Relaxed Phylogenetics and Dating with Confidence

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Introduction

This practical will introduce the BEAST software for Bayesian evolutionary analysis, with a focus on estimating phylogenies and divergence times when you have calibration information from fossil evidence or other prior knowledge.

You will need the following software at your disposal:

- **BEAST** - this package contains the BEAST program, BEAUti, TreeAnnotator and other utility programs. This tutorial is written for BEAST v1.5.x, which has support for multiple partitions. It is available for download from `http://beast.bio.ed.ac.uk/`.

- **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 v1.5.. It is available for download from `http://beast.bio.ed.ac.uk/`.

- **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 v1.3.1. It is available for download from `http://tree.bio.ed.ac.uk/`.

Rates and dates

This tutorial will guide you through the analysis of an alignment of sequences sampled from twelve primate species. The goal is to estimate the phylogeny as well as the rate of evolution on each lineage based on dates of divergence of their host species. The first step will be to convert a NEXUS file with a DATA or CHARACTERS block 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.
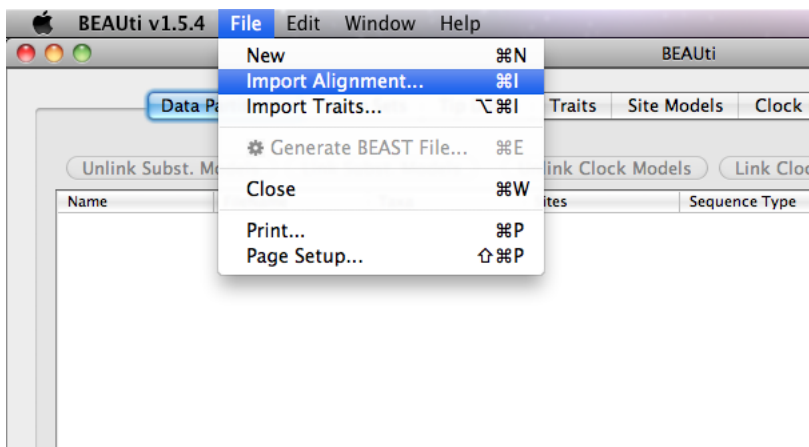
③ The final step is to explore the output of BEAST in order to diagnose problems and to summarize the results.

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 Alignment...` option from the File menu:

Select the file called `primates.nex`. This file contains an alignment of sequences of 12 species of primates. It looks like this (the lines have been truncated):

```
#NEXUS
begin data;
dimensions ntax=12 nchar=898;
format datatype=dna interleave=no gap=-;
matrix
Tarsius_syrichta    AAGTTTCATTGGAGCCACCACTCTTATAATTGCCCATGGCCTCACC
Lemur_catta         AAGCTTCATAGGAGCAACCATTCTAATAATCGCACATGGCCTTACA
Homo_sapiens        AAGCTTCACCGGCGCAGTCATTCTCATAATCGCCCACGGGCTTACA
Pan                 AAGCTTCACCGGCGCAATTATCCTCATAATCGCCCACGGACTTACA
Gorilla             AAGCTTCACCGGCGCAGTTGTTCTTATAATTGCCCACGGACTTACA
Pongo               AAGCTTCACCGGCGCAACCACCCTCATGATTGCCCATGGACTCACA
Hylobates           AAGCTTTACAGGTGCAACCGTCCTCATAATCGCCCACGGACTAACC
Macaca_fuscata      AAGCTTTTCCGGCGCAACCATCCTTATGATCGCTCACGGACTCACC
M_mulatta           AAGCTTTTCTGGCGCAACCATCCTCATGATTGCTCACGGACTCACC
M_fascicularis      AAGCTTCTCCGGCGCAACCACCCTTATAATCGCCCACGGGCTCACC
M_sylvanus          AAGCTTCTCCGGTGCAACTATCCTTATAGTTGCCCATGGACTCACC
```
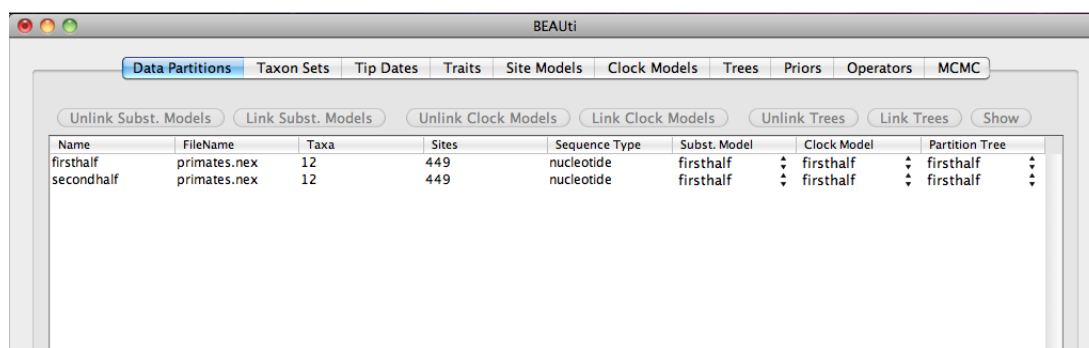
2

```
Saimiri_sciureus   AAGCTTCACCGGCGCAATGATCCTAATAATCGCTCACGGGTTTACT
;
end;

begin assumptions;
charset firsthalf = 1-449;
charset secondhalf = 450-898;
end;
end;
```

Once loaded, the two character partitions are displayed in the main panel:

Defining the calibration nodes

Select the **Taxon Sets** tab at the top of the main window. You will see the panel that allows you to create sets of taxa. Once you have created a taxa set you will be able to add calibration information for its most recent common ancestor (MRCA) later on. Press the small "plus" button at the bottom left of the panel. This will create a new taxon set.

Rename it by double-clicking on the entry that appears (it will initially be called `untitled1`). Call it `ingroup` (it will contain all taxa except the lemur, which will form the outgroup). In the next table along you will see the available taxa. Select all taxa and press the green arrow button. Move the lemur back into the excluded taxa set. Since we know that lemur is the outgroup, we will set select the checkbox in the Monophyletic? column. This will ensure that the ingroup is kept monophyletic during the course of the MCMC analysis.

