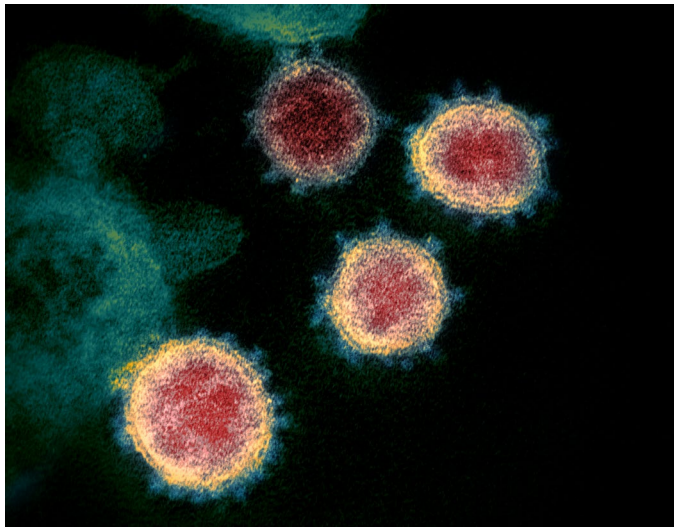
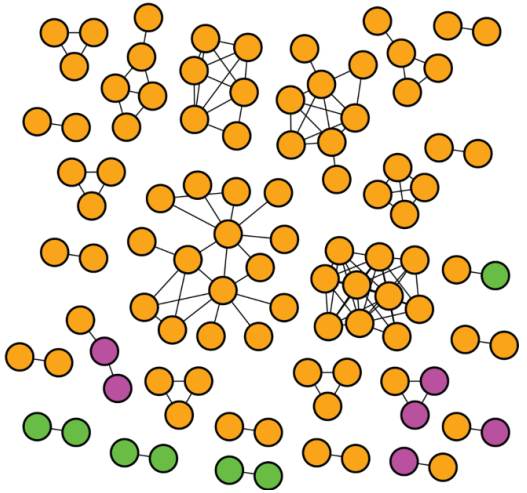
Whole Genome Sequence Analysis of SARS-CoV-2

- Insights and Challenges





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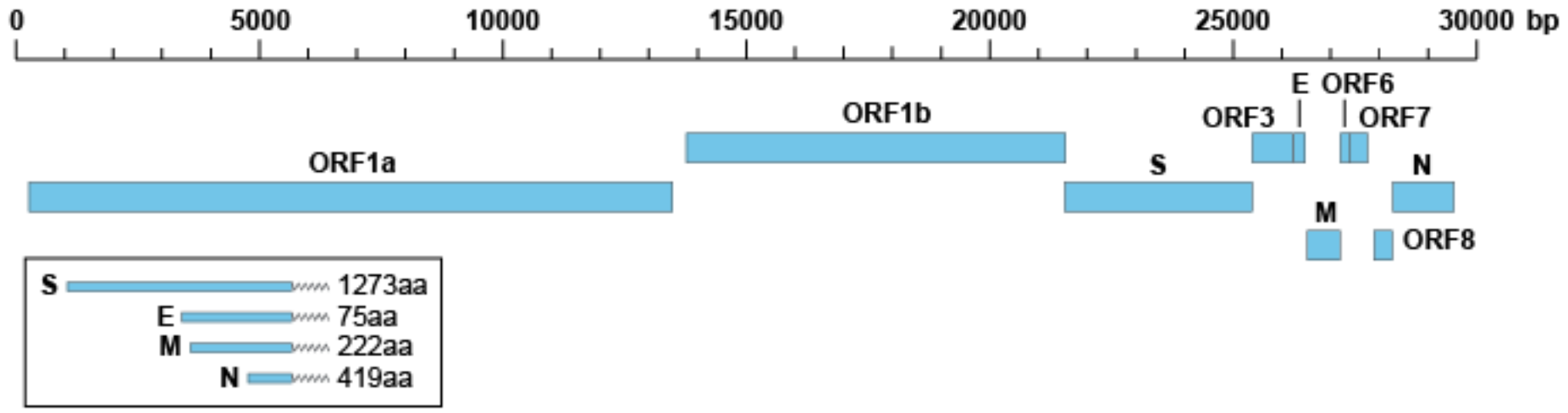
Department of Mathematics, SFU

The SARS-CoV-2 genome

- Positive single-stranded RNA genome
- Genome shares ~82% sequence identity with SARS-CoV and MERS-CoV
- ~30,000bp sequence – Wuhan-Hu-1 reference strain 29,903bp



https://www.novusbio.com/support/sars-cov-research-resources

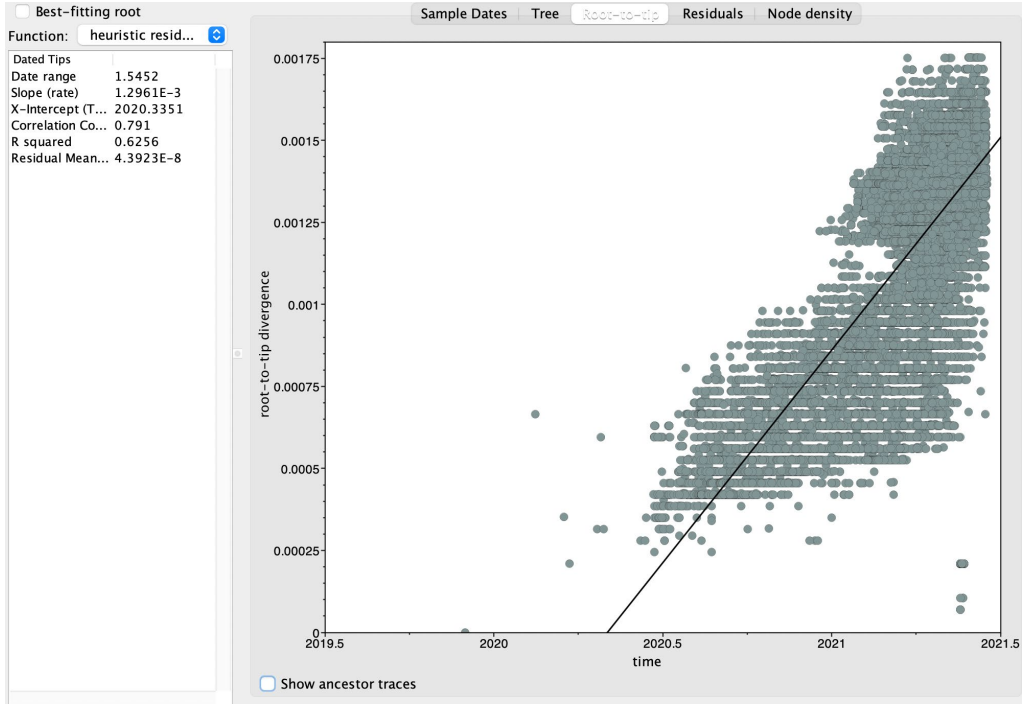
SARS-CoV-2 evolution

- Fast mutation rate (~$8x10^{-4}$ substitutions/site/year Boni *et. al.* 2020) – though relatively slow for an RNA virus
  - HIV (~$5x10^{-3}$ substitutions/site/year)
  - Influenza (~$4x10^{-3}$ substitutions/site/year)

- No evidence for mutation rate differences between lineages

- Large number of isolates and fast mutation rate has led to emergence of a number of lineages

- Multiple convergent mutations including many in the spike protein

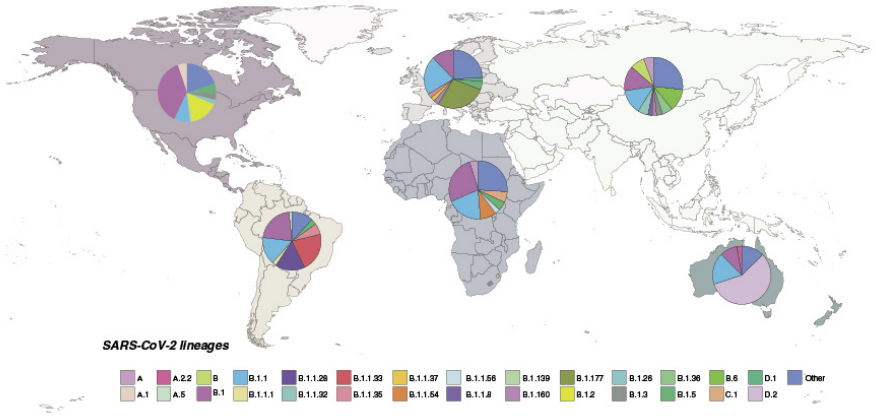


What can we learn from genomic analysis of SARS-CoV-2?

