

Phylogeographic Diffusion in Continuous Space - YFV example

Summary: This tutorial provides a step-by-step explanation on reconstructing the spatial dynamics of the Yellow fever virus (YFV) epidemic in Brazil based on a set of viral genome sequences which have been isolated at different points in time (Faria et al. 2018). YFV is responsible for 29,000 to 60,000 deaths annually in South America and Africa and is the most severe mosquito-borne infection in the tropics. YFV transmission occurs mainly via the sylvatic cycle, in which nonhuman primates are infected by mosquito vectors, but can also occur via an urban cycle, in which humans are infected by *Aedes* spp. mosquitoes that feed mostly on humans. Recently, Brazil has experienced its largest recorded Yellow fever outbreak in decades. In that context, Faria et al. (2018) have analysed a data set of 67 YFV genomes collected between January and April 2017 in order to characterise the dispersal history and dynamics of the virus during this recent outbreak. For this purpose, they have applied the continuous diffusion (Lemey et al. 2010, Pybus et al. 2012) to estimate the ancestral locations of the virus in continuous space. In the present tutorial, we detail how to set this continuous phylogeographic analysis and how to analyse/visualise the outputs.

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Introduction

The first step will be to convert an alignment file in fasta format into a BEAST XML input file. This is done using the program BEAUti (this stands for Bayesian Evolutionary Analysis Utility). This is a user-friendly program for setting the evolutionary model and options for the MCMC analysis. The second step is to actually run BEAST using the input file that contains the data, model and settings. The final step is to explore the output of BEAST in order to diagnose potential problems and to summarize the results.

To undertake this tutorial, you will need to download three software packages in a format that is compatible with your computer system (all three are available for Mac OS X, Windows and Linux/UNIX operating system).



BEAST - this package contains the BEAST (beast) program, BEAUti (beauti) and a couple of utility programs. At the time of writing, the current version is v1.10.4. BEAST releases are available for download from <https://github.com/beast-dev/beast-mcmc/releases>.



BEAGLE - this is a high-performance library that can perform the core calculations at the heart of most Bayesian and Maximum Likelihood phylogenetics packages. It can make use of highly-parallel processors such as those in graphics cards (GPUs) found in many PCs. Binary installers and installation instructions can be found at <https://github.com/beagle-dev/beagle-lib>.



Tracer - this program is used to explore the output of BEAST (and other Bayesian MCMC programs). It graphically and quantitatively summarizes the empirical distributions of continuous parameters and provides diagnostic information. At the time of writing, the current version is v1.7.1. It is available for download from <https://github.com/beast-dev/tracer/>.



FigTree - this is an application for displaying and printing molecular phylogenies, in particular those obtained using BEAST. At the time of writing, the current version is v1.4.3. It is available for download from <http://tree.bio.ed.ac.uk/software/figtree/>.



Spread3 - this is an application for the visualization of phylogeographic analyses performed with BEAST. At the time of writing, the current version is v0.9.7.1. It is available for download from <https://rega.kuleuven.be/cev/ecv/software/Spread3>.

⬇️ All the files needed for this tutorial can be downloaded from [here](#) (/tutorials/workshop_continuous_diffusion_yfv/files/continuousTutorialFiles.zip). If you download this zipped folder, there is no need to download other files/folders linked further in the tutorial.

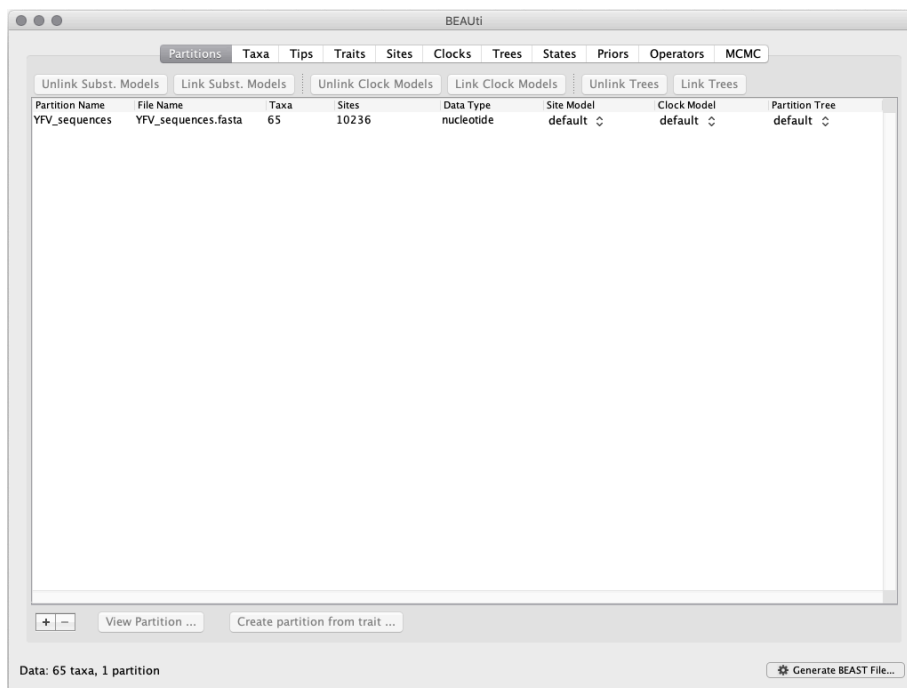
Running BEAUti

The program BEAUti is a user-friendly program for setting the model parameters for BEAST. Run BEAUti by double clicking on its icon.

Loading the sequence data file

 The data file for this tutorial is called 'YFV_sequences.fasta' and [can be downloaded from here \(https://tutorials/workshop_continuous_diffusion_yfv/files/YFV_sequences.fasta\)](https://tutorials/workshop_continuous_diffusion_yfv/files/YFV_sequences.fasta).

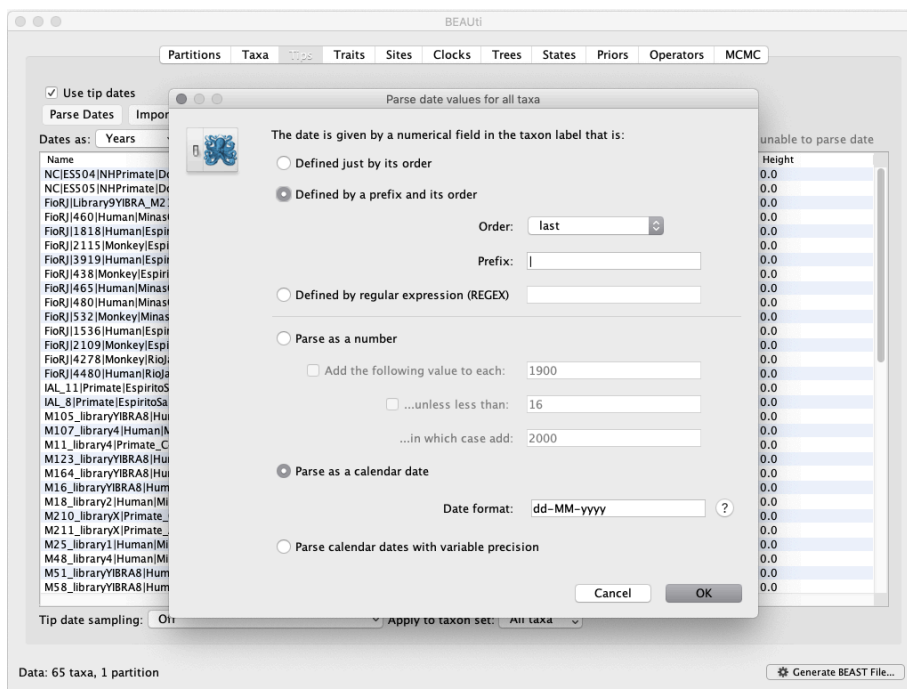
To load the alignment, simply select the **Import Data...** option from the File menu and browse to the 'YFV_sequences.fasta' file to load it. This file contains an alignment of 65 YFV genomes 10,236 nucleotides in length. Once loaded, the sequence data will be listed under **Partitions** as shown in the figure:



Specifying the sampling date information

By default all the taxa are assumed to have a date of zero (i.e. the sequences are assumed to be sampled at the same time). In this case, the YFV sequences have been sampled at various days during 2017. To set tip dates switch to the **Tips** panel using the tabs at the top of the window.