Now repeat the whole procedure creating a set called `Human-Chimp` that contains only `Homo_sapiens` and `Pan` taxa. The screen should look like this:
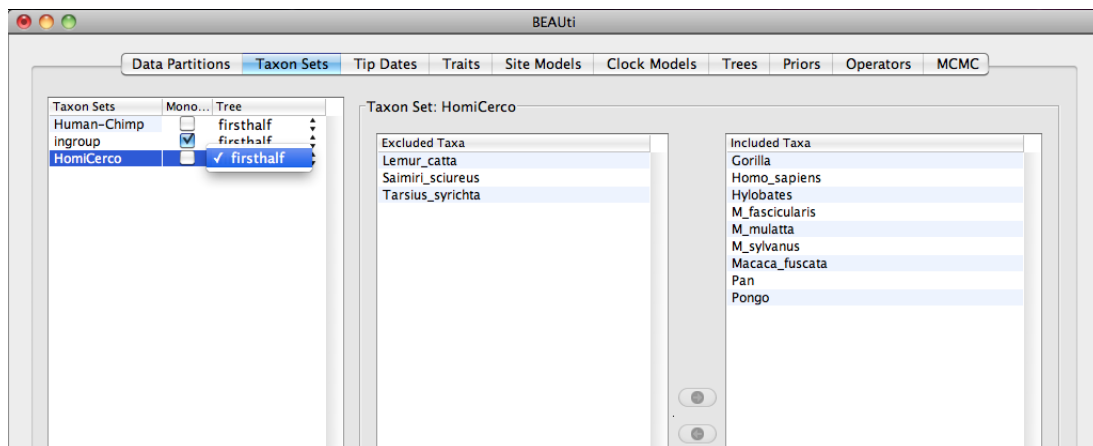
Finally, create a taxon group that contains everything under the hominoid/cercopithecoid split (i.e. everything except Lemur, Saimiri and Tarsius). Call this taxon set something like HomiCerco.

Setting the substitution model

The next thing to do is to click on the **Site Models** 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, or amino acids, or binary data, or general data. The settings that will appear after loading the Primates data set will be the default values so we need to make some changes.

Most of the models should be familiar to you. For this analysis, we will respectively select substitution models listed each time on the left side make the same change: select **Gamma** under the **Site Heterogeneity Model** menu which will allow rate variation between sites in the associated alignment.
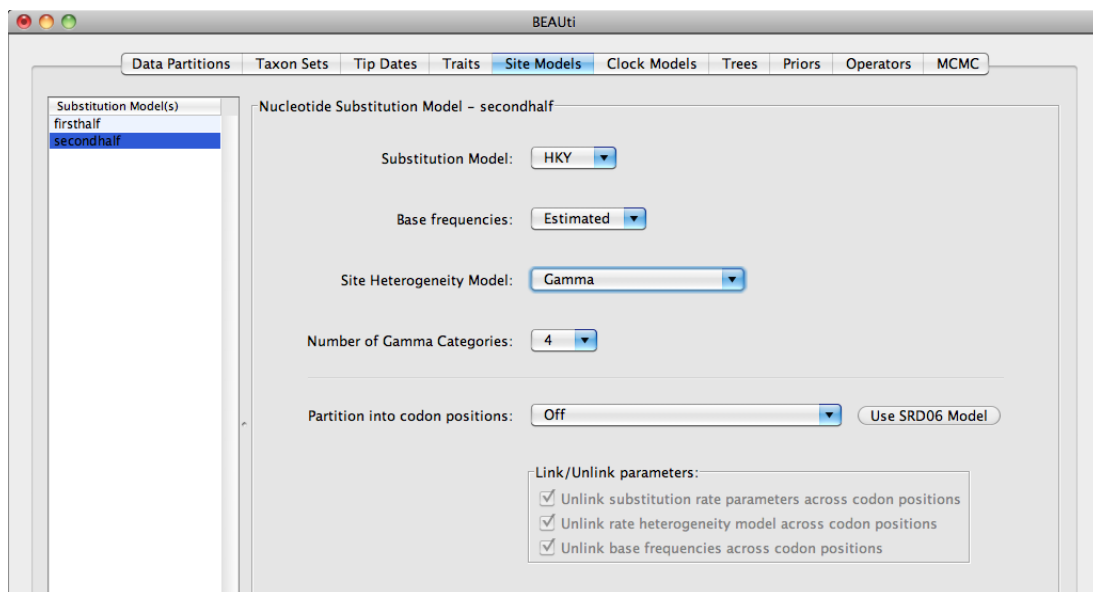
At this point we will need to unlink the substitution model so that each parameter is estimated separately for the two partitions. To do this return to **Data Partitions** panel, select both partitions in the table and click the "Unlink Subst Models" button.
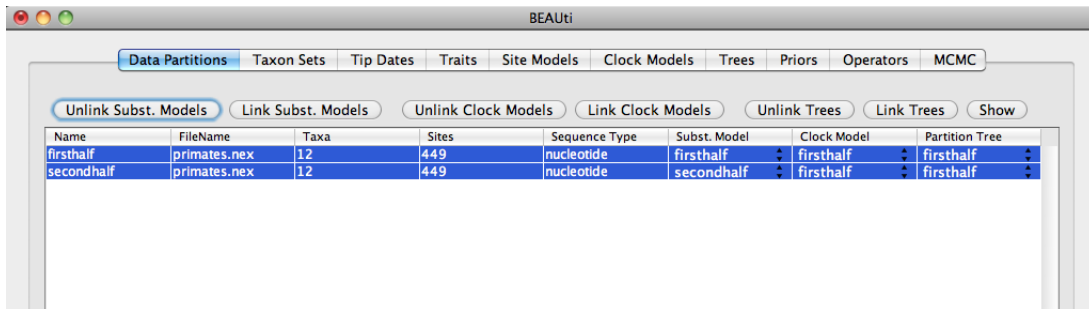
And you can also change the partition model name in its respective panel (e.g. **Clock Models** panel), and make the final partitions as illustrated below:
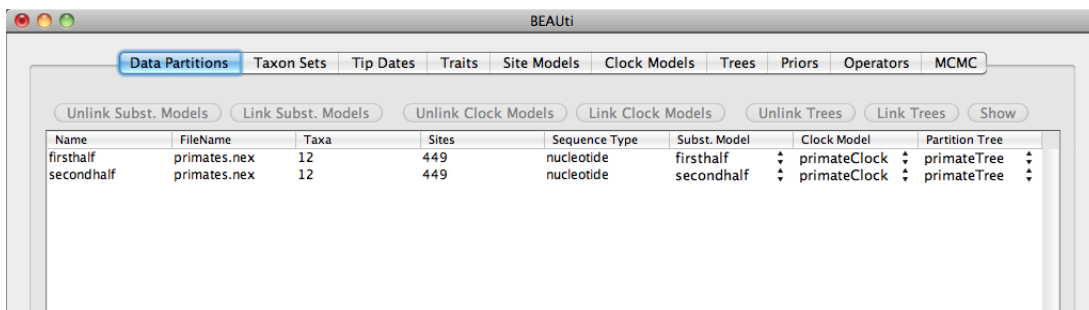

