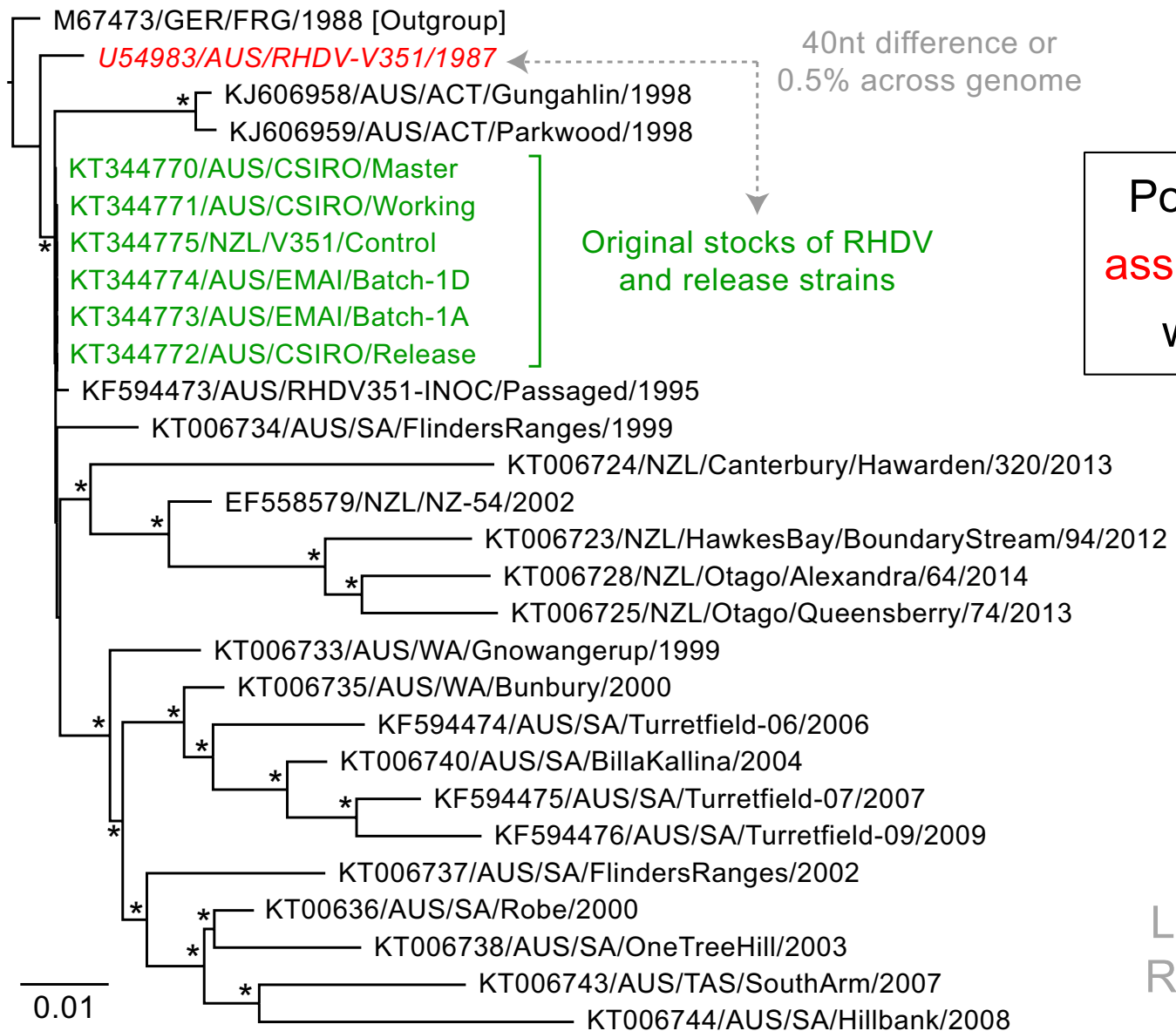
1. **Sampling & Sequencing:** Even through time and location

2. **Gene alignment:** Informative gene & homologous positions

3. **Appropriate model & phylogenetic method:** Statistically robust

4. **Correct topology:** Where is the root?