- First whole genome sequence - Wuhan-Hu-1 - published early in the pandemic (3rd February 2020)

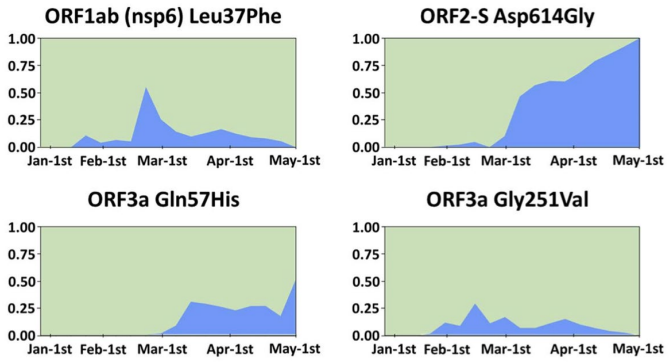
- GISAID initiative holds 2,232,941 SARS-CoV-2 whole genome sequences (as of 5th July 2021)

Lineage assignment



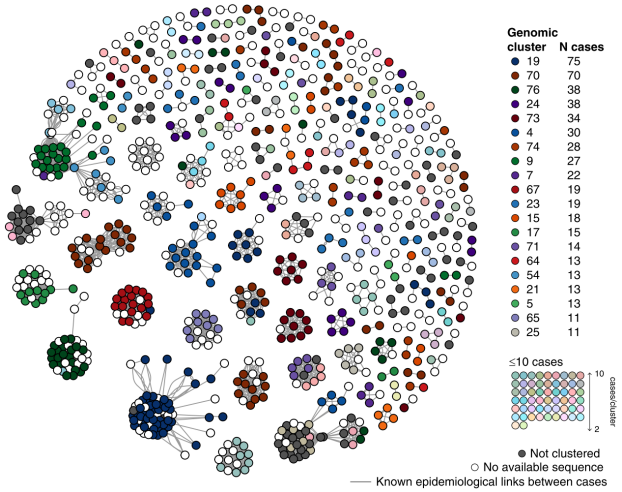
Cella *et. al.* 2021

Mutation detection



Cortey *et. al.* 2020

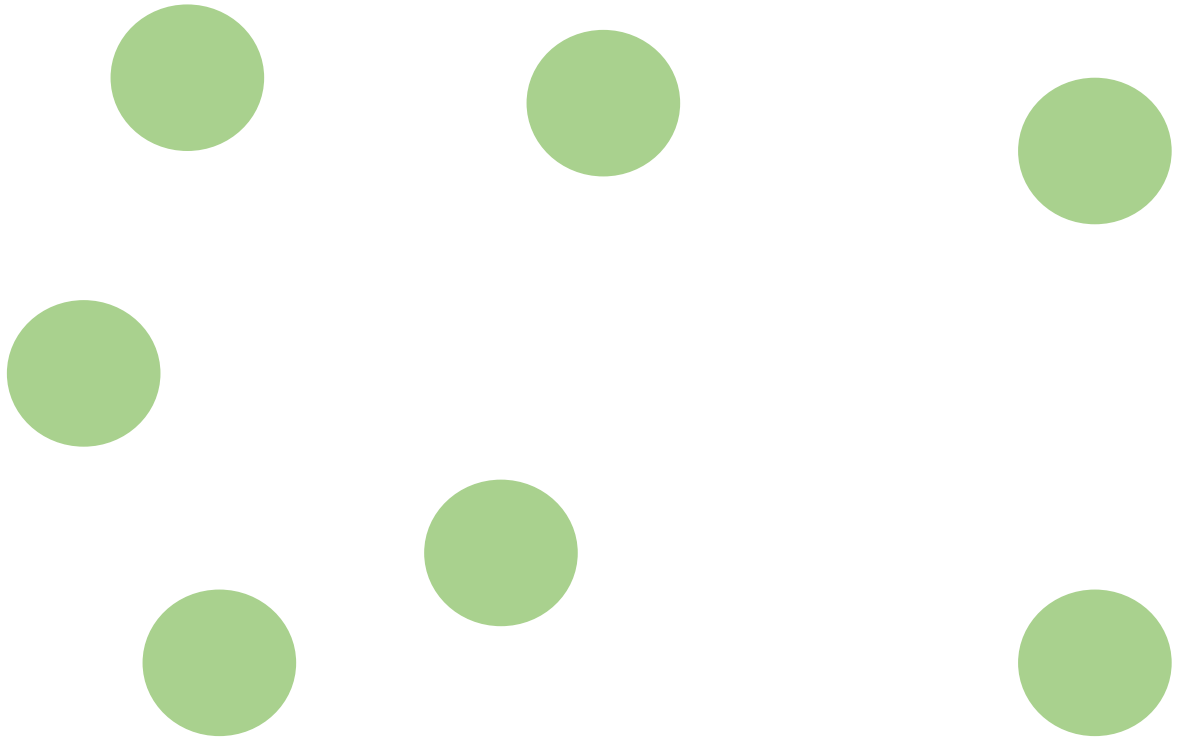
Clustering



Seemann *et. al.* 2020

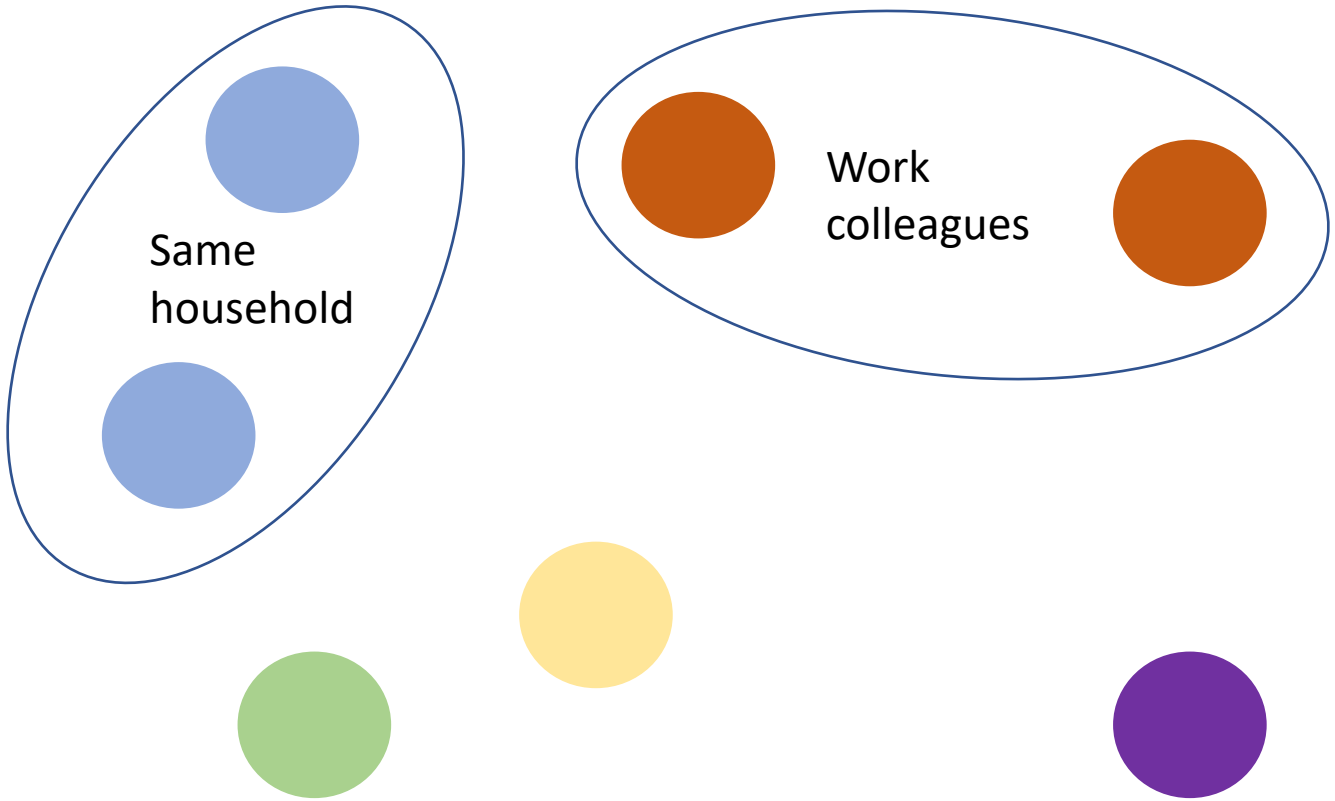
Clustering of SARS-CoV-2 cases

- Traditional epidemiological approach – contact tracing



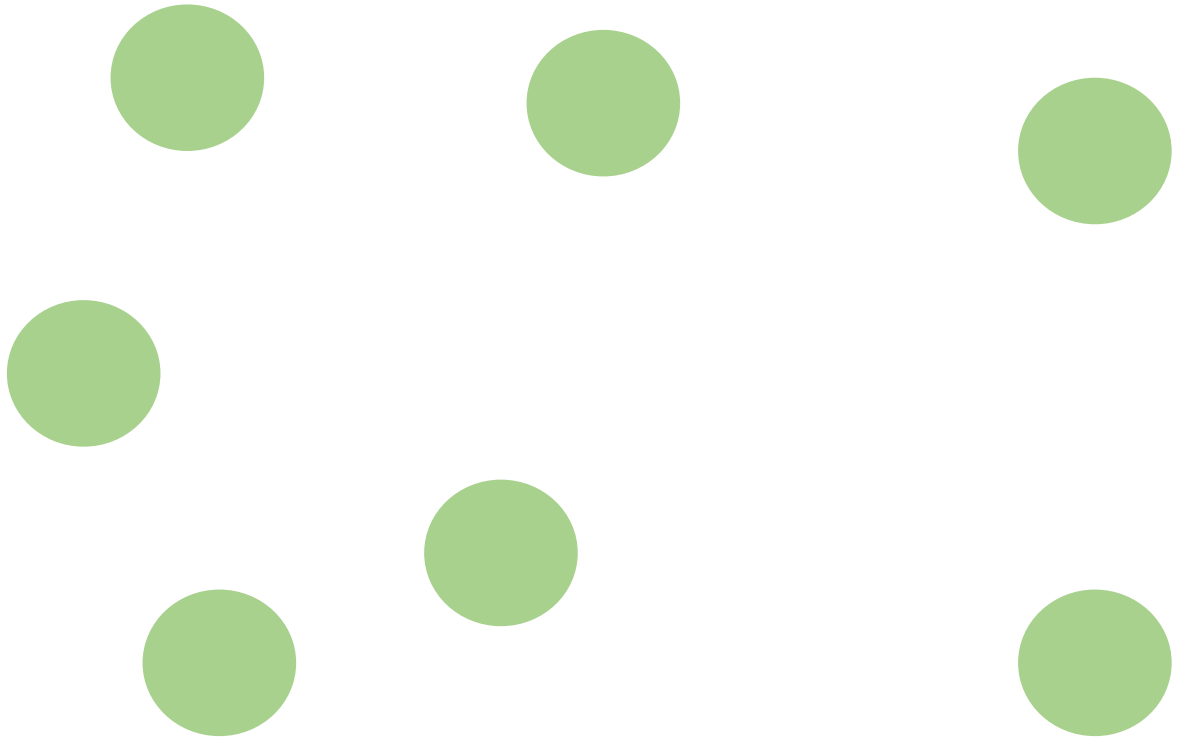
Clustering of SARS-CoV-2 cases

- Conduct questionnaire – 48-hour contacts, household members etc.



