MCMCTree tutorials

Mario dos Reis and Ziheng Yang

It is assumed that you have some basic knowledge of using the command line in Windows or Unix systems (e.g. Linux and MacOS). You need to download and install the PAML package from http://abacus.gene.ucl.ac.uk/software/paml.html. Make sure you have the latest version, currently 4.5. There are executables for Windows (*.exe) but Unix users may need to compile the programs. Please follow the instructions in the PAML website to modify your operating system PATH variable. This is necessary so that you can call the programs from the command line without having to type their full folder (path) location.

MCMCTree performs Bayesian estimation of species divergence times using soft fossil constraints under various molecular clock models[2, 3, 6]. The general theory of molecular dating is given in chapter 7 of Yang[7]. The program uses for input a sequence alignment (nucleotide or protein), a phylogenetic tree with fossil calibrations, and a control file (usually called mcmctree.ctl) that contains the instructions for the program.

Tutorial 1: divergence time of apes

In this tutorial we will analyze a data set of mitochondrial protein-coding genes for 7 ape species. This is the same data set analyzed by Yang and Rannala[6], and is included in the PAML[5] release (examples/DatingSoftBound). File “mtCDNApri123.txt” contains the nucleotide alignment. The alignment is divided into 3 partitions corresponding to the 1st, 2nd and 3rd codon sites. File “mtCDNApri.trees” contains the phylogenetic tree relating the 7 species with the fossil calibrations. The tree file looks like this

```
7 1
((((human, (chimpanzee, bonobo)) '>.06<.08', gorilla),
(orangutan, sumatran)) '>.12<.16', gibbon);
```

The first line has the number of species (7) and the number of trees (1). Then the tree in Newick format is given. The tree must not have branch lengths. The tree has two fossil calibrations, one for the most recent common ancestor of human-chimp: ’$> .06 < .08$’ and another for the most recent common ancestor of the greater apes: '.12 < .16'. The time unit is 100 Million years (Myr). So the first calibration restricts the common ancestor of human-chimp to between 6-8 Myr ago. Calibration bounds in MCMCTree are soft, and there is a small probability (0.025 by default) that the bounds may be violated. Note that the tree does not have a fossil calibration at the root. MCMCTree always needs a calibration on the root of the tree, and when this calibration is not present in the tree file, it must be specified in the control file (variable RootAge).

The control file "mcmctree.ctl" contains all the necessary instructions to run the MCMCTree program. You can open this file with a text editor (e.g. Notepad or TextEdit). The file should look like this

```
          seed = -1
       seqfile = mtCDNApri123.txt
      treefile = mtCDNApri.trees
       outfile = out
         ndata = 3
       usedata = 1    * 0: no data; 1:seq; 2:approximation; 3:out.BV (in.BV)
         clock = 2    * 1: global clock; 2: independent; and 3: correlated rates
       RootAge = '<1.0'  * safe constraint on root age, used if no fossil for root.
         model = 0    * 0:JC69, 1:K80, 2:F81, 3:F84, 4:HKY85
         alpha = 0    * alpha for gamma rates at sites
         ncatG = 5    * No. categories in discrete gamma
     cleandata = 0    * remove sites with ambiguity data (1:yes, 0:no)?
       BDparas = 1 1 0   * birth, death, sampling
   kappa_gamma = 6 2      * gamma prior for kappa
   alpha_gamma = 1 1      * gamma prior for alpha
   rgene_gamma = 2 2     * gamma prior for rate for genes
  sigma2_gamma = 1 10    * gamma prior for sigma^2     (for clock=2 or 3)
      finetune = 1: .1 .1 .1 .1 .1 .1 * auto (0 or 1) : times, rates, etc.
         print = 1
        burnin = 2000
      sampfreq = 2
       nsample = 20000
```

seed: this sets the random seed used by the program. When set to -1 the program will use the computer's current time to set the seed, so every time you run MCMCTree it will start with a different seed and the results of the MCMC will look different. If you need reproducible results, you can set the seed to an odd number or an even number.

seqfile and *treefile*: these are the names of the sequence alignment file and the tree file respectively.

outfile: once the program completes, it will write a summary of the results to this file.

ndata: the number of partitions in the alignment file. In our example, we have three partitions, corresponding to each one of the three codon positions in the mitochondrial proteins.

usedata: when set to 1, the likelihood function is calculated in the normal way, and the MCMC analysis proceeds as usual. When usedata=0, the likelihood is not calculated (it is set to 1), so only the prior is computed. When usedata=2 and =3, approximate likelihood calculation and ML estimation of branch lengths is performed. We will explain these in the next tutorial.

clock: the clock model to use. Here we will work with the independent rates model (clock=2), where the rates follow a log-normal distribution (that is, the logarithm of the rate is normally distributed).

