Phylogeographic analysis reveals that swine influenza virus spreads across Europe via trade of live pigs

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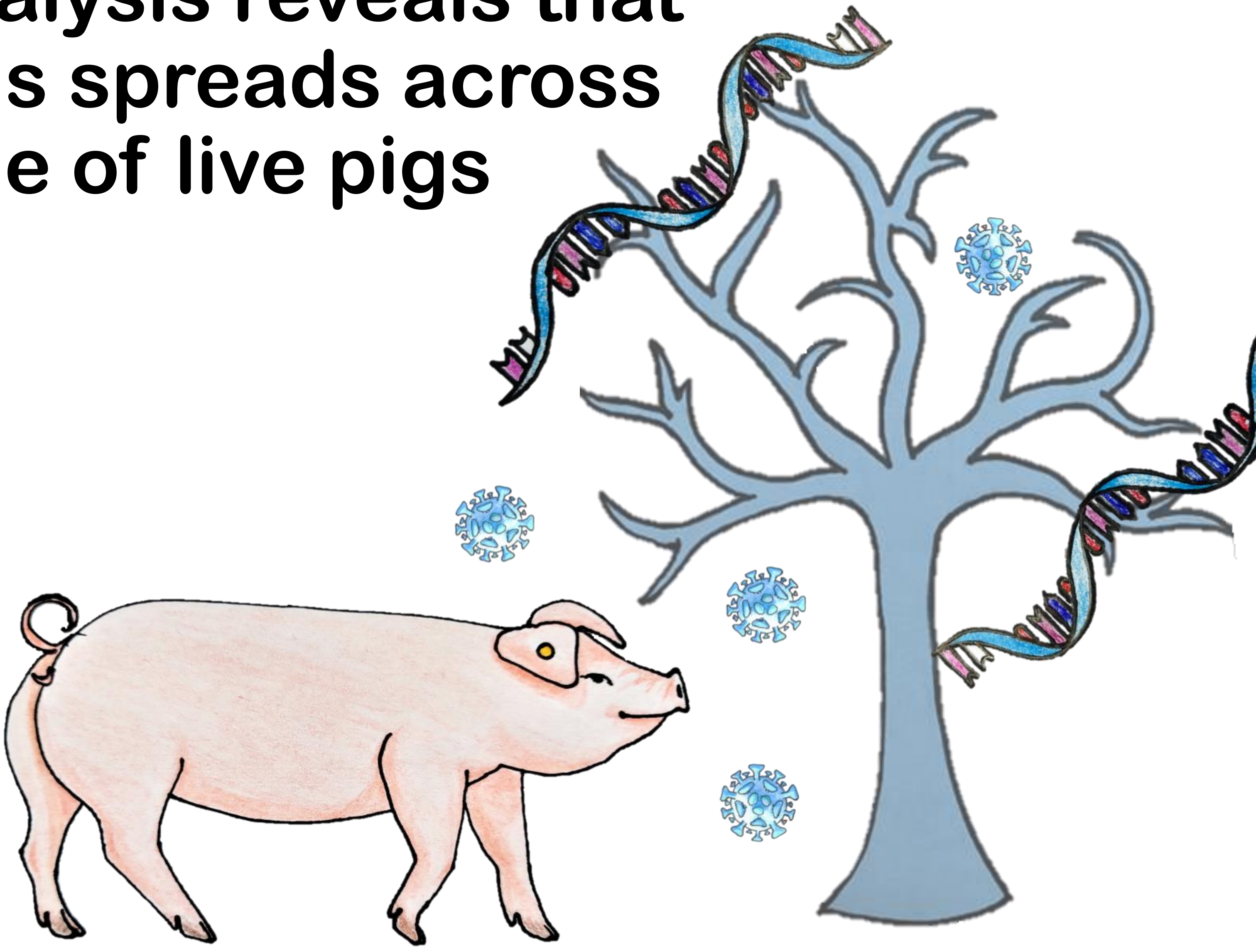

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- ESFLU COST Action
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- Dr. Bram Vrancken
- Dr. Fabiana Gambaro

STSM at ULB Brussels Spatial Epidemiology Lab (SPELL)

Pig trade is a driver of intercontinental and within-US swIAV spread

Apart from shift & drift, there are 3 drivers of swIAV evolution and spread

I. **Humans** are a major source of new strains

II. Virus diversity spreads globally along **international trade routes**

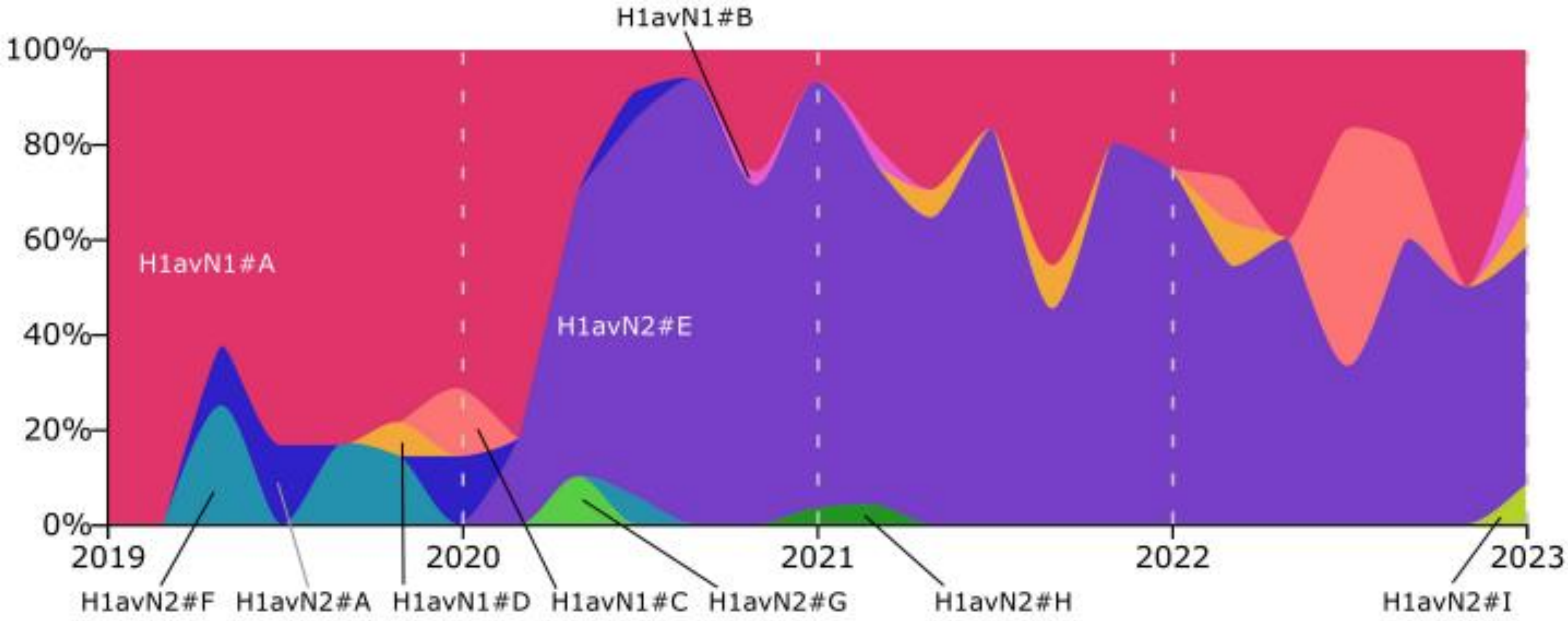
III. "Swineflows" rapidly spread strains long distances **between regions of production**