Clustering of SARS-CoV-2 cases

- Simple genomic approach – SNP threshold



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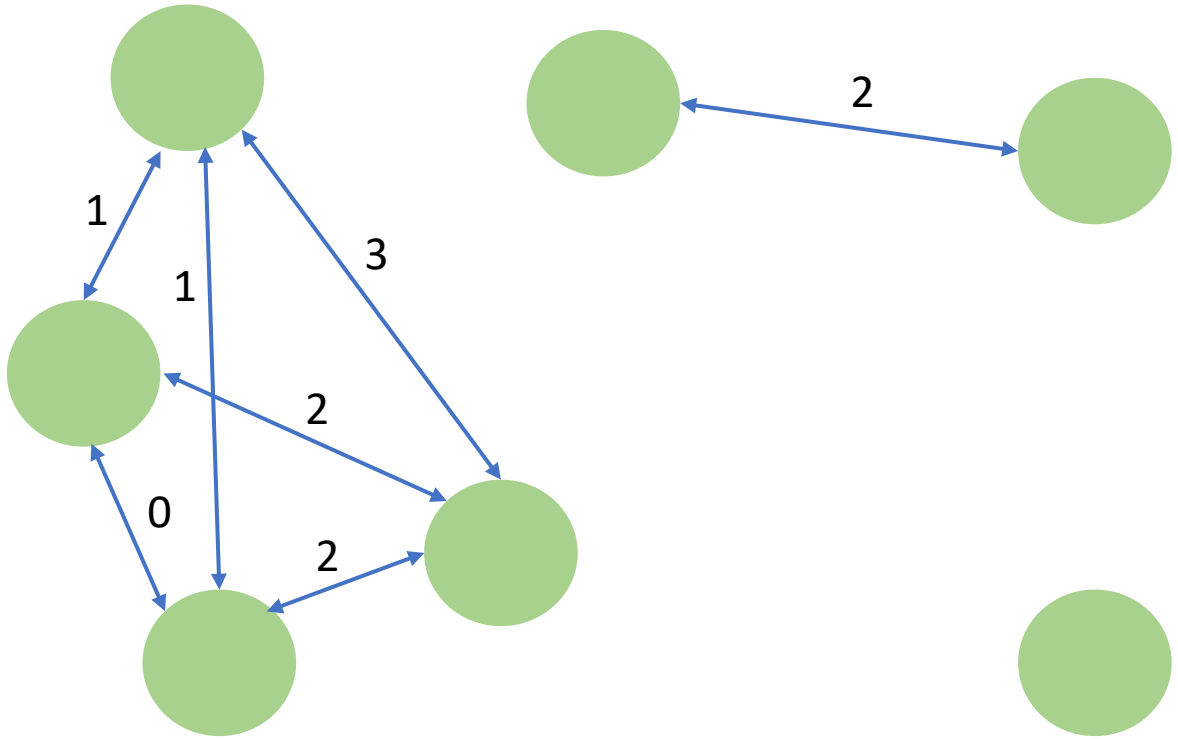
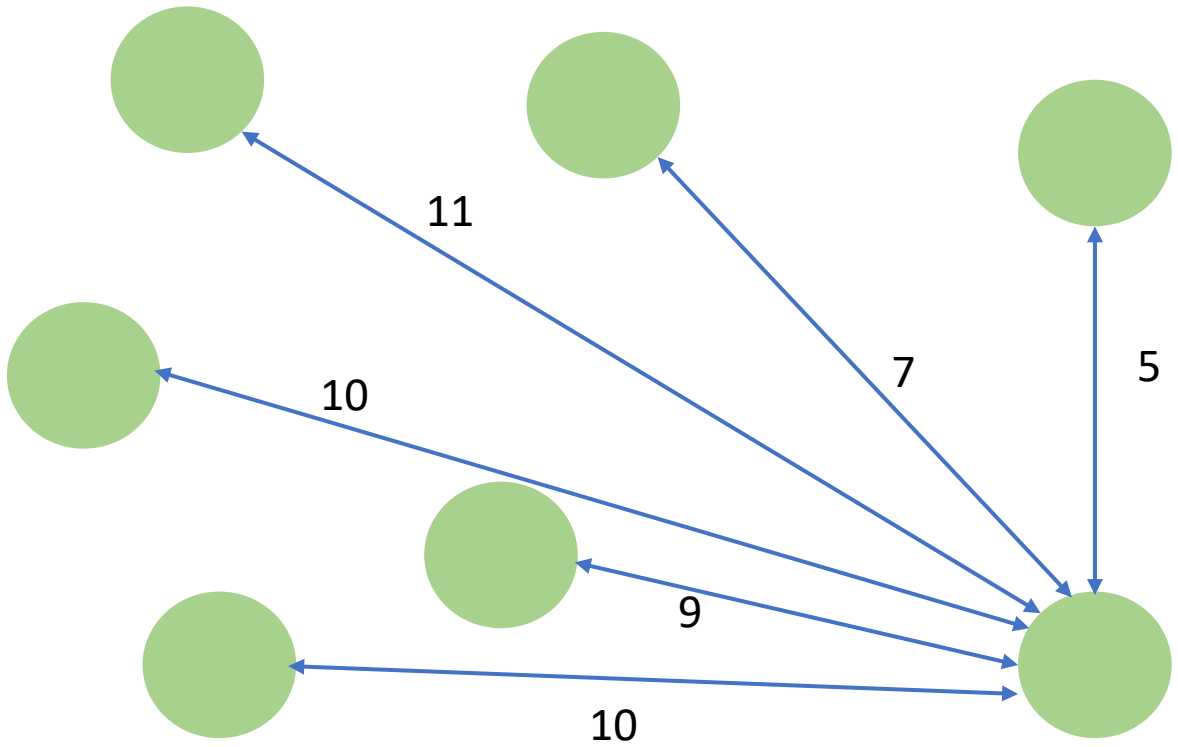


Threshold
2 SNPs

Clustering of SARS-CoV-2 cases

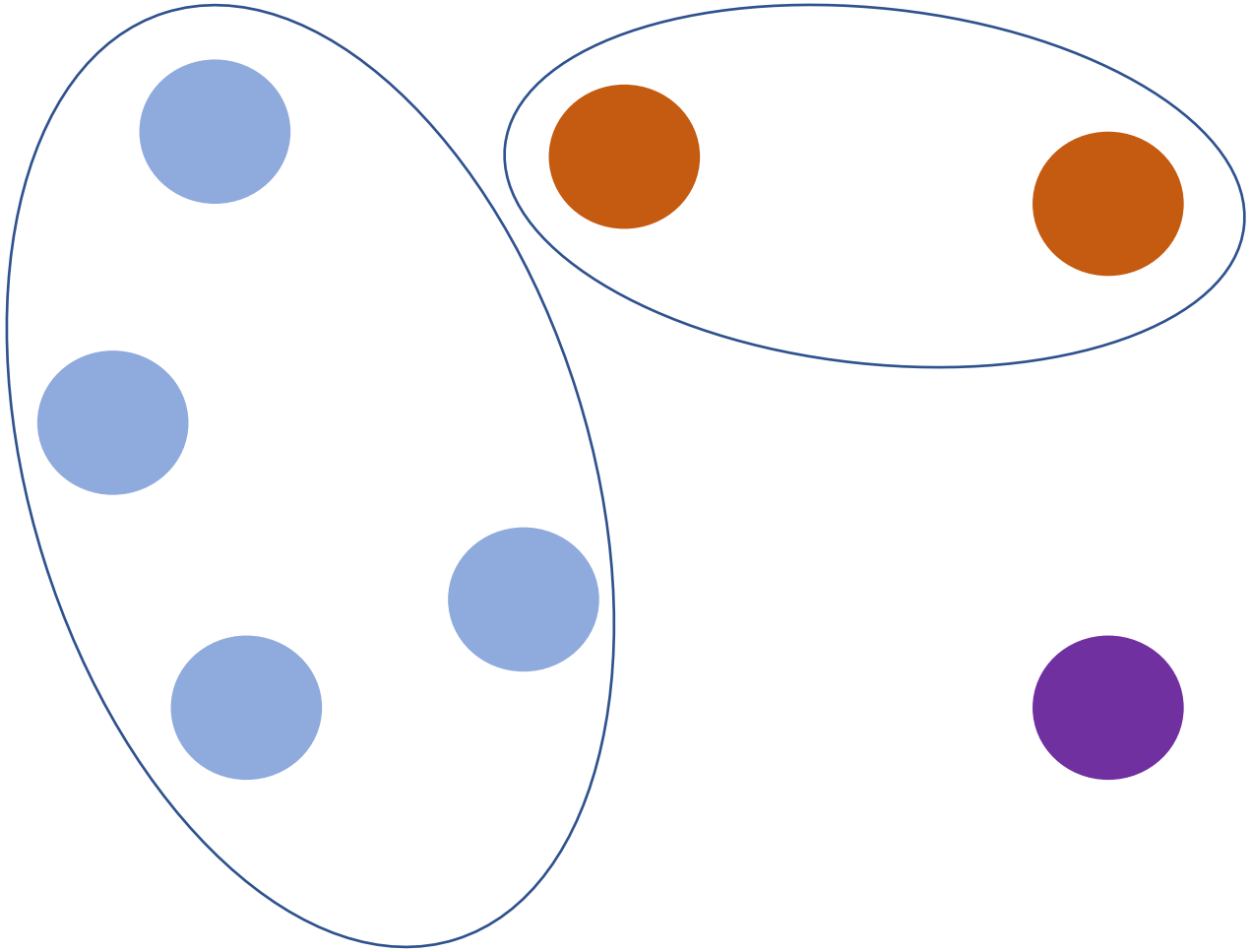
- Simple genomic approach – SNP threshold