RootAge: a calibration to use for the root if this is not provided in the tree file. Here we use '<1.0' or a maximum constraint of 100 Myr for the age of the most recent common ancestor of all apes.

model, *alpha* and *ncatG*: the substitution model to be used In this example we use JC69 (it is very quick to compute). Because alpha=0, We do not use a gamma model of rate variation in this example.

BDparas: parameters controlling the birth-death process. The birth-death process is used to construct the time prior for the nodes in the tree that do not have a fossil calibration. Here we used the default 1 1 0, which generates uniform node age priors.

kappa_gamma and alpha_gamma: gamma priors for the substitution model parameters κ (transition/transversion rate ratio) and α (gamma shape parameter for variable rates among sites).

rgene_gamma: gamma prior for the mean substitution rate. The gamma distribution has mean α/β and variance α/β^2. The first parameter (α) controls the shape of the distribution. Values of $\alpha = 1$ or $= 2$ lead to fairly diffuse priors. It is advisable to set α to one of those two values, and then fix β so that then mean rate is reasonable. In this example we set $\alpha = 2$ and $\beta = 2$ for a mean rate of 1 substitution per 100 Myr. Users of the R program for statistics (www.r-project.org) can easily plot the gamma distribution with

```
> curve(dgamma(x, shape=2, scale=2), from=0, to=10)
```

sigma2_gamma: gamma prior for the rate drift parameter (i.e, the variance of the logarithm of the rate, σ^2). Larger values of σ^2 imply more rate heterogeneity. The prior on σ^2 can have a strong impact on posterior time estimates[2], particularly for short alignments and few loci.

finetune: step sizes for proposals during the MCMC. From version 4.4e, autofinetune has been implemented so setting the step sizes is not as critical as before. We will explain later on how to improve the fine tune if necessary.

print: if set to 1, the output of the MCMC and a summary of the results will be written to the hard disk (the MCMC is written to the mcmc.out file and the summary to the outfile as set above). You want this. If set to 0, results will be printed to the screen only. You don't want that.

burnin, *sampfreq* and *nsample*: in our example, the program will discard the first 2,000 iterations as burn-in, and then it will sample every 2 iterations until it has gathered 20,000 samples. In total, the MCMC will run for $2{,}000+$

$2 \times 20,000 = 42,000$ iterations. Normally, you should gather between 10,000 to 20,000 samples for a good statistical summary. Large sample sizes (say 100,000) tend to waste a lot of hard drive space providing very little statistical improvement. It may also take a long time for the program to summarize the results. If you need to increase the length of the MCMC (to improve convergence), increase sampfreq but keep nsample at a reasonable value.

We are now ready to run the program and look at the results. Open a terminal window (on Windows go to Start > All programs > Accessories > Command prompt) and go to the directory where the tutorial files have been saved. Create a new directory called run01, and copy the tree, alignment and control files into this directory. On my Windows computer, the tutorial files were copied into C:\Users\Mario\Tutorial\run01>. Go into this new directory, and on the command line (terminal window) type

```
C:\Users\Mario\Tutorial\run01> mcmctree mcmctree.ctl
```