Setting the clock model

Second, we will do is to click on the **Clock Models** tab at the top of the main window, and to change the molecular clock model to **Relaxed Clock: Uncorrelated Lognormal** so as to account for lineage-specific rate heterogeneity. Your model options should now look like this:
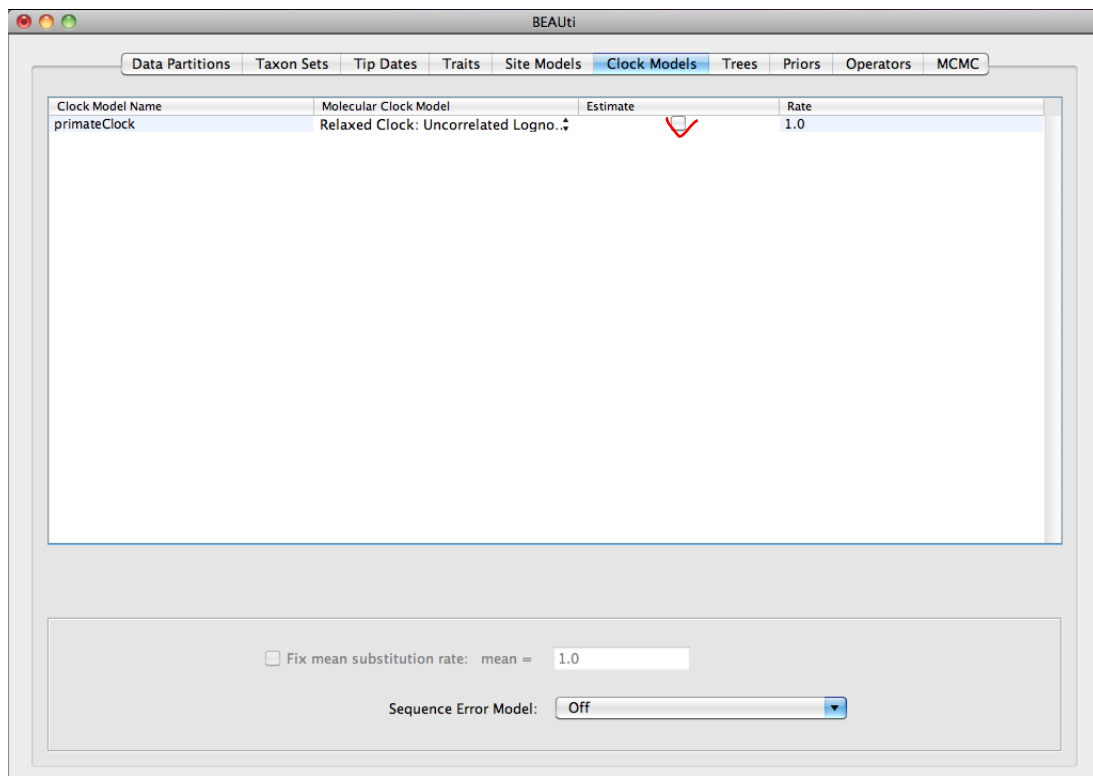
The **Estimate** check box is required to be checked, because we wish to estimate the clock rate (and in doing so the divergence times). But this will be automatically checked, in this case, when we put a proper prior on **tmcra** statistics appeared in **Priors** panel.

Trees

The **Trees** tab allows priors to be specified for each parameter in the model. The first thing to do is to specify that we wish to use the **Yule** model as the tree prior. This is a simple model of speciation that is generally more appropriate when considering sequences from different species. Select this from the **Tree prior** dropdown menu.
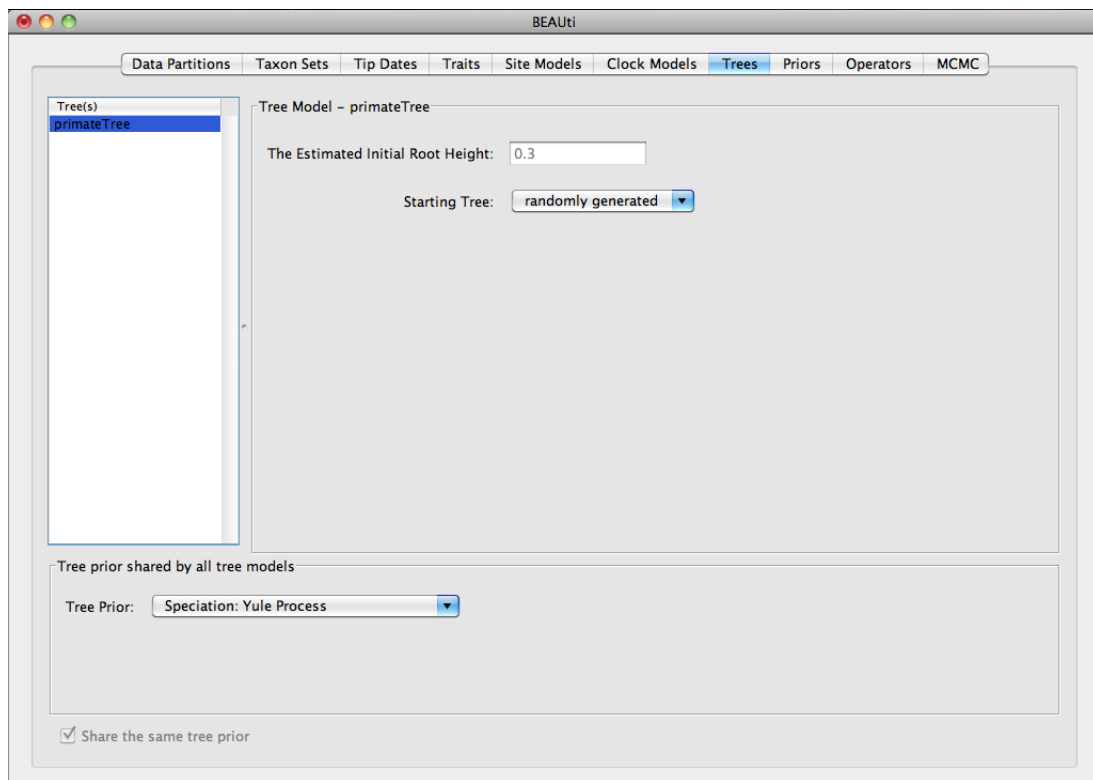
Priors

The **Priors** tab allows priors to be specified for each parameter in the model. The first thing to do is to specify that we wish to use the Yule model as the tree prior. This is a simple model of speciation that is generally more appropriate when considering sequences from different species. Select this from the **Tree prior** dropdown menu.

