Phylogeographic inference in continuous space
A hands-on practical

This chapter provides a step-by-step tutorial for reconstructing the spatial dynamics of the West Nile virus (WNV) invasion across North America based on a set of viral genome sequences which have been isolated at different points in time (heterochronous data) in different US counties (Pybus et al., 2012, PNAS, 109(37), 15066-15071). WNV is a mosquito-borne RNA virus whose primary host is birds, and was first detected in the United States in New York City in August 1999. The data are 104 genomes collected between 1999 and 2008. We will estimate the ancestral locations of the virus in continuous space, the rate of spread during the WNV invasion and test whether the virus spread at a relatively constant rate through time. In addition, we will apply a procedure referred to as 'Renaissance counting' (Lemey et al., 2012, Bioinformatics, 28(24), 3248-3256), to quantify site-specific selection pressures in the form of nonsynonymous/synonymous substitution rate ratios (dN/dS). Renaissance counting maps substitutions throughout evolutionary history in nucleotide space, and then 'counts' the corresponding number of nonsynonymous and synonymous substitutions, their 'neutral' expectations, and applies a empirical Bayes procedure to those counts to arrive at dN/dS estimates.

The first step will be to convert a NEXUS file with a DATA or CHARACTERS block or a FASTA file 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 problems and to summarize the results.

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

- **BEAST** - this package contains the BEAST program, BEAUti and a couple of utility programs. At the time of writing, the current version is v1.8.4. *BEAST* releases are generally available for download from http://beast.bio.ed.ac.uk/, but the latest release can now be found at https://github.com/beast-dev/beast-mcmc/releases.

- ***Tracer*** - this program is used to explore the output of *BEAST* (and other Bayesian MCMC programs). It graphically and quantitively summarizes the distributions of continuous parameters and provides diagnostic information. At the time of writing, the current version is v1.6. It is available for download from http://tree.bio.ed.ac.uk/software/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 v1.4.2. It is available for download from http://tree.bio.ed.ac.uk/software/figtree

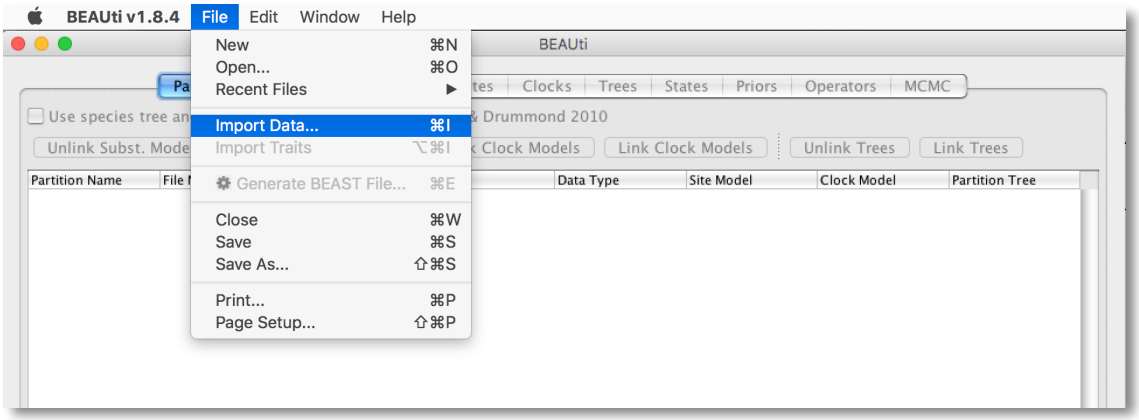
- **SpreaD3** - this is an application for the visualization of phylogeographic analyses performed with *BEAST*. At the time of writing, the current version is v0.9.6. It is available for download from https://rega.kuleuven.be/cev/ecv/software/SpreaD3.

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.

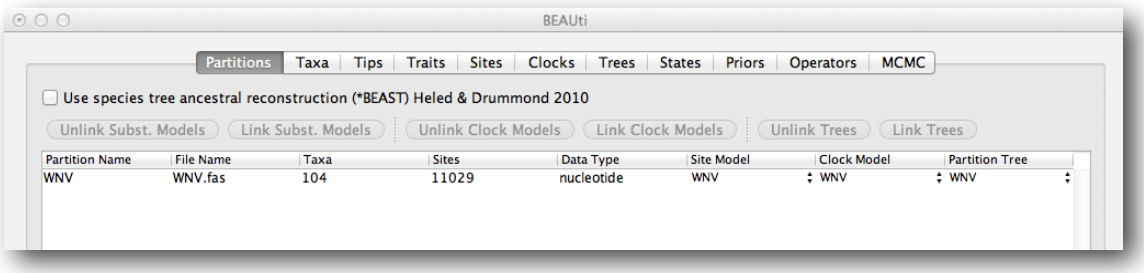
Loading the NEXUS file

To load a NEXUS format alignment, simply select the **Import Data...**. option from the **File** menu.



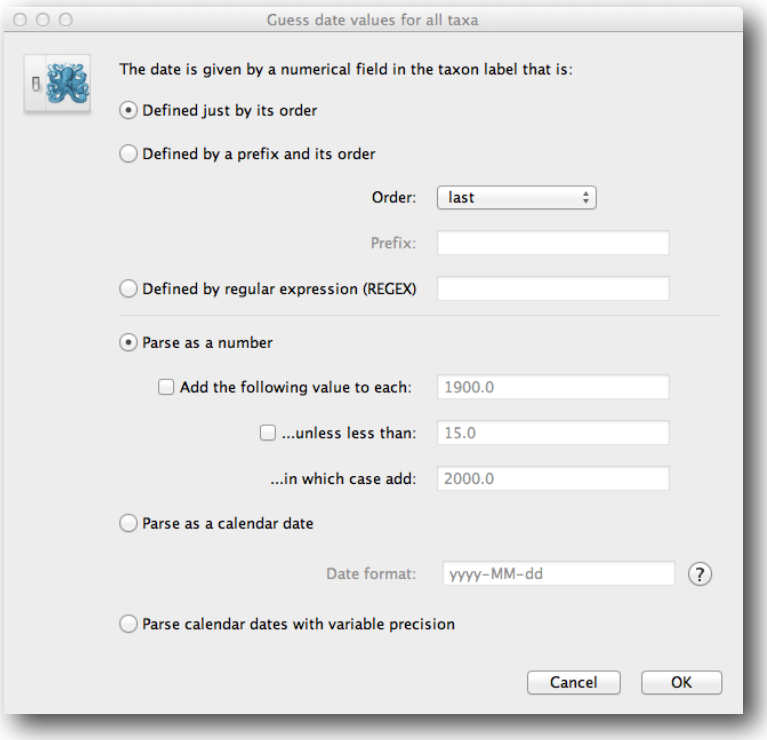
The NEXUS alignment

Select the file called `WNV.fas`. This file contains an alignment of 104 WNV genomes 11029 nucleotides in length. Once loaded, the sequence data will be listed under **Partitions**:



Specifying the sampling date information

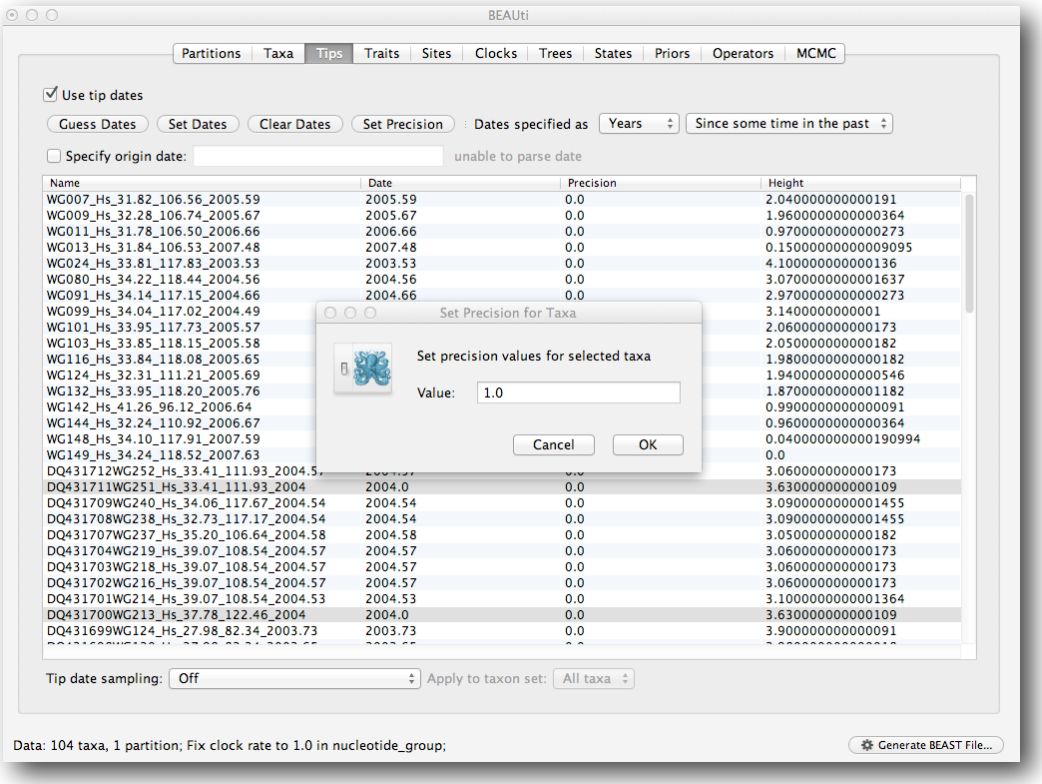
To inform BEAUti/BEAST about the sampling dates of the sequences, go to the **Tips** tab and select the '`Use tip dates`' option. By default all the taxa are assumed to have a date of zero (i.e. the sequences are assumed to be sampled at the same time). In this case, the WNV sequences have been sampled at various dates going back to 1999. The fractional year of sampling is given 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 taxa names contain the calibration information, then a convenient way to specify the dates of the sequences in BEAUti is to use the `Guess Dates` button at the top of the Data tab. Clicking this will make a dialog box appear:



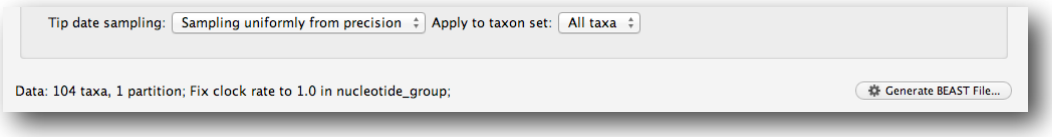
This operation attempts to guess what the dates are from information contained within the taxon names. It works by trying to find a numerical field within each name. If the taxon names contain more than one numerical field then you can specify how to find the one that corresponds to the date of sampling. 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 as ``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:.." option 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. There is also an option to parse calendar dates and one for calendar dates with various precisions. For the H1N1/09 sequences you can keep the default '**Defined just by its order**' and select '**last**' from the drop-down menu for the order and press '**OK**'. The dates will appear in the appropriate column of the main window. You can then 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 would be the case for years such as 2005) 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 '**Precision**' column allows specifying with what precision the sampling times are know. This is useful in our case because some sampling dates are known to the exact day, while others are only known up to the year of sampling (those without decimal in the taxa name or with the .0 decimal in the Date column), and *BEAST* allows to integrate over the uncertainty of the latter. To make use of the ability to adequately accommodate the uncertainty of our sampling dates, select all the taxa (holding down the cmd key) with sampling dates only known up to the year in the Tips window and click on '**Set Precision**'.

