Tutorial using BEAST v2.7.3
MASCOT Tutorial

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Parameter and State inference using the approximate structured coalescent

1 Background

Phylogeographic methods can help reveal the movement of genes between populations of organisms. This has been widely used to quantify pathogen movement between different host populations, the migration history of humans, and the geographic spread of languages or the gene flow between species using the location or state of samples alongside sequence data. Phylogenies therefore offer insights into migration processes not available from classic epidemiological or occurrence data alone.

The structured coalescent allows to coherently model the migration and coalescent process, but struggles with complex datasets due to the need to infer ancestral migration histories. Thus, approximations to the structured coalescent, which integrate over all ancestral migration histories, have been developed. This tutorial gives an introduction into how a MASCOT analysis in BEAST2 can be set-up. MASCOT is short for **M**arginal **A**pproximation of the **S**tructured **CO**alescen**T**(Müller et al. 2018) and implements a structured coalescent approximation (Müller et al. 2017). This approximation doesn't require migration histories to be sampled using MCMC and therefore allows to analyse phylogenies with more than three or four states.

2 Programs used in this Exercise

2.0.1 BEAST2 - Bayesian Evolutionary Analysis Sampling Trees 2

BEAST2 (http://www.beast2.org) is a free software package for Bayesian evolutionary analysis of molecular sequences using MCMC and strictly oriented toward inference using rooted, time-measured phylogenetic trees. This tutorial is written for BEAST v2.7.x (Drummond and Bouckaert 2014).

2.0.2 BEAUti2 - Bayesian Evolutionary Analysis Utility

BEAUti2 is a graphical user interface tool for generating BEAST2 XML configuration files.

Both BEAST2 and BEAUti2 are Java programs, which means that the exact same code runs on all platforms. For us it simply means that the interface will be the same on all platforms. The screenshots used in this tutorial are taken on a Mac OS X computer; however, both programs will have the same layout and functionality on both Windows and Linux. BEAUti2 is provided as a part of the BEAST2 package so you do not need to install it separately.

2.0.3 TreeAnnotator

TreeAnnotator is used to summarise the posterior sample of trees to produce a maximum clade credibility tree. It can also be used to summarise and visualise the posterior estimates of other tree parameters (e.g. node height).

TreeAnnotator is provided as a part of the BEAST2 package so you do not need to install it separately.

2.0.4 Tracer

Tracer (http://tree.bio.ed.ac.uk/software/tracer) is used to summarise the posterior estimates of the various parameters sampled by the Markov Chain. This program can be used for visual inspection and to assess convergence. It helps to quickly view median estimates and 95% highest posterior density intervals of the parameters, and calculates the effective sample sizes (ESS) of parameters. It can also be used to investigate potential parameter correlations. We will be using Tracer v1.7.2.

2.0.5 FigTree

FigTree (http://tree.bio.ed.ac.uk/software/figtree) is a program for viewing trees and producing publication-quality figures. It can interpret the node-annotations created on the summary trees by TreeAnnotator, allowing the user to display node-based statistics (e.g. posterior probabilities). We will be using FigTree v1.4.4.
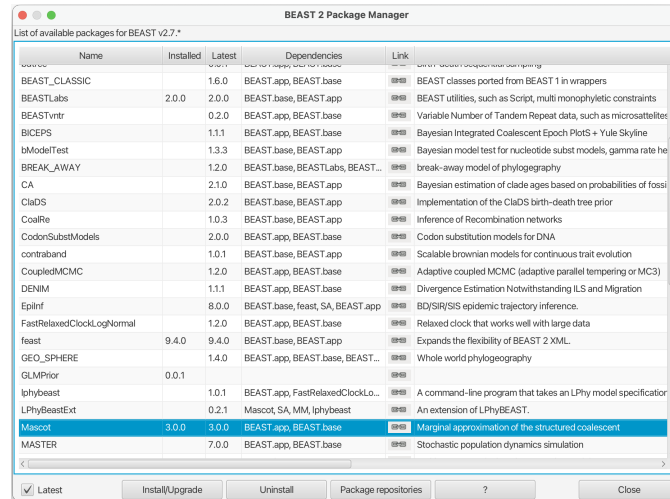
Figure 1: Download the MASCOT package.

3 Practical: Parameter and State inference using the approximate structured coalescent

In this tutorial we will estimate migration rates, effective population sizes and locations of internal nodes using the marginal approximation of the structured coalescent implemented in BEAST2, MASCOT (Müller et al. 2018).

The aim is to:

- Learn how to infer structure from trees with sampling location
- Get to know how to choose the set-up of such an analysis
- Learn how to read the output of a MASCOT analysis

3.1 Setting up an analysis in BEAUti

3.1.1 Download MASCOT

First, we have to download the package MASCOT using the BEAUTi package manager. Go to *File >> Manage Packages* and download the package MASCOT.

MASCOT will only be available in BEAUti once you close and restart the program.