Does this apply to Europe?

pictures by Prof. dr. Martha Nelson

Dominant SIV strain differs between countries and changes over the years

- **France**



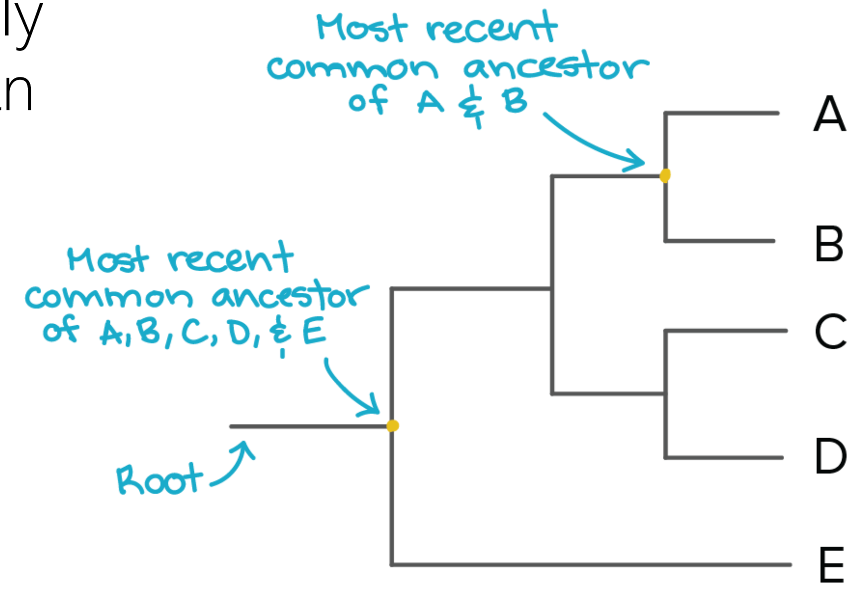
Richard et al. 2024 bioRxiv

Research Questions

- What is the geographical history of swIAV spread in Europe?
- What are the drivers for swIAV spread across European countries?
  - Pig trade as main covariate of interest

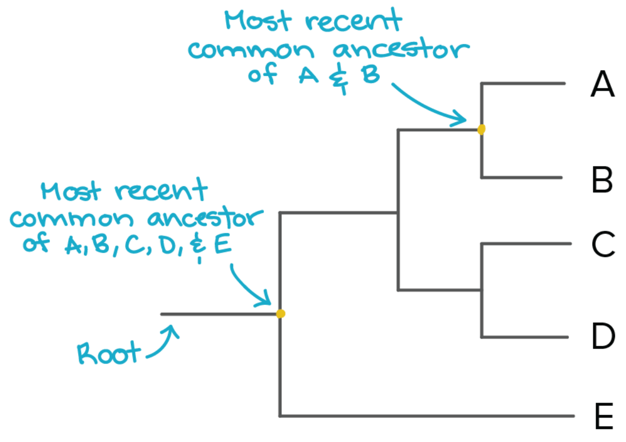
What is phylogeography?

- Principle: **fast evolving viruses** evolve simultaneously with **geographic dispersal**, so the spatial history can be recovered from genomic data
- Estimate the ancestral locations in a phylogenetic tree conditional on the observed locations of viral sequences represented by the tips of the tree



Faria, N. R., Suchard, M. A., Rambaut, A., & Lemey, P. (2011). Toward a quantitative understanding of viral phylogeography. *Current opinion in virology, 1*(5), 423–429

What is phylogeography?



Discrete trait phylogeography:

Rates of location exchange (transitions) along tree, simultaneous with building ‘best’ tree.

Disadvantage: Ancestral locations can only be in the specified locations and locations with a lot of sequences will be overestimated as source location

Advantage: Can be extended to identify predictors for location exchange, using a GLM

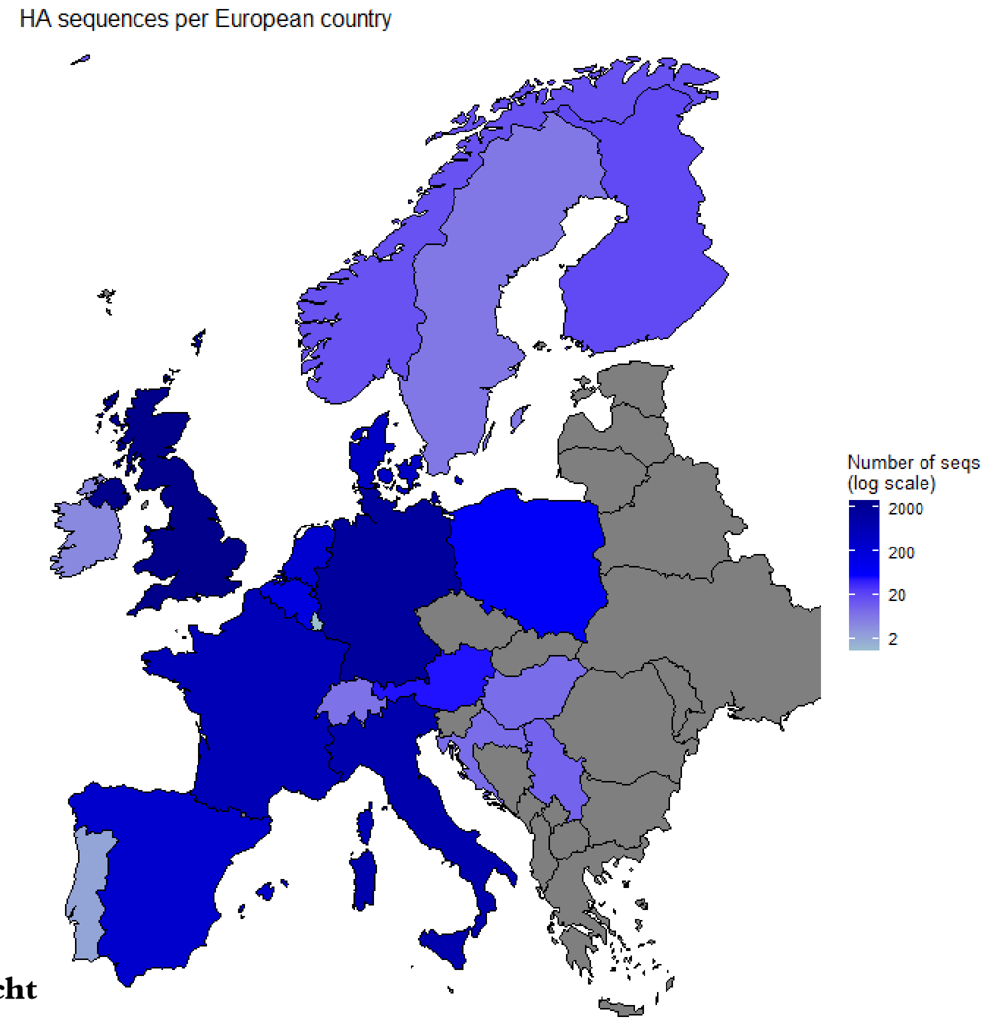
Faria, N. R., Suchard, M. A., Rambaut, A., & Lemey, P. (2011). Toward a quantitative understanding of viral phylogeography. *Current opinion in virology*, *1*(5), 423–429