5. **Rates analysis with root-to-tip distances:** Path-o-Gen (Tempest)

> **Basic formula =**
>   Sequences + Alignment + ModelTest + ML tree

Phylogeny of founder viruses

M67473/GER/FRG/1988 [Outgroup]

U54983/AUS/RHDV-V351/1987 ◄‒‒‒‒‒‒‒‒‒┐

40nt difference or
0.5% across genome

* KJ606958/AUS/ACT/Gungahlin/1998

KJ606959/AUS/ACT/Parkwood/1998

KT344770/AUS/CSIRO/Master

KT344771/AUS/CSIRO/Working

KT344775/NZL/V351/Control — Original stocks of RHDV and release strains

KT344774/AUS/EMAI/Batch-1D

KT344773/AUS/EMAI/Batch-1A

KT344772/AUS/CSIRO/Release

KF594473/AUS/RHDV351-INOC/Passaged/1995

KT006734/AUS/SA/FlindersRanges/1999

* KT006724/NZL/Canterbury/Hawarden/320/2013

* EF558579/NZL/NZ-54/2002

* KT006723/NZL/HawkesBay/BoundaryStream/94/2012

* KT006728/NZL/Otago/Alexandra/64/2014

KT006725/NZL/Otago/Queensberry/74/2013

KT006733/AUS/WA/Gnowangerup/1999

* KT006735/AUS/WA/Bunbury/2000

* KF594474/AUS/SA/Turretfield-06/2006

* KT006740/AUS/SA/BillaKallina/2004

* KF594475/AUS/SA/Turretfield-07/2007

* KF594476/AUS/SA/Turretfield-09/2009

KT006737/AUS/SA/FlindersRanges/2002

* KT00636/AUS/SA/Robe/2000

* KT006738/AUS/SA/OneTreeHill/2003

* KT006743/AUS/TAS/SouthArm/2007

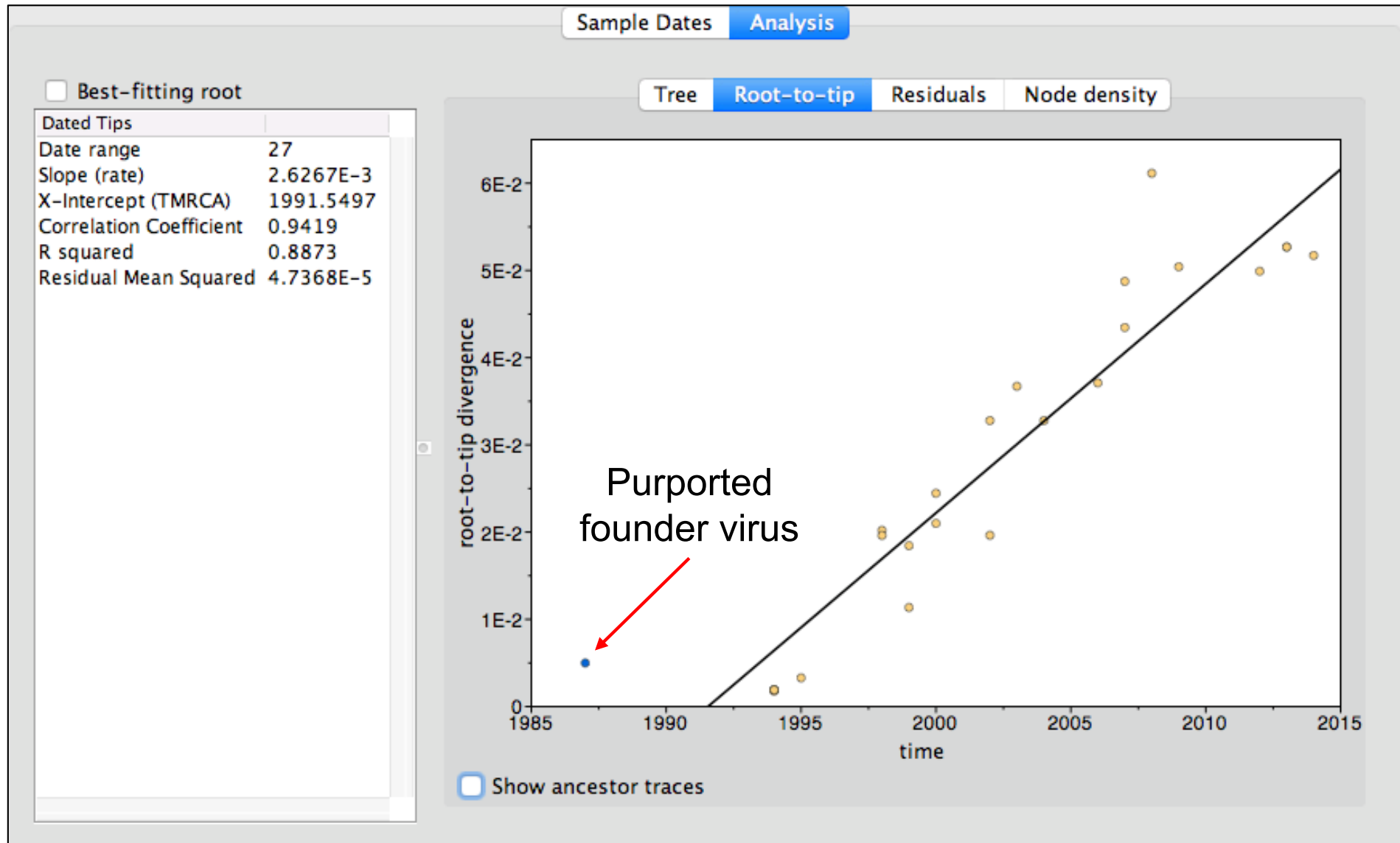KT006744/AUS/SA/Hillbank/2008

0.01

Position of previously
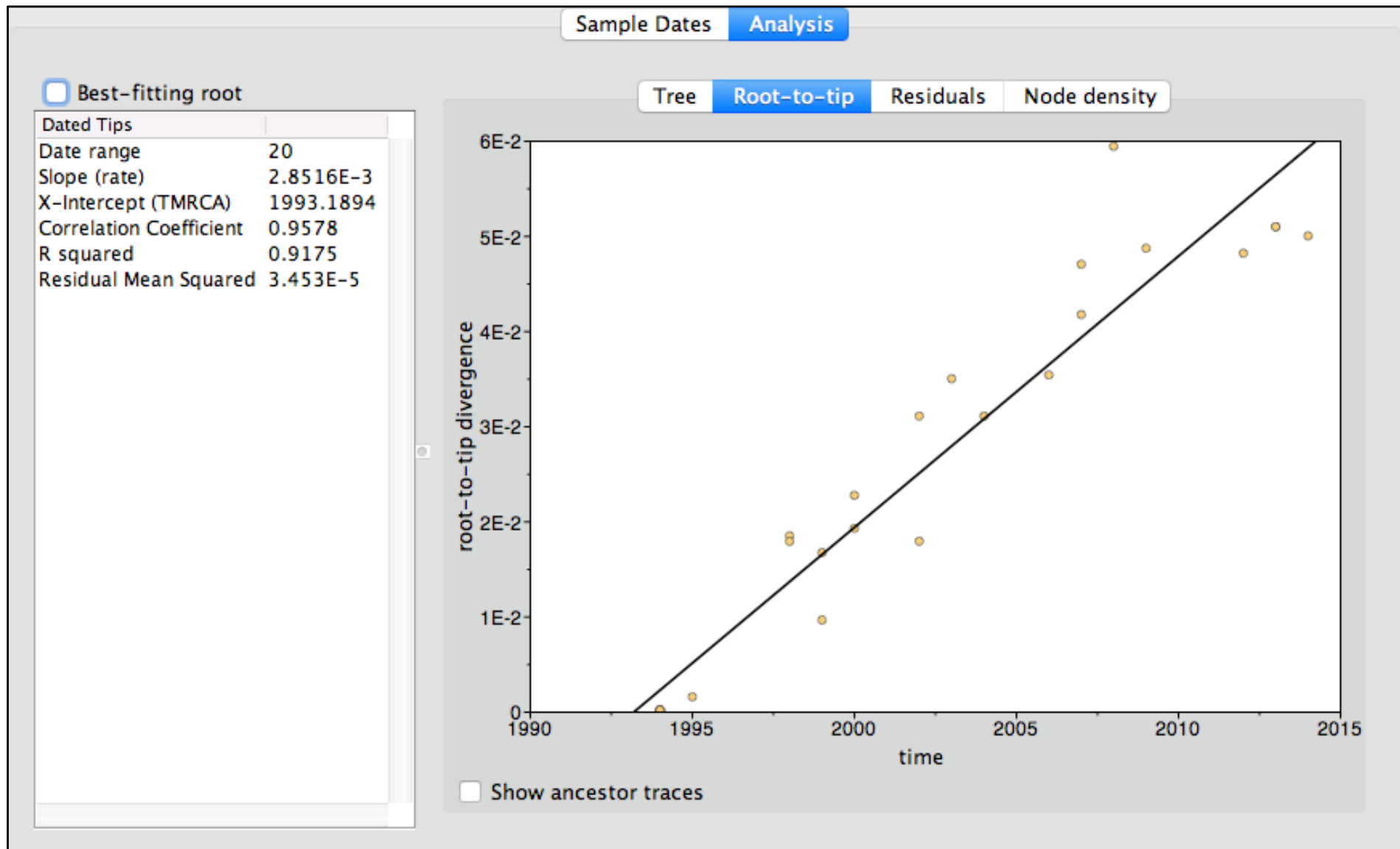assumed 'founder virus'
was clearly wrong