3.1.2 Loading the Influenza A/H3N2 Sequences (Partitions)

The sequences from the *data* folder name *H3N2.nexus* can be either drag and dropped into BEAUti or added using BEAUti's menu system via *File >> Import Alignment*. Once the sequences are added, we need to specify the sampling dates.

3.1.3 Get the sampling times (Tip Dates)

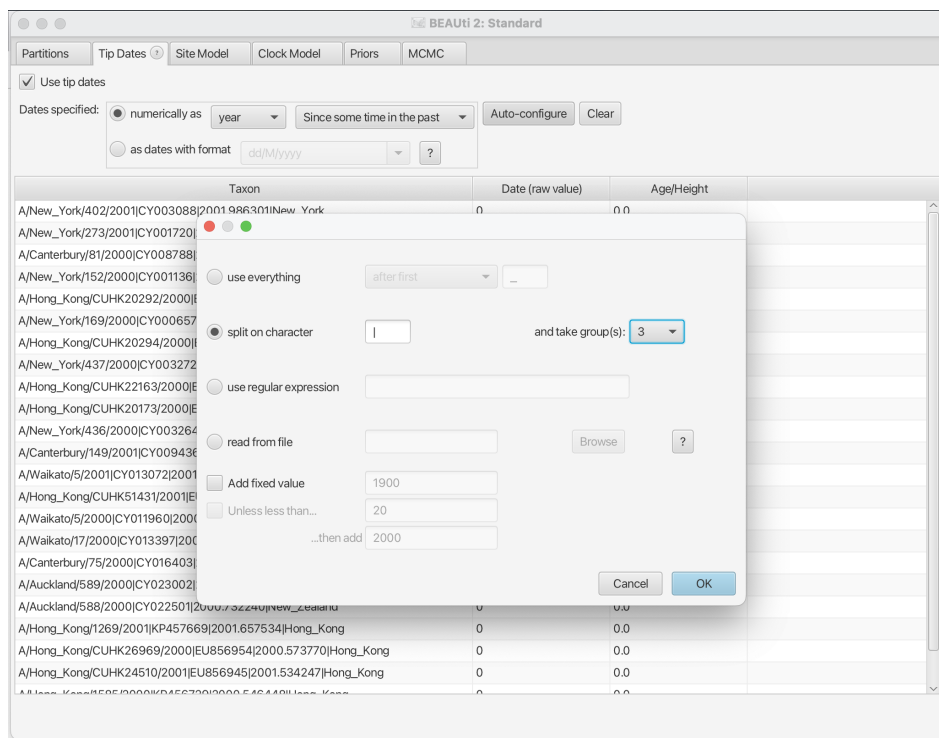Open the "Tip Dates" panel and then select the "Use tip dates" checkbox.

Figure 2: Guess sampling times.

The sampling times are encoded in the sequence names. We can tell BEAUti to use these by clicking the *Auto-configure* button. The sampling times appear following the third vertical bar "|" in the sequence name. To extract these times, select "split on character", enter "|" (without the quotes) in the text box immediately to the right, and then select "3" from the drop-down box to the right, as shown in the figure below.

Clicking "Ok" should now populate the table with the sample times extracted from the sequence names: the column **Date** should now have values between 2000 and 2002 and the column **Height** should have values from 0 to 2. The heights denote the time difference from a sequence to the most recently sampled sequence. If everything is specified correctly, the sequence with Height 0.0 should have Date 2001.9.

3.1.4   Specify the Site Model (Site Model)

Next, we have to specify the site model. For Influenza Hemagluttanin sequences as we have here, HKY is the most commonly used model of nucleotide evolution. It allows for difference in transversion and transition rates. Meaning that changes between bases that are chemically closer related (transitions) are allowed to have a different rate than changes between bases that chemically more distinct (transversion). Additionally, we should allow for different rate categories for different sites in the alignment. This can be done by setting the *Gamma Category Count* to 4, which is just a value that has typically been used. Make sure that estimate is checked next to the shape parameter. To reduce the number of parameters we have to estimate, we can set Frequencies to Empirical.
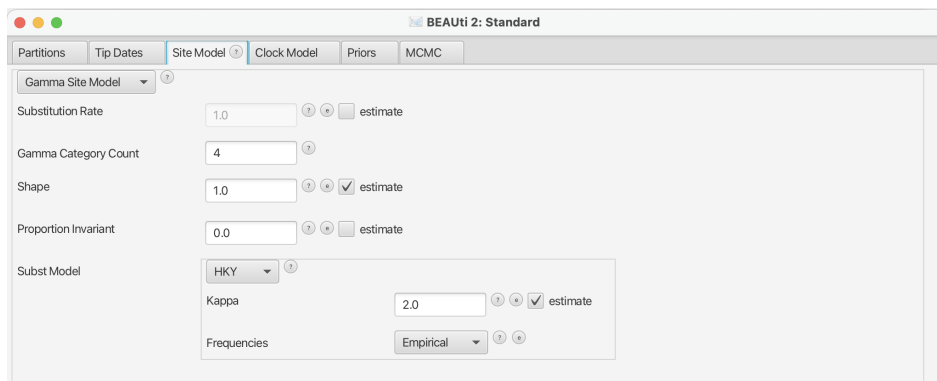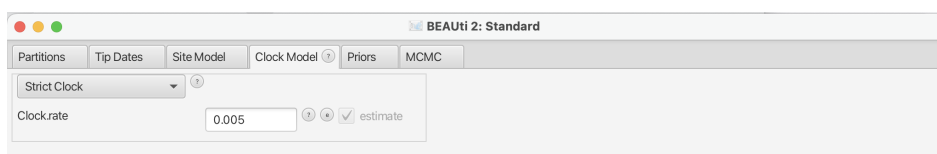
Figure 3: Set the site model.