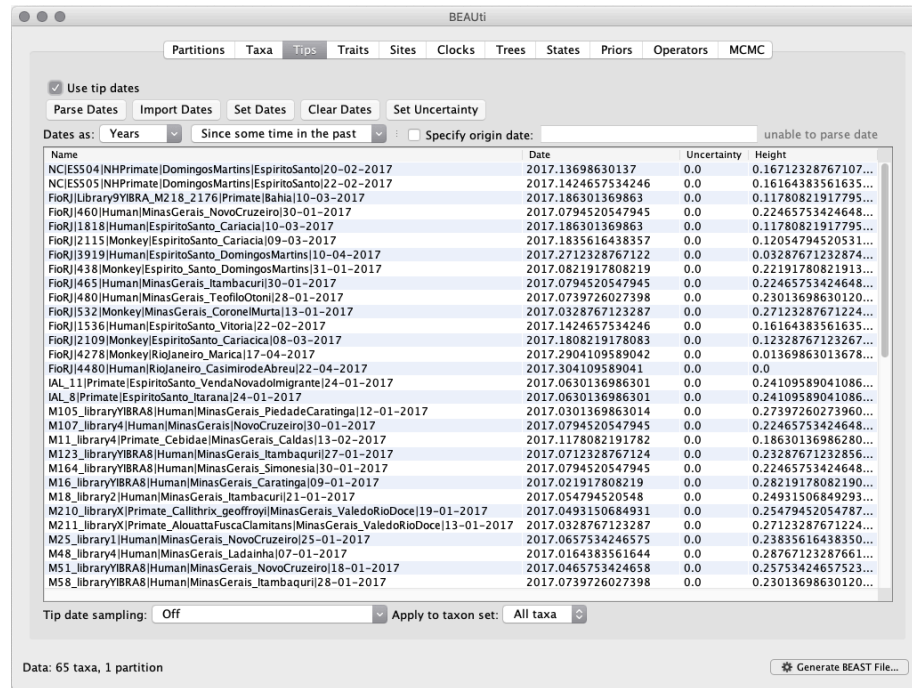
Select the box labelled **Use tip dates**. The actual sampling time in years is encoded in the name of each taxon and we could simply edit the value in the 'Date' column of the table to reflect these. However, if the names contain the calibration information, then a convenient way to specify the dates of the sequences in BEAUti is to use the **Parse Dates** button at the top left of the **Data** panel. Clicking this will make a dialog appear:



This operation attempts to guess what the dates are from information contained within the taxon names. It works by trying to find a numerical field within each name. If the taxon names contain more than one numerical field then you can specify how to find the one that corresponds to the date of sampling. You can (1) specify the order that the date field comes (e.g., first, last or various positions in between) or (2) specify a prefix (some characters that come immediately before the date field in each name) and the order of the field, or (3) define a regular expression (REGEX).

When parsing a number, you can ask BEAUti to add a fixed value to each guessed date. For example, the value **1900** can be added to turn the dates from 2 digit years to 4 digit. Any dates in the taxon names given **00** would thus become **1900**. However, if these **00** or **01**, etc. represent sequences sampled in 2000, 2001, etc., **2000** needs to be added to those. This can be achieved by selecting the **unless less than** and **in which case add:** options adding for example 2000 to any date less than 10. These operations are not necessary in our case since the dates are fully specified at the end of the sequence names is also an option to parse calendar dates and one for calendar dates with various precisions.

For the YFV sequences, dates values have to be parsed as calendar dates by specifying the format **dd-MM-yyyy**. For the other options, select **Defined by a prefix and its order**, select **last** from the dropdown menu for the order, set the **Prefix** to the symbol **|** and press **OK**. The dates will appear in the appropriate column of the main window.




You can now check these and edit them manually as required. At the top of the window you can set the units that the dates are given in (years, months, days) and whether they are specified relative to a point in the past (as is the case for years such as 2017) or backwards in time from the present (as in the case of radiocarbon ages).

The **Height** column lists the ages of the tips relative to time 0 (in our case 2007.63). The **Uncertainty** column allows specifying with what precision the sampling time is known. This is not useful in our case because sampling dates are known to the exact day, but BEAST allows to integrate over the uncertainty for less precise sampling date (e.g., when only the sampling month or year is known).

Specifying the trait information

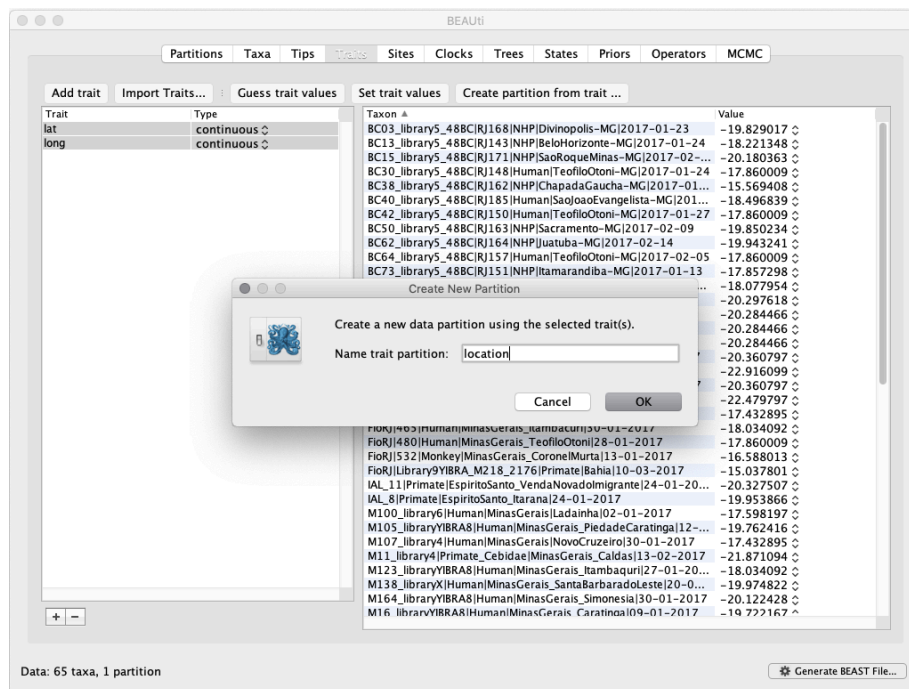
The next thing to do is to click on the **Traits** tab at the top of the main window. A trait can be any characteristic that is inherent to the specific taxon, for example, geographical location or host species. This step will assign a latitude and longitude as bivariate geographical location to each taxa based on the trait specification for each sequence.

 The tab-delimited file associating each taxon with a latitude and longitude 'YFV_coordinates.txt' [can be downloaded from here \(//tutorials/workshop_continuous_diffusion_yfv/files/YFV_coordinates.txt\)](https://tutorials/workshop_continuous_diffusion_yfv/files/YFV_coordinates.txt).

