BEAST Divergence-time Lab

Contents

1 Objective

The goal of this tutorial is to help you become familiar with using fossil data to estimate time-calibrated phylogenetic trees in a Bayesian framework. We will use an example dataset of DNA sequences of crocodylians (crocodiles and alligators) and the software package BEAST version 1.7.5. We will work through the steps necessary for estimating the phylogenetic relationships among crocodiles and alligators, while simultaneously using fossil information and relaxed-clock models to calibrate the branches on the tree to units of time (millions of years).

The stylistic conventions for this tutorial:

Names of files	in this font.
Web site URLs and other clickable links	look like this.
Nested menu items	***Top Name →Mid Name →Lowest***
BEAUTi menu tabs	***tab name***
BEAUTi option fields	`field name`
Field values that you enter	**field value**
Questions for you to answer	***look like this?***

2 Version, Author information, and Acknowledgements

This tutorial was written by Jamie Oaks and Melissa Callahan for BEAST version 1.7.5. Many of the ideas for this lab came from a great tutorial by Tracy Heath.

3 Background Information

Often biologists are interested in estimating trees in which the branch lengths are proportional to time. However, because we only observe the product of time and the rate of evolution (differences among species), time and rate are inextricably linked. As a result, we have to make some assumptions about how the rate of evolution has changed over evolutionary history in order to tease apart the contributions of rate and time, and estimate branch lengths proportional to time. The simplest assumption (i.e., model) is that the rate of substitution is constant over time; a global strict clock (Zuckerkandl and Pauling, 1962). However, we know that in most situations this assumption is violated. As a result, there has been a lot of work done on developing "relaxed-clock" models that allow the rate of substitution to change over time and among lineages. These methods allow us to estimate relative divergence times across a phylogeny (Figure 1).

In order to estimate absolute divergence times, we need extra information to calibrate the rate of evolution across the tree. Paleontological data (e.g., fossils) are often used for this purpose. If we know the date of a fossil, and can estimate its relationships to the extant species, we can use this information to constrain the age of a node in the tree. Combining this approach with a relaxed-clock model allows us to estimate a phylogeny in which the branch lengths are in units of time.

4 Programs Used in This Lab

We will be using the free, open-source software package, BEAST (Bayesian Evolutionary Analysis Sampling Trees; `http://beast.bio.ed.ac.uk`), for estimating divergence times under a relaxed clock. BEAST offers a wide range of models for analyzing molecular data in a Bayesian statistical framework. BEAST also comes with several other utility programs that we will be also using to prepare input files (BEAUTi; Bayesian Evolutionary Analysis Utility) and summarize output files (LogCombiner and TreeAnnotator). We will

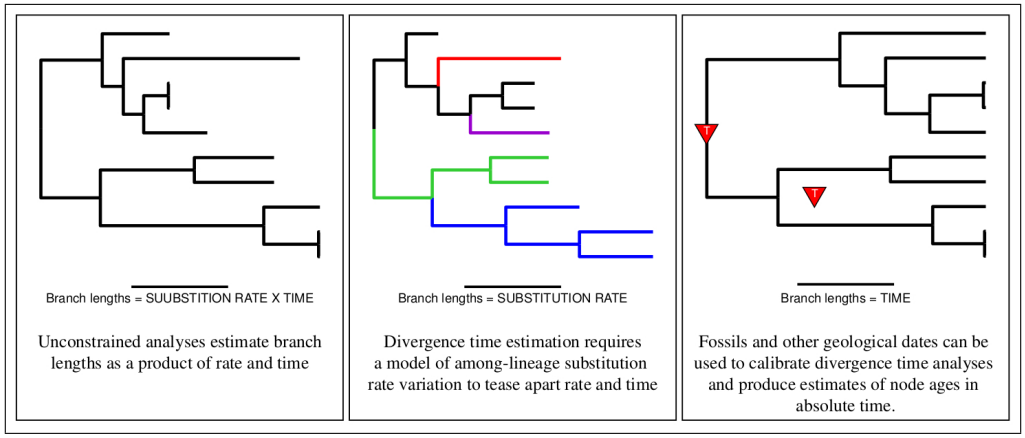


Figure 1: The branch lengths of a phylogenetic tree are the product of rate and time.

also be using the programs Tracer (http://tree.bio.ed.ac.uk/software/tracer) and FigTree (http://tree.bio.ed.ac.uk/software/figtree) for evaluating, summarizing, and viewing results.

5 The Data

We will be analyzing DNA sequence data from 24 species of crocodylians (crocodiles, alligators, caimans, and gharials). The sequences are from Oaks (2011), and encode the protein-coding *cytochrome b* gene from the mitochondrial genome (this is a single gene from a larger multi-locus dataset that is available at http://datadryad.org/resource/doi:10.5061/dryad.5k9s0. You can download a zip archive of the aligned sequence data and other auxiliary files used in this tutorial from http://www.phyletica.com/downloads/div-time-tutorial.zip.

For these data, a likelihood-ratio test rejects a global strict clock model. So, we will use an uncorrelated log-normally distributed (UCLD) relaxed-clock model (Drummond et al., 2006) to account for variation in rates of substitution across the tree in order to estimate branches proportional to time.

Crocodylians have a rich fossil record, and we will be using some of this fossil information to specify priors on the ages of certain nodes in the phylogeny (Figure 2). This will calibrate the branch-specific rates of the relaxed-clock model, and scale the branches of the tree to absolute time. We will use units of millions of years for specifying the node-age calibrations, and so divergence-time estimates will also be in units of millions of years.

6 Tutorial

6.1 Downloads and Installations

Step 1: BEAST is available at http://beast.bio.ed.ac.uk/Main_Page. This tutorial is written for version 1.7.5 of BEAST.

Step 2: Download the div-time-tutorial.zip archive from http://www.phyletica.com/downloads/div-time-tutorial.zip to your desktop, and unzip the archive. You should now have a div-time-tutorial folder on your desktop, and it should contain the files and folders shown in Box 1.