Figure 4: Set the initial clock rate.

3.1.5 Set the clock model (Clock Model)

For rapidly evolving viruses, the assumption of a strict molecular clock is often made, meaning that the molecular clock is the same on each branch of the phylogeny. To decrease the burnin phase, we can set the initial value to 0.005.

3.1.6 Get the sampling locations (Tip Locations)

We first have to choose the tree prior, which in this case is MASCOT. We do this by switching to the "Priors" tab. Search the drop down menu next to *Tree.t:H3N2* and choose MASCOT.

By default, the rate dynamics for this setting is *Constant*, which means that effective population sizes and migration rates are assumed to be constant through time. We next have to define the sampling location of the individual tips.

Initially the column **Location** should be NOT_SET for every sequence. After clicking the *Guess*button, you can split the sequence on the vertical bar "|" again by selecting "split on character" and entering "|" in the box. However, the locations are in the fourth group, so this time choose "4" from the drop-down menu. After clicking the *OK* button, the window should look like the one shown in the figure below:

3.1.7 Specify the priors (Priors)

Now, we need to set the priors for the various parameters of the model. You can find the parameter priors below the tree prior.

First, consider the effective population size parameter *Ne*. Since we have only a few samples per location, meaning little information about the different effective population sizes, we will need an informative prior. In this case we will use a log normal prior with parameters M=0 and S=1. (These are respectively the mean and variance of the corresponding normal distribution in log space.) To use this prior, choose "Log
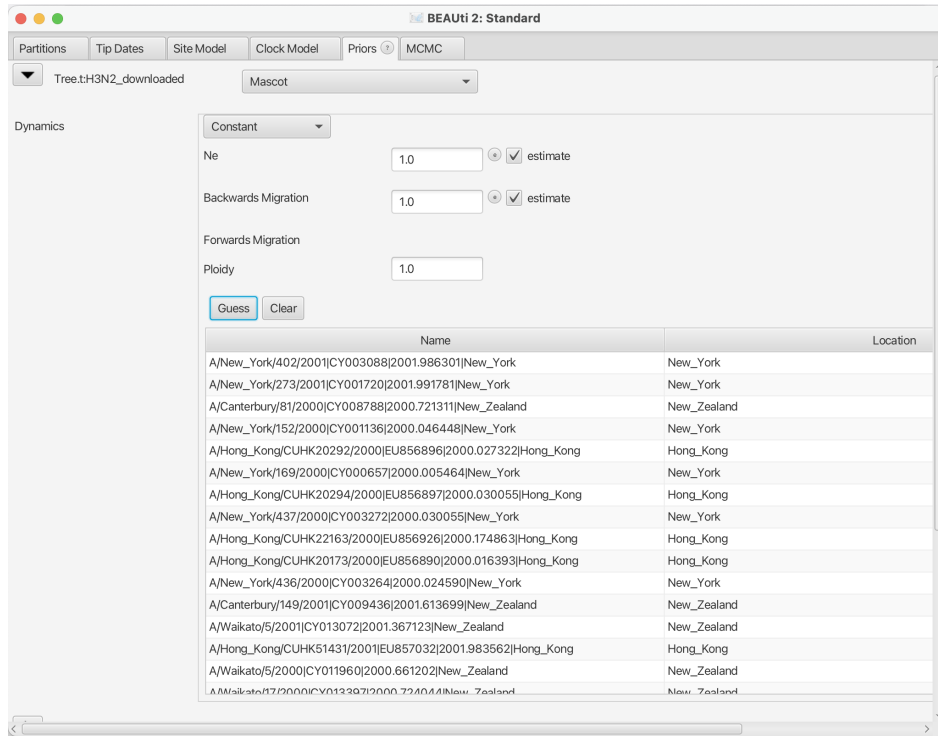
Figure 5: Configuring sample locations.