Threshold
2 SNPs

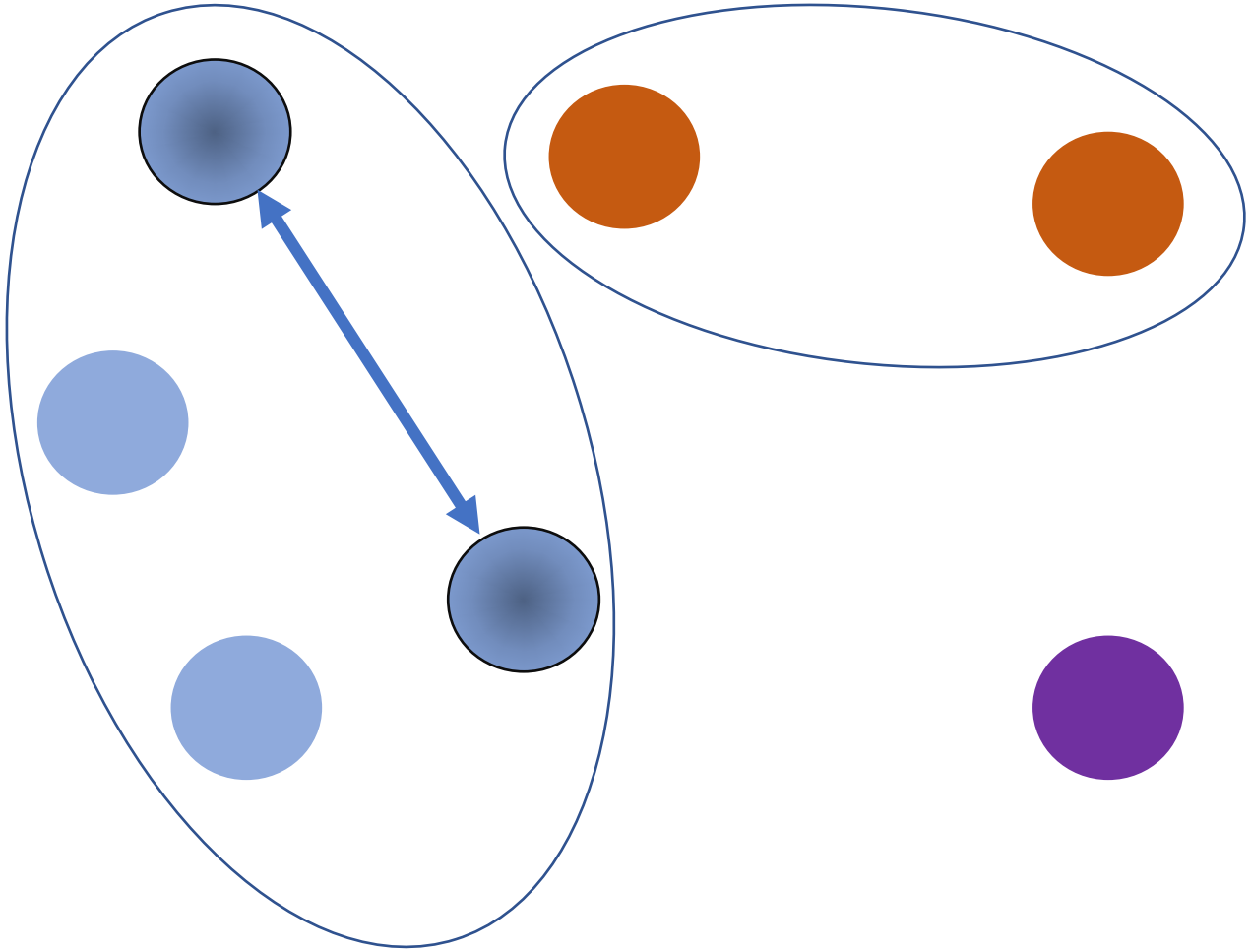
Clustering of SARS-CoV-2 cases

- Link cases under SNP threshold



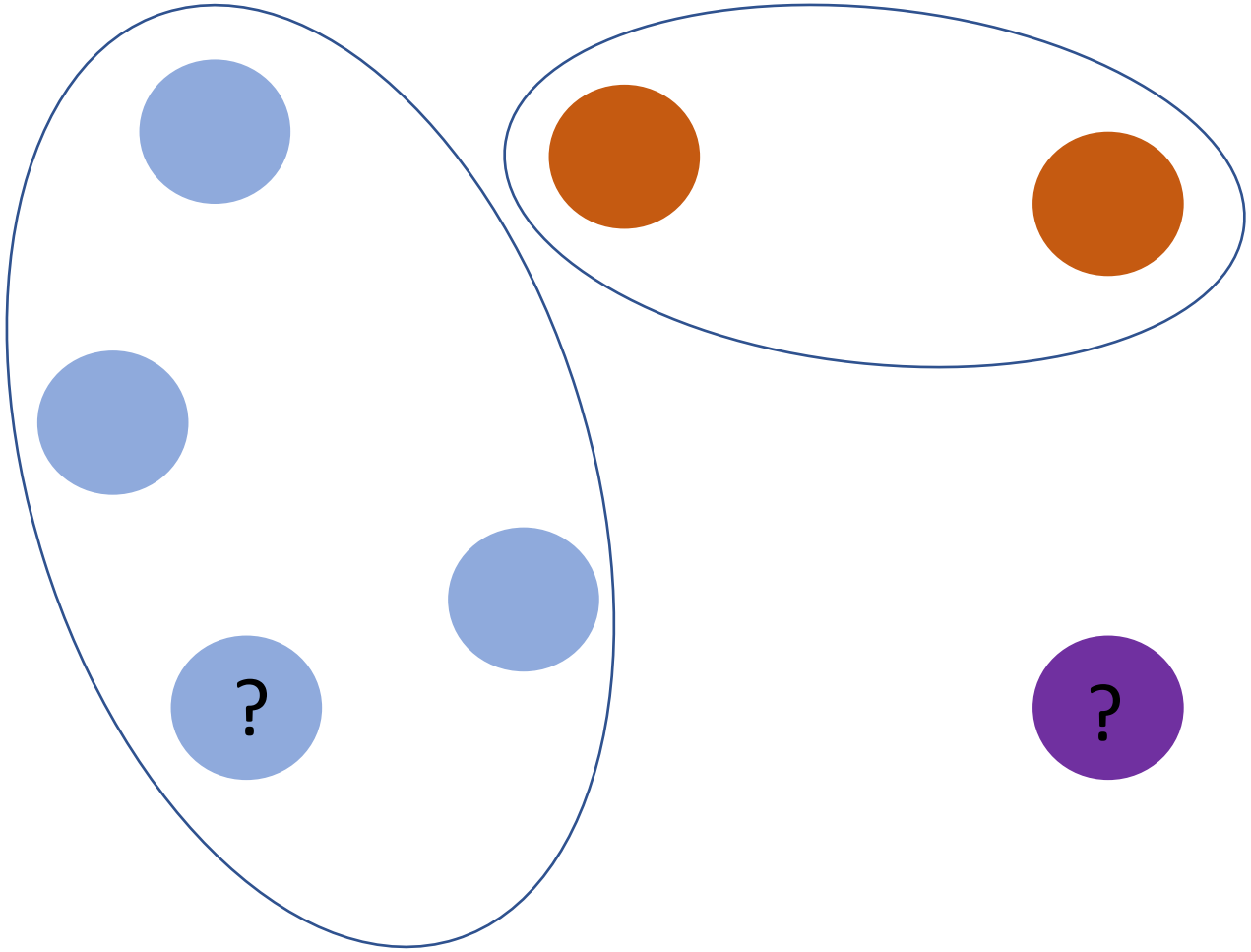
Clustering of SARS-CoV-2 cases

- May find links between cases – same restaurant etc.