We now need to specify a prior distribution for some of the divergence times, based on our prior fossil knowledge. This is known as calibrating our tree. We will actually use two calibrations in this analysis. Click on the button in the table next to `tmrca(human-chimp)`:

A dialog box will appear allowing you to specify a prior for the MRCA of these three species. Select the **Normal** distribution
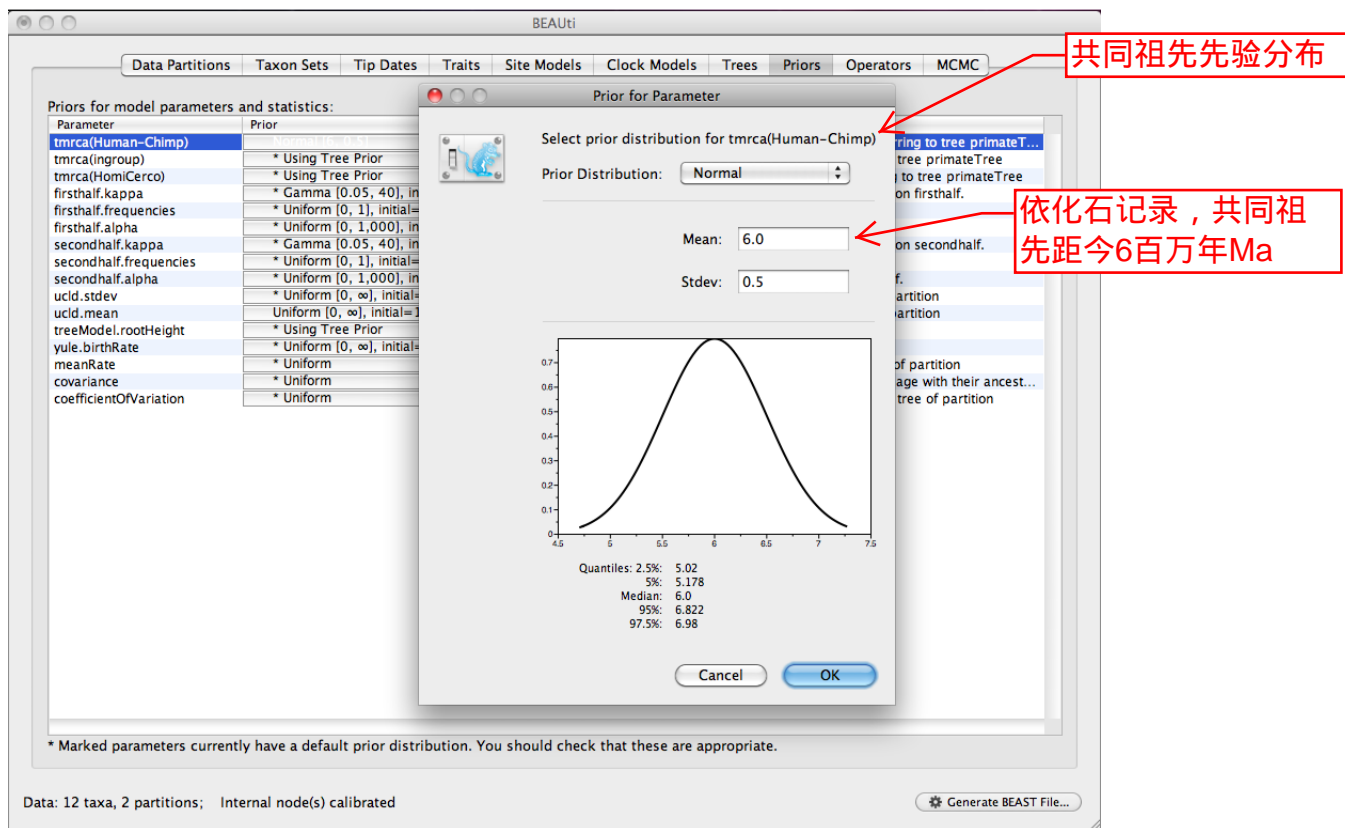
We are going to assume a normal distribution centered at 6 million years with a standard deviation of 0.5 million years. This will give a central 95% range of about 5-7 My. This corresponds to the consensus estimate of the date of the most recent common ancestor of humans and chimps.

Following the same procedure set a calibration of 24 +/- 0.5 million (stdev) for the hominoid-cercopithecoid split.

Although we created a taxon set for the ingroup (`tmrca(ingroup)` in the prior table), we are not going to put an informative prior on this. We can then estimate this divergence time based on the other calibrations. The priors table should now look like this:

Parameter	Prior	Bound	Description
tmrca(Human–Chimp)	Normal [6, 0.5]	[0, 179,7...	tmrca statistic for taxon set Human–Chimp referring to tree pri...
tmrca(ingroup)	* Using Tree Prior	[0, 179,7...	tmrca statistic for taxon set ingroup referring to tree primateTree
tmrca(HomiCerco)	Normal [24, 0.5]	[0, 179,7...	tmrca statistic for taxon set HomiCerco referring to tree primate...
firsthalf.kappa	* Gamma [0.05, 40], initial=1.0	[0, ∞]	HKY transition–transversion parameter of partition firsthalf.
firsthalf.frequencies	* Uniform [0, 1], initial=0.25	[0, 1]	base frequencies of partition firsthalf.
firsthalf.alpha	* Uniform [0, 1,000], initial=0.5	[0, 1,000]	gamma shape parameter of partition firsthalf.
secondhalf.kappa	* Gamma [0.05, 40], initial=1.0	[0, ∞]	HKY transition–transversion parameter of partition secondhalf.
secondhalf.frequencies	* Uniform [0, 1], initial=0.25	[0, 1]	base frequencies of partition secondhalf.
secondhalf.alpha	* Uniform [0, 1,000], initial=0.5	[0, 1,000]	gamma shape parameter of partition secondhalf.
ucld.stdev	* Uniform [0, ∞], initial=0.1	[0, ∞]	uncorrelated lognormal relaxed clock stdev of partition
ucld.mean	Uniform [0, ∞], initial=1.0	[0, ∞]	uncorrelated lognormal relaxed clock mean of partition
treeModel.rootHeight	* Using Tree Prior	[0, ∞]	root height of the tree of partition
yule.birthRate	* Uniform [0, ∞], initial=1.0	[0, ∞]	Yule speciation process birth rate of partition
meanRate	* Uniform	[0, ∞]	The mean rate of evolution over the whole tree of partition
covariance	* Uniform	[−∞, ∞]	The covariance in rates of evolution on each lineage with their a...
coefficientOfVariation	* Uniform	[0, ∞]	The variation in rate of evolution over the whole tree of partition

* Marked parameters currently have a default prior distribution. You should check that these are appropriate.

Data: 12 taxa, 2 partitions;   Internal node(s) calibrated

Setting the MCMC options

Ignore the **Operators** tab as this just contains technical settings effecting the efficiency of the MCMC program (see Notes for details).