What do we need for such an analysis?
1. Sequences

- With country and accurate date of collection
- Representative for diversity of the virus in the country
- Sufficient in number and time period for good clock signal and support

- Preferably full length
- Preferably from ALL countries in Europe

What do we need for such an analysis?
1. Sequences



Subtypes

- H1N1: 4629
- H1N2: 1677
- H3N2: 566
- H3N1: 23

many partial H1 sequences
from 2009-2010
After alignment and cleaning:
N= 3469 H1 seqs

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GISAID – EpiFlu database; NCBI Virus

What do we need for such an analysis?
2. A proxy for the prevalence of swIAV per country

- Aim: prevent over- or underrepresentation of countries → biased transition estimates
- Ideal situation: Prevalence of SIV per country

Table 1. Number and Swine Influenza Virus Status of Field Samples Collected from Domestic Swine with Respiratory Symptoms in 16 European Countries and the French Overseas Department La Réunion during April 2015 until January 2018

Countries	Samples	IAV-Positive Samples		IAV-Pegative Samples		Consignments, Total	IAV-Positive Consignments		IAV-Negative Consignments	
Netherlands (NL)	4.471	1.360	(30.4%)	3.111	(69.6%)	271	185	(68.3%)	86	(31.7%)
Germany (DE)	4.064	1.691	(41.6%)	2.373	(58.4%)	1.550	842	(54.3%)	708	(45.7%)
France (FR)	3.878	1.218	(31.4%)	2.660	(68.6%)	279	151	(54.1%)	128	(45.9%)
Denmark (DK)	2.463	605	(24.6%)	1.858	(75.4%)	146	101	(69.2%)	45	(30.8%)
United Kingdom (UK)	957	140	(14.6%)	817	(85.4%)	59	23	(39.0%)	36	(61.0%)
Spain (ES)	791	260	(32.9%)	531	(67.1%)	61	42	(68.9%)	19	(31.1%)
Poland (PL)	524	67	(12.8%)	457	(87.2%)	13	6	(46.2%)	7	(53.8%)
Belgium (BE)	410	103	(25.1%)	307	(74.9%)	35	15	(42.9%)	20	(57.1%)
Republic of Ireland (IRL)	394	99	(25.1%)	295	(74.9%)	24	15	(62.5%)	9	(37.5%)
Slovakia (SK)	199	23	(11.6%)	176	(88.4%)	4	3	(75.0%)	1	(25.0%)
Serbia (SER)	39	4	(10.3%)	35	(89.7%)	3	2	(66.7%)	1	(33.3%)
Portugal (PT)	33	6	(18.2%)	27	(81.8%)	3	2	(66.7%)	1	(33.3%)
Luxembourg (LU)	25	2	(8.0%)	23	(92.0%)	2	1	(50.0%)	1	(50.0%)
Austria (AT)	23	0	N/A	23	(100.0%)	4	0	N/A	4	(100.0%)
Sweden (SE)	20	0	N/A	20	(100.0%)	1	0	N/A	1	(100.0%)
La Réunion (REU)	19	0	N/A	19	(100.0%)	1	0	N/A	1	(100.0%)
Switzerland (CH)	3	2	(66.7%)	1	(33.3%)	1	1	(100.0%)	0	(0%)
Σ	18.313	5.580	(30.5%)	12.733	(69.5%)	2.457	1.390	(56.6%)	1.067	(43.4%)

Samples were screened with an influenza-generic, M-gene-specific RT-qPCR (Henritzi et al., 2019). "Consignments" consisted of up to 20 samples per farm and were considered positive if at least a single IAV-positive sample was detected. See also Tables S1, S2, and S7.

Henritzi et al. 2020 Cell Host & Microbe

What do we need for such an analysis?
2. A proxy for the prevalence of swIAV per country

Final linear model:

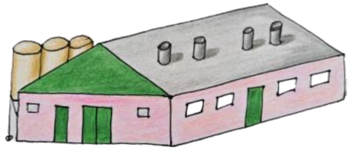
- Farm-level prevalence ~ farmsize * number of farms
  - Adj. $R^2 = 0.53$!
- Predicted prevalence in Italy and Austria (and other EU countries)
- Random subsampling based on proportional rule: N = 721 H1 sequences

EUROSTAT databases

What do we need for such an analysis?

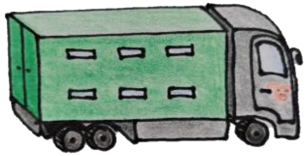
3. Covariates that may be associated with swIAV spread

Pig population



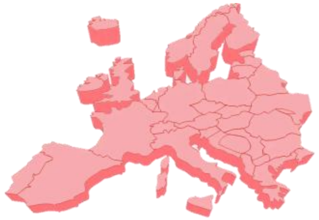
- Number of pigs per km^2
- Number of farms
- Farm size