Normal" from the drop down menu to the right of the *Ne.t:H3N2* parameter label, then click the arrow to the left of the same label and fill in the parameter values appropriately (i.e. M=0 and S=1). Ensure that the "Mean In Real Space" checkbox remains unchecked.

The existing exponential distribution as a prior on the migration rate puts much weight on lower values while not prohibiting larger ones. For migration rates, a prior that prohibits too large values while not greatly distinguishing between very small and very **very** small values is generally a good choice. Be aware however that the exponential distribution is quite an informative prior: one should be careful that to choose a mean so that feasible rates are at least within the 95% HPD interval of the prior. (This can be determined by clicking the arrow to the left of the parameter name and looking at the values below the graph that appears on the right.) We keep the default mean value of 1.

Finally, set the prior for the clock rate. We have a good idea about the clock rate of Influenza A/H3N2 Hemagglutinin. From previous work by other people, we know that the clock rate will be around 0.005 substitution per site per year. To include that prior knowledge, we can set the prior on the clock rate to a Log Normal distribution with mean in **real space** set to 0.005. To specify the mean in real space, make sure that the box "Mean In Real Space" is checked. If we set the S value to 0.25, we say that we expect the clock rate to be with 95% certainty between 0.00321 and 0.00731.

We keep the default priors for the parameters gammaShape and kappa.

3.1.8   Specify the MCMC chain length (MCMC)

Here we can set the length of the MCMC chain and after how many iterations the parameter and trees a logged. For this dataset, 2 million iterations should be sufficient. In order to have enough samples but not
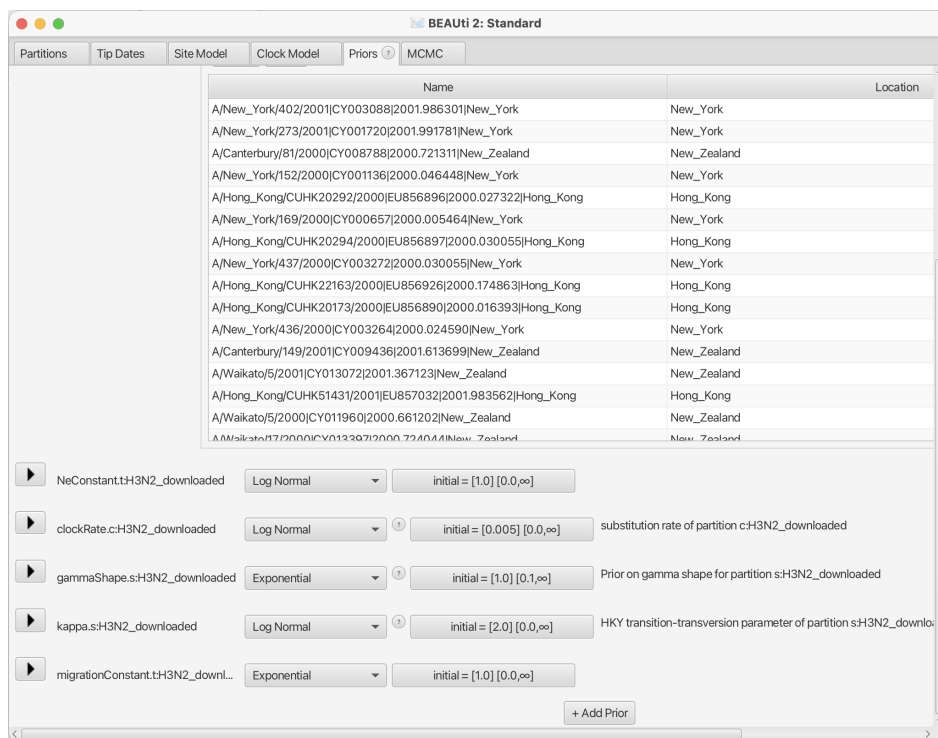
Figure 6: Set up of the prior distributions.

create too large files, we can set the logEvery to 2000, so we have 1001 samples overall. Next, we have to save the *.xml file under *File >> Save as*.

3.1.9 Run the Analysis using BEAST2

Run the *.xml using BEAST2 or use finished runs from the *precooked-runs* folder. The analysis should take about 6 to 7 minutes.

3.1.10 Analyse the log file using Tracer

First, we can open the *.log file in tracer to check if the MCMC has converged. The ESS value should be above 200 for almost all values and especially for the posterior estimates.

Next, we can have a look at the inferred effective population sizes. New York is inferred to have the largest effective population size before Hong Kong and New Zealand. This tells us that two lineages that are in the New Zealand are expected to coalesce quicker than two lineages in Hong Kong or New York.

In this example, we have relatively little information about the effective population sizes of each location. This can lead to estimates that are greatly informed by the prior. Additionally, there can be great differences between median and mean estimates. The median estimates are generally more reliable since they are less influence by extreme values.