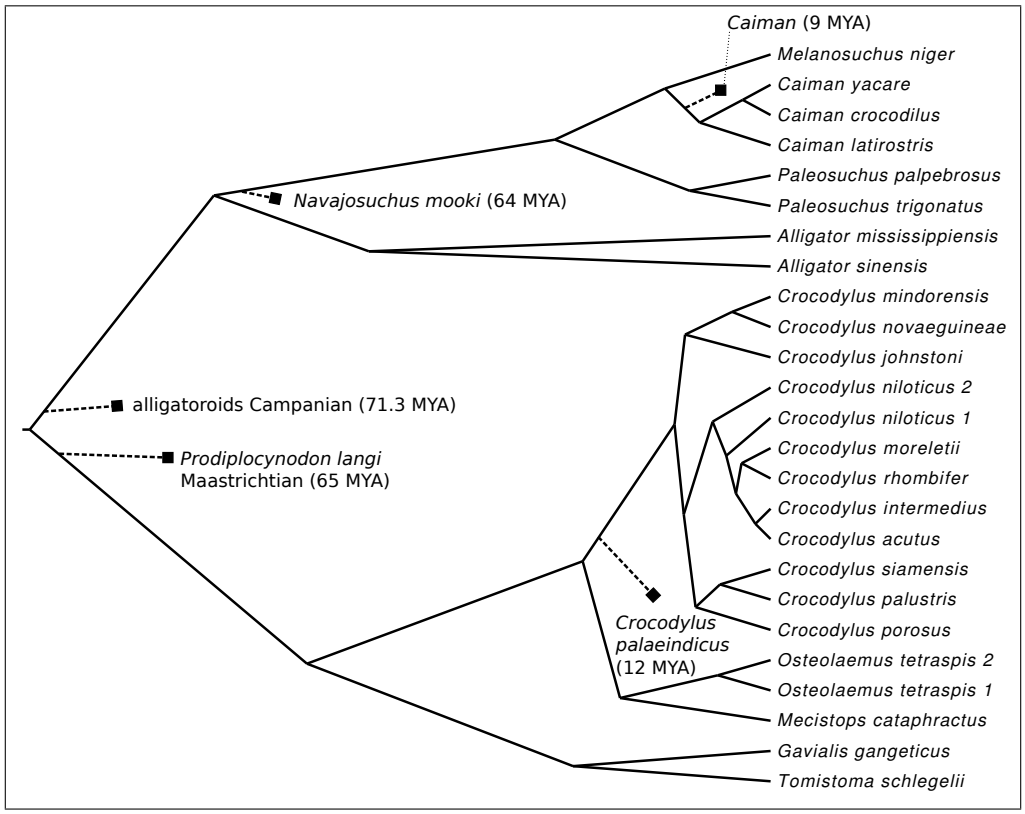


Figure 2: A phylogenetic estimate of Crocodylia with rough fossil placements.

- `div-time-tutorial/`
  - `crocodylia-cytb.nex`
  - `yule.py`
  - `output/`
    - `crocodylia-cytb-run1.log`
    - `crocodylia-cytb-run2.log`
    - `crocodylia-cytb-run1.trees`
    - `crocodylia-cytb-run2.trees`

Box 1: The files required for this tutorial.

6.2 Setting up XML file with BEAUTi

Step 3: Begin by launching the BEAUTi program. If you are using Mac OSX or Windows, you should be able to do this by double clicking on the application. If everything is working correctly, a window should appear that looks something like Figure 3.

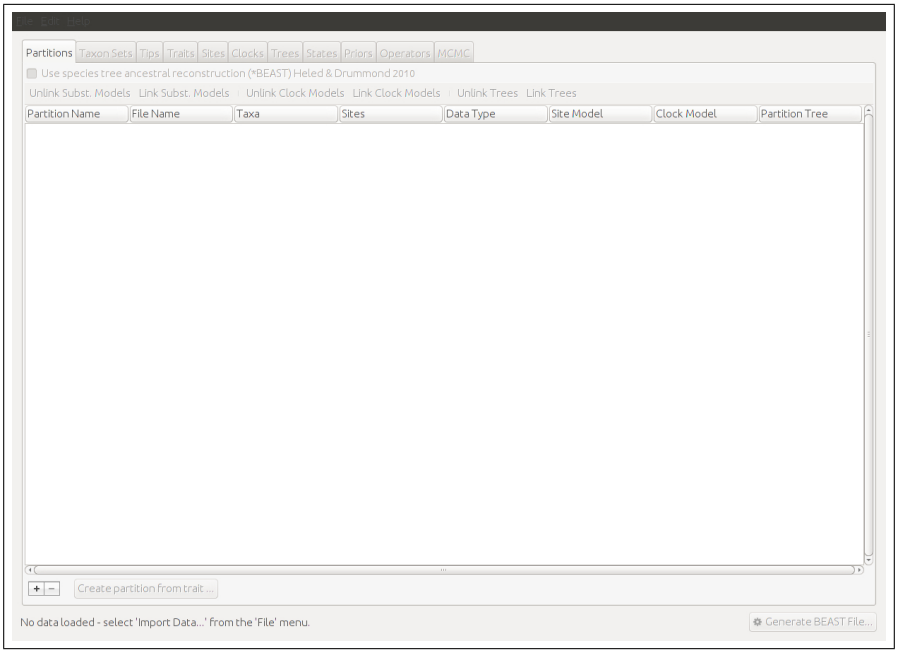


Figure 3: BEAUTi window.

Step 4: Import the sequence data from the file crocodylia-cytb.nex using the drop-down menu ***File*** →***Import Data*** or using the "+" button near the bottom-left corner of the window.

You should be able to confirm that BEAUTi successfully imported 24 sequences of nucleotides of length 1137 (Figure 4).

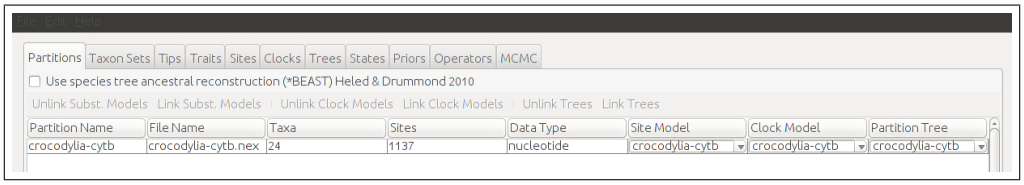


Figure 4: The data successfully loaded by BEAUTi.

Step 5: Double click on the file name crocodylia-cytb.nex to bring up a window displaying the aligned sequences. It is always good practice to make sure everything looks as you expect. The cytochrome b gene is protein-coding, and aligns well across Crocodylia without gaps.

Step 6: Next, we need to define some sets of taxa. Later, we will be able to use each of these sets to place priors on the age of their most recent common ancestor (MRCA). Let's start by defining the set for the clade containing the fossil taxon *Navajosuchus mooki*; this clade has the family name Alligatoridae.

Click on the ***Taxa*** tab. Once in the ***Taxa*** tab, click on the "+" near the bottom-left corner of the window. This will create an untitled taxon set in the left-most box in the window. Change the name of this taxon set to **Alligatoridae** and enter **65** into Age column. This age is simply a starting value for the age of the

MRCA of **Alligatoridae**. It will ensure that the initial tree used to start the analysis is consistent with the lower bound of our fossil calibration (which will be 64 million years).

We do not want to constrain **Alligatoridae** to be monophyletic, so leave the `Mono?` box unchecked. Also, we are confident that *Navajosuchus mooki* is nested within **Alligatoridae**, and so we will leave the `Stem?` box unchecked. Because we only have a single tree, you can leave the `Tree` column unchanged.

Next, we need to highlight the species that belong to **Alligatoridae** within the `Excluded Taxa` window, and move them over to the `Included Taxa` window using the "→" button. **Alligatoridae** includes the following genera:

- *Alligator*
- *Caiman*
- *Melanosuchus*
- *Paleosuchus*

Highlight the species for these genera and move them over to the `Included Taxa` window. If you did everything correctly, your BEAUTi window should look like Figure 5.