Trade



- Live pig trade of different categories of pigs
- Trade of pork

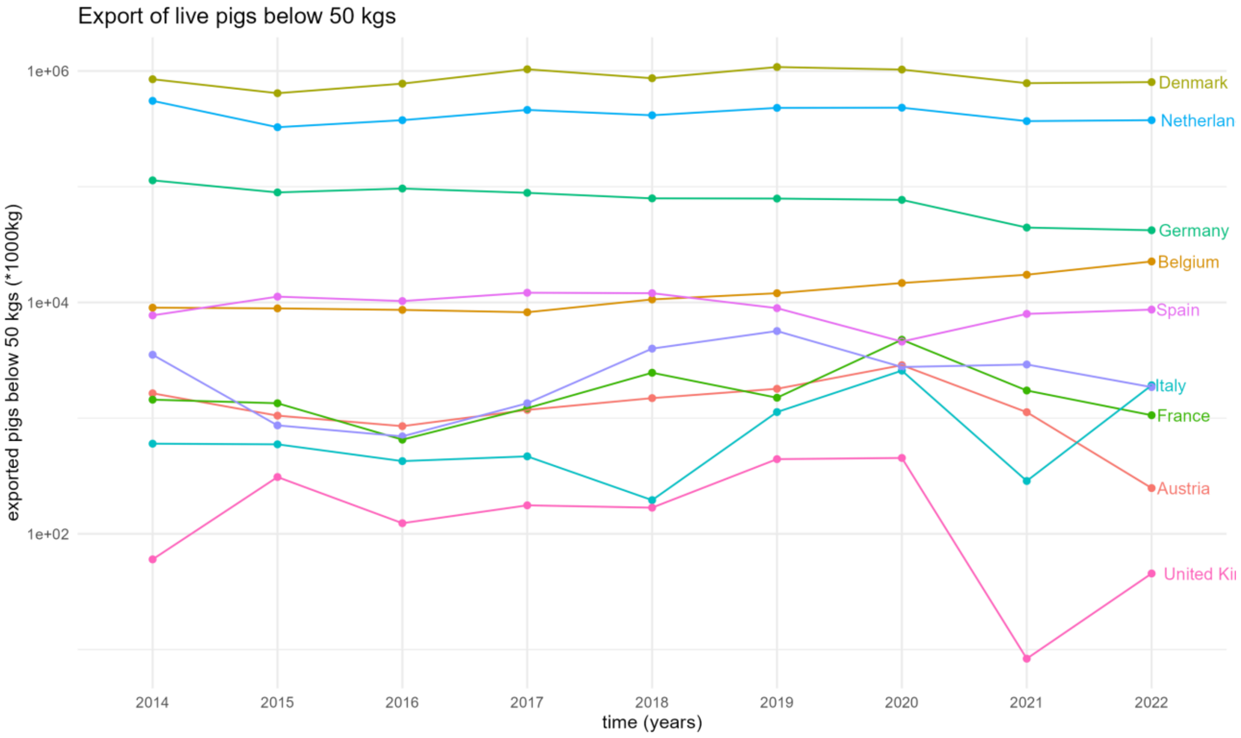
Geographics



- Shared borders
- Distance between countries
- Distance between densely pig-populated areas

Live pig trade (between 10 countries with sufficient sequences)

Breeding
pigs weighing <50 kg
Pigs weighing >50 kg



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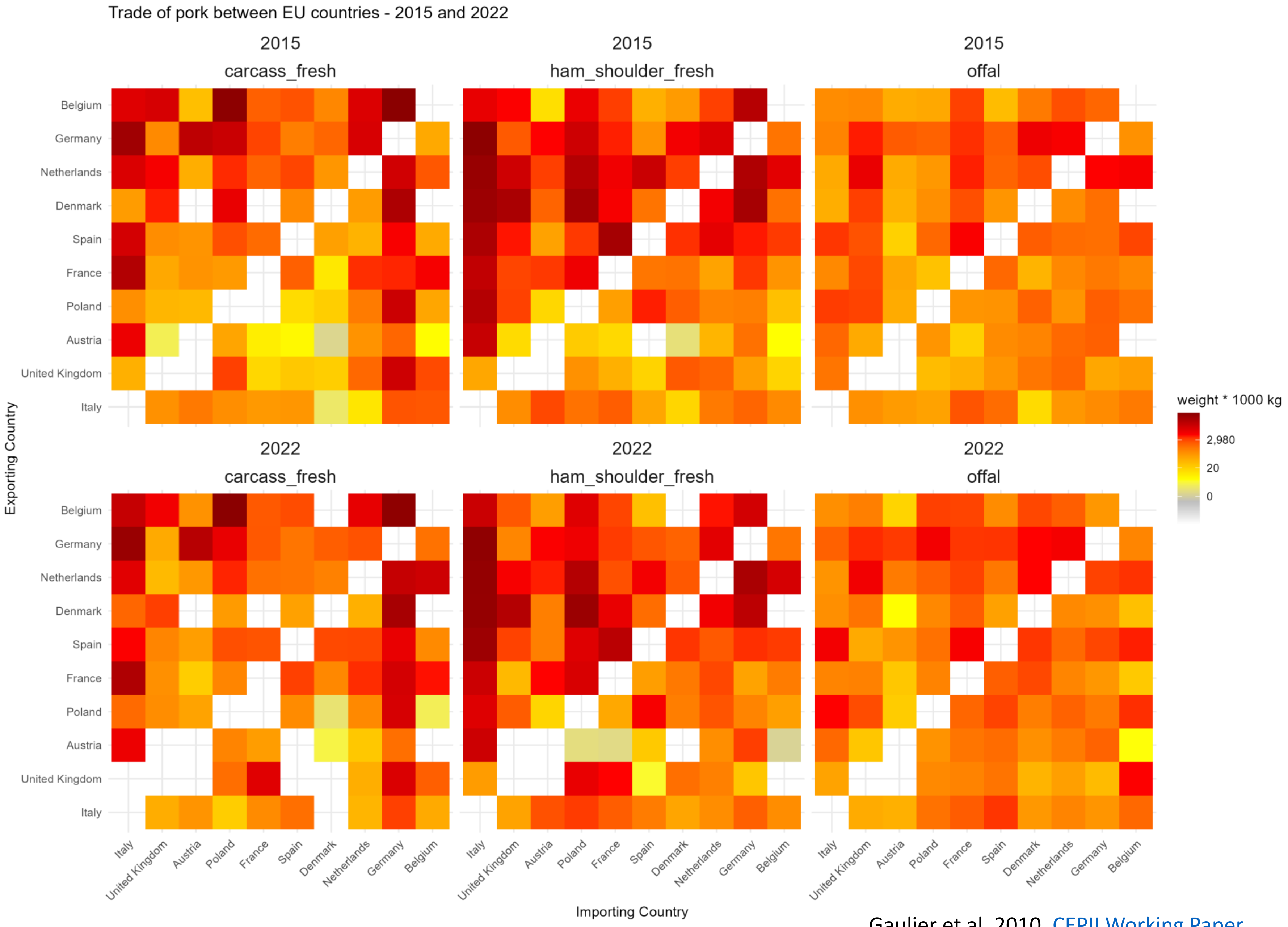
Gaulier et al. 2010, [CEPII Working Paper]

Pork trade

Which pork could lead to transitions of lineages?

- Fresh meat
- Organ meat (offal)

Trade between almost all 10 countries!



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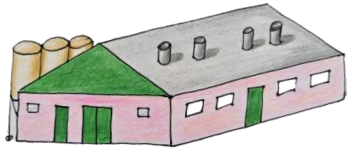
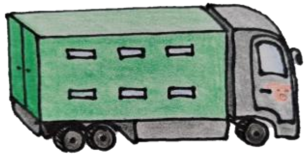
Pig population



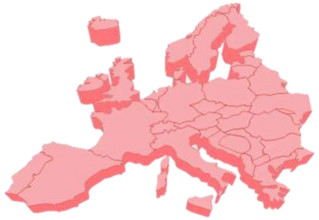
- Number of pigs
- ~~Number of farms~~
- Farm size

Trade



- Trade of breeding pigs + pigs < 50 kg
- Trade of offal (organ meat)

Geographics



- Shared borders
- ~~Distance between countries~~
- Distance between densely pig-populated areas

What do we need for such an analysis?