We can then look at the inferred migration rates. The migration rates have the label b_migration.*, meaning that they are backwards in time migration rates. The highest rates are from New York to Hong Kong. Because they are backwards in time migration rates, this means that lineages from New York are
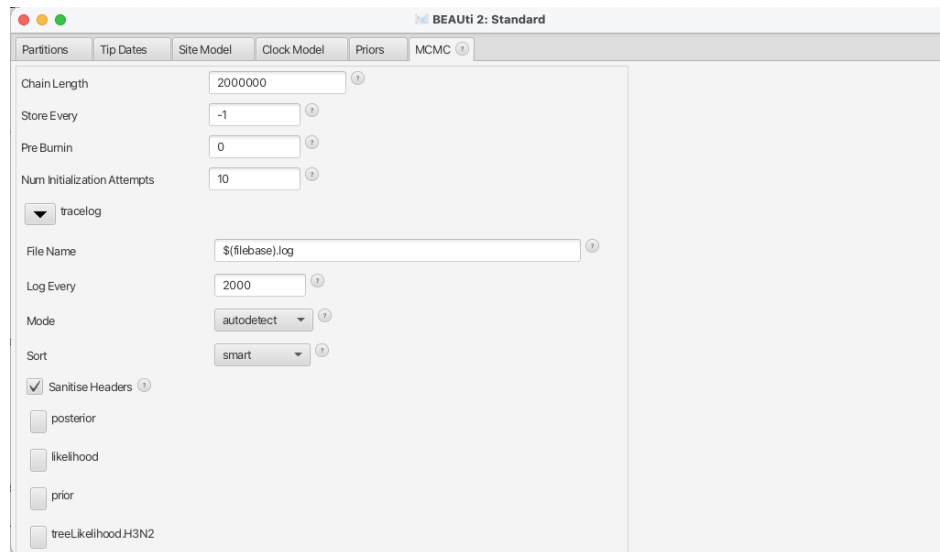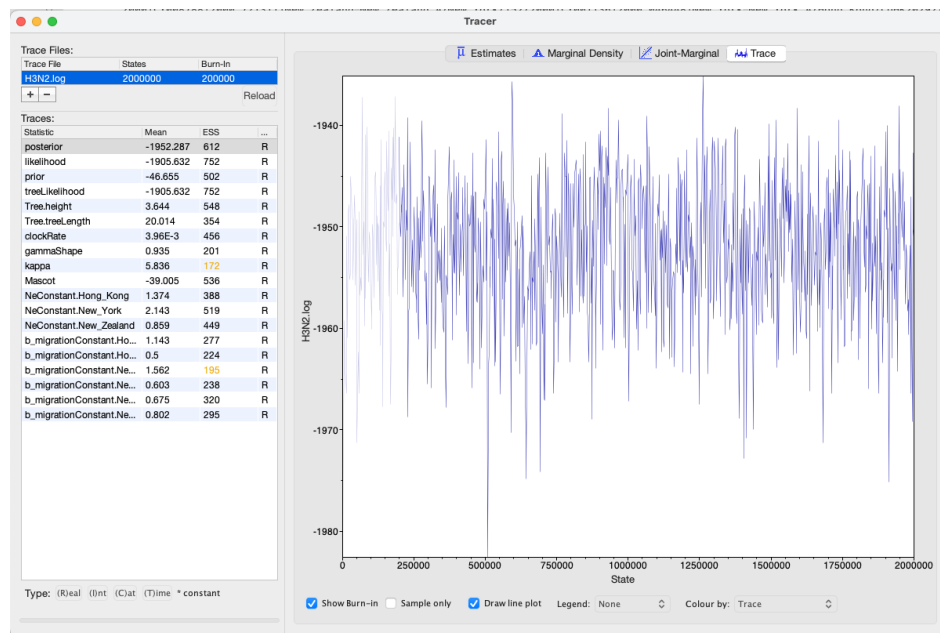
Figure 7: save the *.xml.
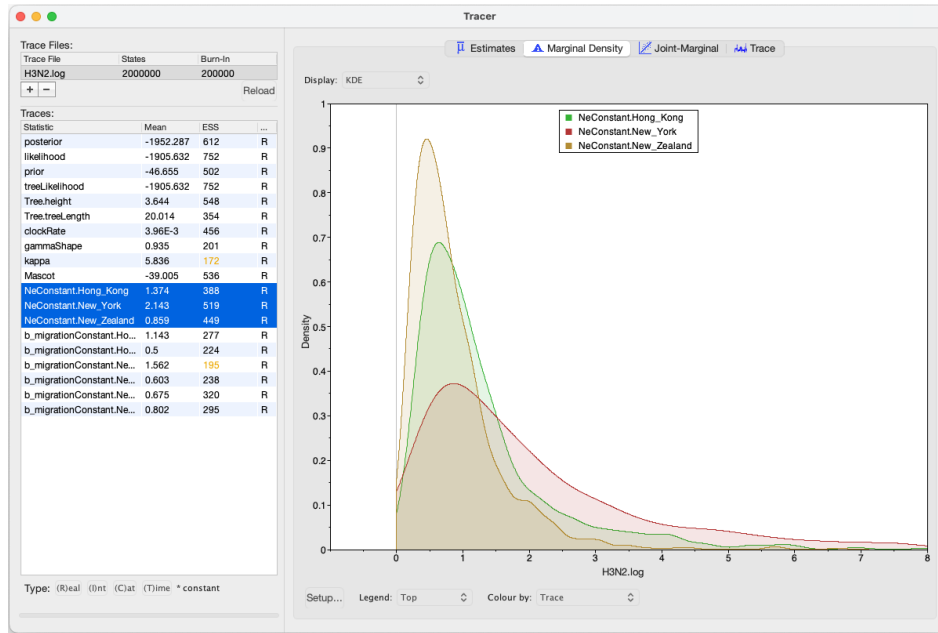