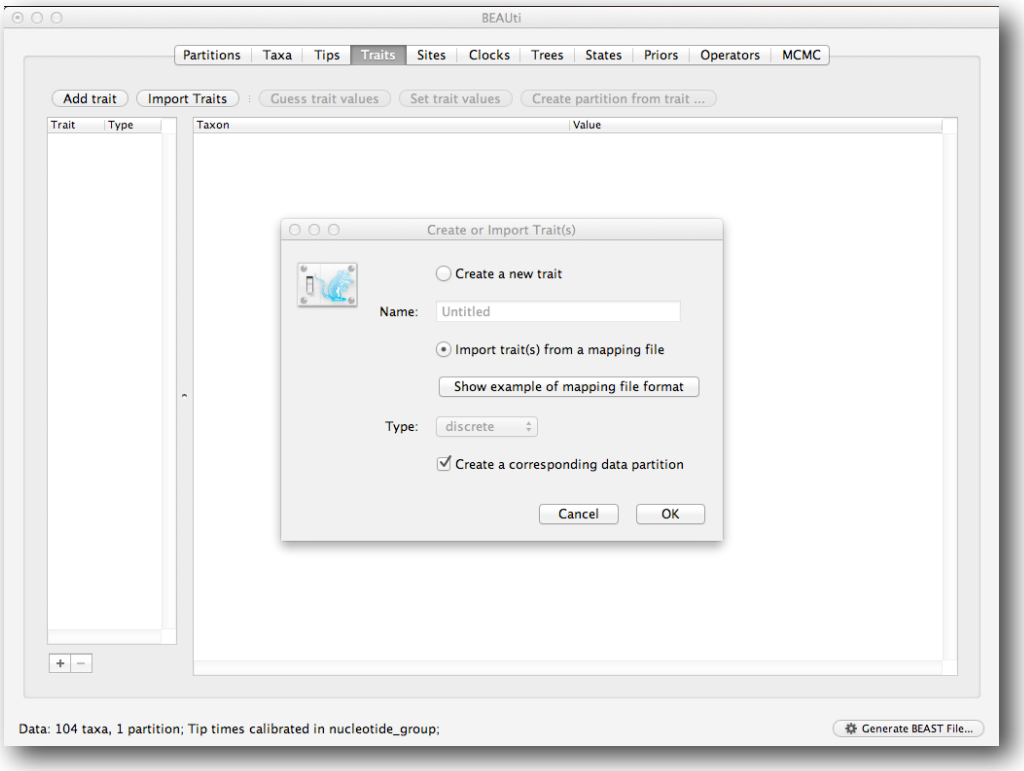


Enter '1.0' as the precision value for 1 year. This will instruct *BEAST* to add a half year to those sampling dates and construct a uniform window of 1 year around this new value. To estimate the respective sampling dates within the constraint of this window in the MCMC analysis, select '**sampling uniformly from precision**' at the bottom left of the Tips panel and keep the '**Apply to taxon set: All taxa**' default.



Specifying the (spatial) trait information for the sequences

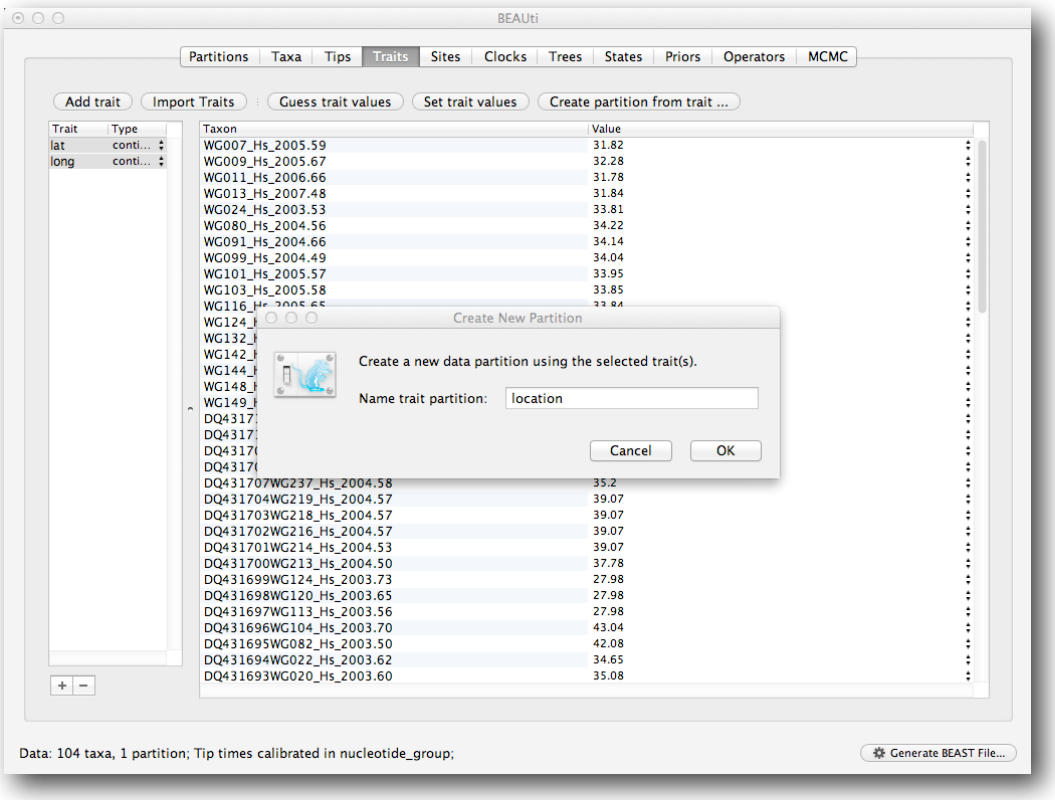
To associate the sequences with the sampling locations, we need to add a new trait under the **Traits** tab (click **Add trait**). This will open a new window to **Create or Import Trait(s)**:



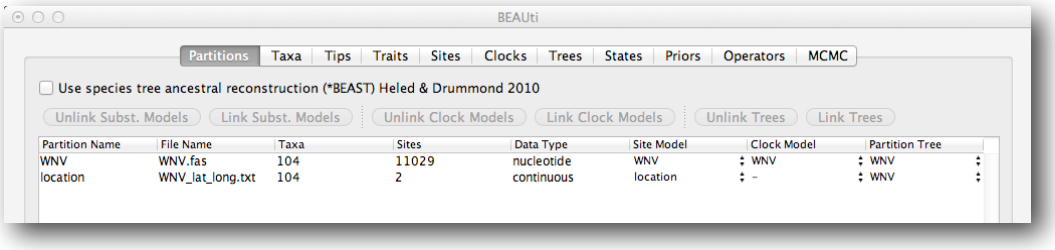
Select **Import trait(s) from a mapping file format**, which will explain how a mapping file should be formatted. Browse to and load the **WNV_lat_long.txt** tab-delimited file which contains latitudes and longitudes for each sequence:

traits	lat	long
WG007_Hs_2005.59	31.82	-106.56
WG009_Hs_2005.67	32.28	-106.74
WG011_Hs_2006.66	31.78	-106.50
WG013_Hs_2007.48	31.84	-106.53
WG024_Hs_2003.53	33.81	-117.83
WG080_Hs_2004.56	34.22	-118.44
WG091_Hs_2004.66	34.14	-117.15
WG099_Hs_2004.49	34.04	-117.02
WG101_Hs_2005.57	33.95	-117.73
WG103_Hs_2005.58	33.85	-118.15
WG116_Hs_2005.65	33.84	-118.08
...		