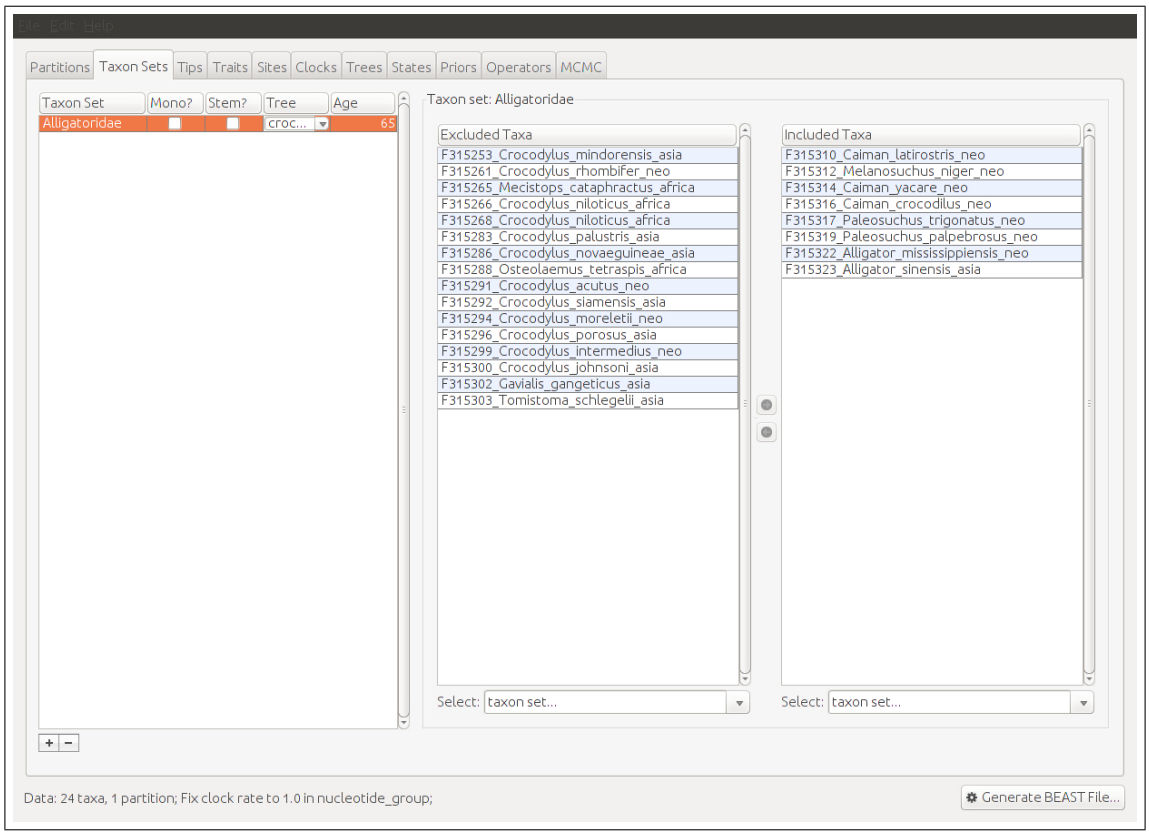


Figure 5: The taxon set **Alligatoridae** correctly defined.

Next, let's define a taxon set for the genus *Crocodylus*, which we will later use to specify an age prior corresponding to the fossil *Crocodylus palaeindicus*. Click on the "+" again to create a new taxon set, and name it **Crocodylus**. Specify a starting age of **13**, and leave the `Mono?` unchecked.

We are confident that *Crocodylus palaeindicus* is more closely related to all *Crocodylus* species than to any other crocodylians. However, we are not confident that it is nested within extant species of *Crocodylus* and suspect it is actually sister to them (as illustrated in Figure 2). As a result, we want to check the `Stem?` box. This specifies that the node we are interested in calibrating is the MRCA of all *Crocodylus* sequences

and their next closest relative (i.e., the stem node of *Crocodylus*). Make sure the **Crocodylus** taxon set is highlighted, and then highlight all the *Crocodylus* species in the `Excluded Taxa` window and move them over to the `Included Taxa` window.

Lastly, we need to define a taxon set for the genus *Caiman*, which we will later use when specifying a calibration informed by the age of the oldest known *Caiman* fossils. Click the "+" to create a new taxon set, name it **Caiman**, specify a starting age of **10**, and leave `Mono?` unchecked. As with *Crocodylus* we don't know if the oldest *Caiman* fossil taxa are nested within or sister to extant *Caiman* species, and so we need to check the `Stem?` box. Highlight the three *Caiman* species and move them over to the `Included Taxa` window.

We will also be specifying a prior for the age of the root node of the tree, but we do not need to define a taxon set for this, because the root node is always defined by BEAUTi (you will see this later).

Before you proceed to the next step, double check the three taxon sets you just defined and make sure you did not make a mistake with their ages or in selecting the species associated with them. Even a single misplaced species can lead to some very bizarre results!

If you did everything correctly, your BEAUTi window should look similar to Figure 6.

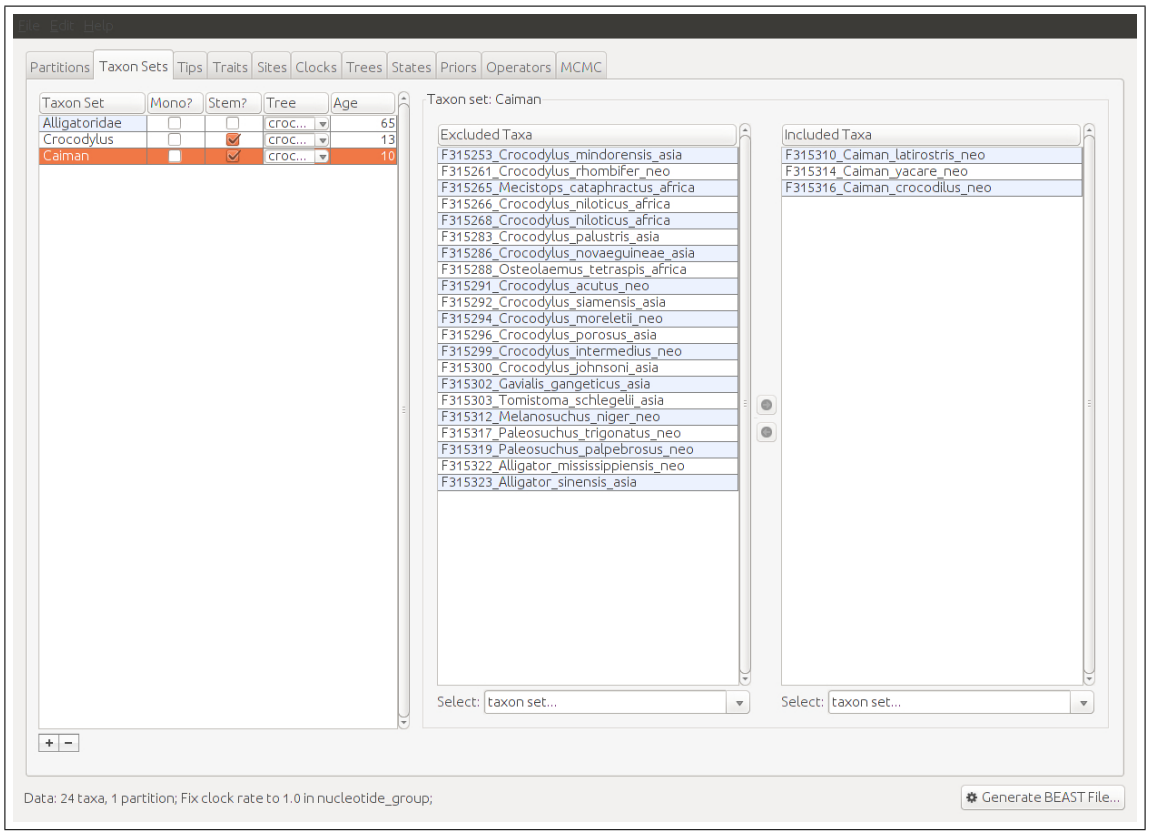


Figure 6: All three taxon sets defined.

Step 7: Next, we need to set up our continuous-time Markov chain (CTMC) model of nucleotide substitution under the ***Sites*** tab. Once in the ***Sites*** tab, and with **crocodylia-cytb** selected in the left `Substitution Model` window, select the following options:

`Substitution Model:` **HKY**
`Base frequencies:` **Estimated**
`Site Heterogeneity Model:` **Gamma**

`Number of Gamma Categories:` **4**
`Partition into codon positions:` **3 partitions: positions 1, 2, 3**

Lastly, check all three `Unlink parameters` options (Figure 7).