Figure 8: Check if the posterior converged.

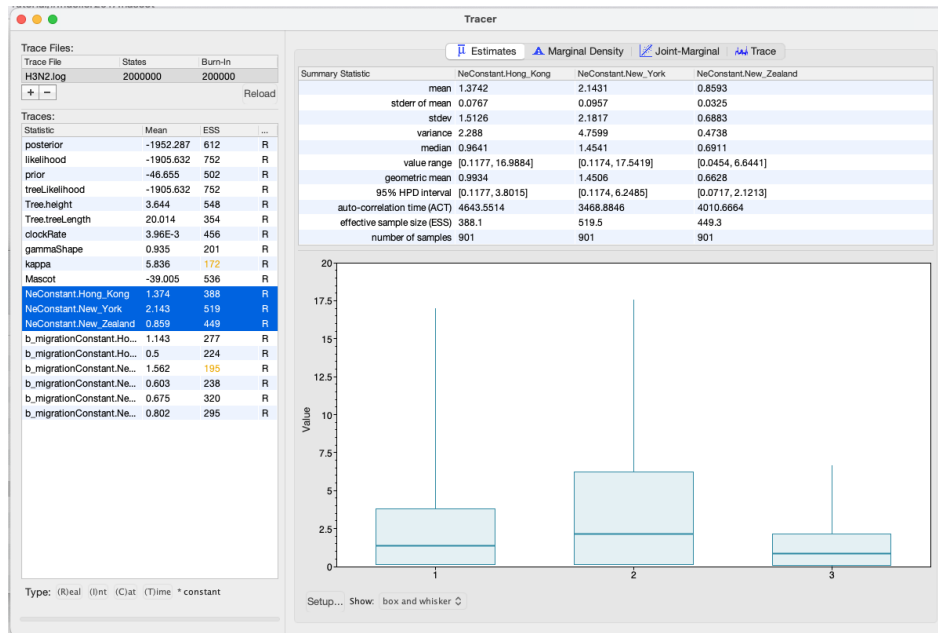Figure 9: Compare the different inferred effective population sizes.

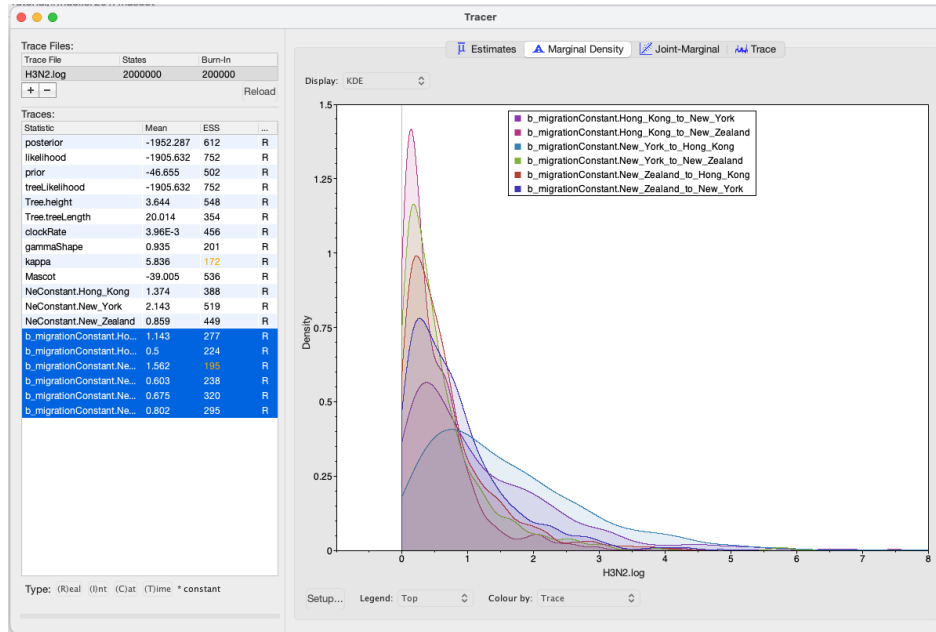Figure 10: Differences between Mean and Meadian estimates.