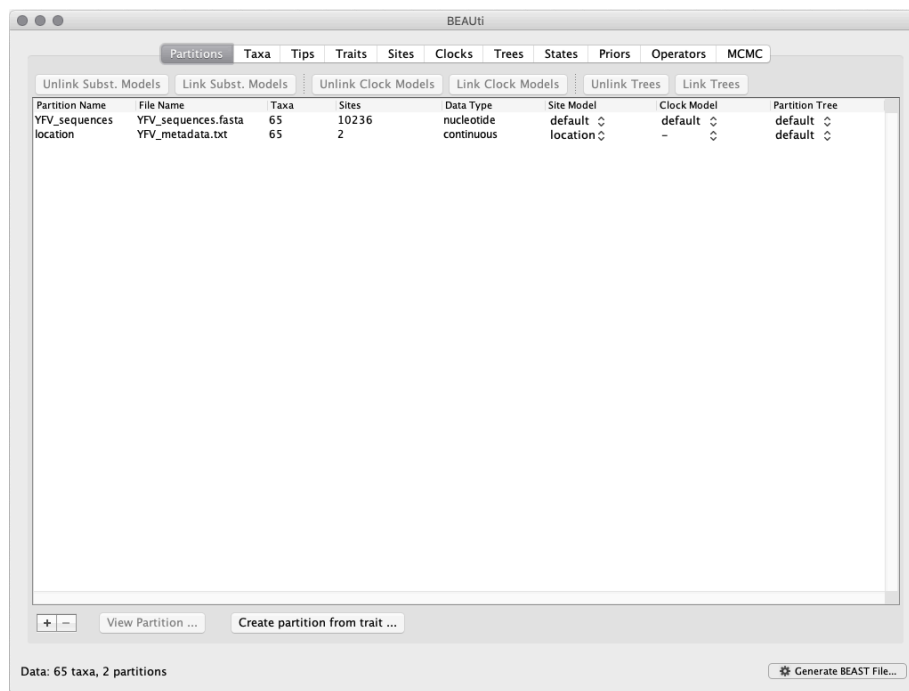
To associate the sequences with the traits, we need to import a new trait under the **Traits** tab (click **Import Traits...**). This will open a new window that allows importing a file with the traits. Browse to and click on the 'YFV_coordinates.txt' tab-delimited file, with the following content:

```
traits lat long
NC|ES504|NHPrimate|DomingosMartins|EspiritoSanto|20-02-2017 -20.360797 -40.65981
NC|ES505|NHPrimate|DomingosMartins|EspiritoSanto|22-02-2017 -20.360797 -40.65981
FioRJ|Library9YIBRA_M218_2176|Primate|Bahia|10-03-2017 -15.037801 -41.934681
FioRJ|460|Human|MinasGerais_NovoCruzeiro|30-01-2017 -17.432895 -41.998972
FioRJ|1818|Human|EspiritoSanto_Cariaciacia|10-03-2017 -20.284466 -40.431258
FioRJ|2115|Monkey|EspiritoSanto_Cariaciacia|09-03-2017 -20.284466 -40.431258
FioRJ|3919|Human|EspiritoSanto_DomingosMartins|10-04-2017 -20.360797 -40.65981
FioRJ|438|Monkey|Espirito_Santo_DomingosMartins|31-01-2017 -20.360797 -40.65981
FioRJ|465|Human|MinasGerais_Itambacuri|30-01-2017 -18.034092 -41.683337
FioRJ|480|Human|MinasGerais_Teofilotooni|28-01-2017 -17.860009 -41.509104
FioRJ|532|Monkey|MinasGerais_CoroneoMurta|13-01-2017 -16.588013 -42.196772
FioRJ|1536|Human|EspiritoSanto_Vitoria|22-02-2017 -20.297618 -40.295777
...
MF170971|Monkey|MinasGerais_SaoRocioDeMinas|NA|30-01-2017 -20.177911 -46.439305
```

After clicking **OK**, select both the 'lat' and 'long' trait in the left window and click on **create partition from trait...** In the window that pops up, enter a name for this partitions, e.g. 'location':



This new 'location' partition with 2 'Sites' and with a continuous 'Data Type' will be shown under the **Partitions** tab:



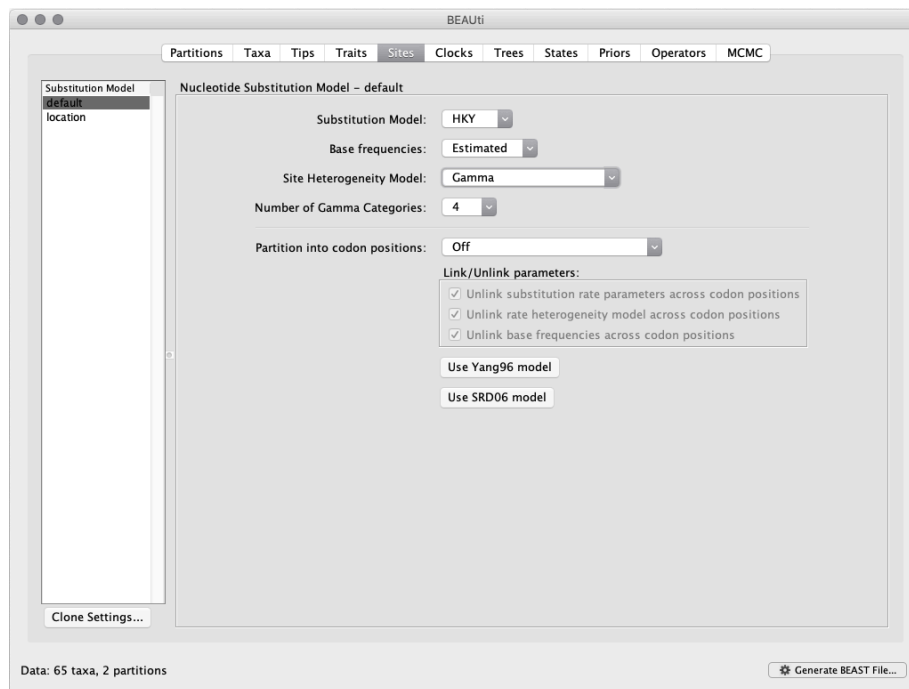
Setting the sequence and trait evolutionary models

The next thing to do is to click on the **Sites** tab at the top of the main window. This will reveal the evolutionary model settings for BEAST. Exactly which options appear depend on whether the data are nucleotides, acids or traits.

This tutorial assumes that you are familiar with the available evolutionary models, however there are a couple of points to note about selecting a model in BEAUti:

Selecting the **Partition into codon positions** option assumes that the data are aligned as codons. This option will then estimate a separate rate of substitution for each codon position, or for 1+2 versus 3, depending on the setting. Selecting the **Unlink substitution model across codon positions** will specify that BEAST should estimate a separate transition-transversion ratio or general time reversible rate π for each codon position. Selecting the **Unlink rate heterogeneity model across codon positions** will specify that BEAST should estimate a set of rate heterogeneity parameters (gamma shape parameter and/or proportion of invariant sites) for each codon position.

For the nucleotide substitution model in this tutorial, keep the default HKY substitution model, keep the base frequencies to be **Estimated**, specify the **Site Rate Heterogeneity** to **Gamma**, and keep the **Partition into codon positions** to **Off**.