The next tab, **MCMC**, provides more general settings to control the length of the MCMC and the file names.

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 data set, the complexity of the model and the quality of answer required. The default value of 10,000,000 is entirely arbitrary and should be adjusted according to the size of your data set. For this data set let's initially set the chain length to 800,000 as this will run reasonably quickly on most modern computers (a few minutes).

The next options specify how often the parameter values in the Markov chain should be displayed on the screen and recorded in the log file. The screen output is simply for monitoring the programs progress so can be set to any value (although if set too small, the sheer quantity of information being displayed on the screen will actually 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 with little extra benefit in terms of the precision of the analysis. Sample too infrequently and the log file will not contain much information about the distributions of the parameters. You probably

For this exercise we will set the screen log to 10000 and the file log to 200. The final two options give the file names of the log files for the sampled parameters and the trees. These will be set to a default based on the name of the imported NEXUS file.

- If you are using windows then we suggest you add the suffix `.txt` to both of these (so, `Primates.log.txt` and `Primates.trees.txt`) so that Windows recognizes these as text files.

Generating the BEAST XML file

We are now ready to create the BEAST XML file. 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. Save the file with an appropriate name (we usually end the filename with `.xml`, i.e., `Primates.xml`). We are now ready to run the file through BEAST.

Running BEAST

Now run BEAST and when it asks for an input file, provide your newly created XML file as input.
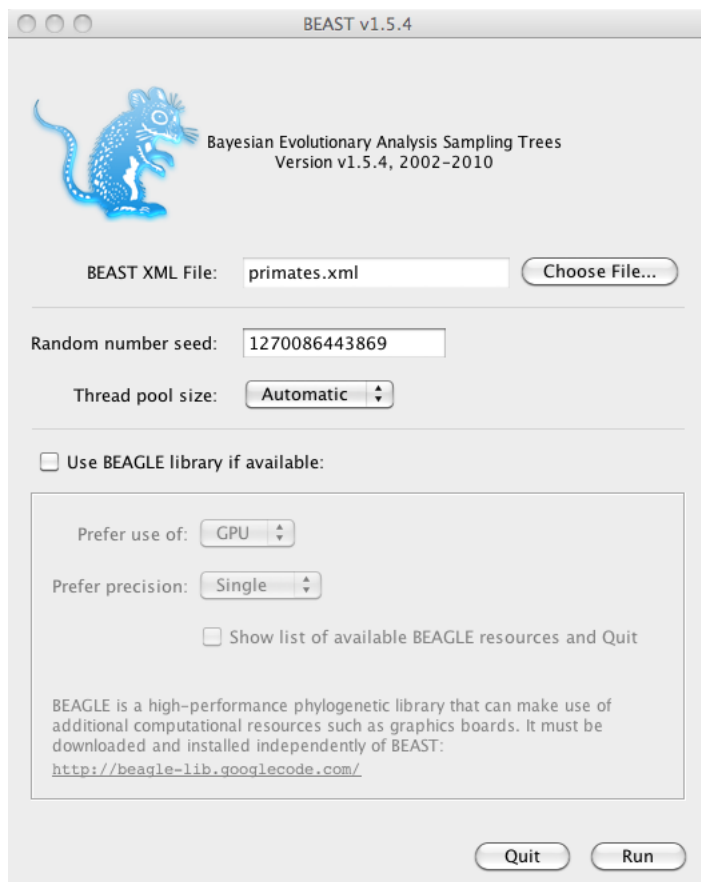
BEAST will then run until it has finished reporting information to the screen. The actual results files are save to the disk in the same location as your input file. The output to the screen will look something like this:

```
            BEAST v1.5.4, 2002-2010
    Bayesian Evolutionary Analysis Sampling Trees
            Designed and developed by
  Alexei J. Drummond, Andrew Rambaut and Marc A. Suchard

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            Downloads, Help & Resources:
              http://beast.bio.ed.ac.uk

  Source code distributed under the GNU Lesser General Public License:
            http://code.google.com/p/beast-mcmc
```

```
                          BEAST developers:
        Alex Alekseyenko, Erik Bloomquist, Joseph Heled, Sebastian Hoehna,
        Philippe Lemey, Gerton Lunter, Sidney Markowitz, Vladimir Minin,
             Michael Defoin Platel, Oliver Pybus, Walter Xie


                            Thanks to:
             Roald Forsberg, Beth Shapiro and Korbinian Strimmer



        Random number seed: 1270078661254


        Parsing XML file: primates.xml
          File encoding: MacRoman
        looking for plugins in/Users/dxie004/Desktop/Practical1a_RelaxedClocks_new/plugins
        Unexpected element in coalescentTree:startingTree:  tmrca in constrainedTaxa, tmrca in constrainedTaxa, tmrca in constrainedT
        Read alignment, ':alignment
          Sequences = 12
              Sites = 898
           Datatype = nucleotide
        Site patterns 'firsthalf.patterns' created from positions 1-449 of alignment 'alignment'
          pattern count = 227
        Site patterns 'secondhalf.patterns' created from positions 450-898 of alignment 'alignment'
          pattern count = 231
        Using Yule prior on tree
        Creating the tree model, 'treeModel'
          initial tree topology = (((((((((Homo_sapiens,M_sylvanus),Pongo),Pan),Tarsius_syrichta),Gorilla),Macaca_fuscata),((Hylobates
          tree height = 163.55349753059264
        Using discretized relaxed clock model.
          over sampling = 1
          parametric model = logNormalDistributionModel
           rate categories = 22
        Creating state frequencies model: Initial frequencies = {0.25, 0.25, 0.25, 0.25}
        Creating HKY substitution model. Initial kappa = 1.0
        Creating state frequencies model: Initial frequencies = {0.25, 0.25, 0.25, 0.25}
        Creating HKY substitution model. Initial kappa = 1.0
        Creating site model.
          4 category discrete gamma with initial shape = 0.5
        Creating site model.
          4 category discrete gamma with initial shape = 0.5
        TreeLikelihood(treeModel) using native nucleotide likelihood core
          Ignoring ambiguities in tree likelihood.
          With 227 unique site patterns.
        Branch rate model used: discretizedBranchRates
        TreeLikelihood(treeModel) using native nucleotide likelihood core
          Ignoring ambiguities in tree likelihood.
          With 231 unique site patterns.
        Branch rate model used: discretizedBranchRates