After clicking **OK**, 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:



The new partition will now also appear in the **Partitions** tab with Data type continuous and 2 'Sites':

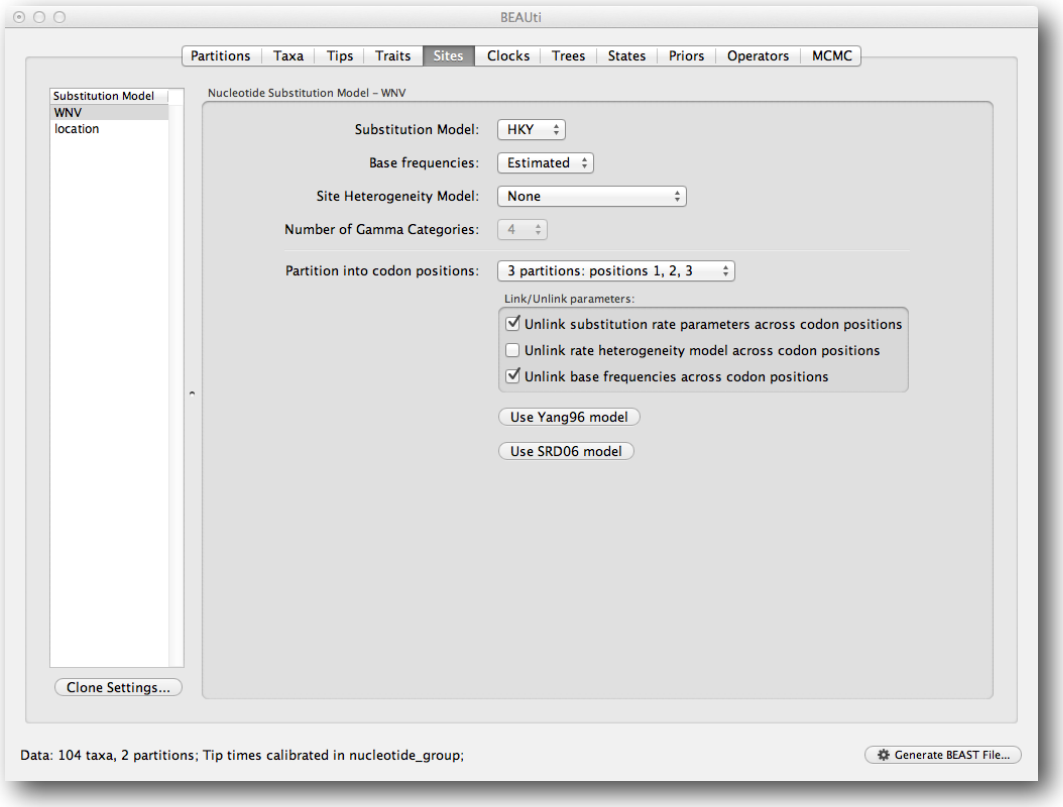


Setting the evolutionary and continuous diffusion model

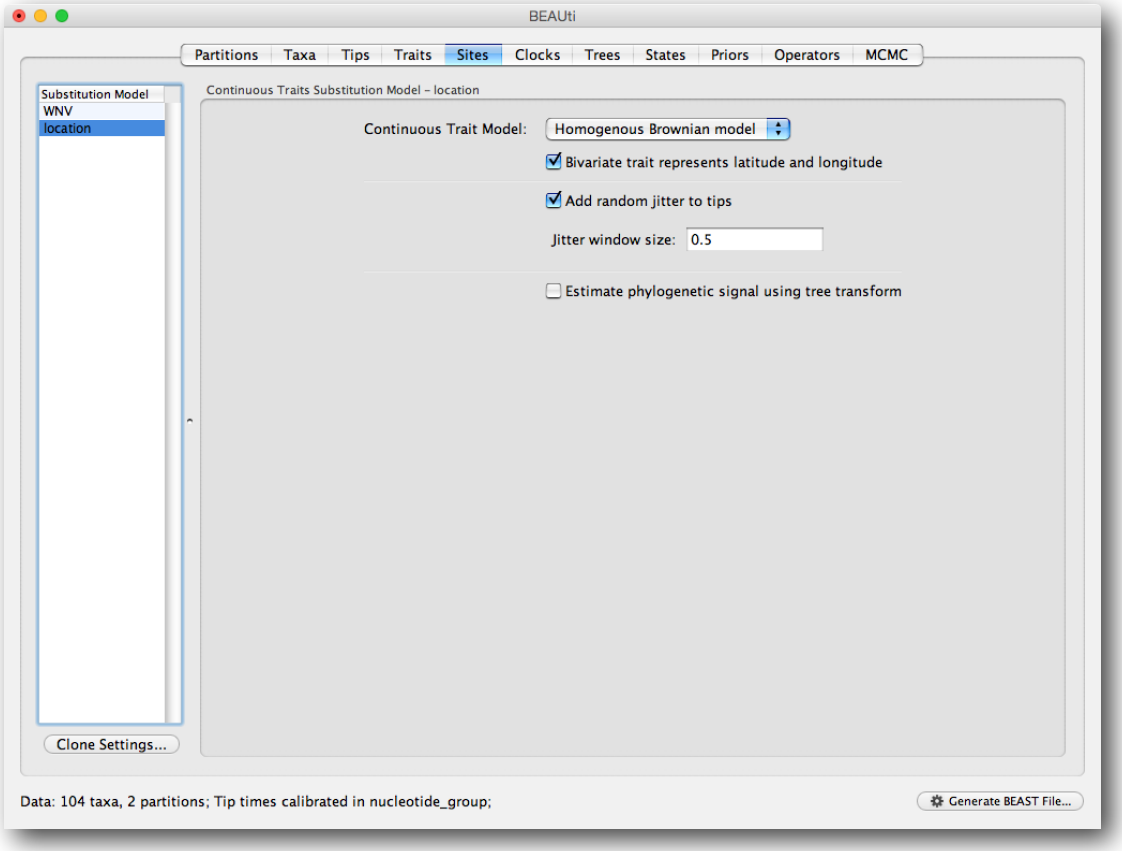
The next thing to do is to click on the **Sites** tab at the top of the main window. This will reveal the evolutionary model settings for BEAST. Exactly which options appear depend on whether the data are nucleotides, amino acids or traits. This tutorial assumes that you are familiar with the evolutionary models available; 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 matrix for each codon position.
- Selecting the Unlink rate heterogeneity model across codon positions will specify that BEAST should estimate 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**, the '**Site Rate Heterogeneity**' to '**None**', and partition the data into 3 partitions for the coding positions (**3 partitions: positions 1,2,3**). Because of the renaissance counting procedure that we will apply to obtain site-specific dN/dS estimates, we cannot model rate heterogeneity within each codon position partition, so we will assume that we capture most among site rate heterogeneity by modeling relative rates among the coding position partitions.

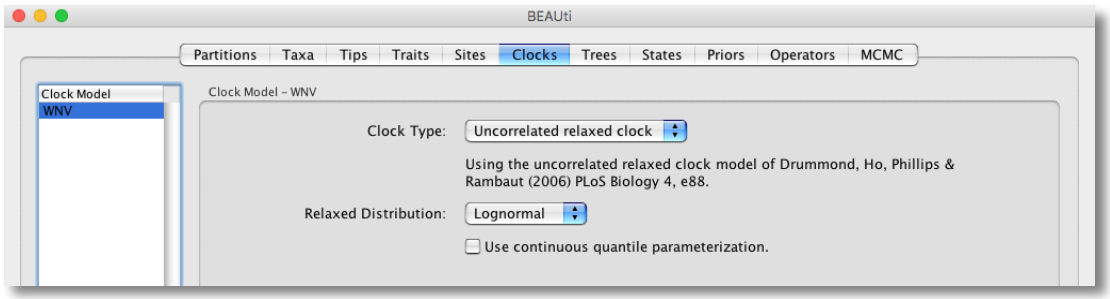


Next, click on **location** in the **Substitution model** window, keep the default **Homogenous Brownian model**, and select **Bivariate trait represents latitude and longitude**. This option generates diffusion statistics that are specific for bivariate spatial traits (with latitude and longitude in 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 0.5. The noise ensures a more appropriate fit of a relaxed random walk model to the data.



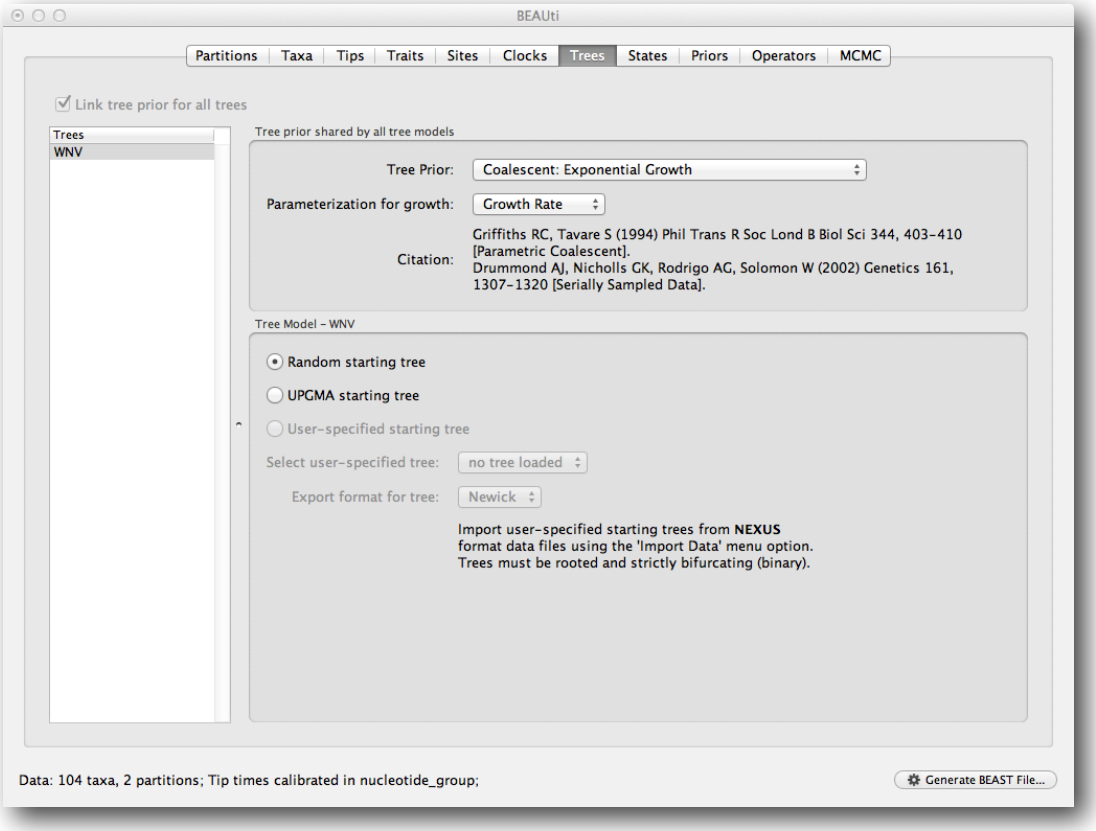
Setting the clock model

Click on the **Clocks** tab at the top of the main window. We will perform our run using the Lognormal relaxed molecular clock (Uncorrelated) model.