4. Learn how to 'tame the BEAST'

- Bayesian analysis – clear tutorials on priors, modeling choices and interpretation

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Contents

Phylogeographic diffusion in discrete space

Summary: This chapter provides a step-by-step tutorial on reconstructing the spatial dispersal and cross-species dynamics of rabies virus (RABV) in North American bat populations based on a set of 372 nucleoprotein gene sequences (nucleotide positions: 594–1353). The data set comprises a total of 17 bat species sampled between 1997 and 2006 across 14 states in the United States (Streicker et al., Science, 2010, 329, 676-679). Following Faria et al. (Phil. Trans. Roy. Soc. B, 2013), two additional species that had been excluded from the original analysis owing to a limited amount of available sequences, *Myotis austroriparius* (Ma) and *Parastrellus hesperus* (Ph), are also included here. We also include a viral sequence with an unknown sampling date (accession no. TX5275, sampled in Texas from *Lasiurus borealis*), which will be adequately accommodated in our inference. The aim of this tutorial is to estimate the ancestral locations of the virus using a Bayesian discrete phylogeographic approach and, at the same time, infer the history of host jumping using the same model approach. Using an extension of the discrete diffusion model, we will then test the factors that underly the host transition dynamics.

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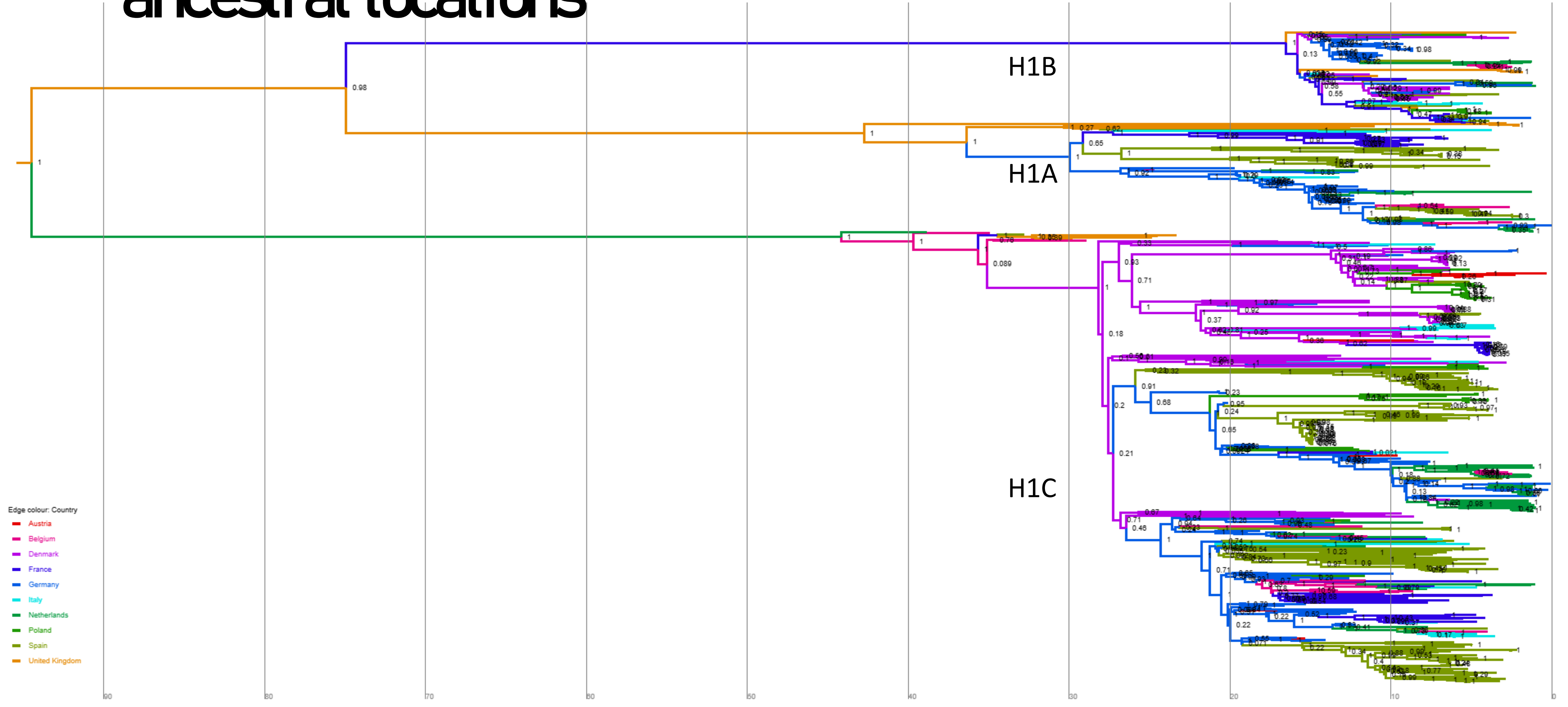
Results: Maximum clade credibility tree with predicted ancestral locations



Results: Covariate live pig trade strongly associated

- Predictors with low posterior inclusion probability
  - Distance between swine-dense region centroids
  - Sharing borders
  - Number of pigs in destination country
  - Farm size
  - Pork trade

- Live pig trade: Inclusion probability of 0.997 to 1 in all models for all gene segments
  Bayes factor > 1000

Discussion

- Quantify number of branching events within vs. between locations?
- Repeat with different random subselection
- Do not forget that many EU countries were not included

- Where are the traded pigs going? How to prevent spread?

Future outlook

- Include reassortment in the phylo-analyses
- Study effect of vaccination practices: Higher vaccination coverage – less spread within and between country?
- Human-to-pig transmission