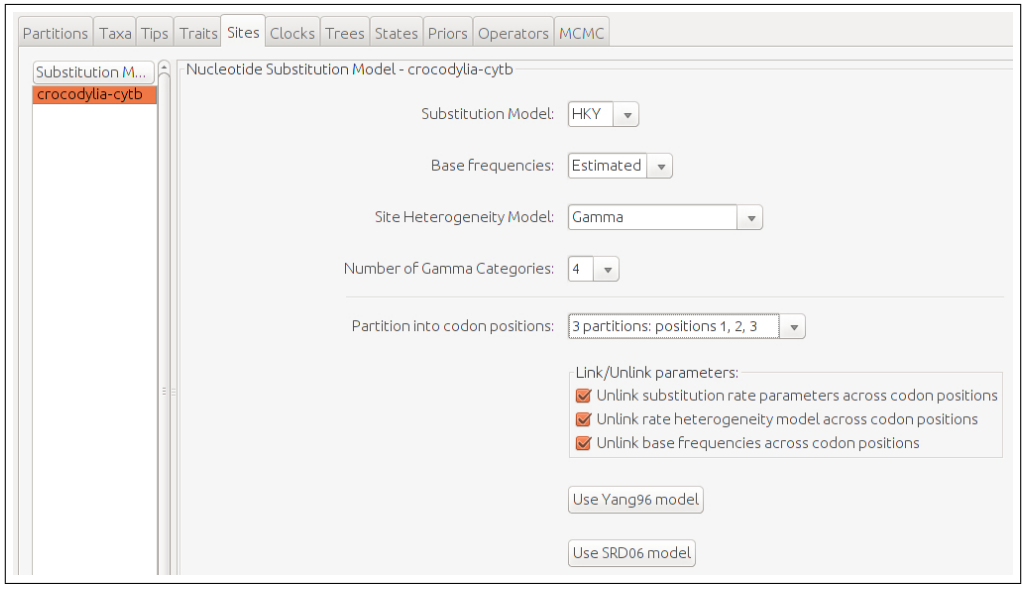


Figure 7: The CTMC model of nucleotide substitution for cytb.

Step 8: Next, we need to move to the ***Clocks*** tab and specify our models of branch rates across the tree. BEAUTi provides one strict-clock and three relaxed-clock options:

Strict clock Assumes a constant rate of substitution across all the branches of the tree.

Lognormal relaxed clock (Uncorrelated) Assumes that the rates of substitution on each branch of the tree are independent and drawn from a single, discretized lognormal distribution (Drummond et al., 2006).

Exponential relaxed clock (Uncorrelated) Assumes that the rates of substitution on each branch of the tree are independent and drawn from a single, exponential distribution (Drummond et al., 2006).

Random local clock Uses Bayesian stochastic search variable selection (BSSVS) to average over local clock models (i.e., it averages over the number of rate changes and their locations) (Drummond and Suchard, 2010).

In general, it is best to compare (or sample over) different clock models. But, for the sake of keeping this tutorial simple, we will select the most commonly used relaxed-clock model for the cytb data. For the **crocodylia-cytb** data, select the **Lognormal relaxed clock (Uncorrelated)**. Make sure to click the `Estimate` box (Figure 8). You do not need to worry about the `Clock Model Group` options in the lower window.

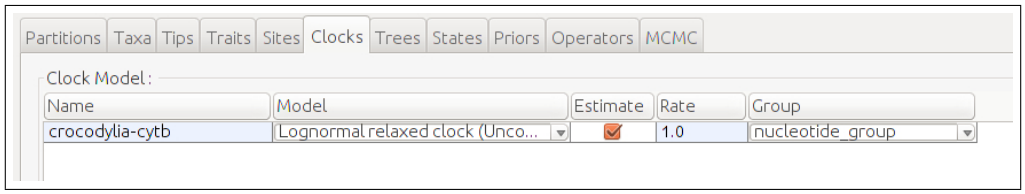


Figure 8: The clock-model settings.

Step 9: Next, let's move to the ***Trees*** tab to specify the prior and starting conditions for our tree. Select the **Speciation: Birth-Death Process** option from the drop-down for the `Tree Prior` option. Select **Random starting tree** in the lower window (Figure 9).

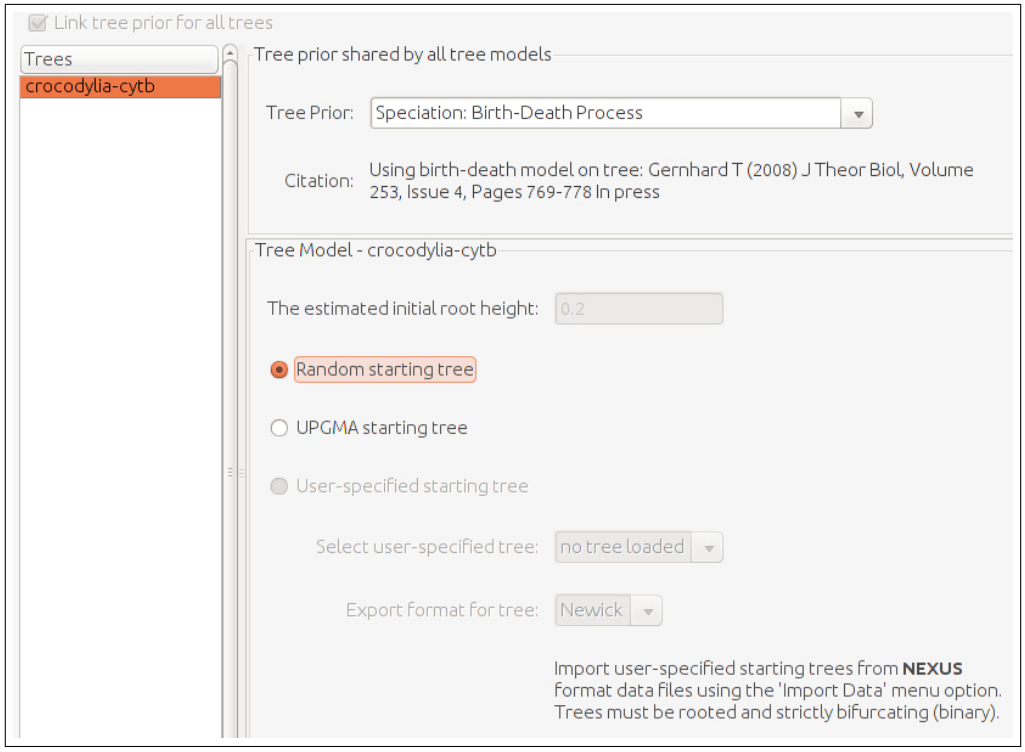


Figure 9: The tree-prior settings.

BEAUTi offers a number of tree prior options. Those labeled **Speciation** are based on stochastic branching processes that assume the tips of the phylogeny are species. The **Coalescent** tree priors are based on stochastic processes of lineage coalescence that assume the tips of the tree are gene copies within a population.