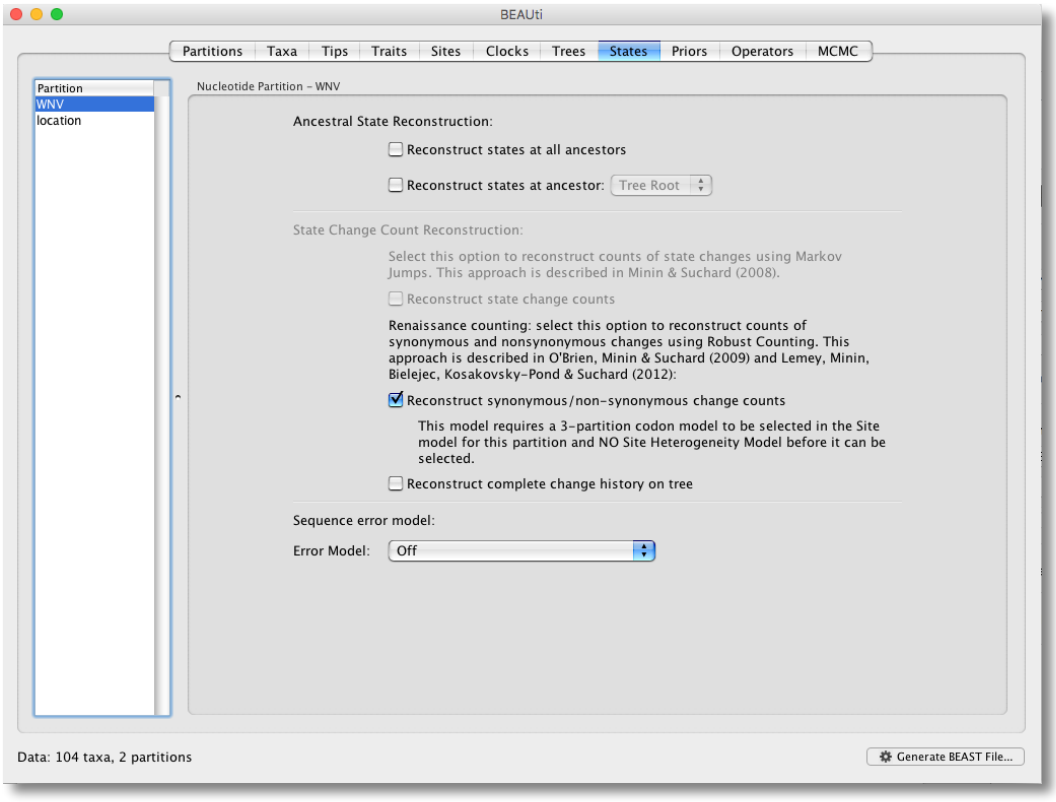
Setting the starting tree and tree prior

Click on the **Trees** tab at the top of the main window. We will select an exponential growth coalescent model as demographic tree prior (Coalescent: Exponential Growth) with standard parametrisation. The tree priors (coalescent and other models) are explained in other lectures/tutorials.

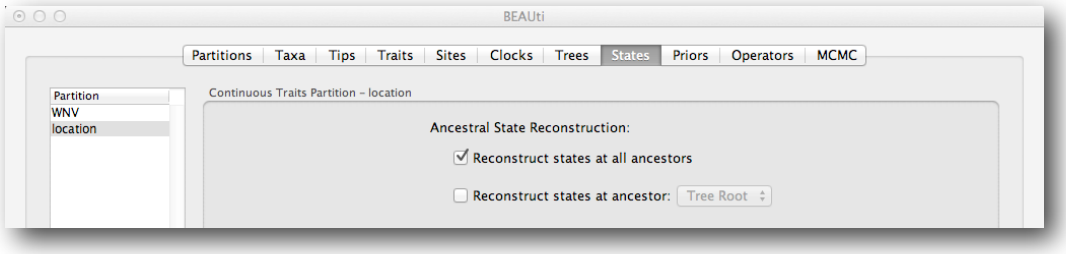


States

In the **states** tab, we can turn on the Renaissance counting procedure by selection '**Reconstruct synonymous/non-synonymous change counts**'.

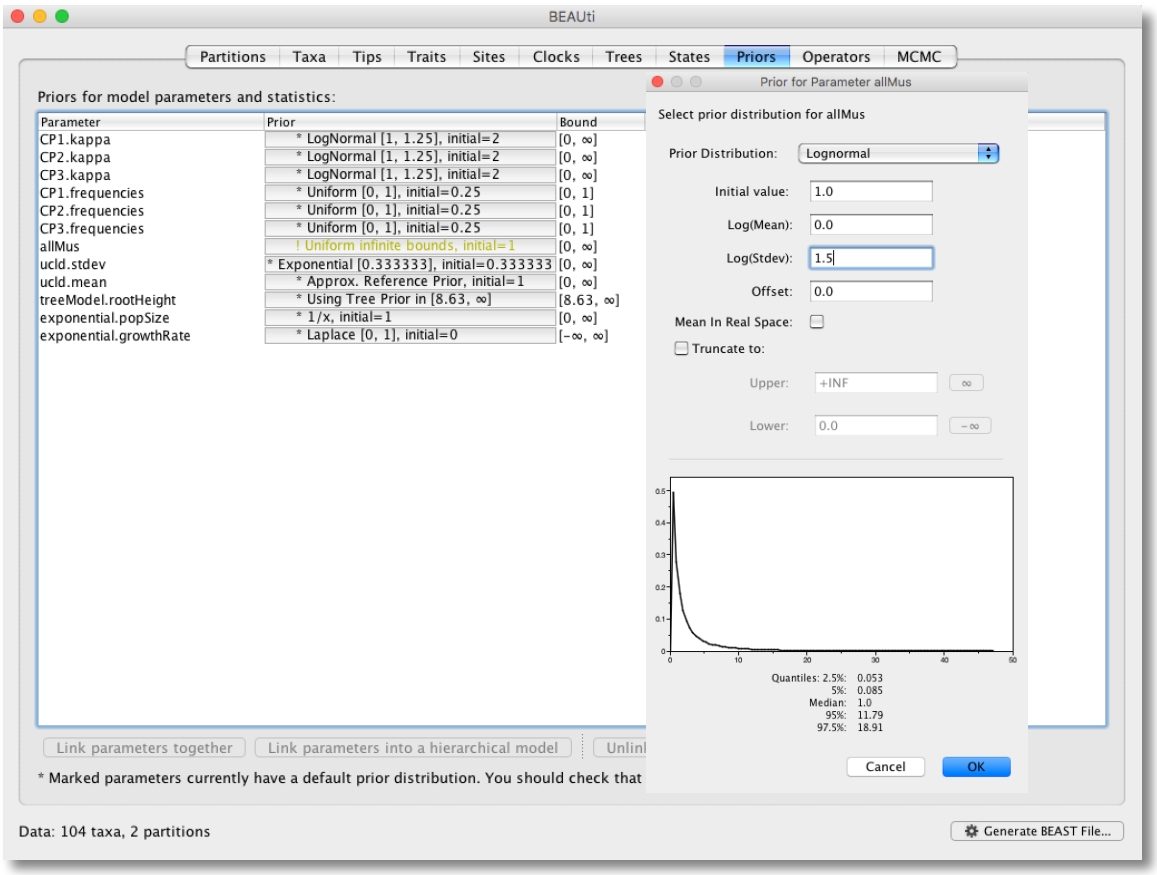


Note also that the Reconstruct states at all ancestors is selected by default for the location Partition.



Setting up the priors

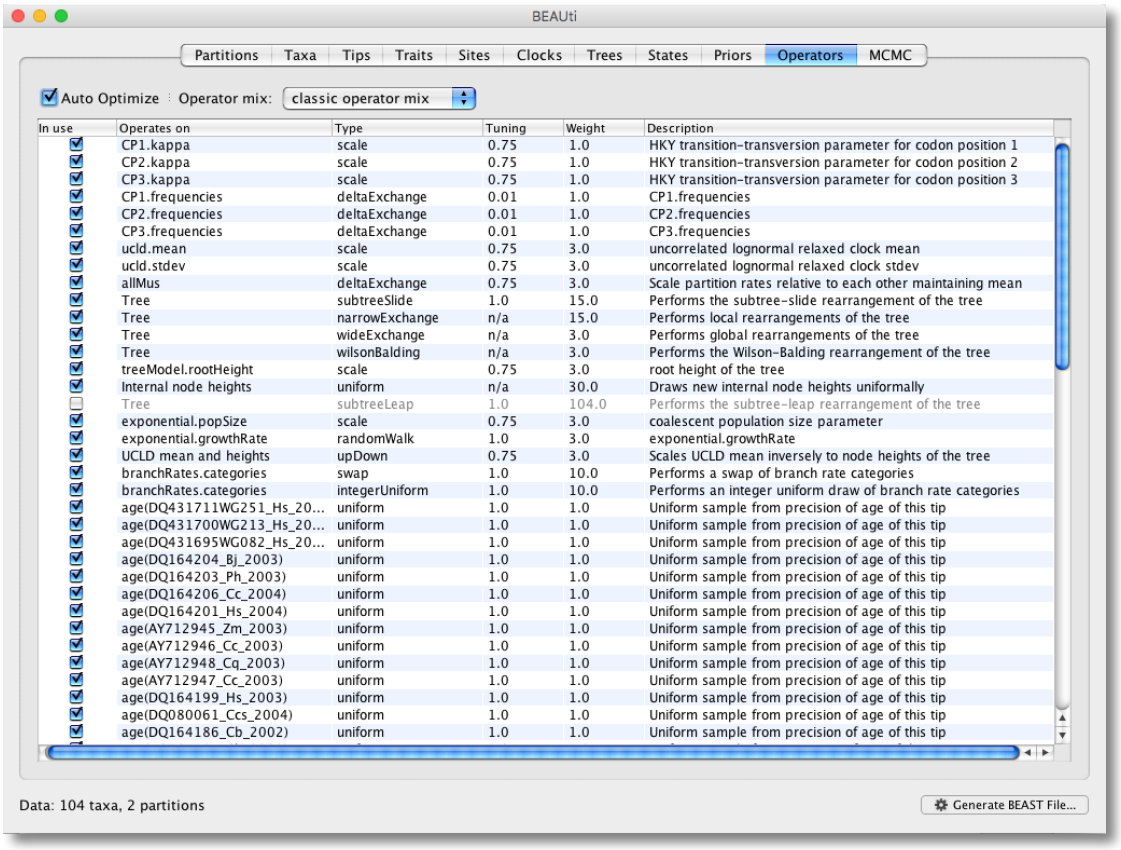
Review the prior settings under the **Priors** tab. Priors that would not be explicitly specified would appear in red, whereas priors that are improper (and hence lead to an improper posterior and improper marginal likelihoods) appear in yellow (e.g. **allMus**). Click on the prior for this parameter and a prior selection 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(mean) 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.