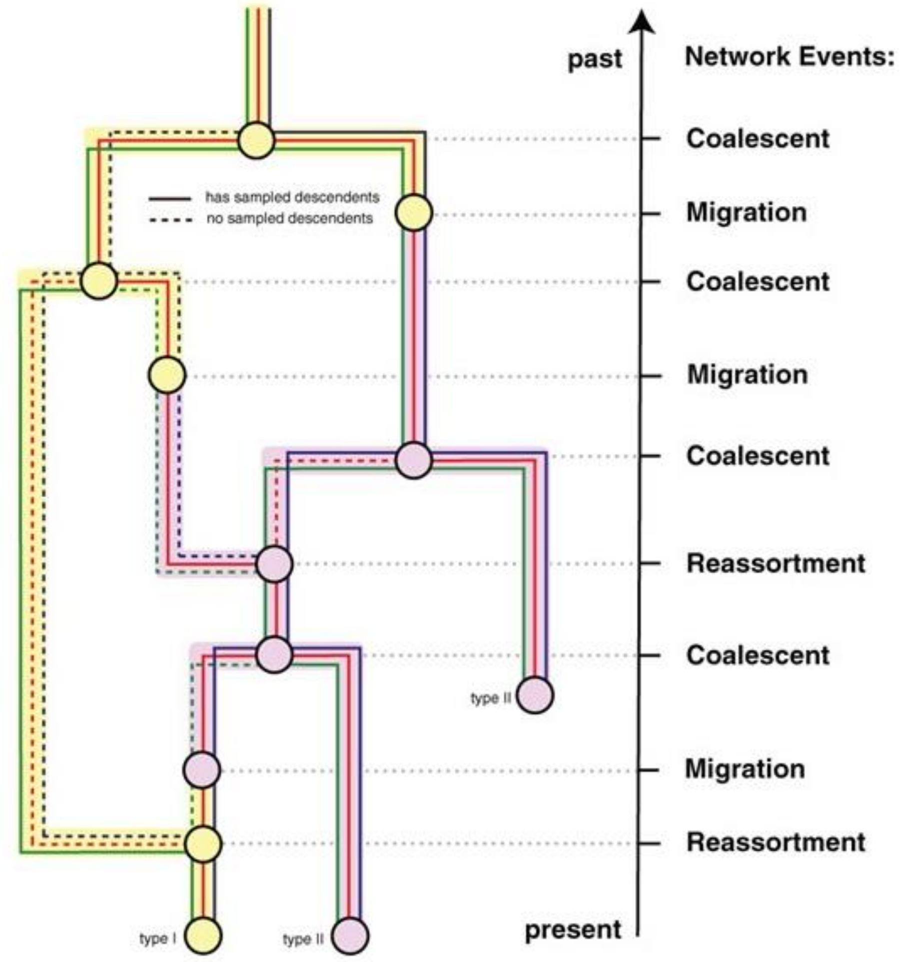


Fig. 5. Example SCoRe network. Network has two types (subpopulations) and three samples: one of type I (light yellow) and two of type II (light purple). It tracks the evolution of three genetic segments, denoted by green, red, and blue and, besides sampling, can have three kinds of events: reassortment, migration, and coalescent. Segments that are not ancestral to our samples are shown in dashed lines. Time increases into the past.

Ugnė Stolz, Tanja Stadler, Nicola F Müller et al.2022. Joint Inference of Migration and Reassortment Patterns for Viruses with Segmented Genomes, [Molecular Biology and Evolution,,]()

Thanks for listening!

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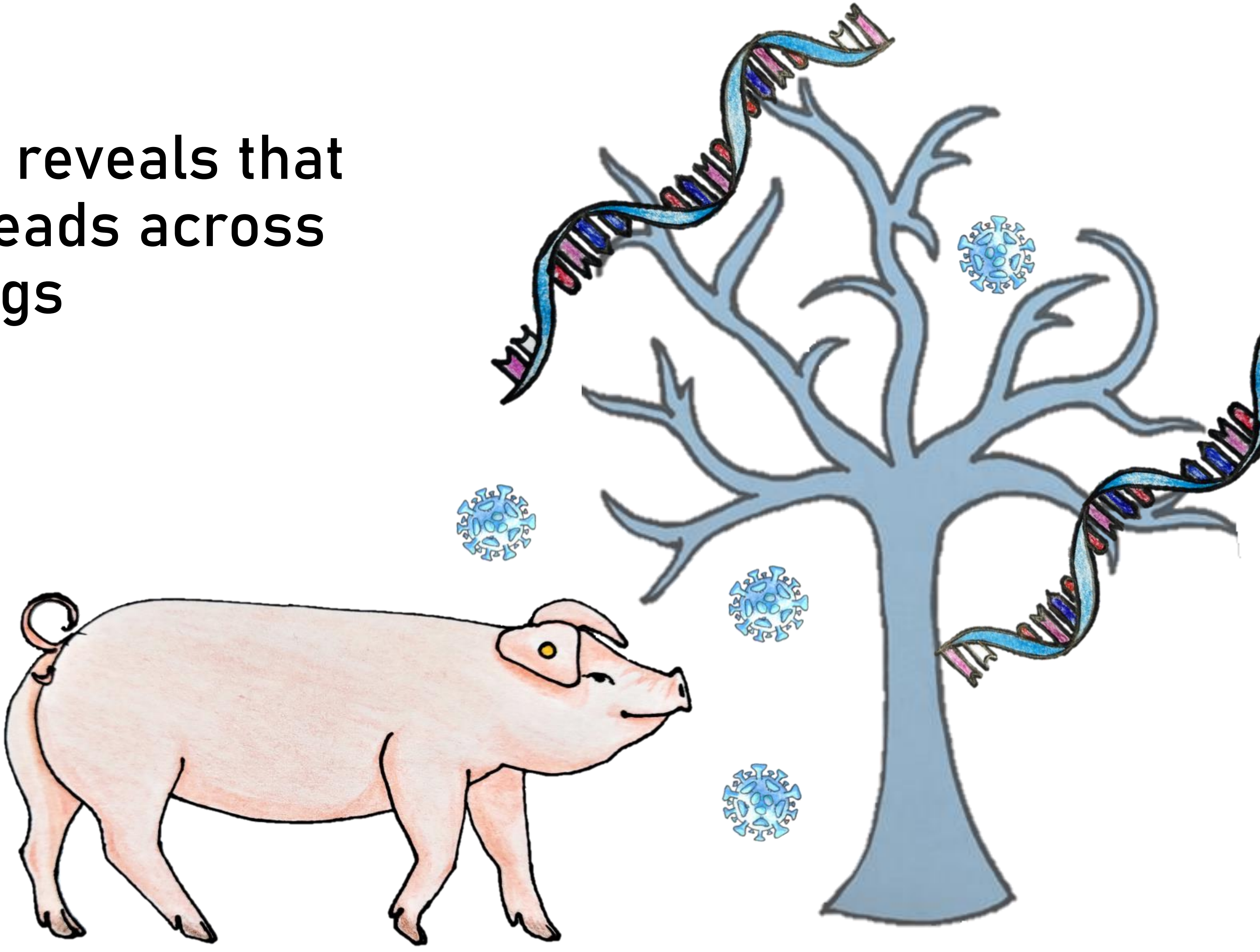