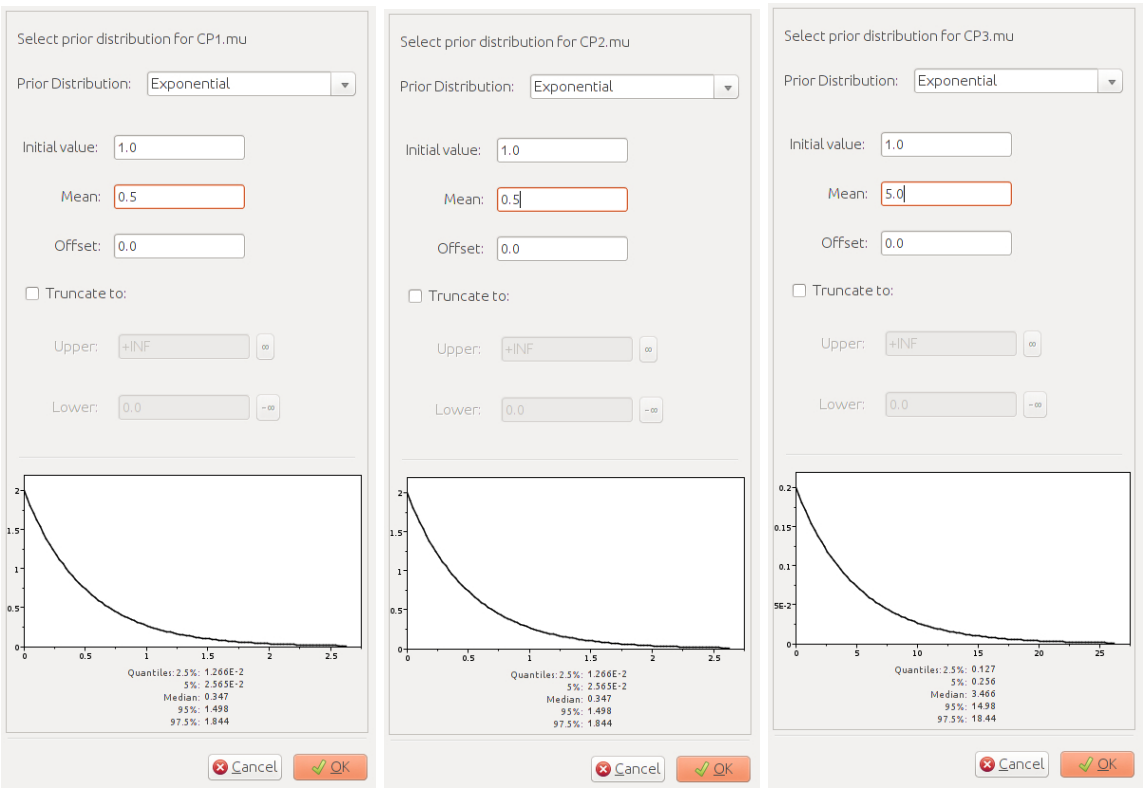
Step 10: Move to the ***Priors*** tab. Here we see that all of the model parameters and statistics that we specified under the other tabs are listed. Now, we can specify prior probability distributions on the substitution-model parameters, relaxed-clock parameters, tree-prior parameters, and the time (age) of the most recent common ancestor (TMRCA) of the taxon sets we specified earlier.

Let's start by selecting priors for the **CP1.mu**, **CP2.mu**, and **CP3.mu** parameters. These are relative-rate parameters that allow sites at the three codon positions to evolve at different rates. Based on our knowledge of the redundancy of the genetic code, we expect the sites at third-codon position to evolve more rapidly than the first and second codon sites *a priori*. So we will specify our priors accordingly.

Click on the `Prior` column for the **CP1.mu** parameter. In the window that pops up, select an **Exponential** `Prior Distribution`, and specify a `Mean` of **0.5**. Do the same for **CP2.mu**. For **CP3.mu**, also select an **Exponential** `Prior`, but set the `Mean` to **5.0** (Figure 10).

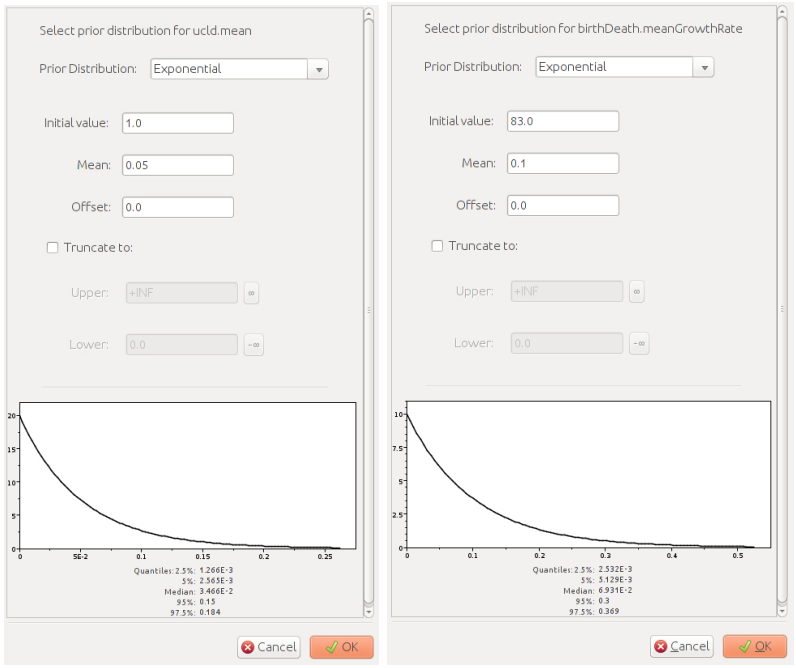
Next, click on the `Prior` column for the **ucld.mean** parameter. This parameter controls the mean of the log-normal distribution from which the rates of each branch of the tree are drawn. Because we will be using fossils to calibrate the overall rate of substitution, we will use a very diffuse prior for this parameter. Select **Exponential** for the `Prior` and specify **0.05** for the `Mean` (Figure 11a). Because we will be specifying node-age priors in units of millions of years, the mean of 0.05 for prior on **ucld.mean** translates to a mean rate of 5% per million years.

The default prior for the **birthDeath.meanGrowthRate** is **Uniform** from **0** to **10000**. This is a very broad prior. Based on our knowledge of the crocodylian fossil record, we can get a rough idea of our prior expectations for this parameter. Given the age of the oldest crocodylian fossil is 71.3 million years, we know the height of our tree is at least that. Given that, we can calculate a pure-birth (Yule process)



(a) CP1.mu. (b) CP2.mu. (c) CP2.mu.

Figure 10: Priors for relative-rate parameters.



(a) ucld.mean. (b) birthDeath.meanGrowthRate.

Figure 11: Priors for clock rates and diversification rate.

rate that has an expected tree height of 71.3 million years. I have included a Python script yule.py in the tutorial download that performs such calculations. This program is hosted via git on GitHub at `https://github.com/joaks1/pyule.git`; license information and documentation are available on the GitHub site.

You do not have to do this now, but if you open a Terminal window and invoke the script as follows:

```
python yule.py height 71.3 24
```

you get the following output:

```
ntips = 24
rate = 0.0389334947792
height = 71.3
length = 590.750974976
```

From these results, we see that the largest we expect a pure-birth rate to be is around 0.04. This allows us to specify a much better prior than the default. Specify an **Exponential** prior for the **birthDeath.meanGrowthRate**, but with a `Mean` of **0.1** (Figure 11b).

You will also specify an **Exponential** prior for the **ucld.stdev** parameter. We will assign you a value to specify for the `Mean` of this distribution. At the end of the lab we will compare are our findings to see how sensitive the analysis is to this prior.

Step 11: Next, we need to specify our node-age priors based on fossil information.

For **tmrca(Alligatoridae)** select **Gamma** for the `Prior Distribution`, and specify **2** for both the `Shape` and `Scale` and **64** for the `Offset` (Figure 12a).