Setting up the operators

Each parameter in the model has one or more 'operators' (these are variously called called *moves*, *proposals* or *transition kernels* by other MCMC software packages such as **MrBayes** and **LAMARC**). The operators specify how 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 while 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 random

proportion and the uniform operator simply picks 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 v1.8.4, different options are available w.r.t. exploring tree space. In this tutorial, we will use the 'classic operator mix', which consists of of 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.



In use	Operates on	Type	Tuning	Weight	Description
☑	CP1.kappa	scale	0.75	1.0	HKY transition-transversion parameter for codon position 1
☑	CP2.kappa	scale	0.75	1.0	HKY transition-transversion parameter for codon position 2
☑	CP3.kappa	scale	0.75	1.0	HKY transition-transversion parameter for codon position 3
☑	CP1.frequencies	deltaExchange	0.01	1.0	CP1.frequencies
☑	CP2.frequencies	deltaExchange	0.01	1.0	CP2.frequencies
☑	CP3.frequencies	deltaExchange	0.01	1.0	CP3.frequencies
☑	ucld.mean	scale	0.75	3.0	uncorrelated lognormal relaxed clock mean
☑	ucld.stdev	scale	0.75	3.0	uncorrelated lognormal relaxed clock stdev
☑	allMus	deltaExchange	0.75	3.0	Scale partition rates relative to each other maintaining mean
☑	Tree	subtreeSlide	1.0	15.0	Performs the subtree-slide rearrangement of the tree
☑	Tree	narrowExchange	n/a	15.0	Performs local rearrangements of the tree
☑	Tree	wideExchange	n/a	3.0	Performs global rearrangements of the tree
☑	Tree	wilsonBalding	n/a	3.0	Performs the Wilson-Balding rearrangement of the tree
☑	treeModel.rootHeight	scale	0.75	3.0	root height of the tree
☑	Internal node heights	uniform	n/a	30.0	Draws new internal node heights uniformally
☐	Tree	subtreeLeap	1.0	104.0	Performs the subtree-leap rearrangement of the tree
☑	exponential.popSize	scale	0.75	3.0	coalescent population size parameter
☑	exponential.growthRate	randomWalk	1.0	3.0	exponential.growthRate
☑	UCLD mean and heights	upDown	0.75	3.0	Scales UCLD mean inversely to node heights of the tree
☑	branchRates.categories	swap	1.0	10.0	Performs a swap of branch rate categories
☑	branchRates.categories	integerUniform	1.0	10.0	Performs an integer uniform draw of branch rate categories
☑	age(DQ431711WG251_Hs_20...	uniform	1.0	1.0	Uniform sample from precision of age of this tip
☑	age(DQ431700WG213_Hs_20...	uniform	1.0	1.0	Uniform sample from precision of age of this tip
☑	age(DQ431695WG082_Hs_20...	uniform	1.0	1.0	Uniform sample from precision of age of this tip
☑	age(DQ164204_Bj_2003)	uniform	1.0	1.0	Uniform sample from precision of age of this tip
☑	age(DQ164203_Ph_2003)	uniform	1.0	1.0	Uniform sample from precision of age of this tip
☑	age(DQ164206_Cc_2004)	uniform	1.0	1.0	Uniform sample from precision of age of this tip
☑	age(DQ164201_Hs_2004)	uniform	1.0	1.0	Uniform sample from precision of age of this tip
☑	age(AY712945_Zm_2003)	uniform	1.0	1.0	Uniform sample from precision of age of this tip
☑	age(AY712946_Cc_2003)	uniform	1.0	1.0	Uniform sample from precision of age of this tip
☑	age(AY712948_Cq_2003)	uniform	1.0	1.0	Uniform sample from precision of age of this tip
☑	age(AY712947_Cc_2003)	uniform	1.0	1.0	Uniform sample from precision of age of this tip
☑	age(DQ164199_Hs_2003)	uniform	1.0	1.0	Uniform sample from precision of age of this tip
☑	age(DQ080061_Ccs_2004)	uniform	1.0	1.0	Uniform sample from precision of age of this tip
☑	age(DQ164186_Cb_2002)	uniform	1.0	1.0	Uniform sample from precision of age of this tip

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 affect the efficiency of the analysis. For most operators (like the subtree slide operator) 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 can be written to 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 can have their operators down-weighted so that they are not changed as often.

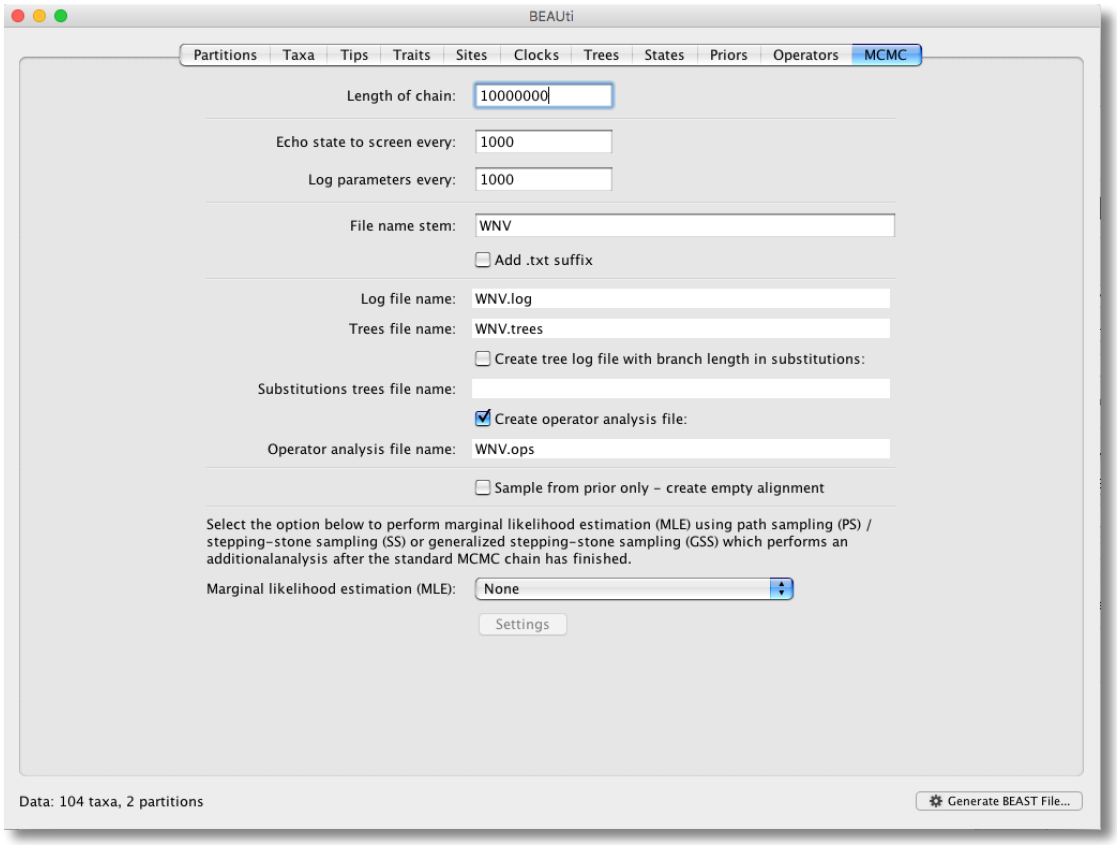
We can keep the default operator settings for the current analysis.

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 depend 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 dataset. We will see later how the resulting log file can be analyzed 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 set to 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 too often will result in very large files (in particular for large trees) 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 so this should be set to something >= chain length / 10,000.



For this dataset let's initially set the chain length to something small like 100,000. Because the renaissance counting procedure, which is executed each time a state is logged, can be computationally expensive for a large coding sequence alignment, we will set the parameter logging to file to 5000 and the state echo to screen every 100 state.

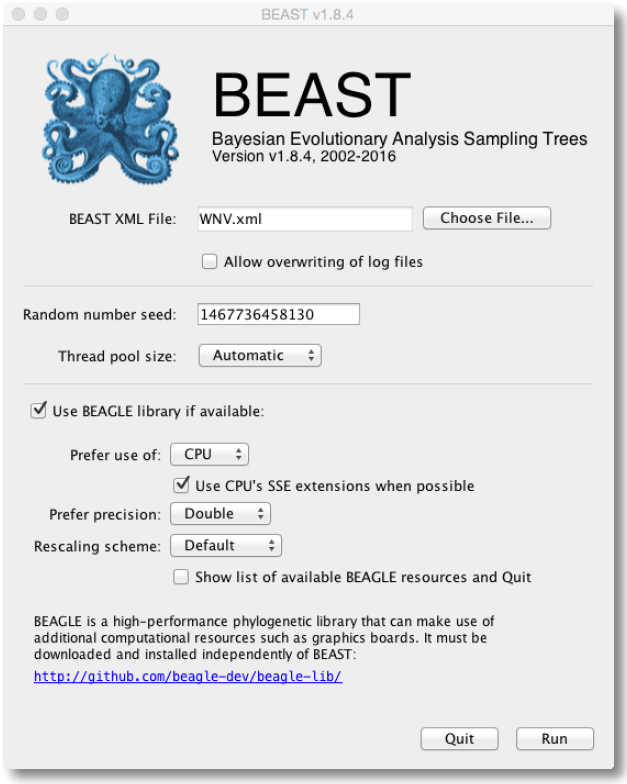
The next option allows the user to set the File stem name; if not set to **WNV_homogeneous**, you can type this in here. The next two options give the file names of the log files for the parameters and the trees. These will be set to a default based on the file stem name. Let's also create an operator analysis file by selecting the relevant option. Finally, an option is available to sample from the prior only, which can be useful to evaluate how divergent our posterior estimates are when information is drawn from the data. Here, we will not select this option, but analyze the actual data.