        Creating swap operator for parameter branchRates.categories (weight=10.0)
        Likelihood is using -1 threads.
        Creating the MCMC chain:
          chainLength=800000
          autoOptimize=true
          autoOptimize delayed for 8000 steps
        # BEAST v1.5.4, Build r3085
        # Generated Thu Apr 01 12:38:20 NZDT 2010 [seed=1270078661254]
        state Posterior     Prior        Likelihood   rootHeight   ucld.mean
        0 -17988.8851  -5960.6020   -12028.2831  163.553       1.00000       -
        10000 -6123.6529   -54.5139     -6069.1390    45.8562       2.06435E-2   -
        20000 -5995.5266   -58.6663     -5936.8603    49.9866       1.52459E-2   0.15 hours/million states
        30000 -5937.3075   -57.7531     -5879.5544    46.0397       9.70747E-3   0.11 hours/million states
        40000 -5898.5427   -55.7572     -5842.7855    41.5696       1.23744E-2   0.1 hours/million states
        50000 -5878.9464   -56.3634     -5822.5830    37.2131       1.04593E-2   0.09 hours/million states
        60000 -5841.3595   -57.0828     -5784.2767    36.8985       1.25161E-2   0.09 hours/million states
        70000 -5821.5282   -58.6466     -5762.8816    47.1654       1.1147E-2    0.08 hours/million states
        80000 -5794.5158   -58.5858     -5735.9300    49.9805       1.16897E-2   0.08 hours/million states
```

```
90000  -5783.5819    -57.4107      -5726.1712   47.6737      1.4616E-2    0.08 hours/million states
100000 -5785.0143    -59.4438      -5725.5705   42.0940      1.77003E-2   0.08 hours/million states


... ...


790000 -5786.0332    -63.5527      -5722.4805   52.7075      1.32915E-2   0.07 hours/million states
800000 -5783.6441    -62.1815      -5721.4626   58.5455      1.23201E-2   0.07 hours/million states

Operator analysis
Operator                                        Tuning  Count    Time   Time/Op  Pr(accept)  Performance suggestion
scale(firsthalf.kappa)                          0.541   748      245    0.33     0.2366      good
firsthalf.frequencies                           0.05    695      251    0.36     0.295       good
scale(firsthalf.alpha)                          0.548   703      268    0.38     0.2731      good
scale(secondhalf.kappa)                         0.532   739      235    0.32     0.2896      good
secondhalf.frequencies                          0.052   716      256    0.36     0.3492      good
scale(secondhalf.alpha)                         0.622   660      235    0.36     0.3636      good
scale(ucld.mean)                                0.694   21202    8048   0.38     0.274       good
scale(ucld.stdev)                               0.616   21469    8204   0.38     0.584       high Try setting scaleFact
subtreeSlide(treeModel)                         3.069   106439   21629  0.2      0.3145      good
Narrow Exchange(treeModel)                              105884   20735  0.2      0.0009      very low
Wide Exchange(treeModel)                                21351    2338   0.11     0.0002      very low
wilsonBalding(treeModel)                                21526    3836   0.18     0.0         very low
scale(treeModel.rootHeight)                     0.736   21130    1663   0.08     0.1517      good
uniform(nodeHeights(treeModel))                         213236   49425  0.23     0.2091      good
scale(yule.birthRate)                           0.284   21382    1168   0.05     0.2949      good
up:ucld.mean down:nodeHeights(treeModel)        0.868   21372    8079   0.38     0.219       slightly high
Try setting scaleFactor to about 0.8761
swapOperator(branchRates.categories)                    71045    18385  0.26     0.4699      slightly high
No suggestions
randomWalkInteger(branchRates.categories)               70778    15364  0.22     0.8972      high Try increasing window
uniformInteger(branchRates.categories)                  70925    15244  0.21     0.5794      high

3.3444 minutes
```
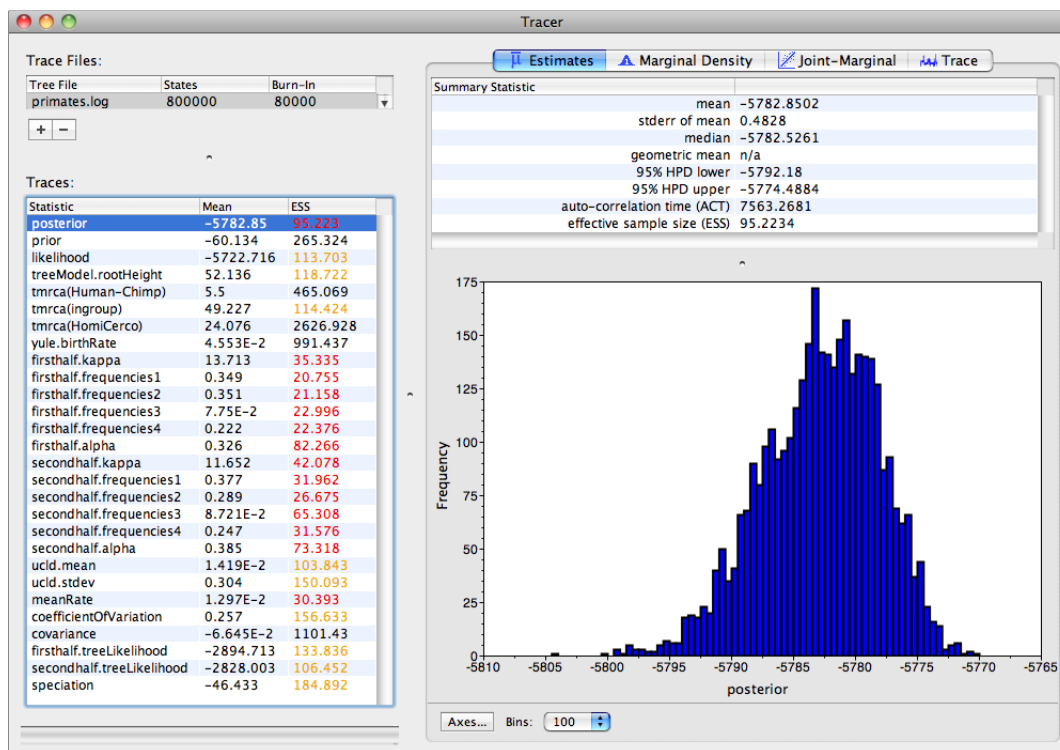
Analyzing the results

Run the program called **Tracer** to analyze the output of BEAST. When the main window has opened, choose **Import Trace File...** from the **File** menu and select the file that BEAST has created called `Primates.log.txt`. You should now see a window like the following:

Remember that MCMC is a stochastic algorithm so the actual numbers will not be exactly the same.

On the left hand side is a list of the different quantities that BEAST has logged. There are traces for the posterior (this is the log of the product of the tree likelihood and the prior probabilities), 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.

Select `meanRate` to look at the rate of evolution averaged over the whole tree. Tracer will plot a (marginal posterior) distribution for the selected parameter and also give you statistics such as the mean and median. The 95% HPD stands for *highest posterior density interval* and represents the most compact interval on the selected parameter that contains 95% of the posterior probability. It can be thought of as a Bayesian analog to a confidence interval.