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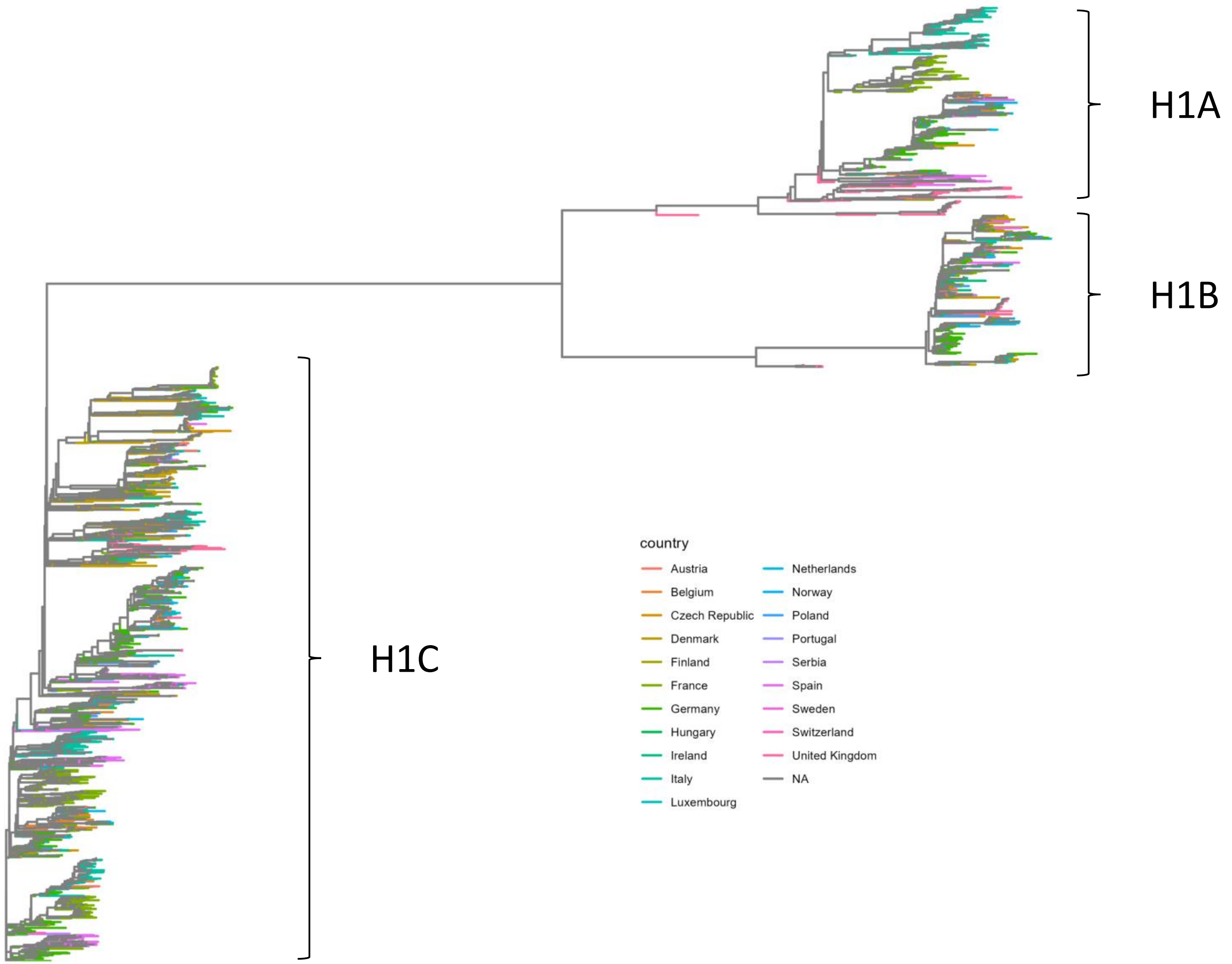
SIV HA Maximum Likelihood tree and temporal signal