For **tmrca(Crocodylus)** select **Exponential** for the `Prior Distribution`, and specify **10** for the mean and **12** for the `Offset` (Figure 12b).

For **tmrca(Caiman)** select **Exponential** for the `Prior Distribution`, and specify **4** for the mean and **9** for the `Offset` (Figure 12c).

For **treeModel.rootHeight** select **Gamma** for the `Prior Distribution`, and specify **78** for the `Initial value`, **1.5** for the `Shape`, **6.0** for the `Scale`, and **71.3** for the `Offset`. Also, make sure `Truncate to` is unchecked (Figure 12d).

After setting all the above priors, your ***Priors*** tab window should look like Figure 13.

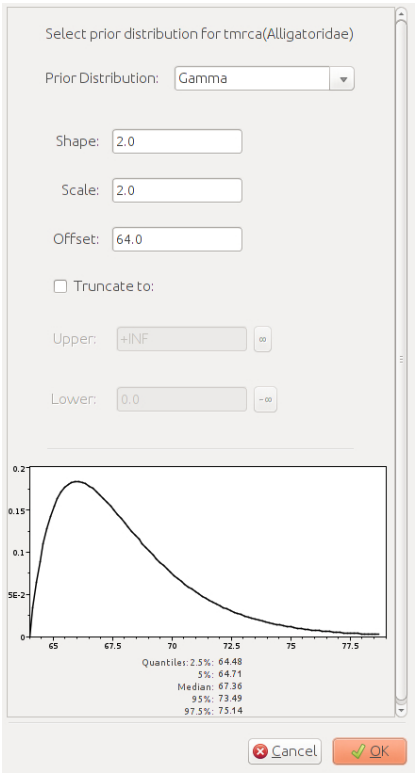
Step 12: Next, move to the ***MCMC*** tab. Change the following settings:

`Length of chain:` **1000000** (1 million)
`Echo state to screen every:` **1000**
`Log parameters every:` **1000**

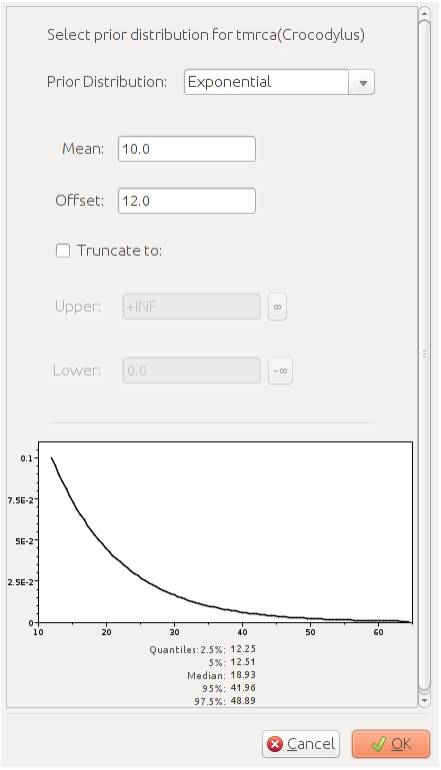
Leave the remaining options at their default values (Figure 14).

Next, click the `Generate BEAST File...` in the bottom-right corner of the window. A subwindow will pop up warning you that some of the priors are still at their default values. You can ignore this and click `Continue`. Another subwindow will appear for specifying the name and location for saving the XML file. You can leave the name the same and save the file to the div-time-tutorial folder on your desktop.

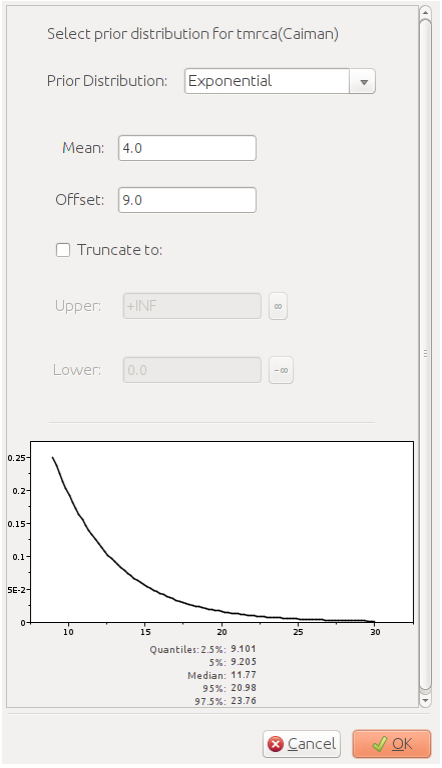
Due to time constraints, in this lab we are running a single, short MCMC chain to sample from the joint posterior distribution of the model we just finished specifying. As a result, the posterior estimates will have a large amount of MCMC sampling error. For a dataset of this size, and a model with this many parameters, we need to run a longer chain in BEAST to get a more robust sample from the posterior. We will not do this today, but in general, you should always run multiple, independent MCMC analyses in order to increase the posterior sample size, and to help assess whether the chains converged to the same stationary distribution. Also, you should always run an MCMC analysis that samples from the joint prior distribution (i.e., an analysis that ignores the data). This allows you to evaluate the interaction of all the priors you



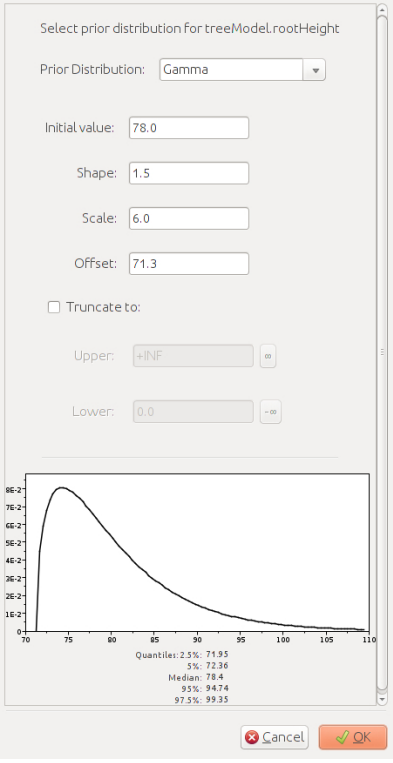
(a) tmrca(Alligatoridae).



(b) tmrca(Crocodylus).