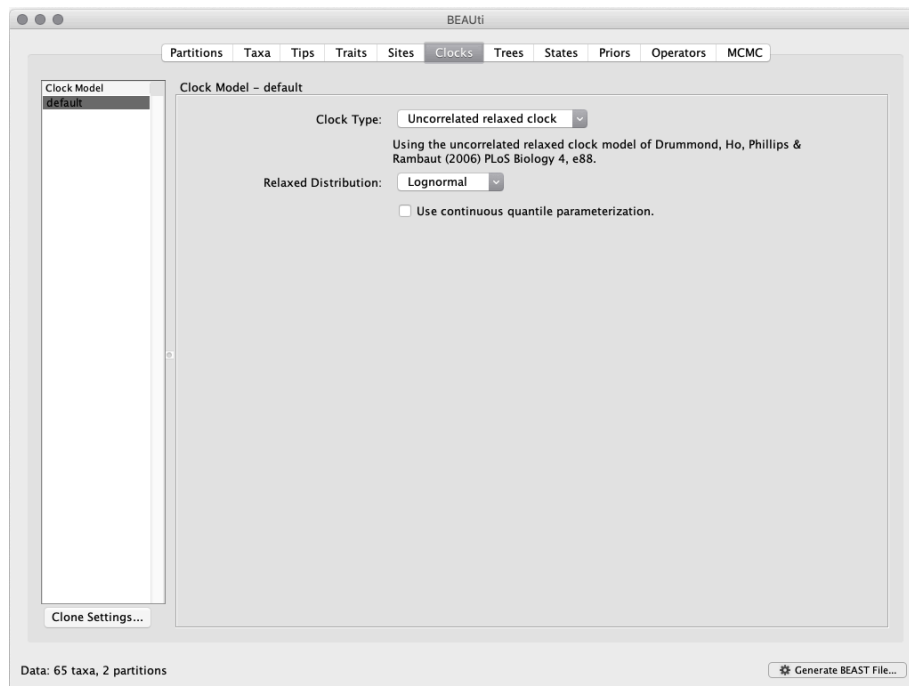


Next, click on `location` in the `Substitution model` window and select the `Cauchy RRW model`, and select `Bivariate trait represents latitude and longitude`. This option generates diffusion statistics that are specific for bivariate spatial traits (with latitude and longitude in that order). Also select the `add random jitter to tips`, which adds noise drawn uniformly at random from a particular jitter window size to duplicated (location) traits. Set the `jitter window size` to 0.01. The noise avoids a poor performance of Brownian diffusion models when not all sequences are associated with unique locations.



Setting the 'molecular clock' model

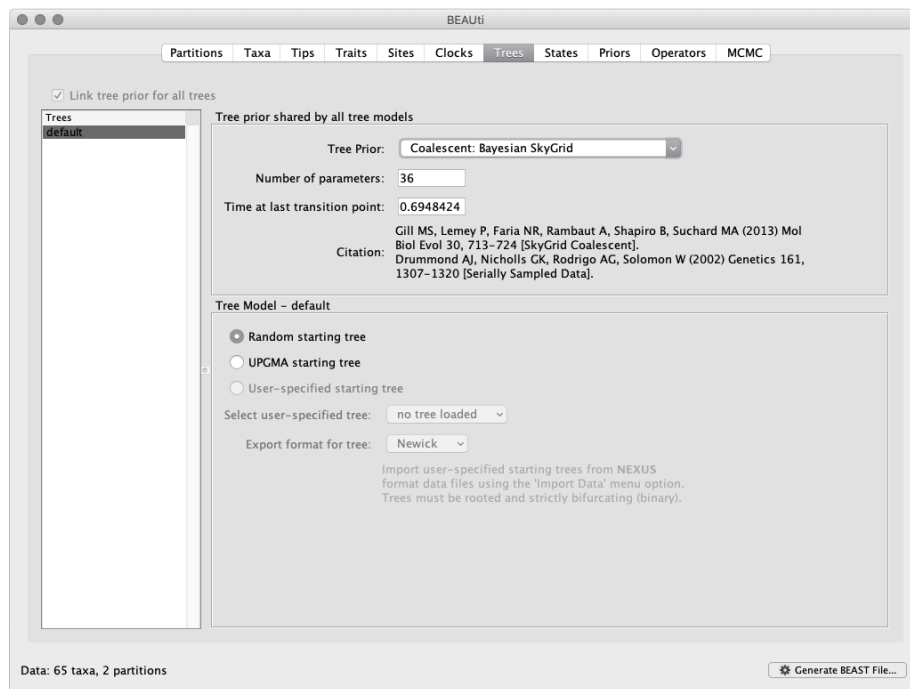
The 'Molecular Clock Model' options in the `Clocks` panel allows us to choose between a strict and a relaxed (uncorrelated lognormal or uncorrelated exponential) clock. We will perform our run using the Lognormal relaxed molecular clock (Uncorrelated) model:



Now move on to the `Trees` panel.

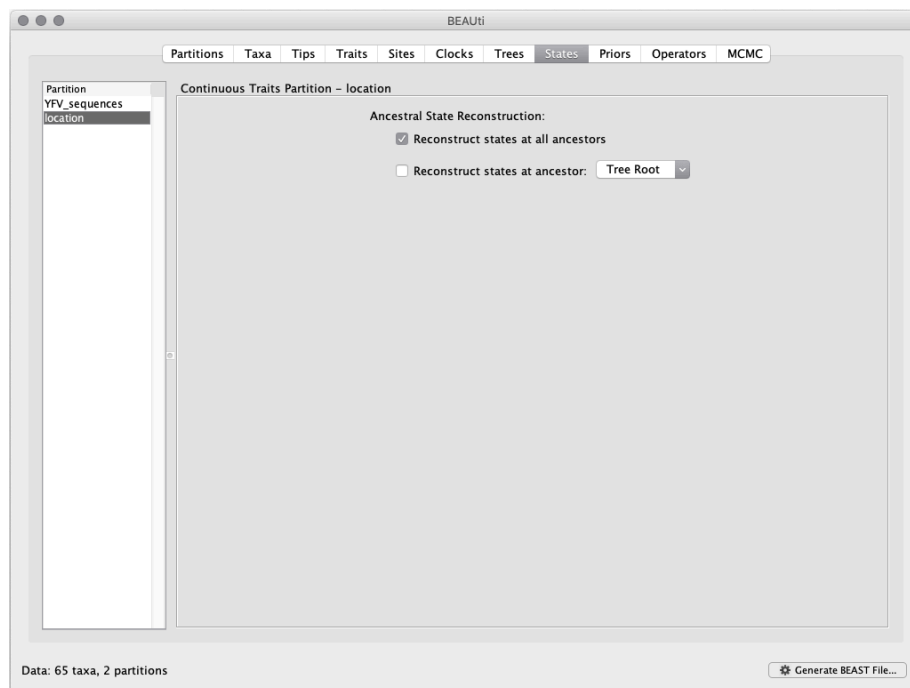
Setting the tree prior

This panel contains settings about the tree. Firstly the starting tree is specified to be 'randomly generated'. The other main setting here is to specify the 'Tree prior' which describes how the population size is expected to change over time according to a coalescent model. The default tree prior is set to a constant size coalescent prior. Here, we will select a flexible skygrid coalescent model as demographic tree prior (**Coalescent: Bayesian SkyGrid**), with 36 grid points (**Number of parameters**) and **A time at last transition point** set to `0.6948424`. By doing so, the grid points actually approximate the number of epidemiological weeks spanned by the duration of the phylogeny (Faria et al. 2018).