Robust tree
+
Sampling dates
↓
Path-o-Gen

Linear Regression of
Root-to-Tip distances

Rates analysis using Path-o-Gen

Rates analysis using Path-o-Gen

Rates analysis using Path-o-Gen

With Czech-V351/1987

Dated Tips	
Date range	27
Slope (rate)	2.6267E-3
X-Intercept (TMRCA)	1991.5497
Correlation Coefficient	0.9419
R squared	0.8873
Residual Mean Squared	4.7368E-5

Without Czech-V351/1987

Dated Tips	
Date range	20
Slope (rate)	2.8516E-3
X-Intercept (TMRCA)	1993.1894
Correlation Coefficient	0.9578
R squared	0.9175
Residual Mean Squared	3.453E-5

- **Rate of evolution increased:** 2.63E-3 vs 2.85E-3 (subs/site/yr)

- **TMRCA more recent:** 1991 vs 1993

- **Better correlation between date & divergence:** 0.94 vs 0.96

- **Correct founder** = Better dating estimates!

Molecular epidemiology

Tracking the Spread and Evolution of RHDV in Australia & NZ

- A common founder virus?

- Unique opportunity to study evolution in parallel

- Genome sequencing of 28 new field isolates from both regions

- Characterised using a viral phylodynamic approach
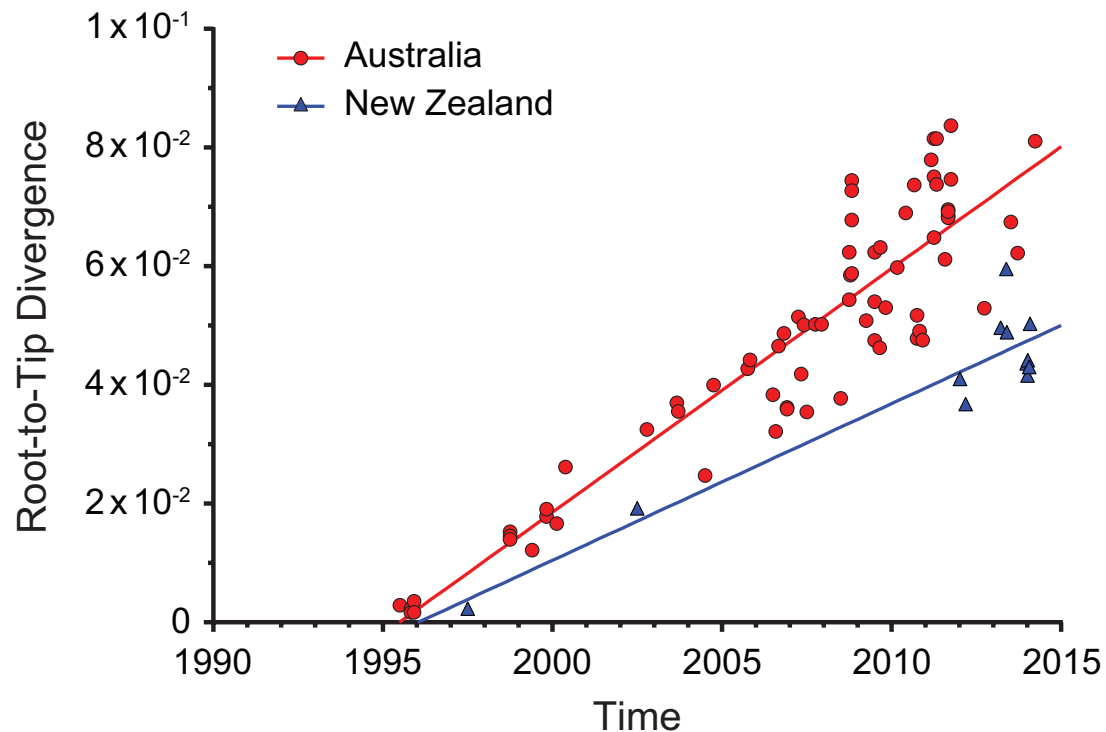
Capsid phylogeny

Phylodynamic inference

- Evidence of clustering by regions (phylogeography):
  - AUS vs NZ
  - AUS East vs West