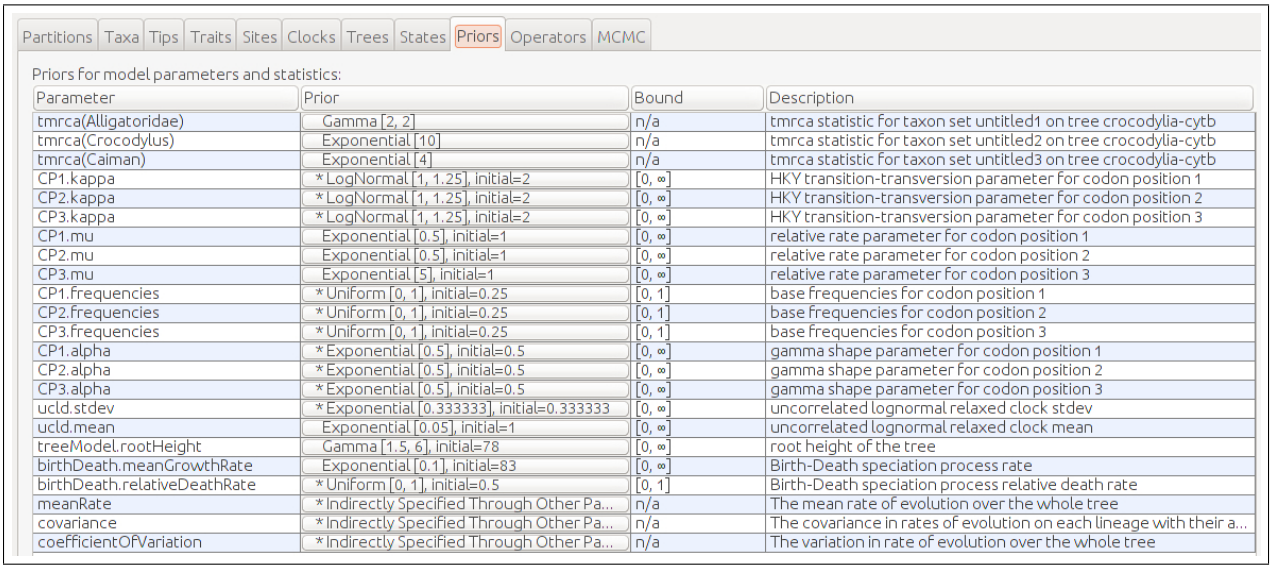


(c) tmrca(Caiman).



(d) treeModel.rootHeight.

Figure 12: Priors for clock rates and diversification rate.

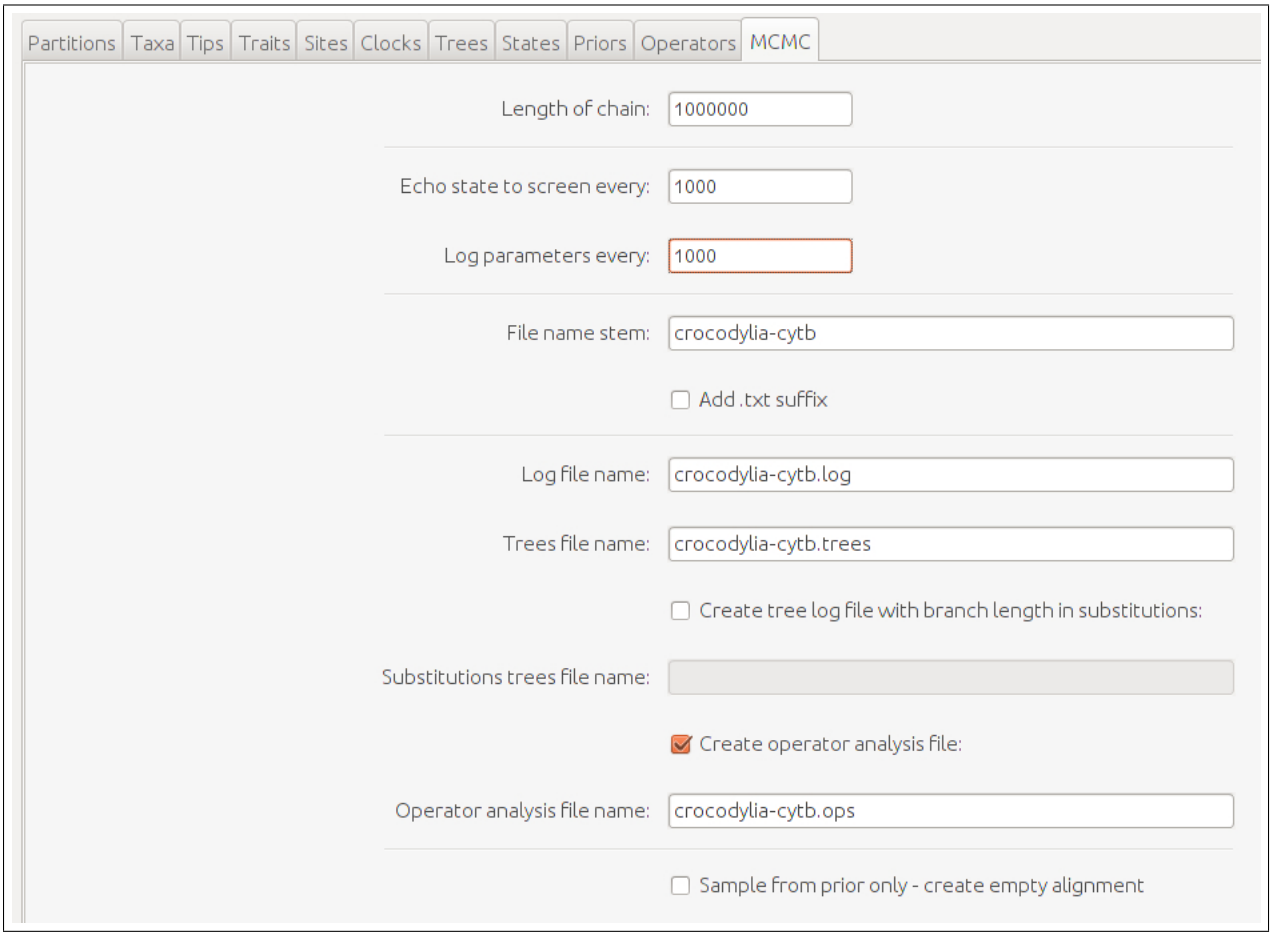


Partitions | Taxa | Tips | Traits | Sites | Clocks | Trees | States | Priors | Operators | MCMC

Priors for model parameters and statistics:

Parameter	Prior	Bound	Description
tmrca(Alligatoridae)	Gamma [2, 2]	n/a	tmrca statistic for taxon set untitled1 on tree crocodylia-cytb
tmrca(Crocodylus)	Exponential [10]	n/a	tmrca statistic for taxon set untitled2 on tree crocodylia-cytb
tmrca(Caiman)	Exponential [4]	n/a	tmrca statistic for taxon set untitled3 on tree crocodylia-cytb
CP1.kappa	* LogNormal [1, 1.25], initial=2	[0, ∞]	HKY transition-transversion parameter for codon position 1
CP2.kappa	* LogNormal [1, 1.25], initial=2	[0, ∞]	HKY transition-transversion parameter for codon position 2
CP3.kappa	* LogNormal [1, 1.25], initial=2	[0, ∞]	HKY transition-transversion parameter for codon position 3
CP1.mu	Exponential [0.5], initial=1	[0, ∞]	relative rate parameter for codon position 1
CP2.mu	Exponential [0.5], initial=1	[0, ∞]	relative rate parameter for codon position 2
CP3.mu	Exponential [5], initial=1	[0, ∞]	relative rate parameter for codon position 3
CP1.frequencies	* Uniform [0, 1], initial=0.25	[0, 1]	base frequencies for codon position 1
CP2.frequencies	* Uniform [0, 1], initial=0.25	[0, 1]	base frequencies for codon position 2
CP3.frequencies	* Uniform [0, 1], initial=0.25	[0, 1]	base frequencies for codon position 3
CP1.alpha	* Exponential [0.5], initial=0.5	[0, ∞]	gamma shape parameter for codon position 1
CP2.alpha	* Exponential [0.5], initial=0.5	[0, ∞]	gamma shape parameter for codon position 2
CP3.alpha	* Exponential [0.5], initial=0.5	[0, ∞]	gamma shape parameter for codon position 3
ucld.stdev	* Exponential [0.333333], initial=0.333333	[0, ∞]	uncorrelated lognormal relaxed clock stdev
ucld.mean	Exponential [0.05], initial=1	[0, ∞]	uncorrelated lognormal relaxed clock mean
treeModel.rootHeight	Gamma [1.5, 6], initial=78	[0, ∞]	root height of the tree
birthDeath.meanGrowthRate	Exponential [0.1], initial=83	[0, ∞]	Birth-Death speciation process rate
birthDeath.relativeDeathRate	* Uniform [0, 1], initial=0.5	[0, 1]	Birth-Death speciation process relative death rate
meanRate	* Indirectly Specified Through Other Pa...	n/a	The mean rate of evolution over the whole tree
covariance	* Indirectly Specified Through Other Pa...	n/a	The covariance in rates of evolution on each lineage with their a...
coefficientOfVariation	* Indirectly Specified Through Other Pa...	n/a	The variation in rate of evolution over the whole tree

Figure 13: The prior settings.