The ancestral states settings

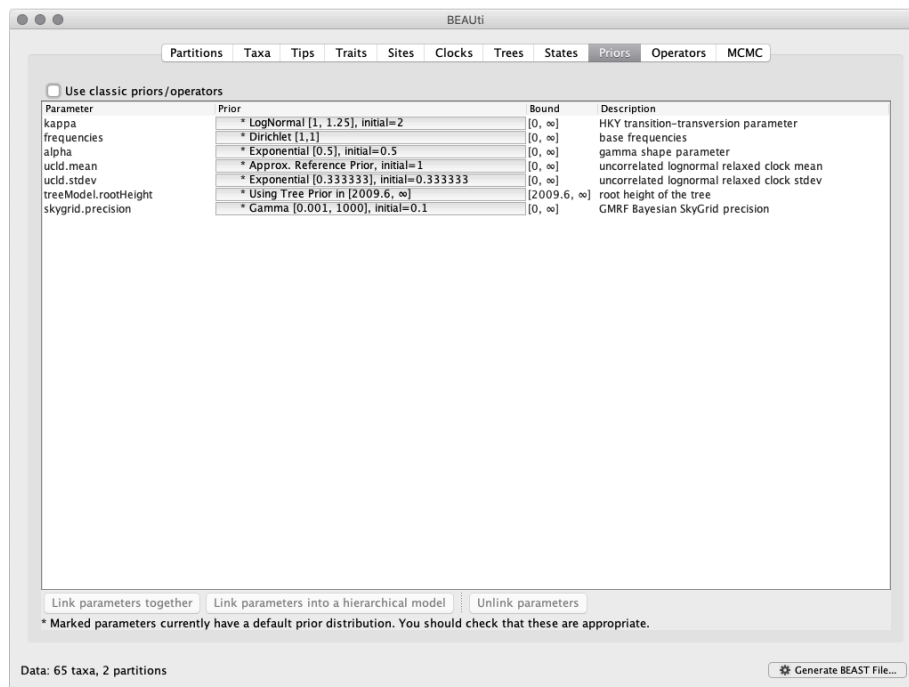
In the **States** panel, check that **location** partition is set to **Reconstruct states at all ancestors** (by default):



Setting up the priors

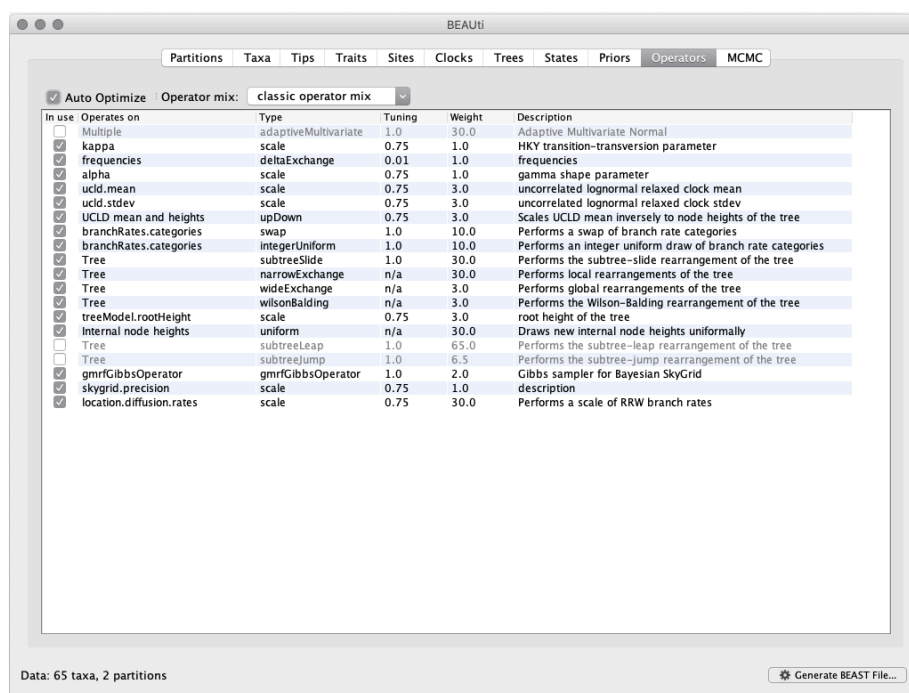
Review the prior settings under the **Priors** tab. This panel has a table showing every parameter of the currently selected model and what the prior distribution is for each. <Priors that would not be explicitly specific would appear in red, whereas priors that are improper (and hence lead to an improper posterior and improper marginal likelihoods) appear in yellow (e.g. allMus). Click on the prior for this parameter and a prior select window will appear. The codon position-specific relative rates (CP1.mu, CP2.mu and CP3.mu), which are constrained to have a mean of 1, still require proper priors. We here specify lognormal distributions with a log of 0.0 and a log(stdev) of 1.5 for these parameters. Notice that the prior setting turns black after confirming this setting by clicking OK.

We can keep the default priors in this case:



Setting up the operators

Each parameter in the model has one or more “operators” (these are variously called moves, proposals or transition kernels by other MCMC software packages such as MrBayes and LAMARC). The operators specify the parameters change as the MCMC runs. The **Operators** tab in BEAUti has a table that lists the parameters, their operators and the tuning settings for these operators:



In the first column are the parameter names. These will be called things like kappa which means the HKY model's kappa parameter (the transition-transversion bias). The next column has the type of operators that are acting on each parameter. For example, the scale operator scales the parameter up or down by a proportion, the random walk operator adds or subtracts an amount to the parameter and the uniform operator simply sets a new value uniformly within a range. Some parameters relate to the tree or to the divergence times of the nodes of the tree and these have special operators. As of BEAST v1.10, different options are available w.r.t. exploring tree space. In this tutorial, we will use the 'classic operator mix', which consists of a set of tree transition kernels that propose changes to the tree. There is also an option to fix the tree topology as well as a 'new experimental mix', which is currently under development with the aim to improve mixing for large phylogenetic trees.

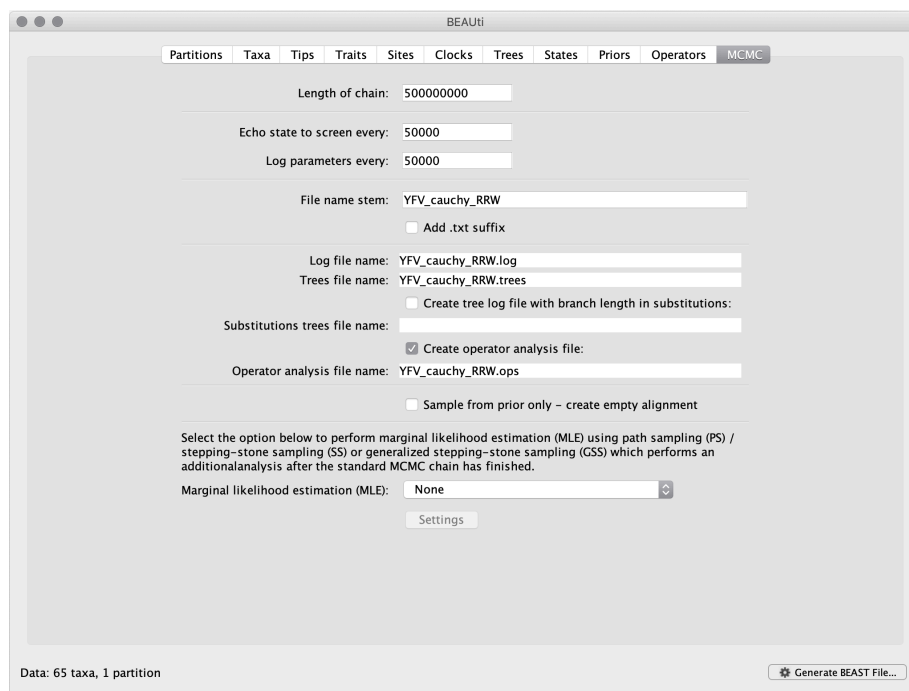
The next column, labelled **Tuning**, gives a tuning setting to the operator. Some operators don't have any tuning settings so have n/a under this column. The tuning parameter will determine how large a move each operator will make which will affect how often that change is accepted by the MCMC which will in turn affect the efficiency of the analysis. For most operators (like random walk and subtree slide operators) a larger tuning parameter means larger moves. However for the scale operator a tuning parameter value closer to 0.0 means bigger moves. At the top of the window is an option called **Auto Optimize** which, when selected, will automatically adjust the tuning setting as the MCMC runs to try to achieve maximum efficiency. At the end of the run a table of the operators, their performance and the final values of these tuning settings will be written to the standard output.

