

Molecular clocks

Martha Luka

2024-02-21

Using clockor2 to infer virus evolution

In this practical we will be use molecular clocks to analyse viral evolution in pre-constructed trees, test the fit of a clock and explore ways to root phylogenetic trees.

Task 1 - Preparation

Navigate to the Data folder (RAGE-workshop-2024/5-phylogenetics_and_maddog/E-tutorial_molecular_clocks/Data) which contains a newick tree (nig-af2-seqs.nwk), fasta file (nig-af2-seqs.fasta) and the respective sequence metadata (nig-af2-seqs-metadata-clockor2.csv).

On your browser, navigate to Clockor2

Task 2 - Upload files

Once on <https://clockor2.github.io>, drag and drop the newick tree file, or click **select file** to navigate to the file on your local computer.

Upload your tree metadata file, which should be a csv file. Headers should be **tip,date,group**. The group is the variable defining the expected monophyletic groups. This could be lineage, state, host etc.

Note: An example file for future reference can be found [here](#).

Click **Parse csv**

Visually inspect your tree and associated output

Task 3 - Analyse

1. Hover over the points on the graph. Do these correlate to where you expect the taxa to be located on the tree?
2. Now click on any taxa on the tree and see it highlighted on the graph.
3. Note the evolution rate of the unrooted tree, and respective R-squared and RMS values.
4. Root the global tree using both the R-squared and RMS values.
5. Note the recalibrated evolutionary rate of your tree, and the R-squared and RMS values. How do they compare to the unrooted values?

Given these values, what is the best way to root this specific tree?

6. Now look again carefully at your clockor2 root-to-tip regression plot. Are there any sequences that look like outliers?
7. Compare the metadata file with the original “raw” data (Data/nig-af2-seqs-RAW-metadata.txt). Can you see any discrepancies in the data associated with the outlier case that might explain why it looks out of place?
8. Now that you have identified the issue, try clockor2 again but with the corrected metadata. This can be found in the file nig-af2-seqs-clockor2-corrected.csv. Note the change to the evolutionary clock estimate and the R-squared value.

Task 4 - Export

You can export your tree in different formats. Rectangular, circular etc

Task 5 - Re-iterate with different trees of your data

You can also compare different trees of your data e.g., changing the group variable!