The MCMC program will start. It will read the alignment, tree and control files and it will first perform some safety checks. When the MCMC itself starts running, the output on the terminal should look like

```
lnL0 = -40215.47
Starting MCMC (np = 48) . . .
finetune steps (time rate mixing para RatePara ...):  0.1000  0.1000  0.1000  0.1000  0.1000
  paras: 6 times, 3 mu, 3 sigma2 (& rates, kappa, alpha)
 -4% 0.16 0.64 0.33 0.00 0.69  0.178 0.149 0.091 0.067 0.032 - 1.438 -34999.3
Current Pjump:      0.16033  0.64117  0.32800  0.00000  0.69267
Current finetune:   0.10000  0.10000  0.10000  0.10000  0.10000
New     finetune:   0.05050  0.31051  0.11113  0.00100  0.37446
 -2% 0.38 0.19 0.40 0.00 0.37  0.160 0.147 0.092 0.066 0.029 - 1.313 -34989.9
Current Pjump:      0.38033  0.18628  0.39600  0.00000  0.36633
Current finetune:   0.05050  0.31051  0.11113  0.00100  0.37446
New     finetune:   0.06743  0.18359  0.15638  0.00001  0.47671
 -1% 0.27 0.37 0.27 0.00 0.30  0.154 0.147 0.092 0.066 0.029 - 1.280 -34995.0
Current Pjump:      0.27133  0.36956  0.27400  0.00000  0.30433
Current finetune:   0.06743  0.18359  0.15638  0.00001  0.47671
New     finetune:   0.06009  0.23632  0.14090  0.00000  0.48476
  0% 0.29 0.27 0.32 0.00 0.30  0.155 0.145 0.094 0.067 0.029 - 1.332 -34996.7  0:02
Current Pjump:      0.29367  0.26978  0.32200  0.00000  0.29833
Current finetune:   0.06009  0.23632  0.14090  0.00000  0.48476
New     finetune:   0.05862  0.20922  0.15316  0.00000  0.48163
  5% 0.32 0.31 0.25 0.00 0.31  0.158 0.147 0.092 0.066 0.029 - 1.280 -34997.5  0:04
 10% 0.31 0.32 0.27 0.00 0.30  0.158 0.146 0.093 0.067 0.029 - 1.303 -35000.8  0:06
 15% 0.32 0.32 0.28 0.00 0.30  0.157 0.146 0.093 0.067 0.029 - 1.308 -34994.6  0:08
 20% 0.32 0.32 0.28 0.00 0.31  0.157 0.146 0.093 0.067 0.029 - 1.311 -34993.5  0:10
```

The initial likelihood is $\text{lnL0} = -40,215.47$. Our rooted tree of 7 species has $7-1=6$ internal nodes and $7 \times 2 - 2 = 12$ branches. Therefore we are estimating 6 divergence times; 3 mean mutation rates and 3 rate drift parameters, one for each one of our 3 partitions (codon sites); and $12 \times 3 = 36$ branch rates. In total we are estimating 48 parameters.

Now let's look at the first line of the MCMC proper:

```
-4% 0.16 0.64 0.33 0.00 0.69  0.178 0.149 0.091 0.067 0.032 - 1.438 -34999.3
```

The negative percentage (-4%) indicates that we are in the burn-in stage of the MCMC. The next 5 numbers are the acceptance proportions. They are printed in the order times, rates, mixing, substitution model parameters, and rate parameters. For example, 16% of all proposed times were accepted during this stage of the MCMC (i.e. 84% were rejected), while 64% of the rates proposed were accepted. A good MCMC analysis should have acceptance proportions close to 30% (20-40% being a good range and 15-70% being acceptable). You can see that the program goes through various rounds of finetune improvement until the acceptance proportions get very close to 30%:

```
0% 0.29 0.27 0.32 0.00 0.30  0.155 0.145 0.094 0.067 0.029 - 1.332 -34996.7  0:02
```