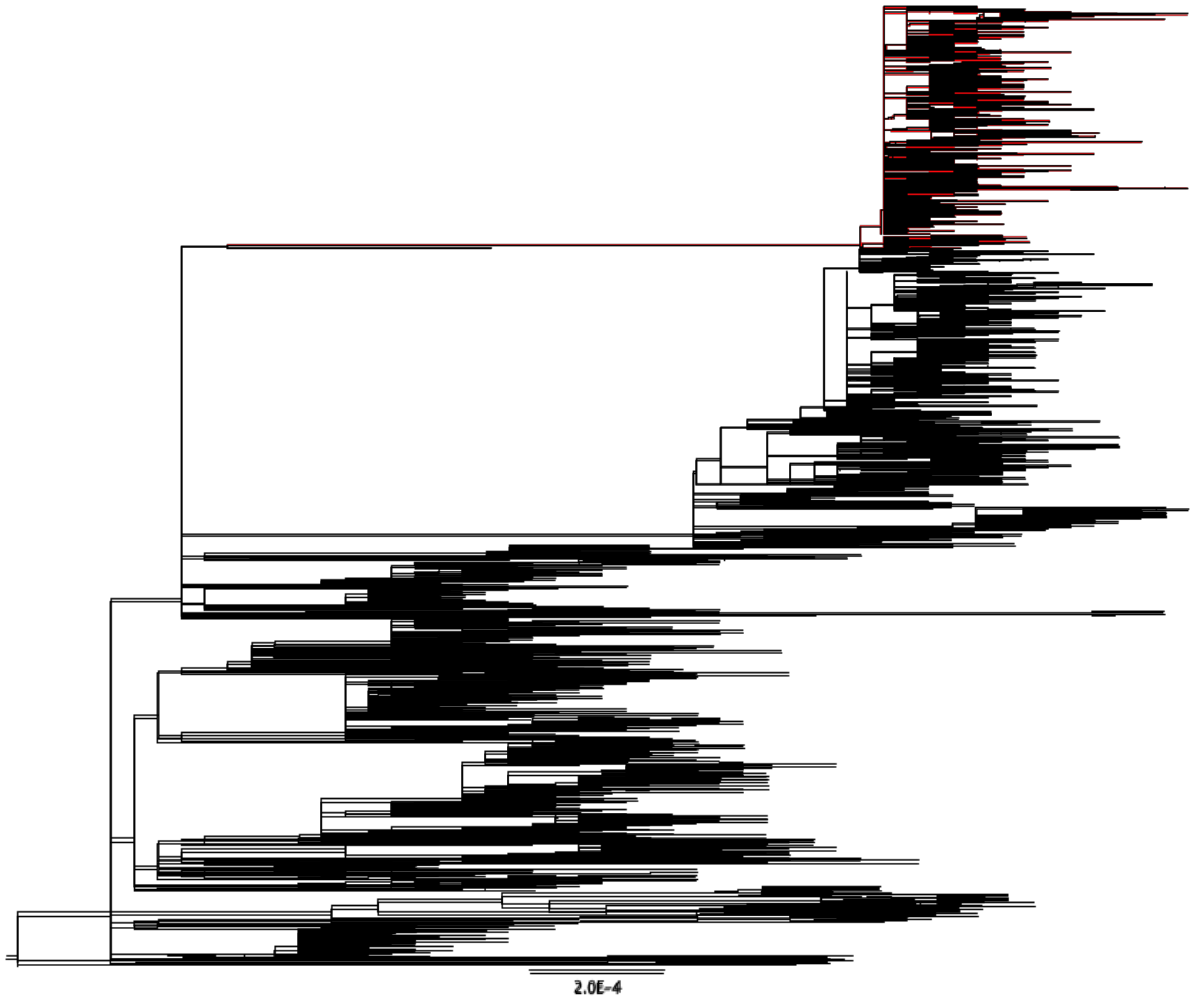
Clustering of SARS-CoV-2 cases

- Still may require some epi investigation but can reduce or direct efforts



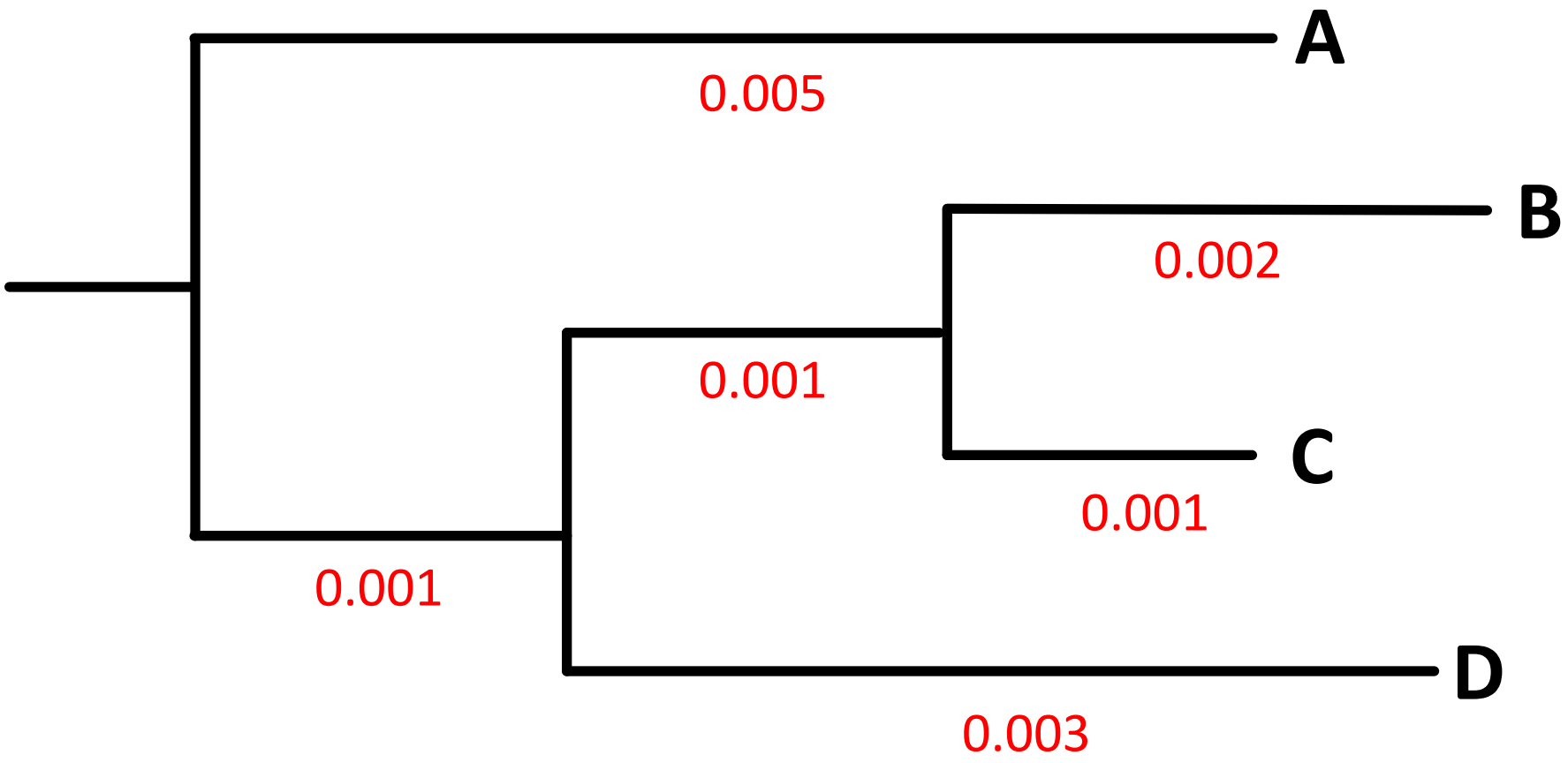
Clustering of SARS-CoV-2 cases using genomics (phylogenetics)

- Maximum likelihood (ML) tree
- Branch length represents genetic diversity
- Uses models of nucleotide substitution to infer evolutionary relationships - not all mutations are the same (transitions vs transversions etc.)



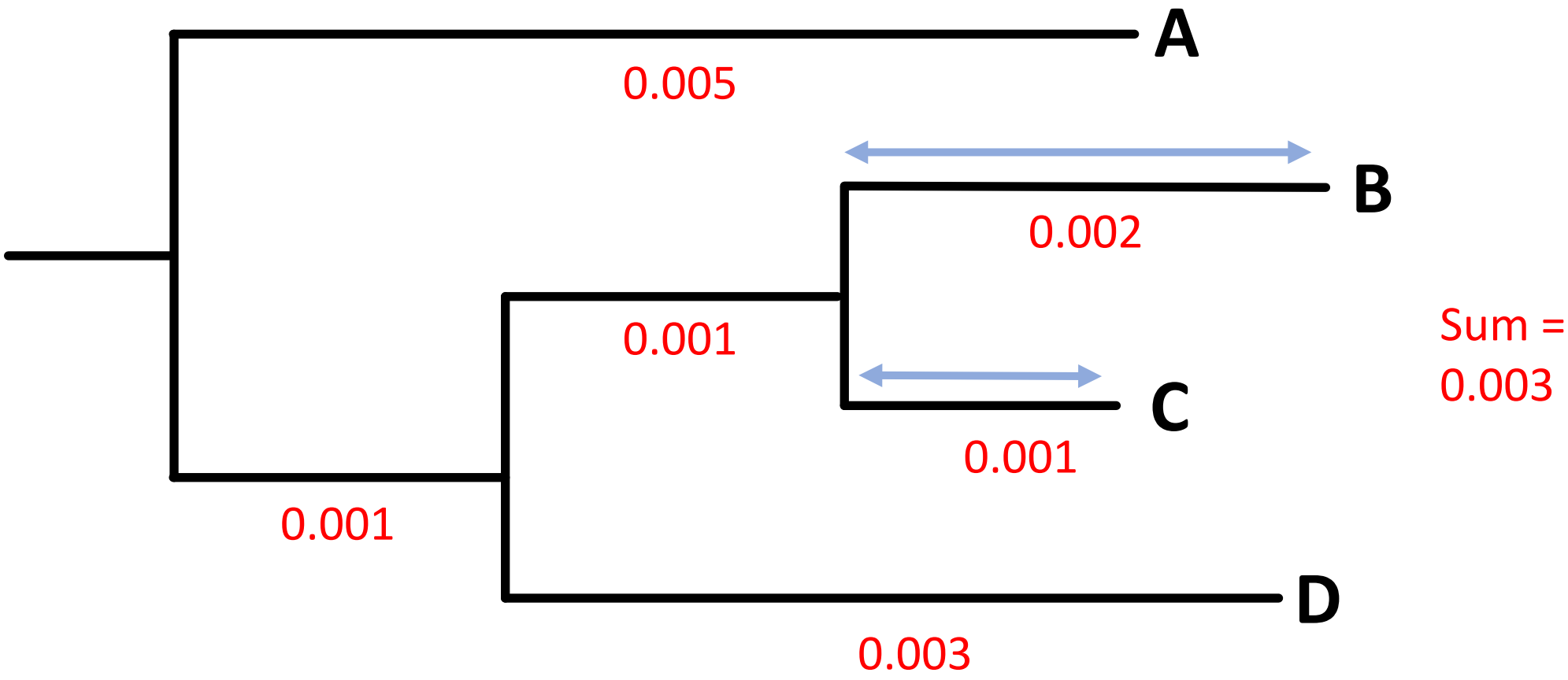
Phylogenetic clustering

- TreeCluster (Balaban et. al. 2020)



Phylogenetic clustering

- TreeCluster (Balaban et. al. 2020) – patristic distance (sum of branch lengths)