The next column, labelled **Weight**, specifies how often each operator is applied relative to the others. Some parameters tend to be sampled very efficiently - an example is the kappa parameter - these parameters have their operators down-weighted so that they are not changed as often. We will start by using the default settings for this analysis. As of BEAST v1.8.4, different options are available w.r.t. exploring tree space. In this tutorial, we will use the **classic operator mix**, which consists of a set of tree transition kernels that propose changes to the tree. There is also an option to fix the tree topology as well as a **new experimental mix** which is currently under development with the aim to improve mixing for large phylogenetic trees. We can keep the default operator settings for the analysis in this tutorial.

Setting the MCMC options

The **MCMC** tab in BEAUti provides settings to control the MCMC chain. Firstly we have the **Length of chain**. This is the number of steps the MCMC will make in the chain before finishing. How long this should be depends on the size of the dataset, the complexity of the model and the precision of the answer required. The default value of 10,000,000 is entirely arbitrary and should be adjusted according to the size of your data. We will see later how the resulting log file can be analysed using Tracer in order to examine whether a particular chain length is adequate.

The next couple of options specify how often the current parameter values should be displayed on the screen and recorded in the log file. The screen output is simply for monitoring the program's progress so can be any value (although if set too small, the sheer quantity of information being displayed on the screen will slow the program down). For the log file, the value should be set relative to the total length of the chain. Sampling often will result in very large files with little extra benefit in terms of the precision of the estimates. Sample too infrequently and the log file will not contain much information about the distributions of the parameters. You probably want to aim to store no more than 10,000 samples.



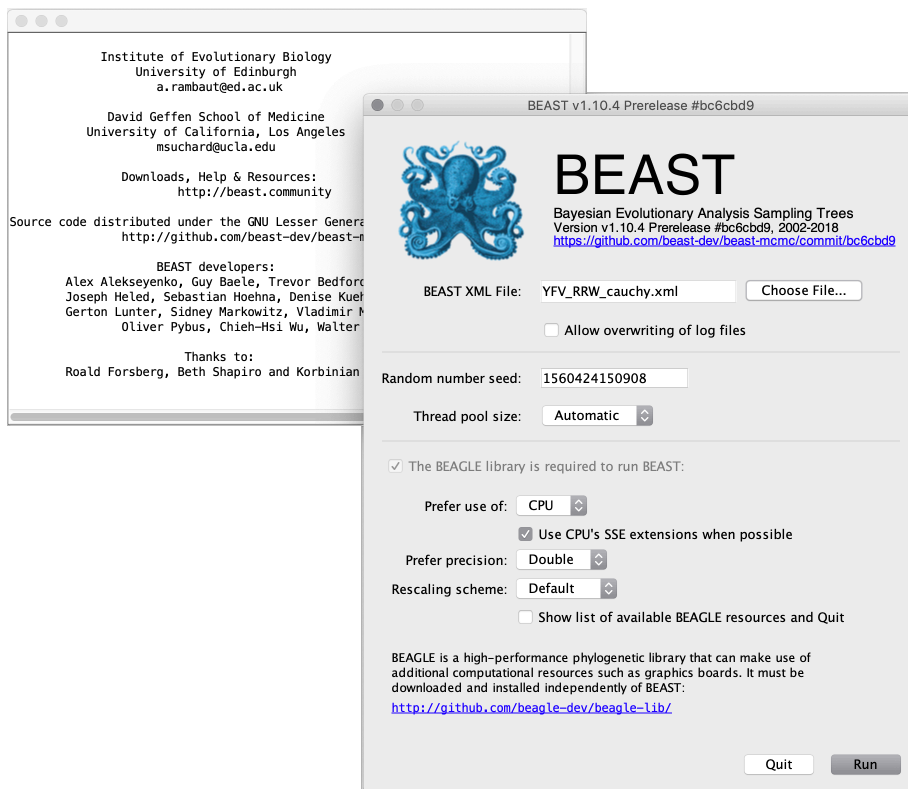
For this dataset let's set the chain length to 500,000,000 and the parameter logging to file as well the state echo to screen every 50,000 state.

The next option allows the user to set the File stem name; set this to 'YFV_RRW_cauchy' ('RRW_RRW_cauchy' for 'relaxed random walk Cauchy diffusion model'). The next two options give the file names of the log for the parameters and the trees. These will be set based on the file stem name. You can also log the operator analysis to a file. An option is also available to sample from the prior only, which can be useful to evaluate divergent our posterior estimates are when information is drawn from the data. Here, we will not select this option, but analyze the actual data.

Finally, one can select to perform marginal likelihood estimation to assess model fit, which is not needed in this exercise. So, at this point we are ready to generate a BEAST XML file and to use this to run the Bayesian evolutionary analysis. To do this, either select the **Generate BEAST File...** option from the **File** menu or click the similarly labelled button at the bottom of the window. Continue and choose a name for the file (for example, 'YFV_RRW_cauchy.xml' by adding the xml extension to the file name stem) and save the file. For convenience, you can leave the BEAUti window open so that you can change the values and re-generate the BEAST file if necessary.

Running BEAST

Once the BEAST XML file has been created the analysis itself can be performed using BEAST. The exact instructions for running BEAST depends on the computer you are using, but in most cases a dialog box will appear in which you select the XML file:

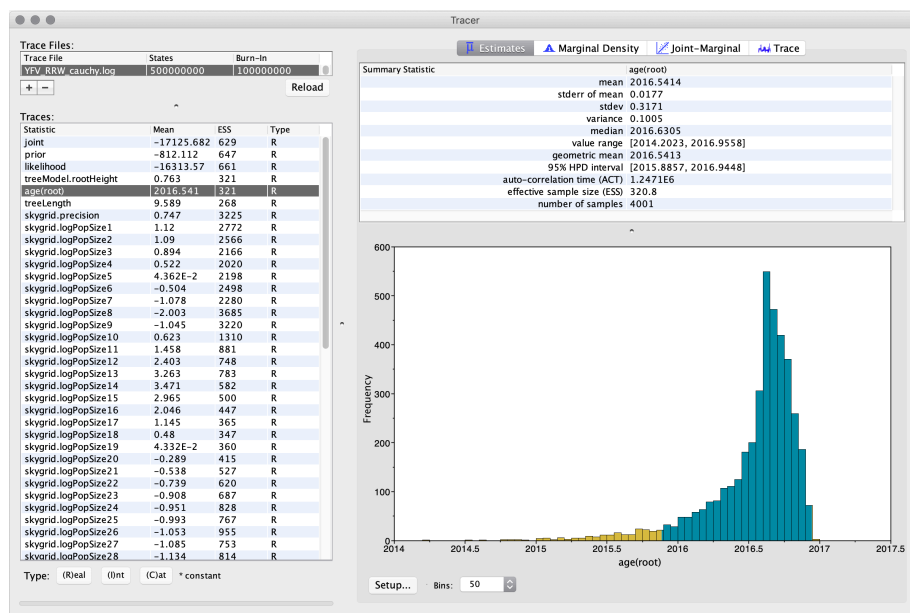


Press the 'Choose File' button and select the XML file you just created and press 'Run'. When you have installed the BEAGLE library (<https://github.com/beagle-dev/beagle-lib>), you can use this in conjunction with B to speed up the calculations. If not installed, unselect the use of the BEAGLE library. If the command line version of BEAST is being used then the name of the XML file is given after the name of the BEAST executable analysis will then be performed with detailed information about the progress of the run being written to the screen. When it has finished, the log file and the trees file will have been created in the same location as your file.