Partitions | Taxa | Tips | Traits | Sites | Clocks | Trees | States | Priors | Operators | MCMC

Length of chain: 1000000

Echo state to screen every: 1000

Log parameters every: 1000

File name stem: crocodylia-cytb

☐ Add .txt suffix

Log file name: crocodylia-cytb.log

Trees file name: crocodylia-cytb.trees

☐ Create tree log file with branch length in substitutions:

Substitutions trees file name:

☑ Create operator analysis file:

Operator analysis file name: crocodylia-cytb.ops

☐ Sample from prior only - create empty alignment

Figure 14: The MCMC settings.

have specified for the various parameters, and also gives you an idea of how much the data are influencing certain parameter estimates. We will not do this today, but you do this by checking the `Sample from prior only-create empty alignment` box and creating another XML file (make sure you change the name!).

Later, when your analysis is running, we will look at results from multiple, longer chains and from a chain that sampled only from the prior.

6.3 Running the XML file with BEAST

Step 13: Launch the BEAST program. If you are using Mac OSX or Windows, you should be able to do this by double clicking on the application. After the BEAST window appears, click the `Choose File...` button, and select the XML file you just created (Figure 15). Click `Run`. The analysis should take about 20 minutes.

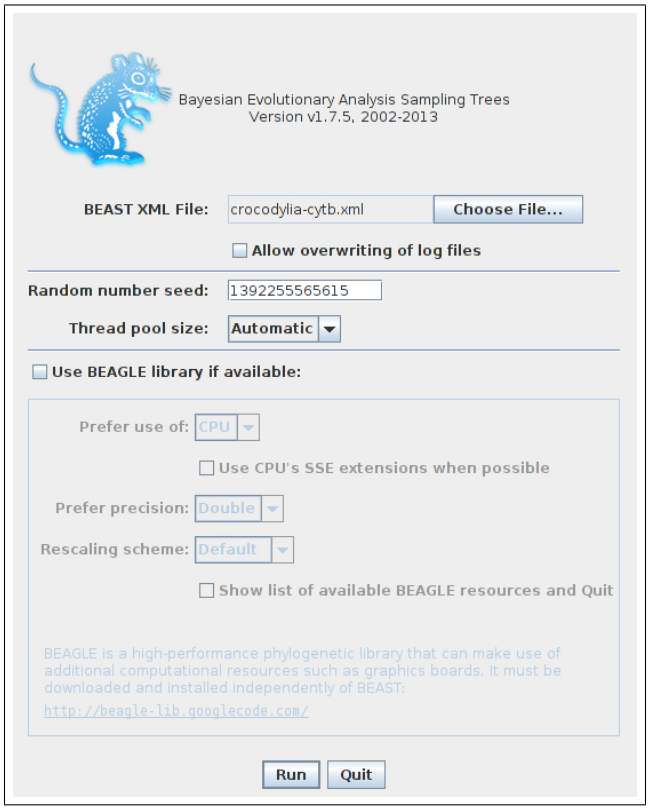


Figure 15: The BEAST GUI window.

6.4 Inspecting previous results with Tracer

In the div-time-tutorial folder, there is a subfolder called output containing .log and .trees files from analyses I ran previously.

Step 14: Launch the Tracer program. Load the crocodylia-cytb-run1.log and crocodylia-cytb-run2.log in the output directory into Tracer using ***File →Import Trace File...*** or the "+" button.

Use Tracer to inspect the behavior of the two MCMC chains.

Does it look like the chains reached stationarity?

Does it look like both chains converged to the same stationarity distribution?

What do we call this stationary distribution?

Now, load the crocodylia-cytb-prior.log file into Tracer. This log file is from the analysis that sampled only from the prior distribution. Use Tracer to compare the prior and posterior samples.

Are any of the parameter estimates similar between the prior and posterior? Which ones?

6.5 Summarizing the trees with LogCombiner and TreeAnnotator

Once you have reviewed the log files from the independent runs in Tracer and determined that they have reached stationarity, you can combine the sampled trees into a single tree file.

Step 15: Launch the LogCombiner program. Change the `File type:` to `Tree Files`. Load the crocodylia-cytb-run1.trees and crocodylia-cytb-run2.trees file in the output directory into LogCombiner using the "+" button. Select the `Choose File...` button and specify the output directory and a file name, crocodylia-cytb-runs1and2.trees. Specify an appropriate burn-in value based on what you saw in Tracer. Click `Run`

Now you have a single tree file with all the trees from the two independent runs called crocodylia-cytb-runs1and2.trees. TreeAnnotator will summarize the trees and identify the topology with the best posterior support, and summarize the age estimates for each node in the tree.

Step 16: Launch the TreeAnnotator program. Specify the burnin value as **0** (we removed the burn-in with LogCombiner). For the `Target tree type` field, choose **Maximum clade credibility tree**. For the `Node heights` field, choose **Median heights**. Select the `Input Tree File` button and select the file crocodylia-cytb-runs1and2.trees. Select the `Output File` button and specify the output directory and a file name, crocodylia-MCC.tre. Click `Run`

6.6 Visualizing the tree in FigTree

Step 17: Launch the FigTree program, and load the crocodylia-MCC.tre file you just created with TreeAnnotator. Check the `Scale Axis` option in the left column, and check the ***Scale Axis*** →***Reverse axis*** box. Check the `Node Bars` option and select **height_95%_HPD** for the ***Node bars*** →***Display*** field.

What is the age of the most recent common ancestor of all* Crocodylus *species?

What is the age of the stem node for* Crocodylus*?

6.7 Inspecting your results with Tracer

Step 18:

If your analysis has finished, launch the Tracer program and load the log file created by BEAST.

What was the mean you specified for the prior on ucld.stdev?

What is your estimate of the mean and 95% HPD interval for the age of the stem node for* Crocodylus *(hint: the tmrca(Crocodylus) statistic)?

Compare your estimate with your classmates that used a different prior on ucld.stdev. Are the results sensitive to this prior? Is there a trend?

7 Quick Version of the Tutorial

Step 1: Download and install BEAST.

Step 2: Download the data and other files from Google Drive.

Step 3: Launch BEAUTi.

Step 4: Import the data in crocodylia-cytb.nex.

Step 5: Inspect the alignment.

Step 6: Define taxon sets.

Step 7: Define Markov-chain models of substitution

Step 8: Define clock models.

Step 9: Select the tree prior.

Step 10: Select priors for parameters.

Step 11: Select priors for node ages!

Step 12: Specify MCMC settings and generate BEAST XML files.

Step 13: Run the XML file in BEAST.

Step 14: Inspect previous results with Tracer.

Step 15: Combine tree files using LogCombiner.

Step 16: Summarize the trees using TreeAnnotator.

Step 17: Look at the summary tree in FigTree.

Step 18: Inspect the results of your short analysis with Tracer.

8 Additional Information/Resources

Great resources for divergence-time estimation include a tutorial and slides by Tracy Heath!

References

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