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. BEAUti will ask you to review the prior settings one more time before saving the file. Continue and choose a name for the file (for example, **WNV_homogeneous.xml**) and save the file..

For convenience, leave the BEAUti window open so that you can change the values and re-generate the BEAST file as required later in this tutorial.

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 standard file dialog box will appear in which you can select the input XML file. If the command line version is being used then the name of the XML file is given after the name of the **BEAST** executable. Press the 'Choose File' button and select the XML file you just created and press 'Run'. When you have installed the **BEAGLE** library (http://beagle-lib.googlecode.com/), you can use this in conjunction with **BEAST** to speed up the calculations. If not installed, unselect the use of the *BEAGLE* library. When clicking Run, the analysis will 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 XML file.

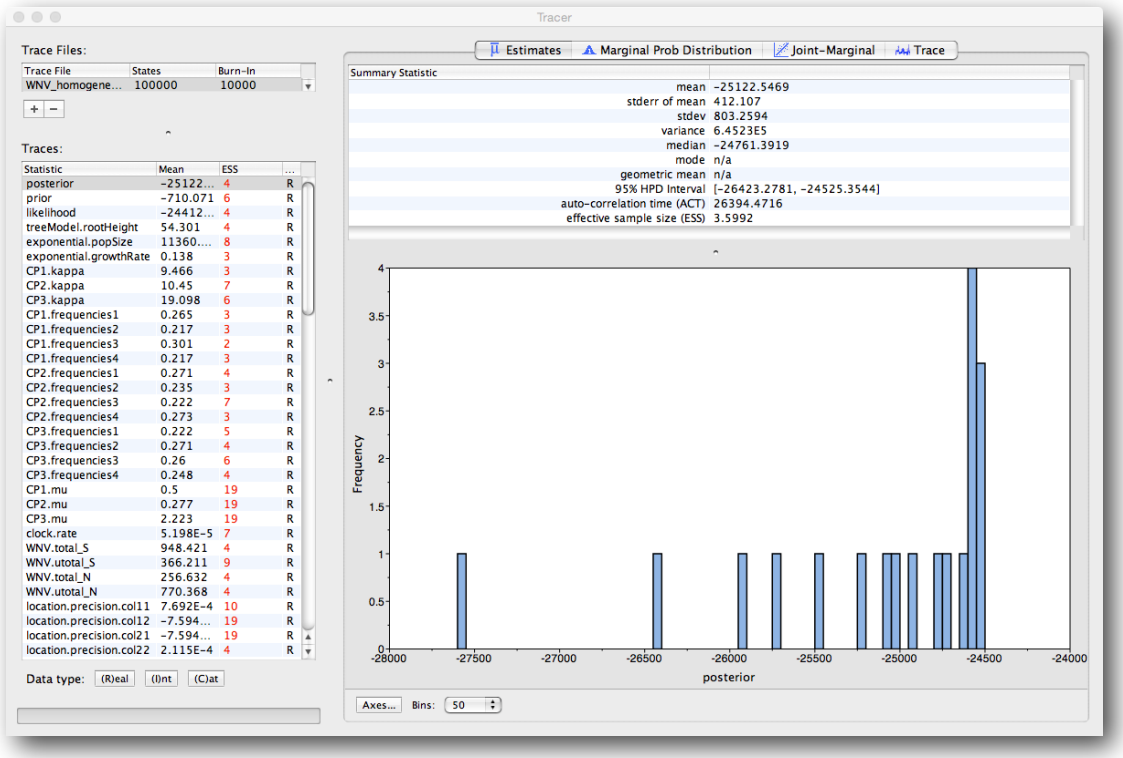


Analyzing the BEAST output

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. Please see the README text file that was distributed with the version you downloaded. Once running, 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. 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. There are traces for a quantity proportional to posterior (this is the product of the data likelihood and the prior probabilities, on the log-scale), and the continuous parameters. 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 posterior 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 of mean - 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).

Geometric mean - The central tendency or typical value of the set of 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).

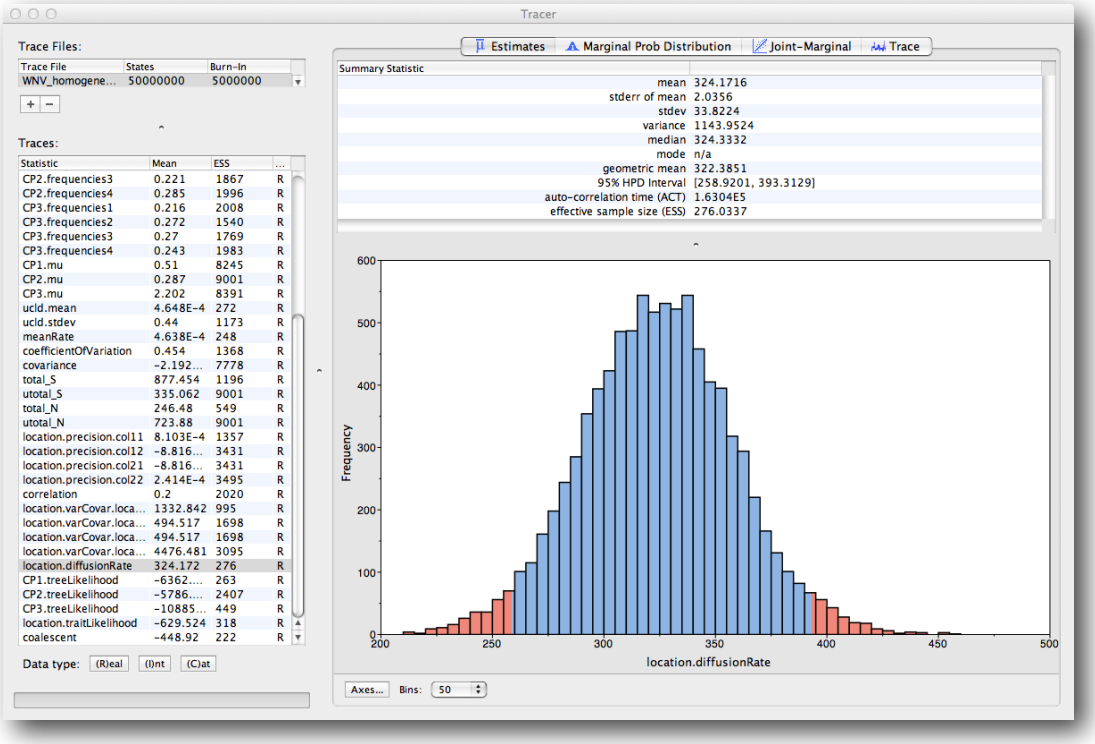
Effective Sample Size (ESS) - The effective sample size (ESS) is the number of independent samples that the trace is equivalent to. This is calculated as the chain length (excluding the burn-in) divided by the ACT.

Note that both the number of samples and the effective sample sizes (ESSs) for all the traces 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 the trace contained a lot of correlated samples and thus may not represent the posterior distribution well. In the bottom right of the window is a frequency plot of the samples which - as expected given the low ESSs - is extremely rough. Inspecting the **Trace** of many continuous parameters shows that the chain is still in the burn-in phase (the posterior values are still increasing/decreasing over most of the chain), and therefore cannot be used to summarize posterior distributions.

The simple response to this situation is that we need to run the chain longer. For this exercise, output files for longer runs are made available. Import the log file for the long run of the homogenous model and reassure yourself that the MCMC run has

reached stationarity: there are no obvious trends 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.

As we are happy with the behavior of posterior probability we can now move on to our statistic of interest: the dispersion rate. Select **location.diffusionRate** in the left-hand table. This shows a plot of the posterior probability density of this statistic that keeps track of the rate of diffusion by measuring the distance covered along each branch (based on the spatial coordinates inferred at the parent and descendent node of each branch), summing this distance for the complete tree and dividing this by the tree length. It uses the great circle distance between the two coordinates, which will provide an estimate in km/yr. You should see a plot similar to this in the **Estimates** tab:

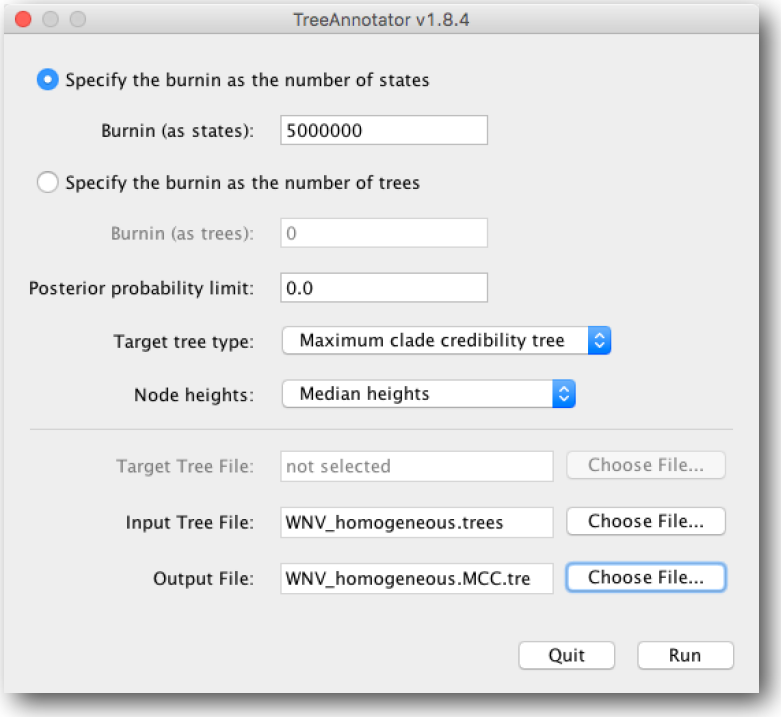


At what rate has WNV invaded North America?