- South Australia as a known 'source' population

- No lineages from apparent 're-release' strains
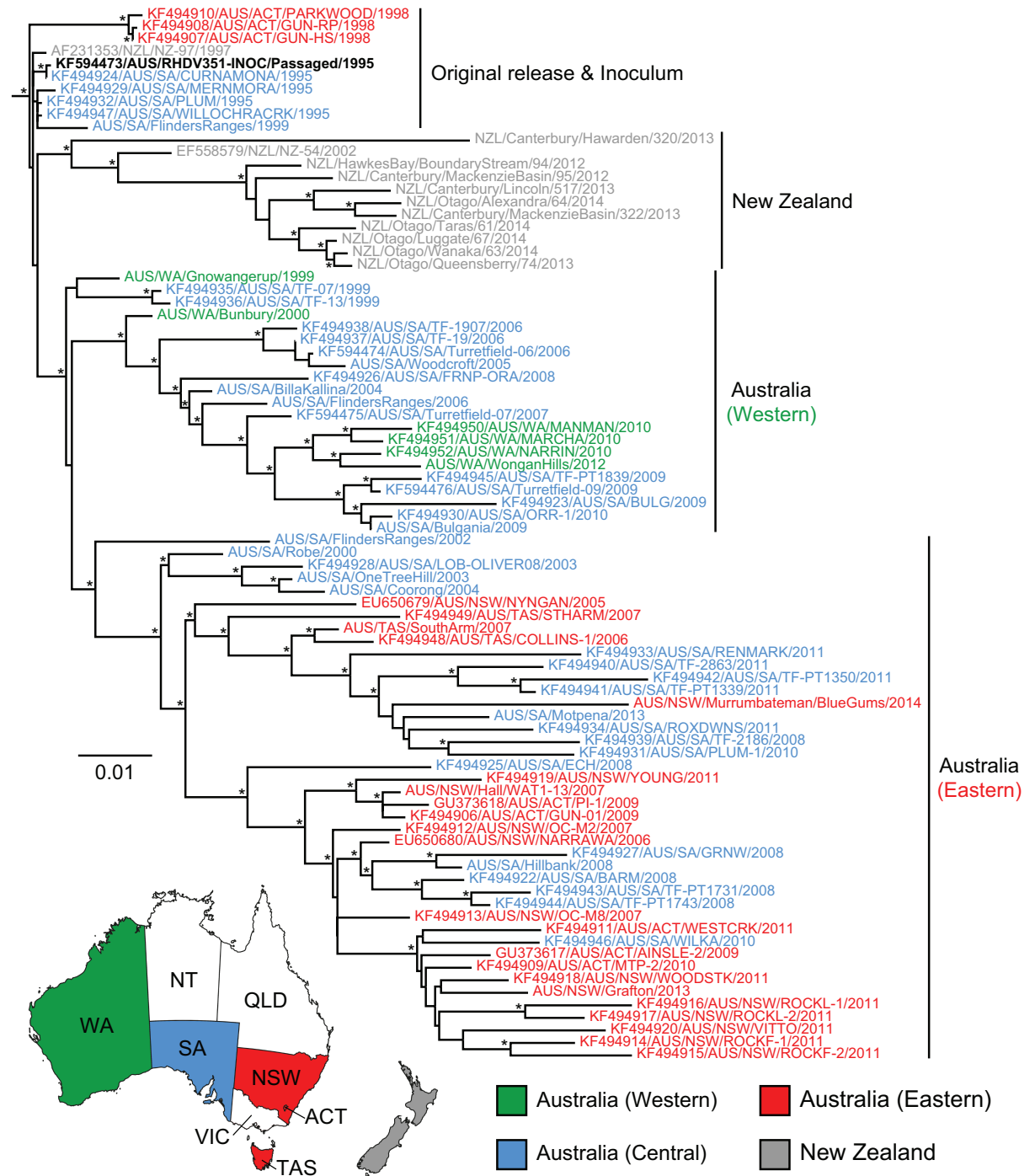
Rates of evolution with Path-o-gen

Export the raw data for more detailed analyses

Best-fit values	Australia	New Zealand
Slope (rate)	$4.1 \times 10^{-3} \pm 2.1 \times 10^{-4}$	$2.6 \times 10^{-3} \pm 3.3 \times 10^{-4}$
X-intercept (TMRCA)	1995.49	1996.04
Correlation coefficient	0.92	0.93

- Linear regression of root-to-tip distances versus time

- Split by different lineages

- Strong temporal structure

- Apparent, lower rate of evolution in NZ compared to Australia

How else can you use Path-o-gen?

1. **Quick assessment of temporal structure**

   - Strict clock will have good correlation between dates and divergence

2. **Spotting outliers**

   - Possible recombinant strains

   - Mis-labelled sequences and dates

   - Any artifacts likes vaccine strains

QUESTIONS?