Questions

What is the rate of molecular evolution in Primates (include the HPD interval)?

*What sources of error does this estimate include?*

The `coefficientOfVariation` statistic gives a summary of how much the rate of evolution varies from lineage to lineage (expressed as a proportion of the mean rate).

Does the rate of evolution differ substantially amongst different lineages in the tree?


Selecting the `treeModel.rootHeight` parameter gives the marginal posterior distribution of the age of the root of entire tree.

How old is the root of the tree (give the mean and the HPD range)?

Select the `treeModel.rootHeight` parameter and the next three (hold shift whilst selecting). This will show a display of the age of the root and the three MRCAs we specified in BEAUti. The two parameters that we used to calibrate the tree (`tmrca(HomiCerco)` and `tmrca(human-chimp)`) will have posterior distributions very similar to the prior distributions that we specified.
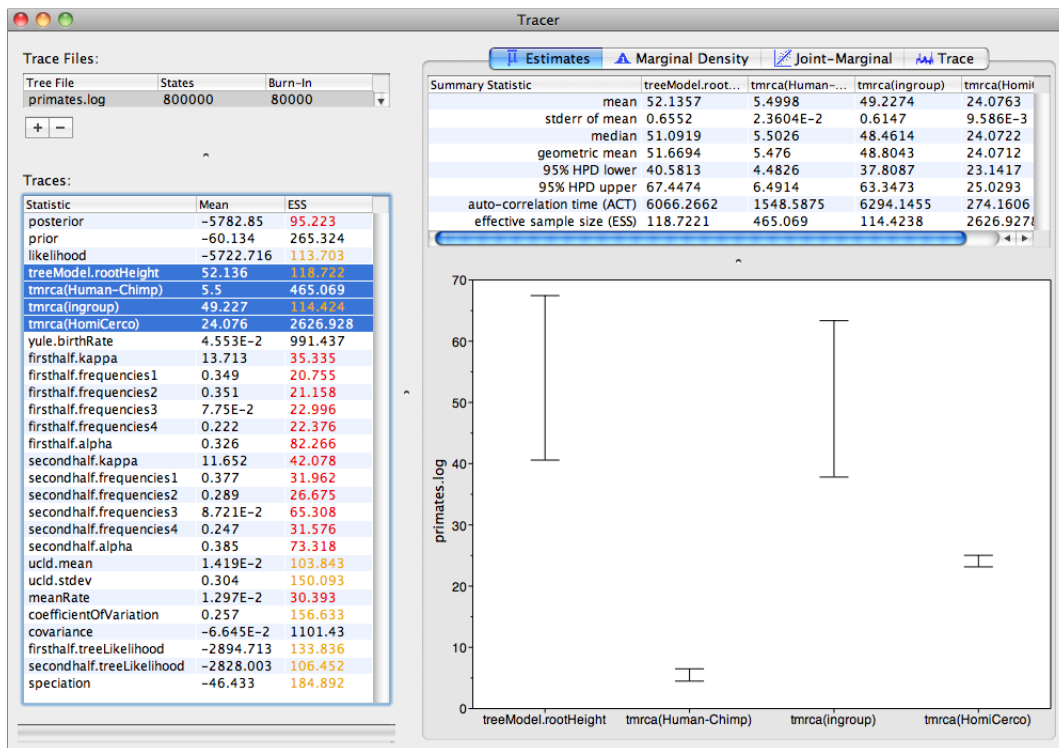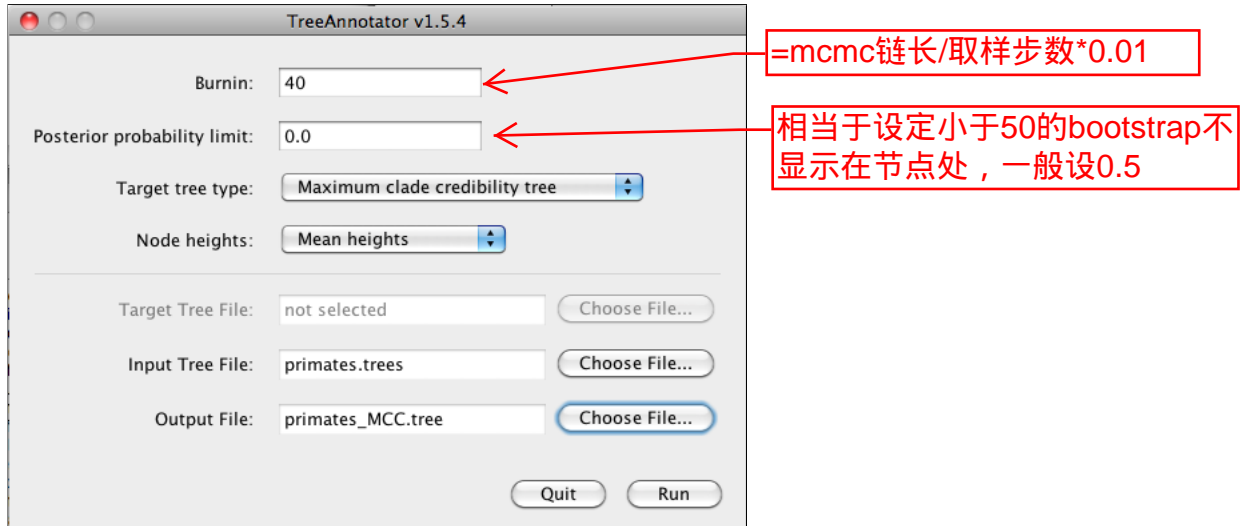
If you switch the tab at the top of the window to **Marginal Density** then you will get a plot of the marginal posterior densities of each of these date estimates overlayed:
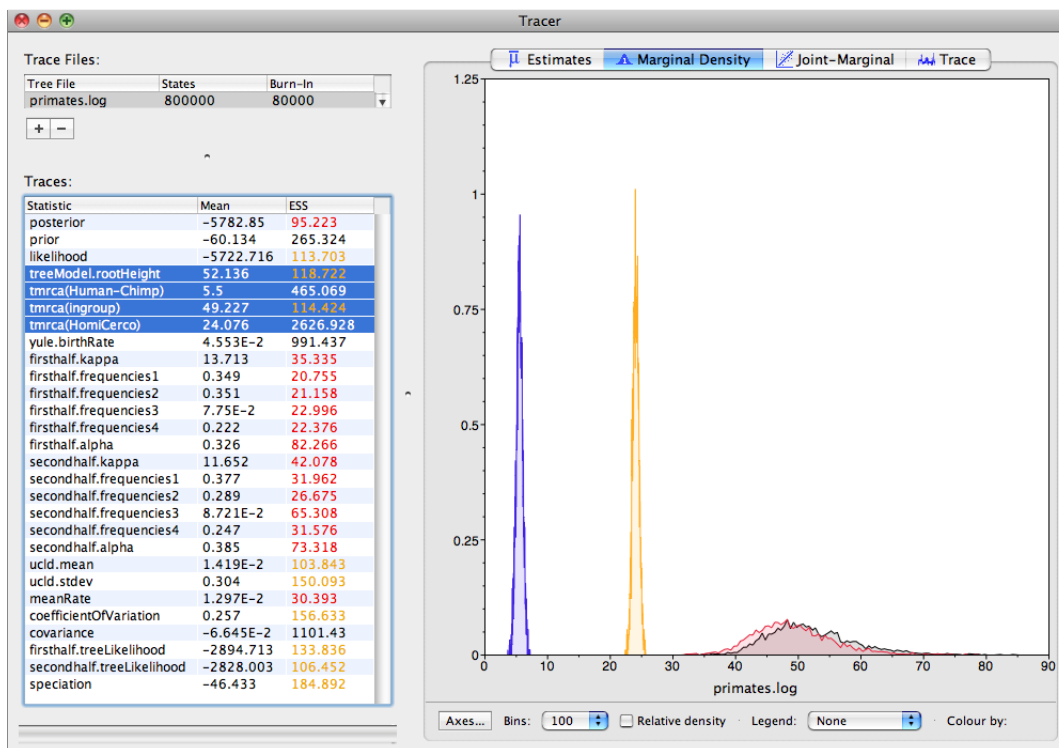
Obtaining an estimate of the phylogenetic tree