Analyzing the BEAST output

To analyze the results of running BEAST we are going to use the program Tracer. The exact instructions for running Tracer differs depending on which computer you are using. Double click on the Tracer icon; once ru Tracer will look similar irrespective of which computer system it is running on.

Select the **Import Trace File...** option from the **File** menu. If you have it available, select the log file that you created in the previous section ('YFV_RRW_cauchy.log'). Alternative, drag and drop your log file i Tracer window. The file will load and you will be presented with a window similar to the one below. Remember that MCMC is a stochastic algorithm so the actual numbers will not be exactly the same.



On the left hand side is the name of the log file loaded and the traces that it contains. Selecting a trace on the left brings up analyses for this trace on the right hand side depending on tab that is selected. When first opened, the 'age(root)' trace is selected and various statistics of this trace are shown under the **Estimates** tab.

In the top right of the window is a table of calculated statistics for the selected trace. The statistics and their meaning are described in the table below.

- Mean - The mean value of the samples (excluding the burn-in).
- Stdev - The standard error of the mean. This takes into account the effective sample size so a small ESS will give a large standard error.
- Median - The median value of the samples (excluding the burn-in).
- 95% HPD Lower - The lower bound of the highest posterior density (HPD) interval. The HPD is the shortest interval that contains 95% of the sampled values.
- 95% HPD Upper - The upper bound of the highest posterior density (HPD) interval.

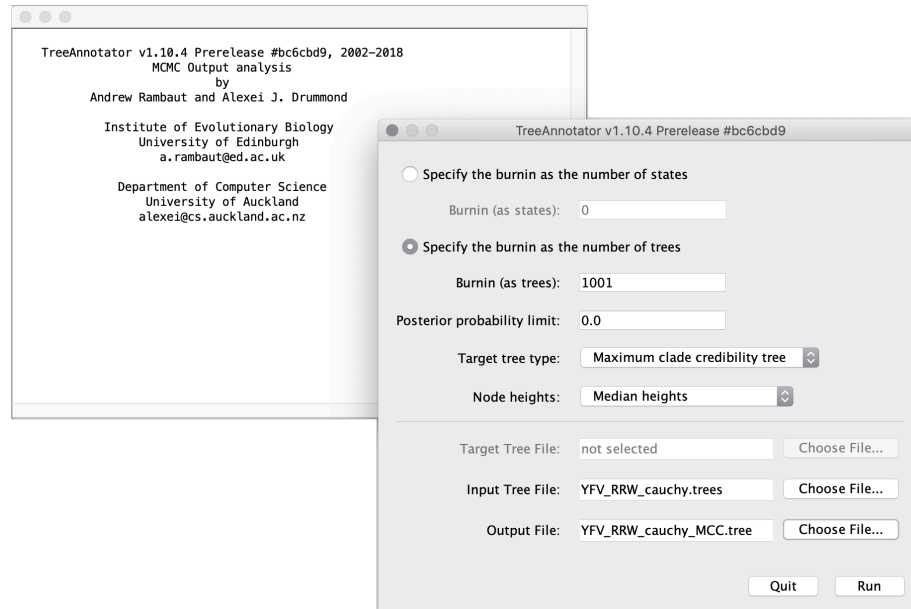
- Auto-Correlation Time (ACT) - The average number of states in the MCMC chain that two samples have to be separated by for them to be uncorrelated (i.e. independent samples from the posterior). The ACT is estimated from the samples in the trace (excluding the burn-in).
- Effective Sample Size (ESS) - The effective sample size (ESS) is the number of independent samples that the trace is equivalent to. This is calculated as the chain length (excluding the burn-in) divided by the \hat{A}

Note that when the effective sample sizes (ESSs) are small (ESSs less than 100 are highlighted in red by Tracer and values > 100 but < 200 are in yellow), this is not good. A low ESS means that your samples are equivalent to only few uncorrelated samples trace and this may not represent the posterior distribution well. A simple response to this kind of situation would be to run the chain for longer. Here, there are no obvious \hat{A} in the plot which would suggest that the MCMC has not yet converged, and there are no large-scale fluctuations in the trace which would suggest poor mixing.

Summarizing the trees

We have seen how we can diagnose our MCMC run using Tracer and produce estimates of the marginal posterior distributions of parameters of our model. However, BEAST also samples trees (either phylogenies or genealogies) at the same time as the other parameters of the model. These are written to a separate file called the 'trees' file. This file is a standard NEXUS format file. As such it can easily be loaded into other software to examine the trees it contains. One possibility is to load the trees into a program such as PAUP* and construct a consensus tree in a similar manner to summarizing a set of bootstrap trees. In this case, the \hat{A} values reported for the resolved nodes in the consensus tree will be the posterior probability of those clades.

In this tutorial, however, we are going to use a tool that is provided as part of the BEAST package to summarize the information contained within our sampled trees. The tool is called TreeAnnotator and once running, will be presented with a window like the one below.



TreeAnnotator takes a single 'target' tree and annotates it with the summarized information from the entire sample of trees. The summarized information includes the average node ages (along with the HPD intervals), posterior support and the average rate of evolution on each branch (for relaxed clock models where this can vary). The program calculates these values for each node or clade observed in the specified 'target' tree. It offers the following options:

- Burnin - This is the number of steps in the MCMC chain, Burnin (as states), or the number of trees, Burnin (as trees), that should be excluded from the summarization.
- Posterior probability limit - This is the minimum posterior probability for a node in order for TreeAnnotator to store the annotated information. The default is 0.0 so every node, no matter what its support, will have information summarized. Make sure this value remains 0.0 as every node will require location annotation for further visualization.
- Target tree type - This has three options **Maximum clade credibility tree** or **User target tree**. For the latter option, a NEXUS tree file can be specified as the Target Tree File, below. Select the first option, TreeAnnotator will examine every tree in the Input Tree File and select the tree that has the highest product of the posterior probabilities of all its nodes.
- Node heights - This option specifies what node heights (times) should be used for the output tree. If the **Keep target heights** is selected, then the node heights will be the same as the target tree. Node heights can also be summarised as a Mean or a Median over the sample of trees. Sometimes a mean or median height for a node may actually be higher than the mean or median height of its parental node (because of particular ancestral-descendent relationships in the MCC tree may still be different compared to a large number of other trees sampled). This will result in artifactual negative branch lengths, but can be avoided with the **Common Ancestor heights** option. Let's use the default Median heights for our summary tree.
- Target Tree File - If the **User target tree** option is selected then you can use **Choose File...** to select a NEXUS file containing the target tree.