Figure 11: Compare the inferrred migration rates.

inferred to be likely from Hong Kong if we're going backwards in time. In the inferred phylogenies, we should therefore make the observation that lineages ancestral to samples from New York are inferred to be from the Hong Kong backwards.

3.1.11 Make the MCC tree using TreeAnnotator

Next, we want to summarize the trees. This we can do using treeAnnotator. Open the programm and then set the options as below. You have to specify the *Burnin precentage*, the *Node heights*, *Input Tree File* and the *Output File*. Use the typed trees in the file *H3N2.H32.trees* as *Input Tree File*. After clicking *Run* the programm should summarize the trees.

3.1.12 Check the MCC tree using FigTree

In each logging step of the tree during the MCMC, MASCOT logs several different things. It logs the inferred probability of each node being in any possible location. In this example, these would be the inferred probabilities of being in Hong Kong, New York and New Zealand. Additonally, it logs the most likely location of each node.

After opening the MCC tree in FigTree, we can visualize several things. To color branches, you can go to *Appearance >> Colour by* and select **max**. This is the location that was inferred to be most often the most likely location of the node.

We can now determine if lineages ancestral to samples from New York are actually inferred to be from Hong Kong, or the probability of the root being in any of the locations.

To get the actual inferred probabilities of each node being in any of the 3 locations, you can go to *Node Labels >> Display* an then choose Hong_Kong, New_York or New_Zealand. These are the actual inferred probabilities of the nodes being in any location.
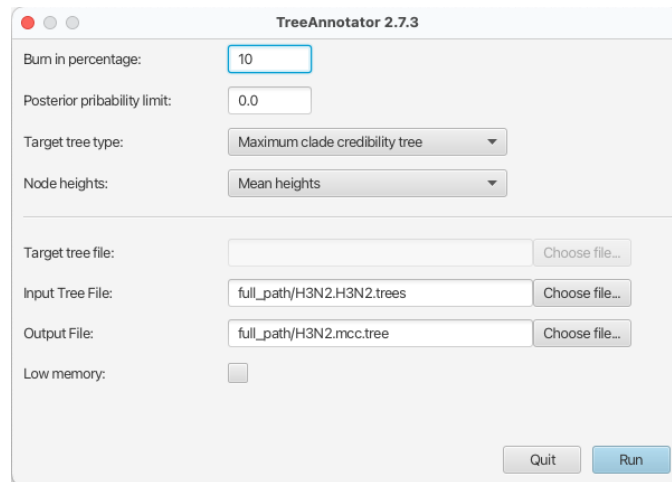
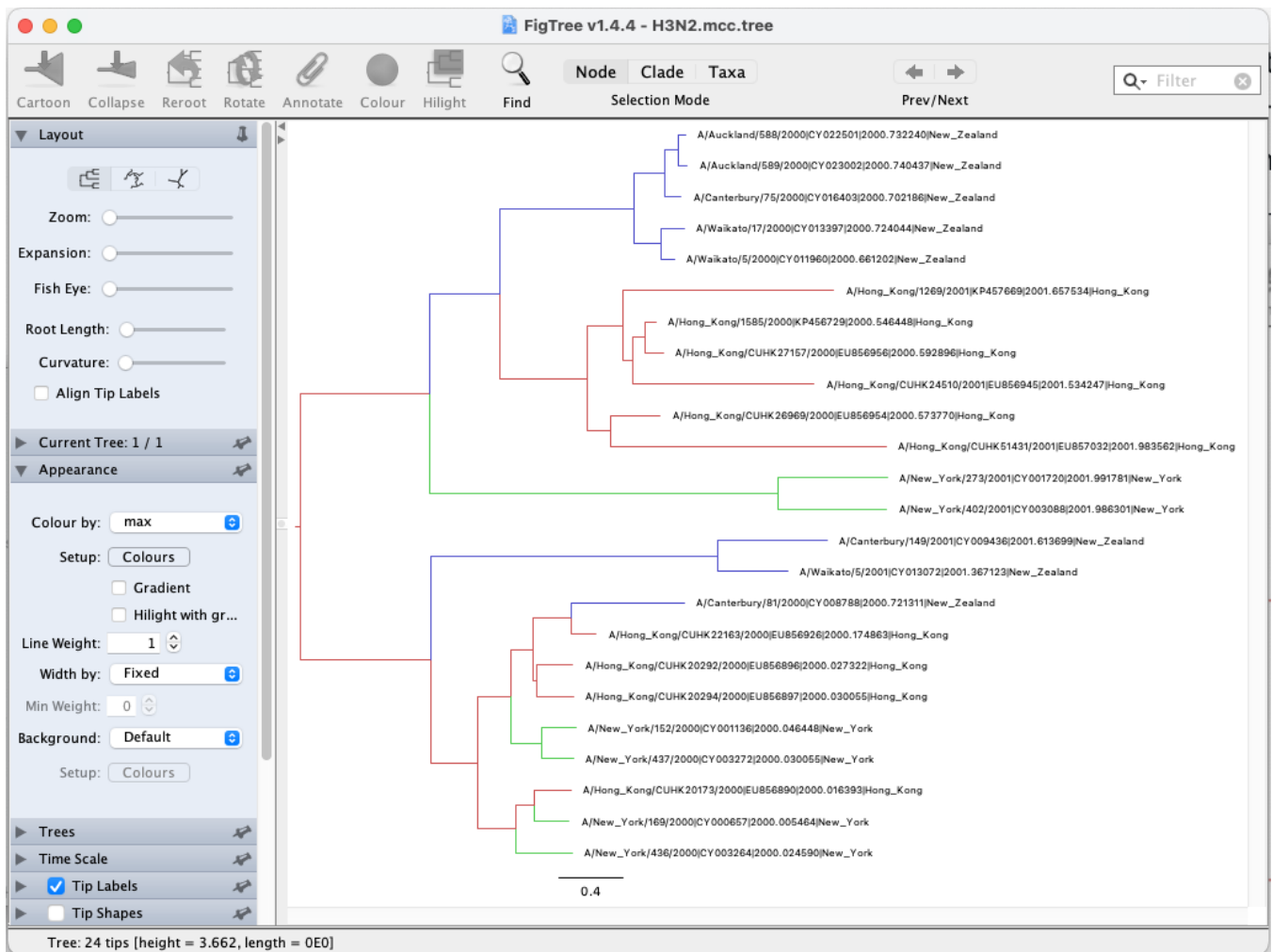Figure 12: Make the maximum clade credibility tree.