BEAST also produces a sample of plausible trees along with its sample of parameter estimates. These need to be summarized using the program **TreeAnnotator** (see Notes for details). This will take the set of trees and find the best supported one. It will then annotate this summary tree with the mean ages of all the nodes and the HPD ranges. It will also calculate the posterior clade probability for each node. Run the TreeAnnotator program and set it up to look like this:

TreeAnnotator v1.5.4	
Burnin:	40
Posterior probability limit:	0.0
Target tree type:	Maximum clade credibility tree
Node heights:	Mean heights
Target Tree File:	not selected — Choose File...
Input Tree File:	primates.trees — Choose File...
Output File:	primates_MCC.tree — Choose File...
	Quit Run

=mcmc   /   *0.01

50   bootstrap
0.5

The burnin is the number of trees to remove from the start of the sample. Unlike **Tracer** which specifies the number of steps as a burnin, in **TreeAnnotator** you need to specify the actual number of trees. For this run, you specified a chain length of 800,000 steps sampling every 200 steps. Thus the trees file will contain 4000 trees and so to specify a 1% burnin use the value 40.

The **Posterior probability limit** option specifies a limit such that if a node is found at less than this frequency in the sample of trees (i.e., has a posterior probability less than this limit), it will not be annotated. The default of 0.5 means that only nodes seen in the majority of trees will be annotated. Set this to zero to annotate all nodes.

For **Target tree type** you can either choose a specific tree from a file or ask TreeAnnotator to find a tree in your sample. The default option, **Maximum clade credibility tree**, finds the tree with the highest product of the posterior probability of all its nodes.

Choose **Mean heights** for node heights. This sets the heights (ages) of each node in the tree to the mean height across the entire sample of trees for that clade.

For the input file, select the trees file that BEAST created (by default this will be called `Primates.trees`) and select a file for the output (here we called it `Primates_MCC.tree`).

Now press Run and wait for the program to finish.

Viewing the Tree

Finally, we can look at the tree in another program called **FigTree**. Run this program, and open the `Primates.MCC.tree` file by using the Open command in the File menu. The tree should appear. You can now try selecting some of the options in the control panel on the left. Try selecting **Node Bars** to get node age error bars. Also turn on **Branch Labels** and select **posterior** to get it to display the posterior probability for each node. Under **Appearance** you can also tell FigTree to colour the branches by the rate. You should end up with something like this:
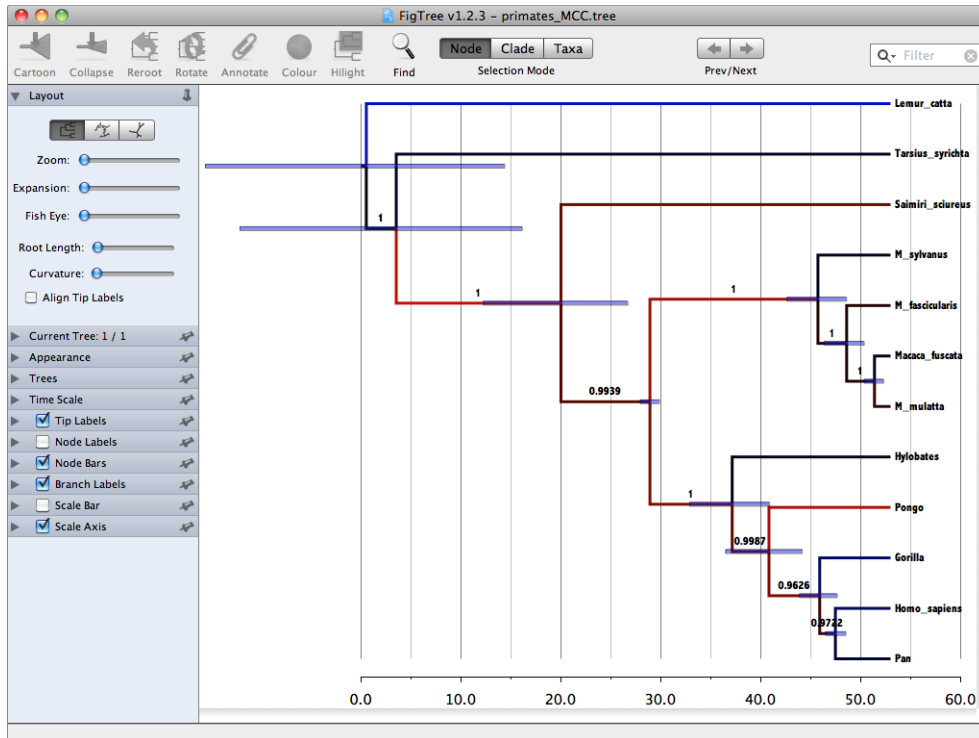
Which branch has the fastest rate of evolution and what is the estimated rate?

Which branch has the slowest rate of evolution and what is the estimated rate?

Are these two rate estimates significantly different? How would you answer this question?

Comparing your results to the prior

Using BEAUti, set up the same analysis but under the MCMC options, select the **Sample from prior only** option. This will allow you to visualize the full prior distribution in the absence of your sequence data. Summarize the trees from the full prior distribution and compare the summary to the posterior summary tree.