The JC69 model has no parameters and so the acceptance proportion is 0%. This is fine, no parameters are being proposed therefore none are being accepted! The next five numbers are the mean divergence times for five nodes. The first number (0.155) is the age of the root. At this stage, the MCMC is estimating the average time for the ancestor of apes to be 15.5 Myr ago. After the dash we see one branch rate, the likelihood ($-34,996.7$) and the time it has taken the MCMC to run up to that point: 2 seconds (0:02). The rest of the output looks like

```
 25% 0.32 0.32 0.29 0.00 0.31  0.157 0.146 0.093 0.067 0.029 - 1.313 -34998.0  0:11
 30% 0.31 0.32 0.30 0.00 0.30  0.156 0.145 0.093 0.067 0.029 - 1.318 -34992.6  0:13
 35% 0.32 0.32 0.30 0.00 0.30  0.156 0.146 0.093 0.067 0.029 - 1.316 -34989.3  0:15
 40% 0.32 0.32 0.29 0.00 0.30  0.156 0.146 0.093 0.067 0.029 - 1.318 -35004.4  0:17
 45% 0.32 0.33 0.29 0.00 0.30  0.156 0.146 0.093 0.067 0.029 - 1.315 -34993.0  0:19
 50% 0.32 0.33 0.29 0.00 0.30  0.156 0.146 0.093 0.067 0.029 - 1.315 -34996.8  0:21
 55% 0.32 0.33 0.29 0.00 0.30  0.156 0.145 0.093 0.067 0.029 - 1.316 -34998.6  0:23
 60% 0.32 0.33 0.29 0.00 0.30  0.156 0.145 0.093 0.067 0.029 - 1.317 -34992.6  0:25
 65% 0.32 0.33 0.29 0.00 0.30  0.156 0.145 0.093 0.067 0.029 - 1.318 -34992.3  0:27
 70% 0.32 0.33 0.29 0.00 0.30  0.156 0.145 0.093 0.067 0.029 - 1.318 -34997.5  0:28
 75% 0.32 0.33 0.29 0.00 0.30  0.156 0.145 0.093 0.067 0.029 - 1.316 -34994.6  0:30
 80% 0.32 0.33 0.29 0.00 0.30  0.156 0.145 0.093 0.067 0.029 - 1.317 -34994.3  0:32
 85% 0.32 0.33 0.29 0.00 0.30  0.156 0.145 0.093 0.067 0.029 - 1.318 -34998.6  0:34
 90% 0.32 0.33 0.29 0.00 0.30  0.156 0.145 0.093 0.067 0.029 - 1.317 -34997.2  0:36
 95% 0.32 0.33 0.29 0.00 0.30  0.156 0.145 0.093 0.067 0.029 - 1.317 -34993.1  0:38
100% 0.32 0.33 0.29 0.00 0.30  0.156 0.145 0.093 0.067 0.029 - 1.318 -34998.9  0:40
```

It is important to look at the values in each column (the acceptance proportions, times and rates). They should be stable throughout the MCMC run. If the acceptance proportions are changing too much (specially at the beginning), it means that the burn-in was not long enough, so you should increase the burnin variable in the control file and run the analysis again. If the age of the root wanders too much, specially if it seems to be wandering in a particular direction (getting older or younger as the MCMC progresses), increase the burning and sampfreq in the control file. Similarly examine the behavior of the other times, the rates and the likelihood and modify the length of the MCMC if necessary.

Achieving convergence in an MCMC analysis is a tricky business. Even if the acceptance proportions, the times and rates look stable, convergence of the MCMC is not guaranteed. The only way to check whether convergence has been achieved is by repeating the analysis. Create a new directory and call it run02, and copy the necessary files into this new directory. Run the analysis again and then compare the output of the two analyses. They should be similar (but not identical).

Once the MCMC has finished (it reached 100%) the program will summarize the results and will print the summary to the screen. The program will also generate several output files: out, SeedUsed, mcmc.out and FigTree.tre. The out file contains a summary of the results. Open this file with your favorite text editor (Notepad, TextEdit, etc.). There is a lot of rubbish printed at the beginning of the file which you can usually ignore. Scroll down the file until you see six phylogenetic trees printed to the screen:

```
Species tree for TreeView.  Branch lengths = posterior mean times; 95% CIs = label
((((1_human, (2_chimpanzee, 3_bonobo) 12 ) 11 , 4_gorilla) 10 , (5_orangutan, 6_su ...
((((human: 0.067071, (chimpanzee: 0.028581, bonobo: 0.028581): 0.038490): 0.025788 ...
((((human: 0.067071, (chimpanzee: 0.028581, bonobo: 0.028581) 0.023-0.035: 0.03849 ...
rategram locus 1:
((((human: 0.389222, (chimpanzee: 0.427481, bonobo: 0.394052): 0.391420): 0.442241 ...
rategram locus 2:
((((human: 0.158087, (chimpanzee: 0.132350, bonobo: 0.145101): 0.129604): 0.164731 ...
rategram locus 3:
((((human: 1.969288, (chimpanzee: 1.926311, bonobo: 1.653482): 2.067122): 1.553286 ...
```

The first tree simply contains node labels. The second tree contains branch lengths in time units. The third tree contains branch lengths in time units, plus credibility intervals for the ages of the nodes. The last three trees have substitution rates instead of branch lengths, each tree representing each locus, in our example, each one of the three codon positions. After the trees, the means and 95% credibility intervals for the 48 estimated parameters are printed to the screen. For example