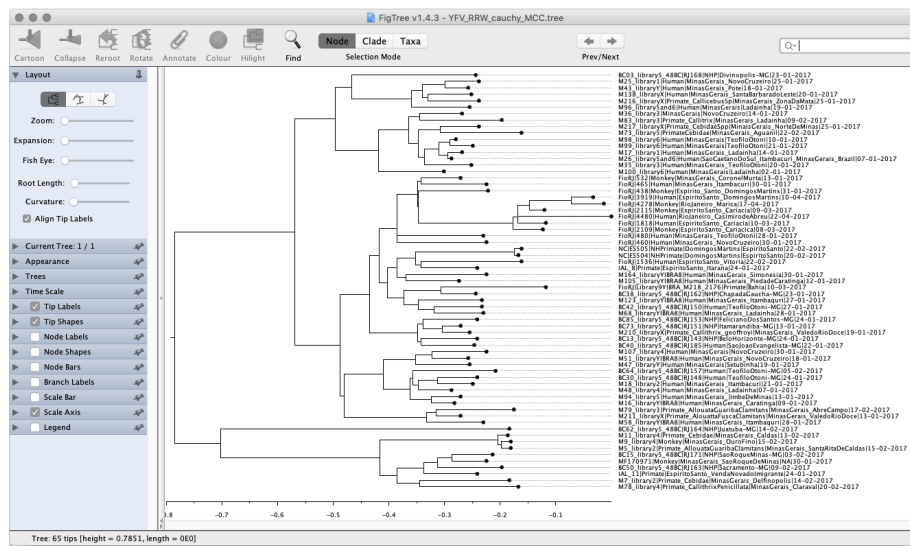
Input Tree File - Use the **Choose File...** button to select an input trees file. This will be the trees file produced by BEAST.

- **Output File** - Select a name for the output tree file (e.g., 'YFV_RRW_cauchy_MCC.tree').

Once you have selected all the options, above, press the **Run** button. TreeAnnotator will analyse the input tree file and write the summary tree to the file you specified. This tree is in standard NEXUS tree file format and can be loaded into any tree drawing package that supports this. However, it also contains additional information that can only be displayed using the FigTree program.

Viewing the annotated tree

Run FigTree and select the **Open...** command from the **File** menu. Select the tree file you created using TreeAnnotator in the previous section. The tree will be displayed in the FigTree window. On the left hand side of the window are the options and settings which control how the tree is displayed.



In the **Layout** panel select the check-box **Align Tip Labels** to increase clarity. We can also plot a time scale axis for this evolutionary history (select **Scale Axis** and deselect **Scale bar**).

Visualizing Bayesian phylogeographic reconstructions using Spred3

Spred3, i.e. Spatial Phylogenetic Reconstruction of Evolutionary Dynamics using Data-Driven Documents (D3), is a software to visualize the output from Bayesian phylogeographic analysis and constitutes a user-friendly application to analyze and visualize pathogen phylogenetic reconstructions resulting from Bayesian inference of sequence and trait evolutionary processes. Spred3 allows to visualise on custom maps and generate HTML pages for display in modern-day browsers such as Firefox, Safari and Chrome. One of the functionalities of Spred3 that relate to the continuous phylogeographic analysis performed previously include visualizing location-annotated MCC trees. A detailed tutorial for this particular step is available here [\[2\]](#).

Brief instructions can be found in the quick how-to summary below. Compare the animated version for both the homogeneous and RRW model: do you notice any difference?

A quick how-to summary

Run BEAUti

- Load a NEXUS format alignment by selecting the **Import Data...** option from the File menu. Select the file called 'YFV_sequences.fasta'.
- In the **Tips** tab, select the **Use tip dates** option and use the **Parse Dates** button. In the new window, select the option **Defined by a prefix and its order**, and the **last** for the order. Final the **Prefix** to the symbol **|** and press **OK**. The dates will appear in the appropriate column of the main window.
- In the **Traits** tab, click **Import trait**. Browse to and load the 'YFV_coordinates.txt' tab-delimited file
- After loading the file, select both the 'Lat' and 'Long' traits in the panel on the left and select **Create partition from trait...** Enter the name 'location' for this partition.
- In the **Sites** tab, keep the default HKY substitution model, keep the base frequencies to be Estimated, and the 'Site Rate Heterogeneity' to 'Gamma'.
- Click on location in the **Substitution model** window on the left and keep the default 'Homogenous Brownian model' and select **Bivariate trait represents latitude and longitude** and a **J** set to '0.01'.
- In the **Clocks** tab, select the Lognormal relaxed molecular clock (Uncorrelated) model.
- In the **Trees** tab, select the skygrid model as the demographic tree prior (**Coalescent: Bayesian SkyGrid**), with 36 grid points (**number of parameters**) and **A time at last transition point** to **0.6948424** (to define grid points approximating the number of epidemiological weeks spanned by the duration of the phylogeny).
- In the **MCMC** tab, set the chain length to 500,000,000 and the sampling frequencies to 50,000. Set the File name stem to 'YFV_RRW_cauchy' and generate the beast file ('YFV_RRW_cauchy.xml').

Run BEAST and load the xml file.

Analyze the output using Tracer. Analyze the output file for the longer runs.

Summarize the trees for the longer run using treeAnnotator.

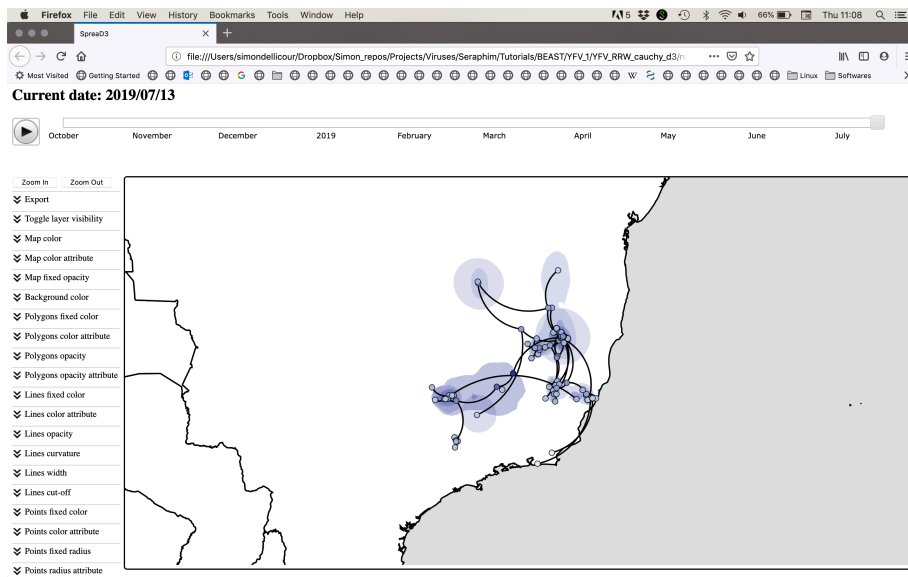
Visualize the tree in FigTree.

Run Spred3.

We will first summarise an MCC tree and then summarise the information in the entire tree distribution.

- Select as input type in the **Data** panel: **MCC tree with CONTINUOUS traits**, load your MCC tree file.
- Select 'location1' as **Latitude attribute** name and 'location2' as **Longitude attribute** name.
- Set the **most recent sampling date** to 2017-04-22.
- Load a GeoJSON 'South_America.json'.
- Keep all other default settings and click **Output** to generate a JSON file.
- Go to the **Rendering** panel, keep the D3 renderer as the renderer of choice, and load the generated JSON file.
- Click **Render to D3** to generate the HTML page and a browser window will open automatically.

This visualizes the MCC tree and the uncertainty for its node locations:



Conclusion and Resources

This tutorial only scratches the surface of the analyses that are possible to undertake using BEAST. It has hopefully provided a relatively gentle introduction to the fundamental steps that will be common to all BEAST analyses and provide a basis for more challenging investigations. BEAST is an ongoing development project with new models and techniques being added on a regular basis. The BEAST website provides details of a mailing list that is used to announce new features and to discuss the use of the package. The website also contains a list of tutorials and recipes to answer particular evolutionary questions using BEAST as well as a description of the XML input format, common questions and error messages.

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