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 in order to examine the trees it contains. One possibility is to load the trees into a program such as MrBayes or PAUP* and construct a consensus tree in a similar manner to summarizing a set of bootstrap trees. In this case, the support 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, you 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), the posterior support and the average rate of evolution on each branch (for models where this can vary). The program calculates these values for each node or clade observed in the specified 'target' tree.

- **Burn-in** - 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. For the example above, with a chain of 50,000,000 steps, a 10% burnin corresponds to 5,000,000 steps. Alternatively, sampling every 50,000 steps results in 1,000 trees in the file, and to obtain at the same 10% burnin, the number of trees needs to be set to 100.

- **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 all nodes will have information summarized. Make sure this value remains 0.0 in order to summarize all nodes in the target tree, which is necessary for further phylogeographic visualizations.

- **Target tree type** - This has two options '**Maximum clade credibility**' or '**User target tree**'. For the latter option, a NEXUS tree file can be specified as the Target Tree File, below. For the former option, TreeAnnotator will examine every tree in the Input Tree File and select the tree that has the highest sum 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 particular ancestral-descendent relationships in the MCC tree may still be different compared to a large number of other tree sampled). This will result in artifactual negative branch lengths, but can be avoided by the 'Common Ancestor heights' option. Keep the default median node heights for the time being.

- **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., **WNV_homogeneous.tre**).

Once you have selected all the options above, press the '**Run**' button. TreeAnnotator will analyze the input tree file and write the summary tree to the file you specified. This tree is in standard NEXUS tree file format so may 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

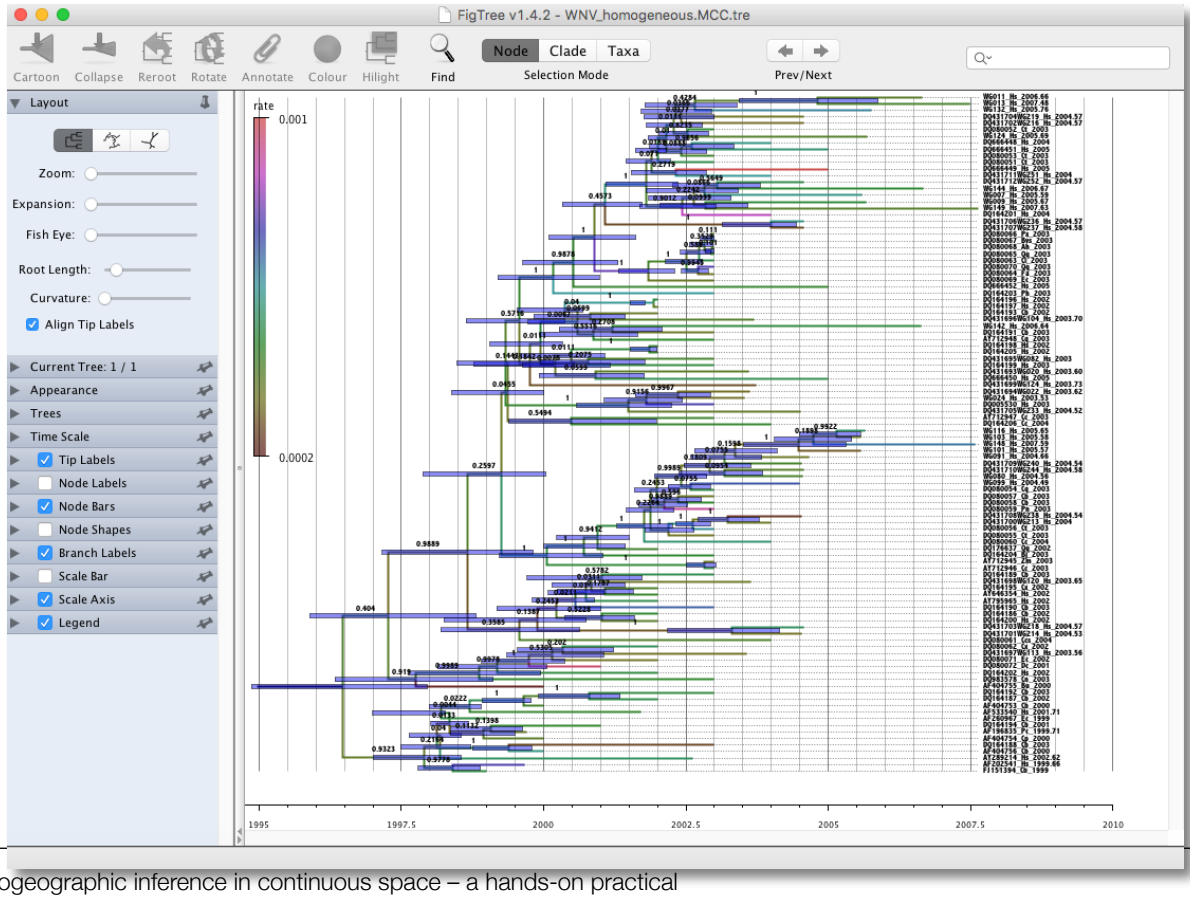
Run FigTree and select the **Open...** command from the **File** menu. Select the tree file you created using TreeAnnotator in the previous section. The tree will be displayed in the FigTree window. On the left hand side of the window are the options and settings which control how the tree is displayed. In this case we want to display the posterior probabilities of each of the clades present in the tree and estimates of the age of each node. In order to do this you need to change some of the settings.

First open the '**Branch Labels**' section of the control panel on the left. Now select '**posterior**' from the '**Display**' popup menu. The posterior probabilities won't actually be displayed until you tick the check-box next to the '**Branch Labels**' title.

We now want to display bars on the tree to represent the estimated uncertainty in the date for each node. TreeAnnotator will have placed this information in the tree file in the shape of the 95% highest posterior density (HPD) intervals (see the description of HPDs, above). Open the '**Node Bars**' section of the control panel and select to display the 95% HPDs of the node heights, afterwards select the check-box in order to turn the node bars on. We can also plot a time scale axis for this evolutionary history (select '**Scale Axis**' and deselect '**Scale bar**'). For appropriate scaling, open the '**Time Scale**' section of the control panel, set the '**Offset**' to 2007.63, the scale factor to -1.0. and '**Reverse Axis**' under '**Scale Axis**'.

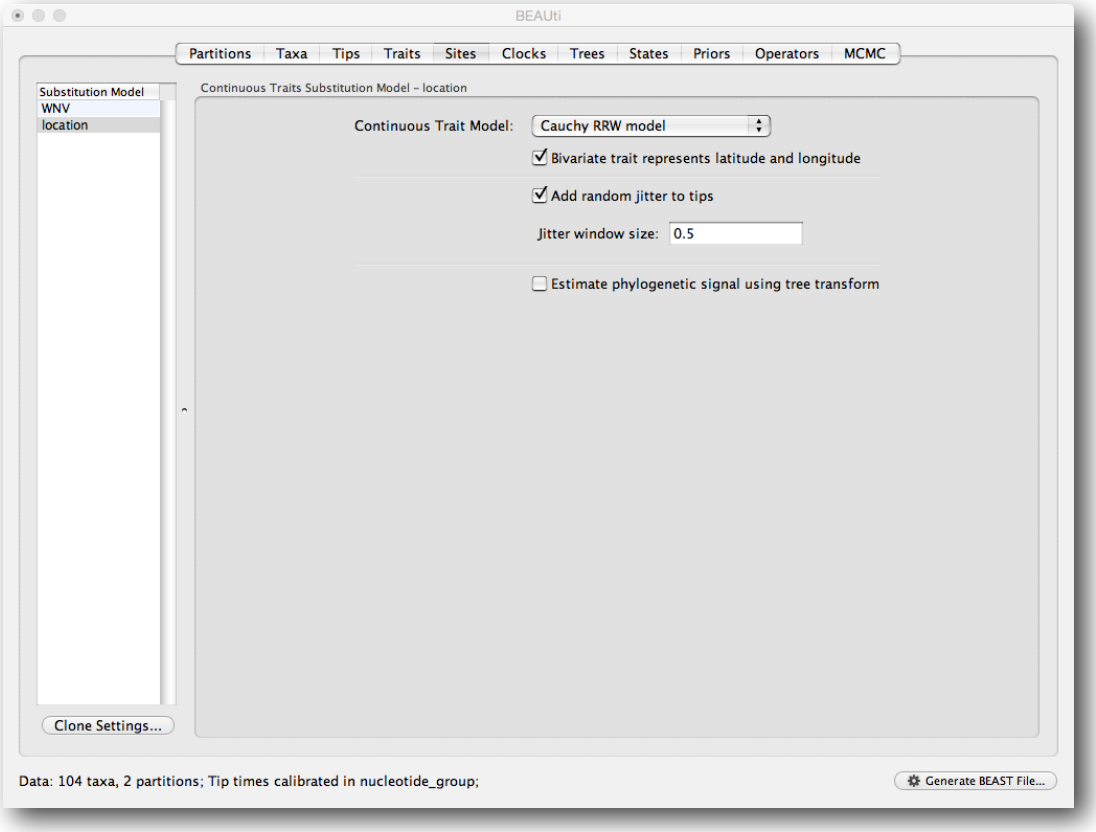
In the '**Layout**' panel select the check-box '**Align Tip Labels**' for clearness.

Finally, open the '**Appearance**' panel, alter the '**Line Weight**' to draw the tree with thicker lines and colour the branches according to the '**rate**'. Select the '**Legend**' option and choose 'rate' as an attribute to display a legend for the branch coloring. None of the options actually alter the tree's topology or branch lengths in anyway so feel free to explore the options and settings. You can also save the tree and this will save all your settings so that when you load it into FigTree again it will be displayed exactly as you selected.