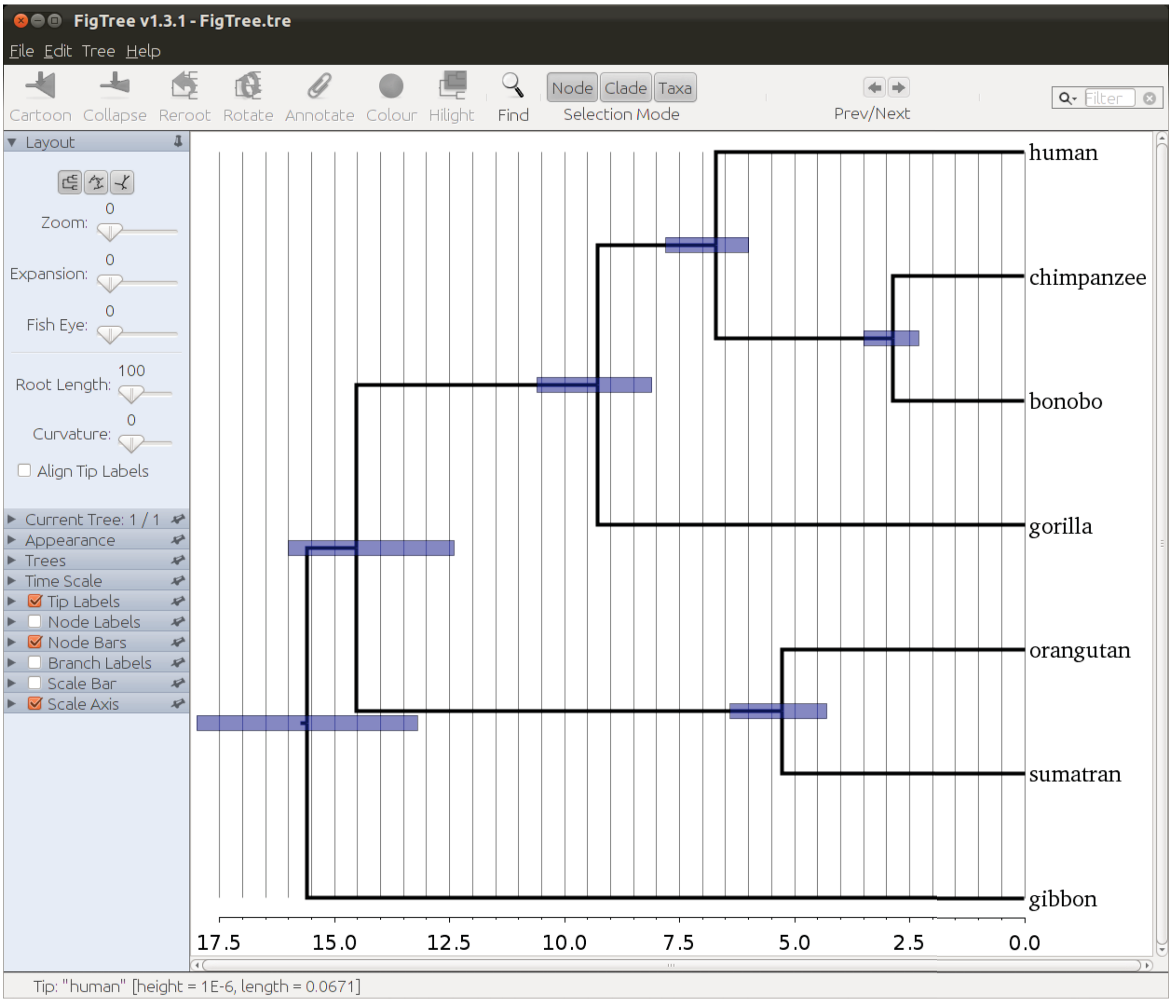
```
t_n8 0.1560 (0.1315, 0.1805) (Jeffnode 12)
```

is the age (time) for node 8 (the root of the tree). The jeffnode is the node number used by the program Multidivtime, written by Jeff Thorne[4].

File mcmc.out contains the raw output of the MCMC. In our example, this file has 50 columns and 20,002 lines. The first column is the generation (iteration) number of the sample, the next 48 columns correspond to each of the 48 parameters analyzed, and the last column has the likelihood. The number of lines corresponds to the number of samples taking during the MCMC. The mcmc.out file is suitable for analysis with the Tracer program (http://beast.bio.ed.ac.uk/Tracer).

File SeedUsed contains the random seed used to initialize the MCMC. If you copy the value in the file (in my case it is 949119895) into the seed variable of the control file, you can run the analysis again and get identical results.