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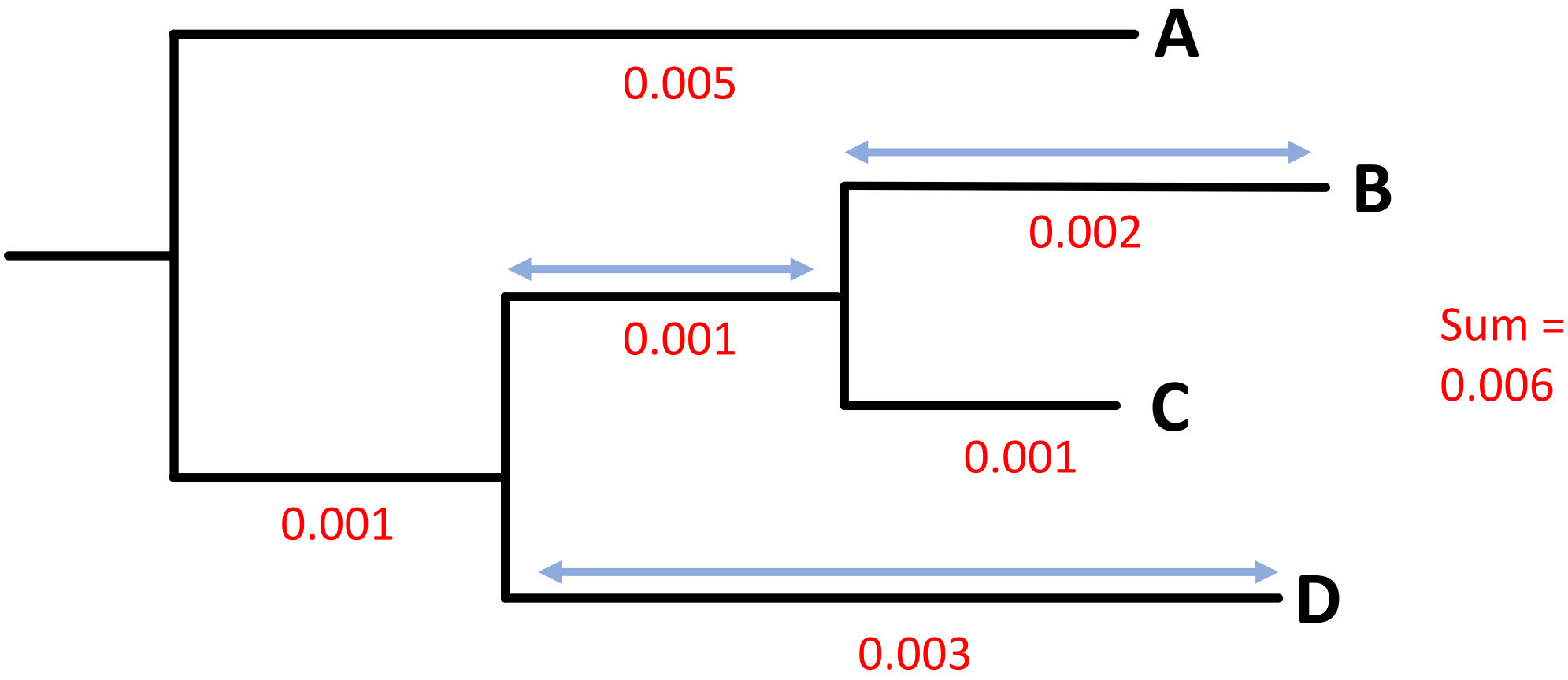

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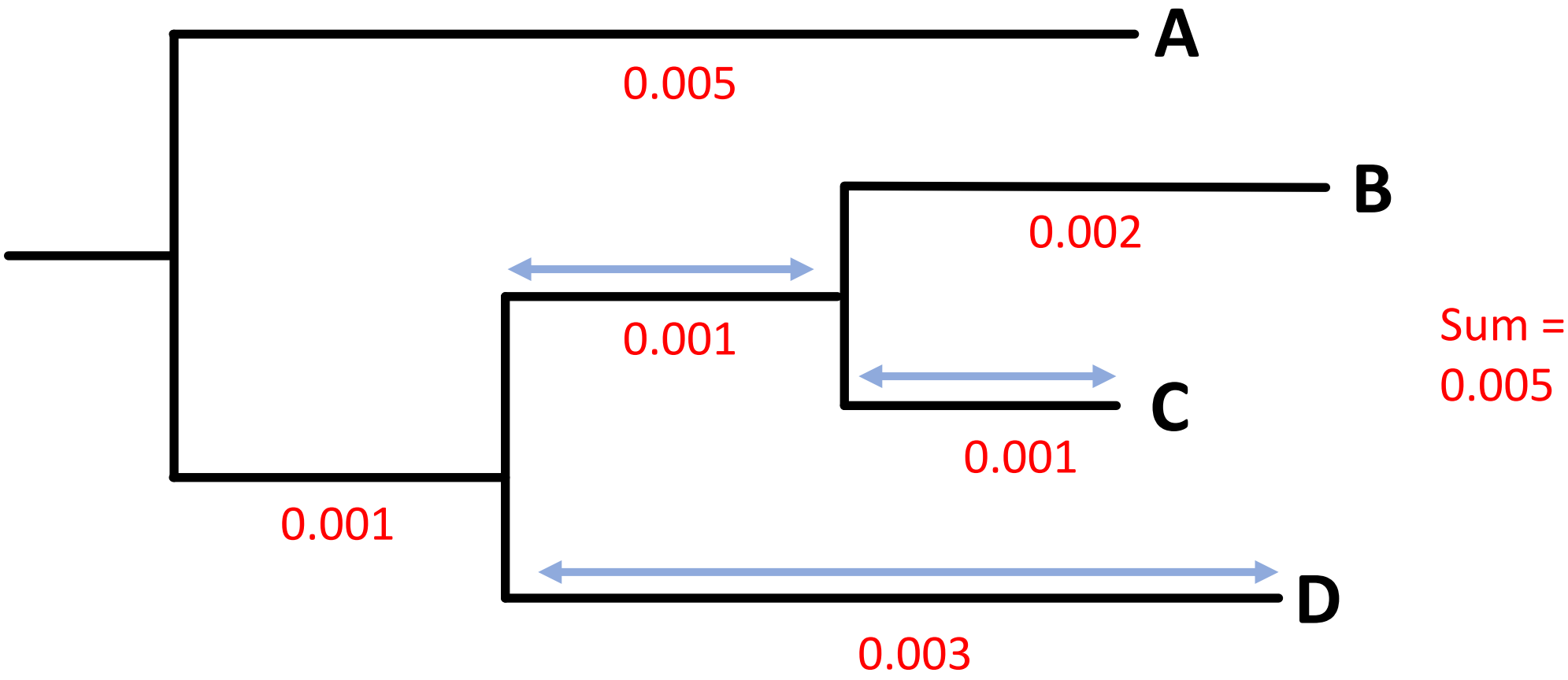