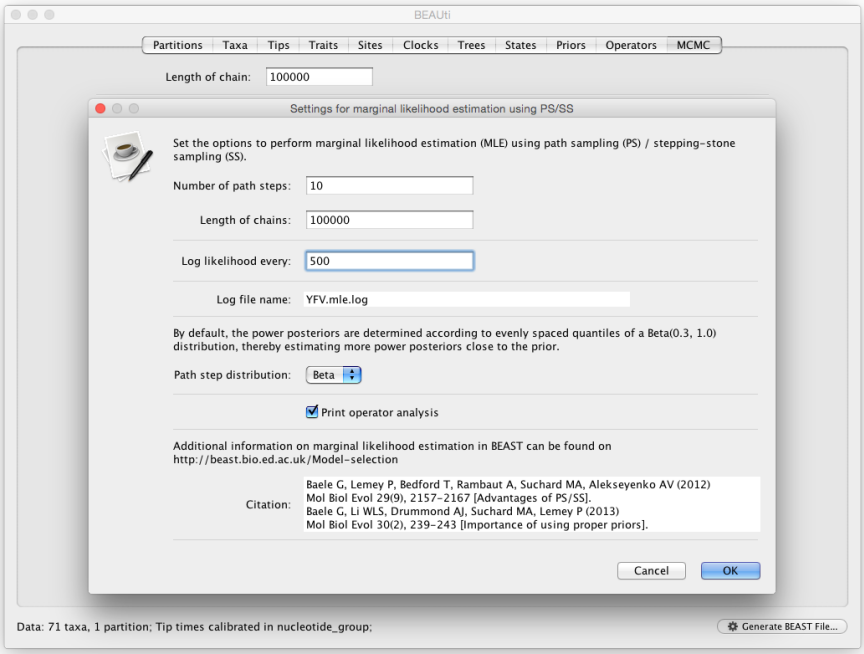


Evaluating diffusion rate variation

To investigate whether the rate of spread was roughly constant throughout the rabies epidemic we can fit models that allow for branch-specific rate variation in the diffusion process (termed 'relaxed random walks', RRWs) and test whether these result in improvements in model fit compared to the standard homogeneous Brownian diffusion process. To this purpose, we have also analyzed the same data under a Cauchy RRW. This can be set under the **Sites** tab for the location **Substitution model**:



For the different models, we can estimate marginal likelihoods (MLE) using path sampling (PS) and stepping stone sampling, which have recently been implemented in BEAST (Baele at al., 2012, 2013). Typically, PS/SS model selection is performed after doing a standard MCMC analysis. PS and SS sampling can then start where the MCMC analysis has stopped (i.e. you should have run the MCMC analysis long enough so that it has converged towards the posterior before attempting a PS/SS analysis), thereby eliminating the need for PS and SS to first converge towards the posterior. To set up such analyses, we can return to BEAUti and select "path sampling (PS) / stepping-stone sampling (SS)" from the "Perform marginal likelihood estimation (MLE)" menu, which performs and additional analysis after the standard MCMC chain has finished." in the MCMC panel. Click on "settings" to specify the PS/SS settings.



We need to set the number of steps (in this case 50) for the path between the posterior and the prior, the length of the MCMC chain (in this case 1,000,000) for each step that estimates a specific power posterior, and the logging frequency for each MCMC sampling (in this case every 1,000). Note that using these settings, the marginal likelihood estimation will take approximately the time it takes to complete a standard MCMC run of 50,000,000 generations for this data. The powers for the different power posteriors are defined using evenly spaced quantiles of a Beta(α,1.0) distribution, with α here equal to 0.30, as suggested in the stepping-stone sampling paper (Xie et al. 2011) since this approach is shown to outperform a uniform spreading suggested in the path sampling paper (Lartillot and Philippe 2006). Note that currently additional research is being performed on how to use a more recently developed marginal likelihood estimator, i.e. generalised stepping-stone sampling (Fan et al., 2011; Baele et al., 2016), to compare trait diffusion models.

Based on the settings above, the homogeneous Brownian analysis results in marginal likelihood estimates of -24182.20 and -24176.83 for PS and SS respectively. For the Cauchy RRW, we get -24074.30 and -24072.10 for the same estimators. Is there significant evidence for diffusion rate heterogeneity? Does this have a big effect on the mean dispersal rate estimate?

The rate variation among lineages can be depicted in the MCC tree using a colour annotation. Summarize the MCC tree for the Cauchy RRW model and under '**Appearance**' in **FigTree**, select to **Colour by location.rate**.

Visualizing Bayesian phylogeographic inference using SpreaD3

SpreaD3, 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 phylodynamic reconstructions resulting from Bayesian inference of sequence and trait evolutionary processes. **SpreaD3** allows to visualise on custom maps and generates HTML pages for display in modern-day browsers such as Firefox, Safari and Chrome. One of the functionalities of **SpreaD3** that relate to the continuous phylogeographic analysis performed previously include visualizing location-annotated MCC trees. A detailed tutorial for this particular step is available at https://rega.kuleuven.be/cev/ecv/software/SpreaD3_tutorial#sectionFourThree. We have also provided a PDF version of the entire **SpreaD3** tutorial. 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?

Quantifying site-specific selection through Renaissance Counting dN/dS estimates.

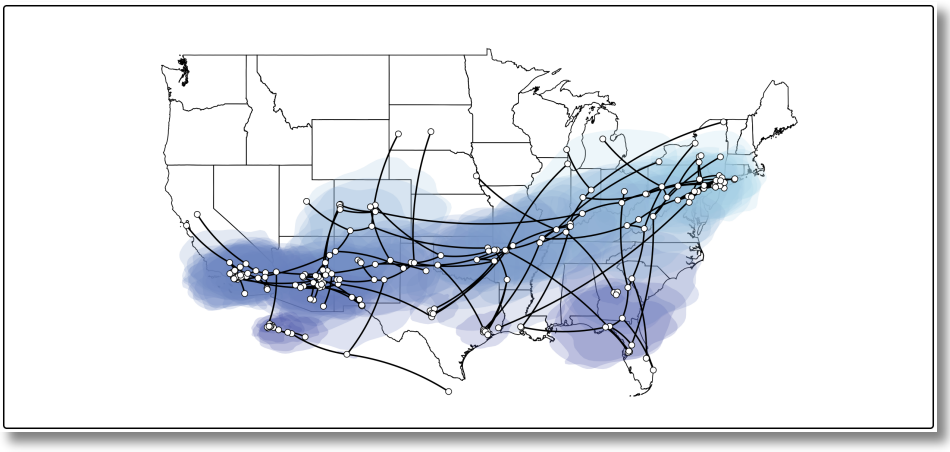
As part of our WNV analysis, we performed a Renaissance Counting procedure to obtain site-specific dN/dS estimates. These estimates are logged in the `WNV_homogeneous.dNdS.log` file and summarized in `WNV_homogeneous.dNdS.summary.txt`. In this summary, each codon site is listed with its mean dN/dS estimate and credible intervals (CPD Low and CPD Up). In addition, a classification is provided based on these estimates according to three categories: negatively selected represented by '-', neutral, represented by '0' and positively selected represented by '+'. Finally, when sites are considered to be significantly negatively or positively selected (based in the frequency by which 1 is covered by the credible intervals of the dN/dS estimate), this is indicated with a '*'. In general, the mean dN/dS estimates are low and the variation in their values is fairly discrete. This is due to the sparsity of the number of substitutions in this data, in particular nonsynonymous substitutions. So negative selection is the dominating evolutionary force in the WNV history. There are however five sites that are estimated to be under (not so strong) positive selection (449, 1367, 1991, 2209, 2518, 2522 and 2842). Were any of these sites also detected to be positively selected in the following study: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3667762/ ?

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 **WNV.fas**.
  - In the **Tips** tab, select the **Use tip dates** option and use the **Guess Dates** button. *Keep* the default **Defined just by its order**, set order to **last**, and **Parse as a number: add the following value to each: 1900.0, ...unless less than 13.0, ... in which case add: 2000.0.** Select all the taxa (holding down the cmd key) with sampling dates only known up to the year in the Tips window (those with a .0 decimal) and click on '**Set Precision**'. Enter '**1.0**' as the precision value for 1 year. Select '**sampling uniformly from precision**' at the bottom left of the Tips panel and keep the '**Apply to taxon set: All taxa**' default.
  - In the **Traits** tab, click **Add trait**. This will open a new window to **Create or Import Trait(s). Select Import trait(s)** from a mapping file format, Browse to and load the **LatLong.txt** tab-delimited file
  - After clicking **OK**, 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**, the '**Site Rate Heterogeneity**' to '**None**', and partition the data into 3 partitions for the coding positions (**3 partitions: positions 1,2,3**).
- Click on **location** in the **Substitution model** window and keep the default **Homogenous Brownian model** and select 'Bivariate trait represents latitude and longitude'.
- In the **Clocks** tab, select the **Lognormal relaxed molecular clock (Uncorrelated)** model.
- In the **Trees** tab, select the exponential growth model as a flexible demographic tree prior (**Coalescent: Exponential Growth**) and keep the default random starting tree.
- In the **states** tab, turn on the Renaissance counting procedure by selection '**Reconstruct synonymous/non-synonymous change counts**'
- In the **Priors** tab, set the **ucld.mean** prior to a **gamma** distribution with **shape** = 0.001 and **scale** = 1000.
- In the **MCMC** tab, set the chain length to 100,000 and both the sampling frequencies to 100. Set the **File name stem** to **WNV_homogeneous** and generate the beast file (WNV_homogeneous.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** (**burn-in** = 500).
- Visualize the tree in **FigTree**.
- Run **SpreaD3**.
  - Select as input type in the Data panel: **MCC tree with CONTINUOUS traits**, load your MCC tree file.
  - Select 'location1' as Latitute attribute name and 'location2' as Longitude attribute name.
  - Set the **most recent sampling date** to 2007-07-15.
  - Load a GeoJSON file of the United States.
  - 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.



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 the 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.

- The BEAST website: **http://beast.bio.ed.ac.uk**/ (or **https://github.com/beast-dev/beast-mcmc**)
- Tutorials: **http://beast.bio.ed.ac.uk/Tutorials**
- Phylogeography: **http://www.phylogeography.org**
- Frequently asked questions: **http://beast.bio.ed.ac.uk/FAQ**

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