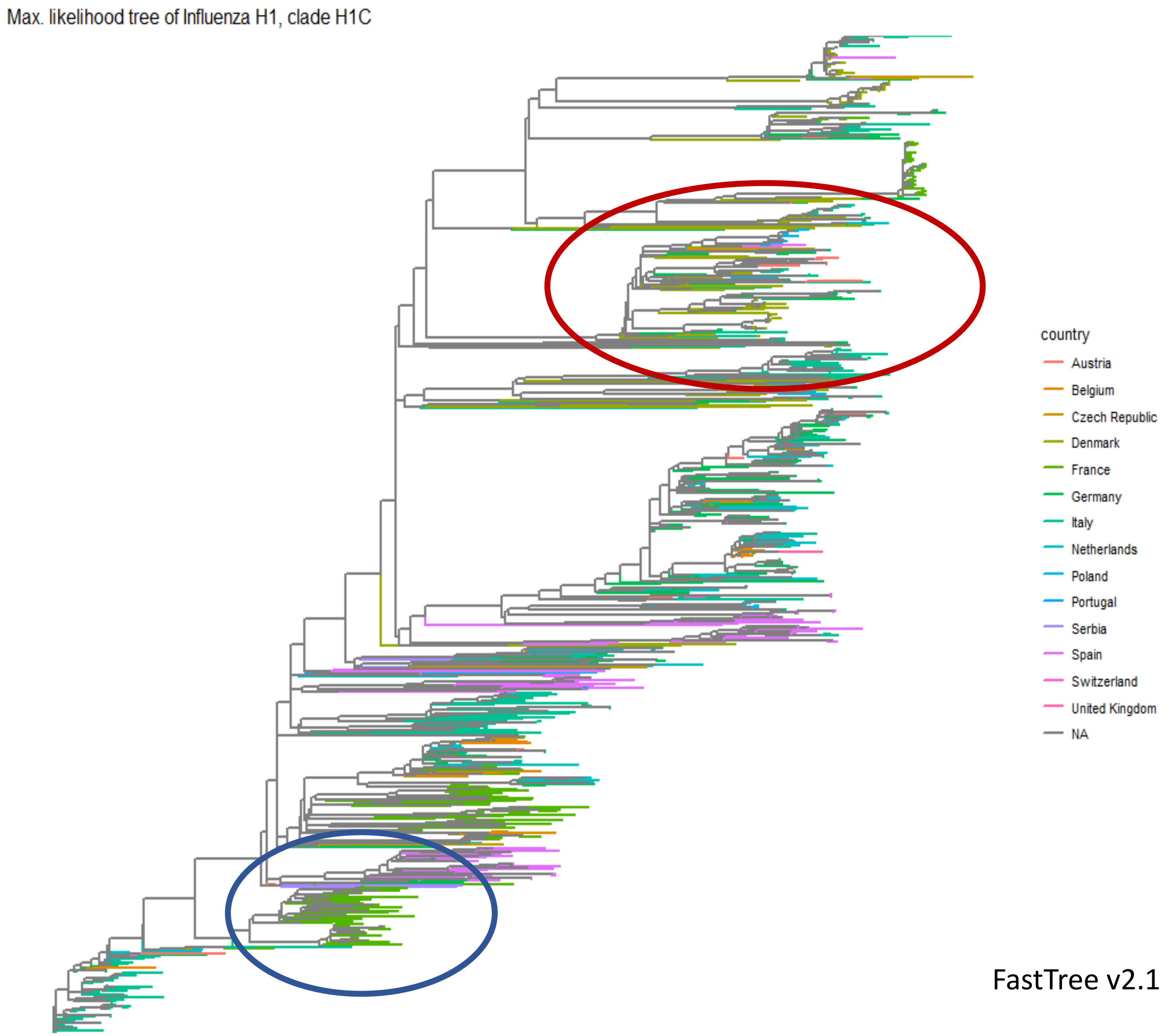


FastTree v2.1 (GTR+g) – TempEst v1.5.3

SIV HA Maximum Likelihood tree and temporal signal

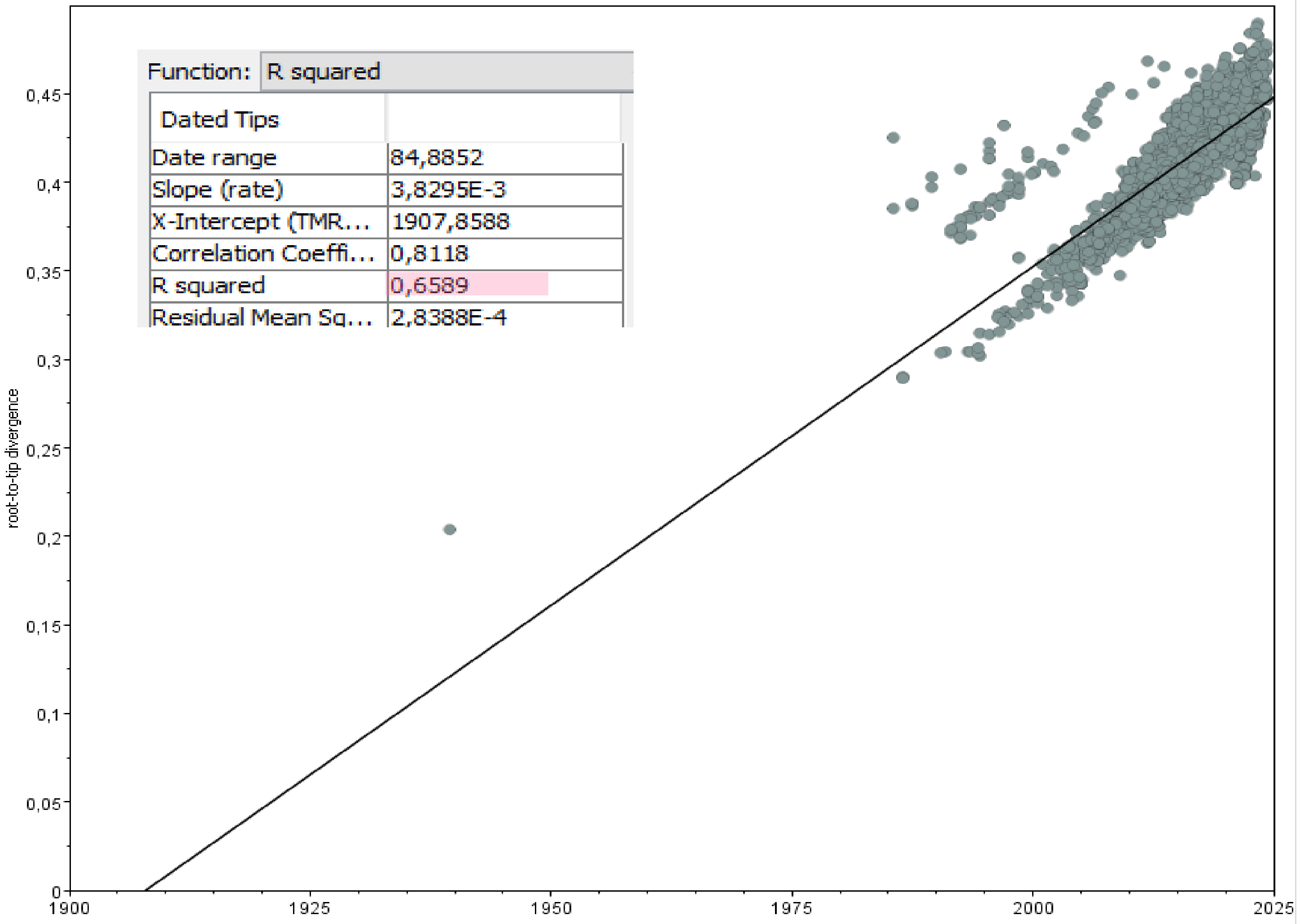
H1A

H1C



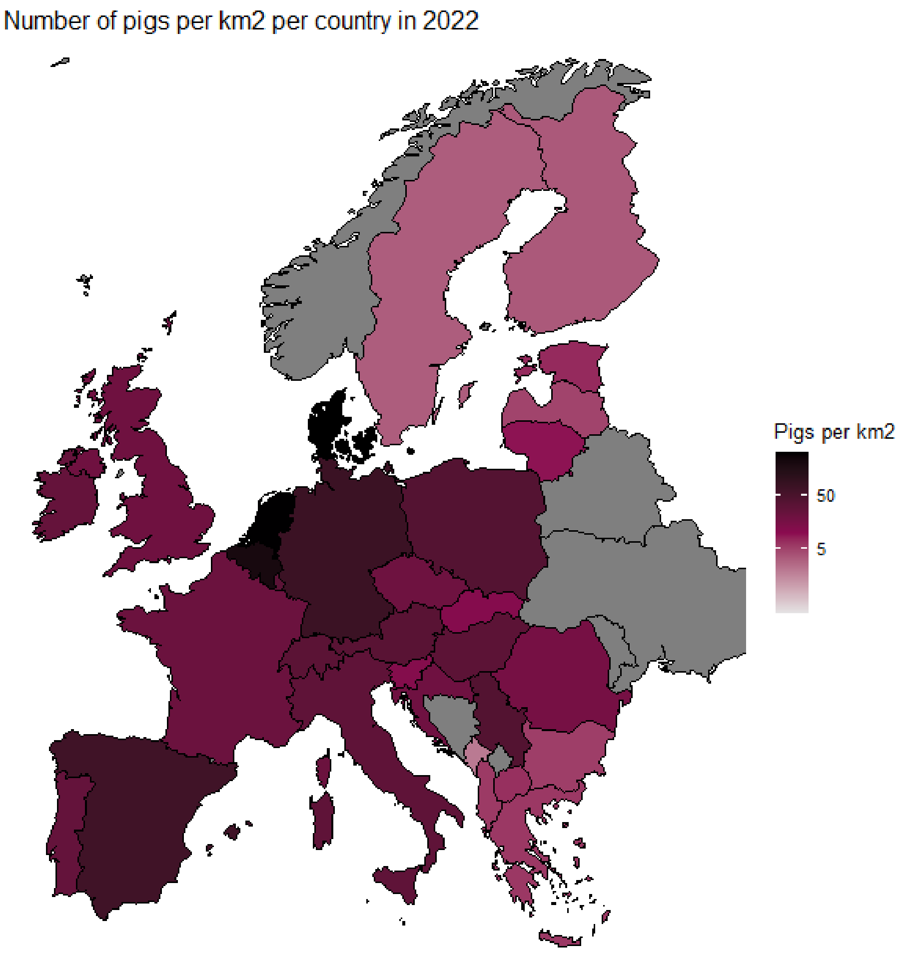
FastTree v2.1

SIV HA Maximum Likelihood tree and temporal signal



FastTree v2.1 (GTR+g) – TempEst v1.5.3

Pig population in Europe



Farm size of commercial farms

Ranges from 11 (Serbia) to 4509 (Denmark) and continues to increase over the years…

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Eurostat - Pig population - annual data [apro_mt_lspig__custom_12819695]