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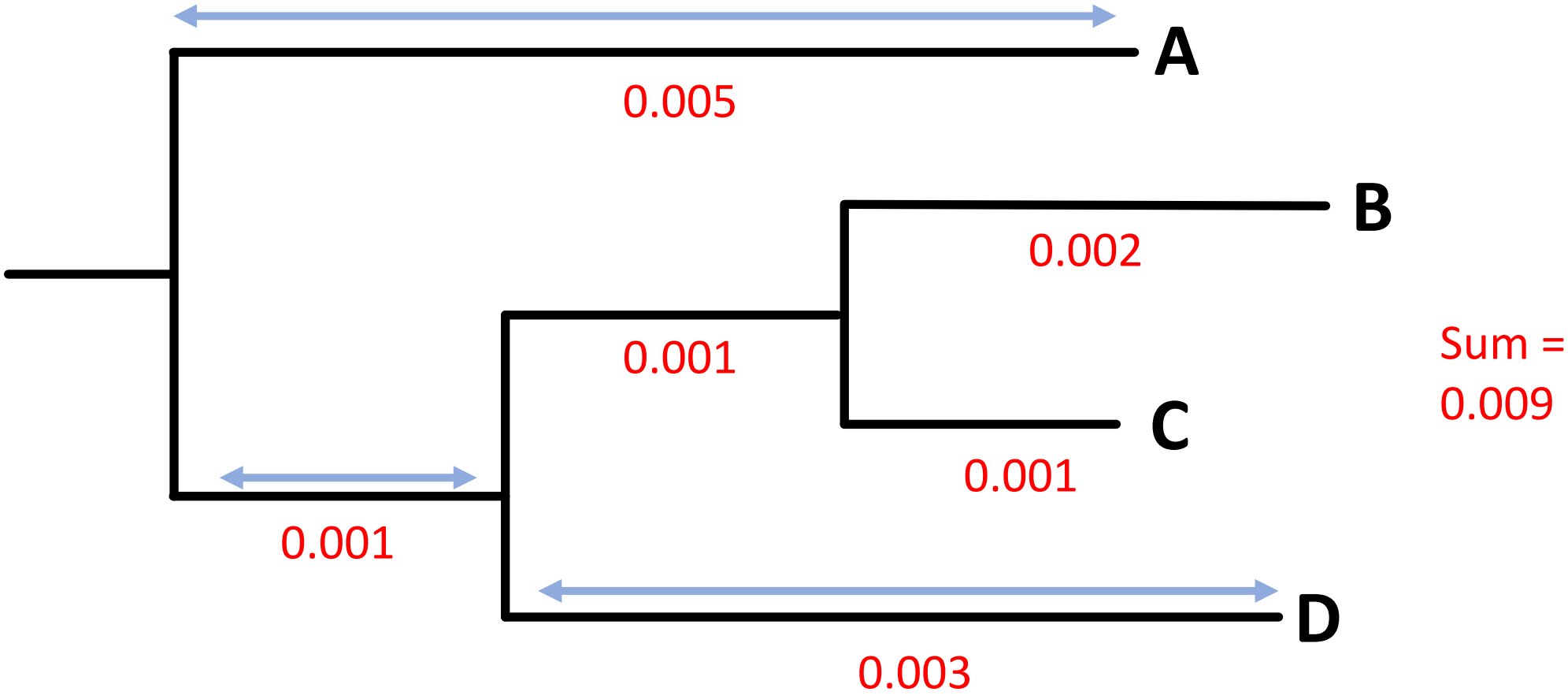
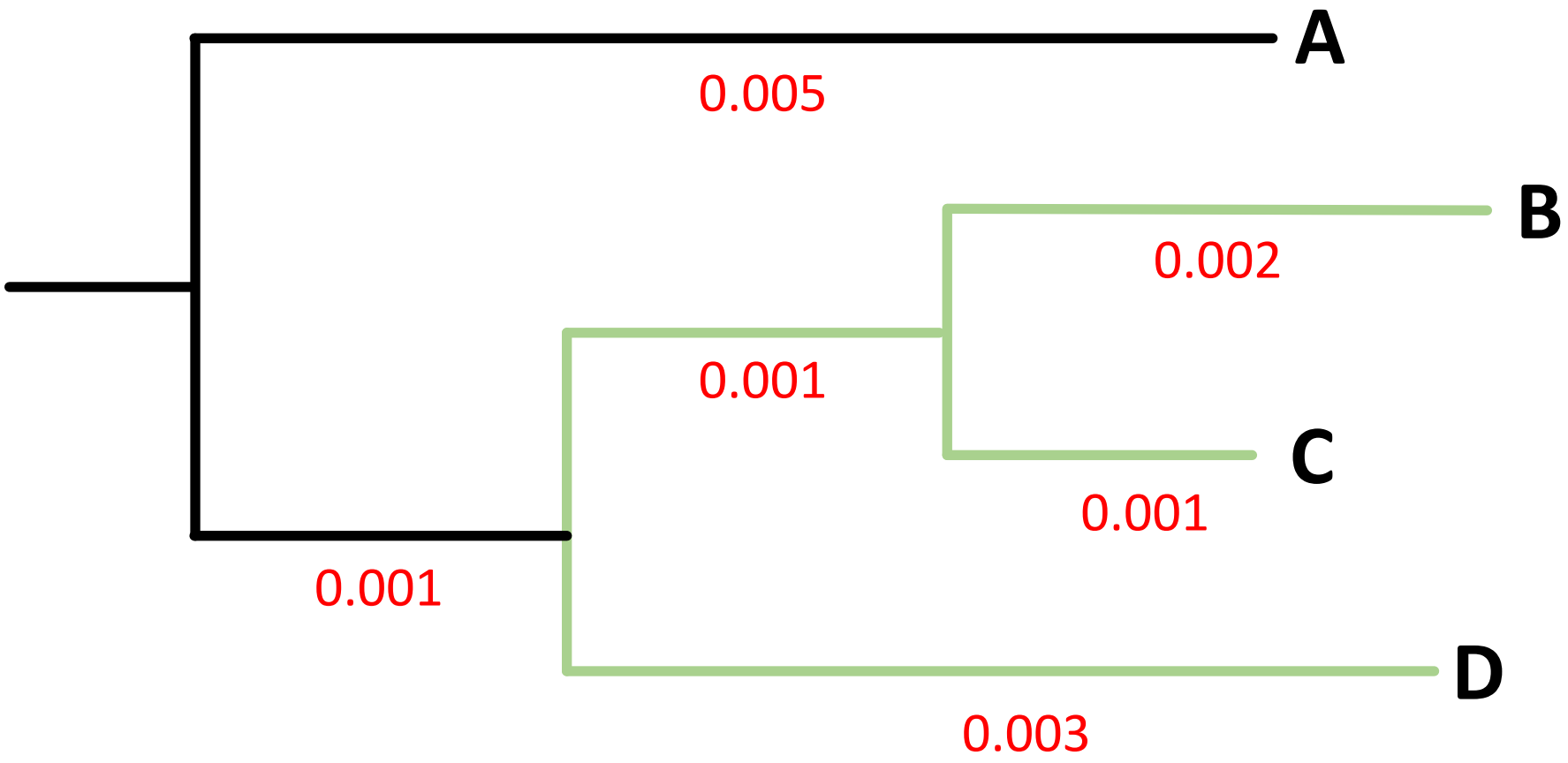