Figure 13: Compare the inferred node probabilities.

It should however be mentioned that the inference of nodes being in a particular location makes some simplifying assumptions, such as that there are no other locations (i.e. apart from the sampled locations) where lineages could have been.

Another important thing to know is that currently, we assume rates to be constant. This means that we assume that the population size of the different locations does not change over time. We also make the same assumption about the migration rates through time.

3.1.13 Errors that can occur (Work in progress)

One of the errors message that can occur regularly is the following: *too many iterations, return negative infinity.* This occurs when the integration step size of the ODE's to compute the probability of observing a phylogenetic tree in MASCOT is becoming too small. This generally occurs if at least one migration rate is really large or at least one effective population size is really small (i.e. the coalescent rate is really high). This causes integration steps to be extremely small, which in turn would require a lot of time to compute the probability of a phylogenetic tree under MASCOT. Instead of doing that, this state is rejected by assigning its log probability the value negative infinity.

This error can have different origins and a likely incomplete list is the following:

1. The priors on migration rates put too much weight on really high rates. To fix this, reconsider your priors on the migration rates. Particularly, check if the prior on the migration rates make sense in comparison to the height of the tree. If, for example, the tree has a height of 1000 years, but the prior on the migration rate is exponential with mean 1, then the prior assumption is that between any two states, we expected approximately 1000 migration events.
2. The prior on the effective population sizes is too low, meaning that the prior on the coalescent rates (1 over the effective population size) is too high. This can for example occur when the prior on the effective population size was chosen to be $1/X$. To fix, reconsider your prior on the effective population size.
3. There is substantial changes of the effective population sizes and/or migration rates over time that are not modeled. In that case, changes in the effective population sizes or migration rates have to be explained by population structure, which can again lead to some effective population sizes being very low and some migration rates being very high. In that case, there is unfortunately not much that can be done, since MASCOT is not an appropriate model for the dataset.
4. There is strong subpopulation structure within the different subpopulations used. In that case, reconsider if the individual sub-populations used are reasonable.

4 Useful Links

If you interested in the derivations of the marginal approximation of the structured coalescent, you can find them here (Müller et al. 2017). This paper also explains the mathematical differences to other methods such as the theory underlying BASTA. To get a better idea of how the states of internal nodes are calculated, have a look in this paper (Müller et al. 2018).

- MASCOT source code: https://github.com/nicfel/Mascot
- Bayesian Evolutionary Analysis with BEAST 2 (Drummond and Bouckaert 2014)
- BEAST 2 website and documentation: http://www.beast2.org/
- Join the BEAST user discussion: http://groups.google.com/group/beast-users

Version dated: June 9, 2023

Relevant References

Drummond, AJ and RR Bouckaert. 2014. *Bayesian evolutionary analysis with BEAST 2.* Cambridge University Press,

Müller, NF, D Rasmussen, and T Stadler. 2018. Mascot: parameter and state inference under the marginal structured coalescent approximation. *Bioinformatics* bty406.

Müller, NF, DA Rasmussen, and T Stadler. 2017. The structured coalescent and its approximations. *Molecular Biology and Evolution* msx186.