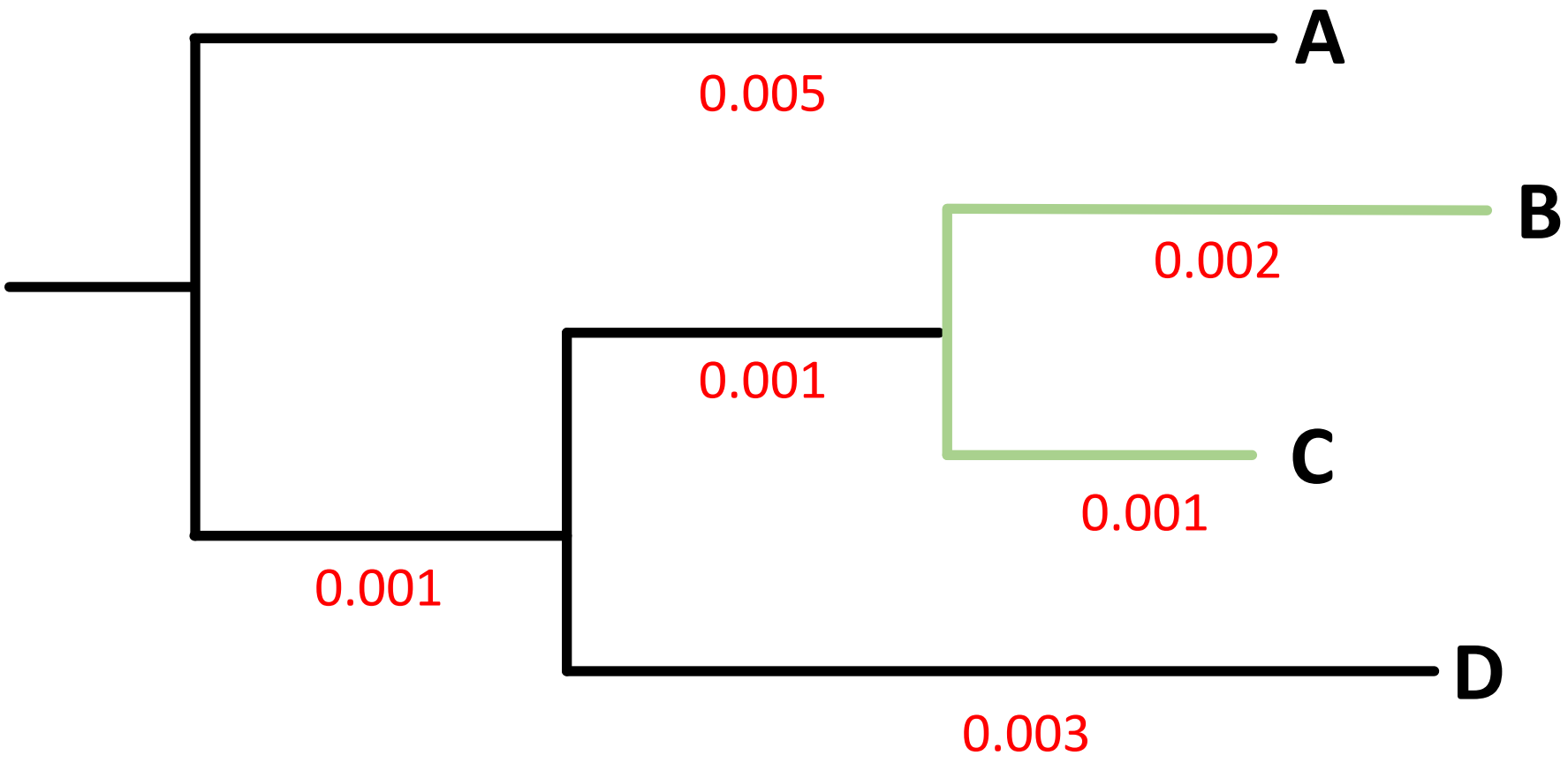
Phylogenetic clustering

- TreeCluster (Balaban et. al. 2020) – pairwise threshold = 0.005



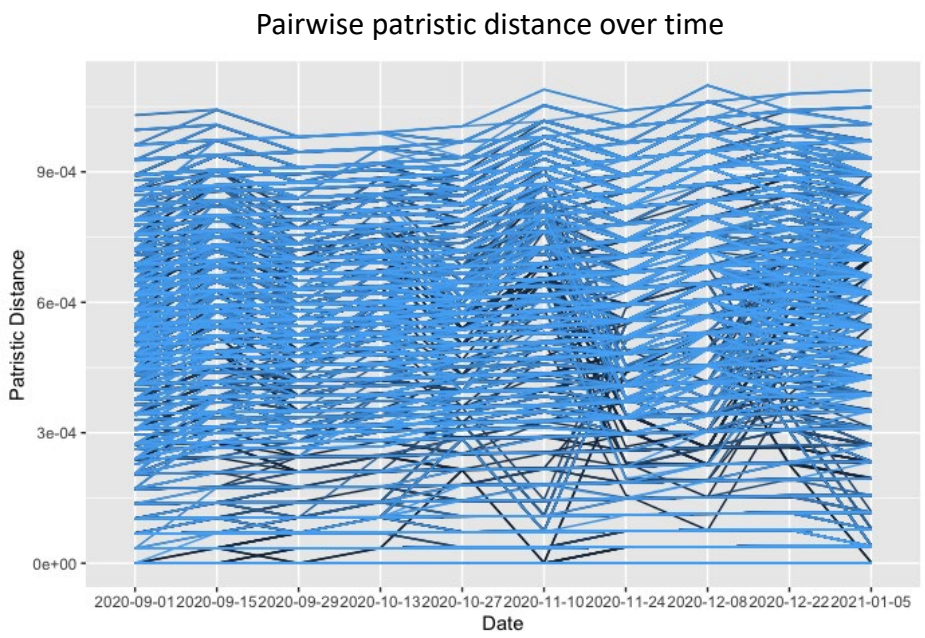
Phylogenetic clustering

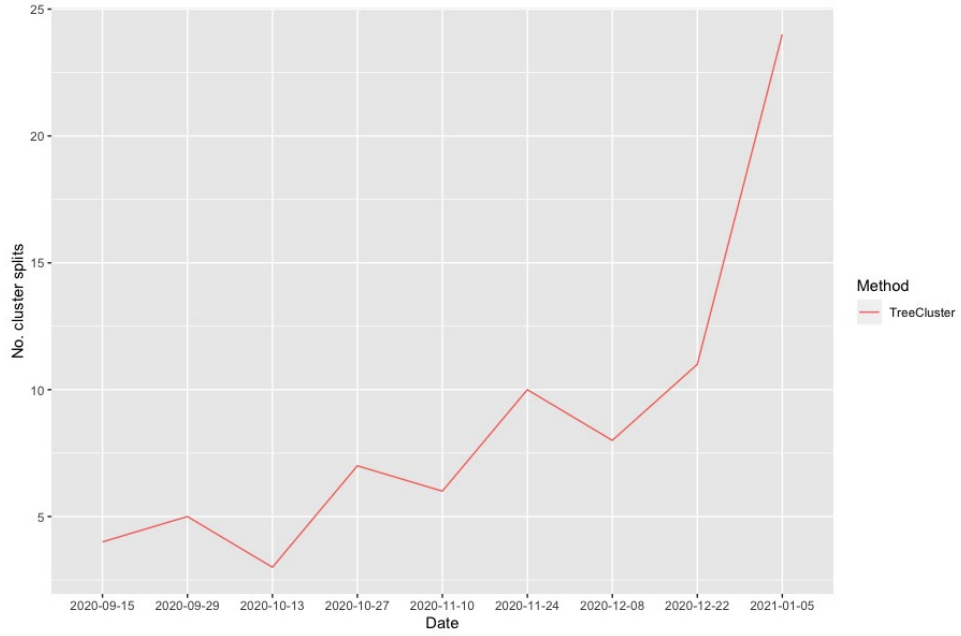
- TreeCluster (Balaban et. al. 2020) – max clade threshold = 0.005



Issues with phylogenetic clustering

- Developed for HIV – higher diversity through time – more stable phylogeny
- SARS-CoV-2 often low diversity in tree – very large clusters
- Instability of clusters with max clade threshold





Genomic clustering with logit function

- Uses pairwise patristic distance from phylogenetic tree, coupled with other variables (e.g. dates) to calculate probability of cases being linked - P ∈ (0,1)

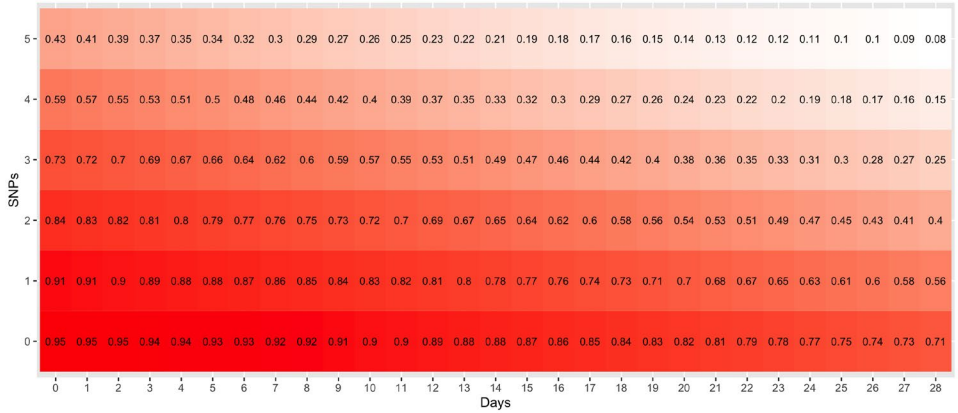
$$\ell = \log \frac{p}{1-p} = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_n x_n \quad (1)$$

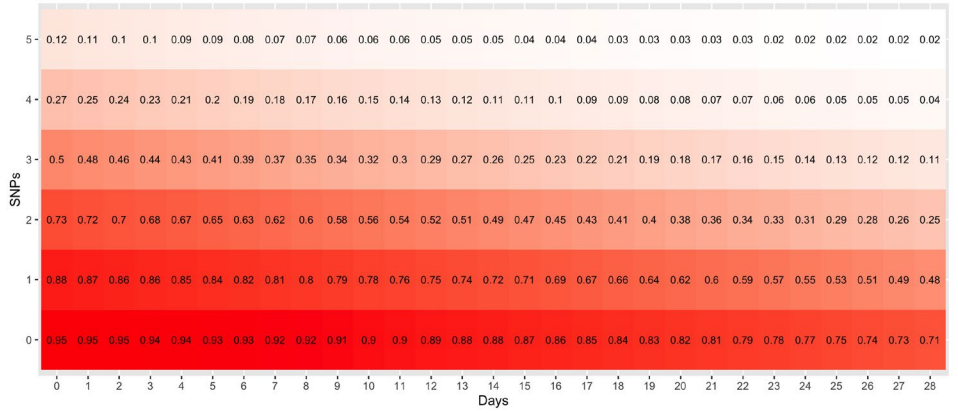
ℓ = log-odds
p = probability of Y = 1 (sequences are linked)
β_i = beta coefficient parameters
x_1 = distance predictor variable (pairwise SNP or genetic distance)
x_2 = time predictor variable (days between collection dates)

- Set max pairwise threshold to link cases and form clusters

Genomic clustering with logit function

- Can set betas accordingly and adjust for different lineages (e.g. P1 very low diversity, stricter beta on genomic diversity)



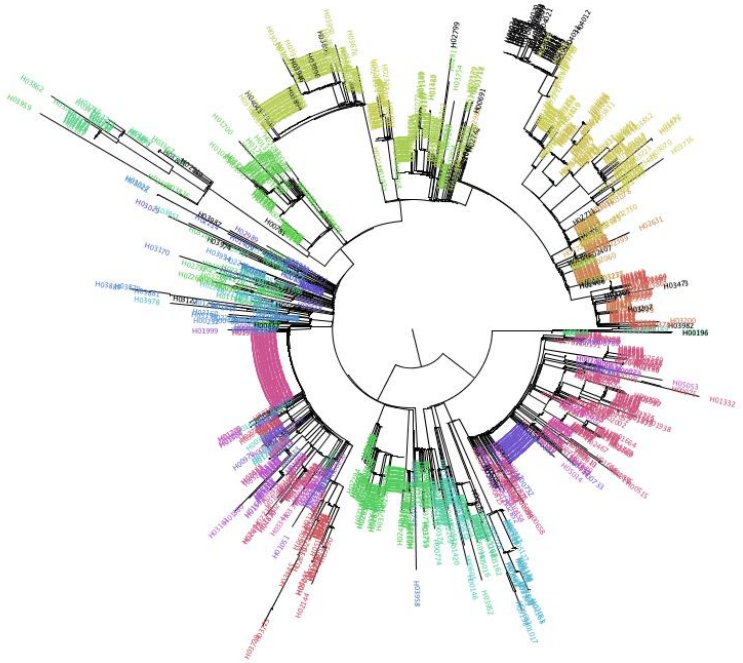


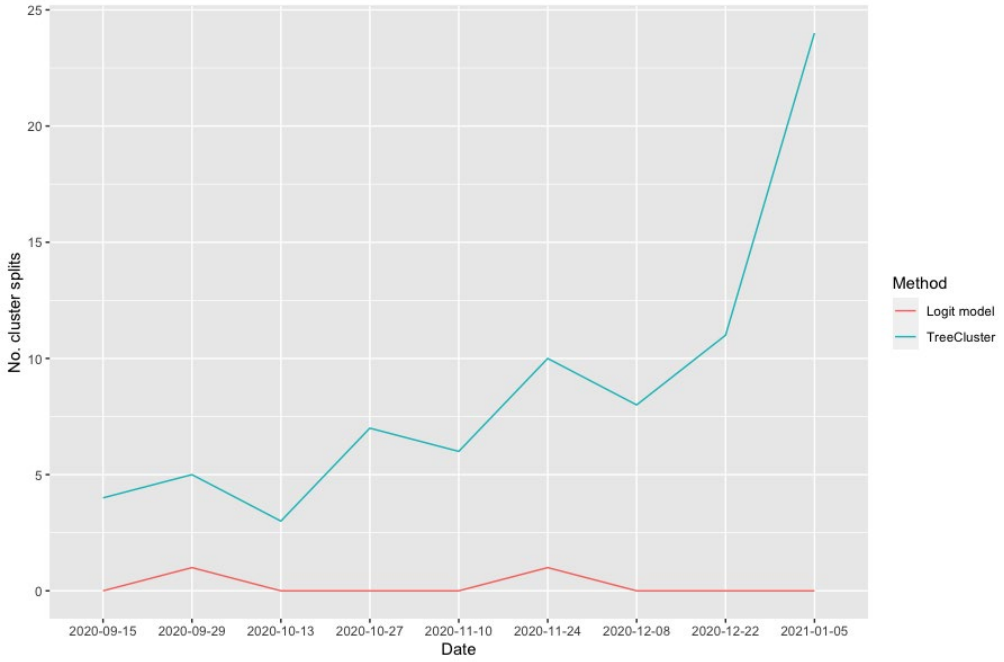
- Add any important explanatory variables that may help define clusters

Results – logit model

- Stable SARS-CoV-2 clusters that can inform epidemiological investigation

- Easy to implement and interpret in public health settings





Limitations and improvements – logit model

- Long chain clustering still causes large clusters if only considering patristic distance - still need additional data to refine clusters

- Slower to run than TreeCluster - around 5 minutes for 20,000 sequences compared to a few seconds

- Requires good quality data – genomic and epidemiological

Acknowledgements

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