File FigTree.tre has a version of the posterior tree in nexus format, suitable for the Fig Tree program of Andrew Rambaut (http://tree.bio.ed.ac.uk/software/figtree/). Note that this is a text file, and at the end of it there are some notes about options you may use for Figtree. This is a screen shot of our ape phylogeny as plotted by Figtree



Tutorial 2: divergence time of apes with approximate likelihood calculation

For large alignments, calculation of the likelihood function during the MCMC is computationally expensive, and estimation of divergence times is very slow. Thorne et al.[4] suggested using an approximate method to calculate the likelihood that improves the speed of the MCMC dramatically. The approximate method is explained in detail by dos Reis and Yang[1]. As of MCMCTree v4.5, a modification of Thorne's method has been introduced, and the arcsine-based approximation is now the default[1].

Estimation of divergence times using the approximate method follows two steps. In the first step the branch lengths are estimated by maximum likelihood, together with the gradient and Hessian (i.e. vector of first derivatives and matrix of second derivatives) of the likelihood function at the maximum likelihood estimates. The gradient and Hessian contain information about the curvature of the likelihood surface. In the second step, estimation of divergence times proceeds using MCMC, but using the gradient and Hessian to construct an approximation to the likelihood function by Taylor expansion[1].

Go to the same folder where you run the previous tutorial. Create a new folder called "Hessian", and copy the tree, alignment and control files into this folder. Open the control file "mcmctree.ctl" using your favorite text editor. Set the usedata variable to 3:

```
usedata = 3    * 0: no data; 1:seq; 2:approximation; 3:out.BV (in.BV)
```

Now run the MCMCTree program:

```
C:\Users\Mario\Tutorial\Hessian> mcmctree mcmctree.ctl
```

MCMCTree will create three temporary files for each partition in the alignment: tmp1.txt containing the alignment for the first partition, tmp1.tree with the tree for the first alignment, tmp1.ctl a control file for the BASEML program to analyze tmp1.tree and tmp1.txt, and so on. MCMCTree will then call BASEML three times to perform maximum likelihood estimation of branch lengths, gradient and Hessian for the three partitions.

Now look for a filled called "out.BV". This file contains the three sets of branch lengths, gradient and Hessian for each partition. The beginning of the file should look like

```
7
(((human: 0.025136, (chimpanzee: 0.013241, bonobo: 0.010461): 0.014365): 0.013406, ...
0.025520 0.013406 0.025136 0.014365 0.013241 0.010461 0.029460 0.041605 0.022252 ...
0.000000 0.000000 0.000000 0.000000 0.000000 0.000000 0.000000 0.000000 0.000000 ...
Hessian
-96520.86 -3713.45 -10369.47 -11757.41 -13729.28 -20652.92 -8997.32 -4832.46 ...
-3713.45 -176562.71 -4315.44 -143.38 -13722.26 -9111.27 -21294.71 -7017.87 ...
```

The first line is the number of species (7), then there is the (unrooted) tree with branch lengths, then the vector of $2 \times 7 - 3 = 11$ branch lengths, then the gradient (usually all zero values) and the Hessian matrix ($11 \times 11 = 121$ values). If you scroll down the file, you will see another block with another tree, set of branch lengths, etc. corresponding to the second partition; and further down, a final block corresponding to the third partition. Every time BASEML finishes analyzing a partition, it writes the tree, gradient and Hessian to a file called rst2. MCMCTree collects the rst2 files and joins them together in the larger out.BV file.

Return to the parent directory (in my example, C:\Users\Mario\Tutorial), and create a new folder called "approx01". Into this folder copy the tree, alignment, control and out.BV files. Go into the new folder, and rename file out.BV as in.BV. Open the mcmctree.ctl file and modify the usedata variable:

```
usedata = 2    * 0: no data; 1:seq; 2:approximation; 3:out.BV (in.BV)
```

and then run the program

```
C:\Users\Mario\Tutorial\approx01> mcmctree mcmctree.ctl
```

MCMCTree will now perform divergence time estimation, but this time using the gradient and Hessian to approximate the likelihood. As with any MCMC, you need to run the analysis again to check for convergence. Create a new folder and call it approx02 and repeat the MCMC step of the analysis (it is not necessary to repeat the first step of estimation of branch lengths, gradient and Hessian). Compare the results obtained with the approximate method with those from the exact method. They should be very similar. Compare the time used by both type of analyses (look for a Time used line in the out file of each analysis).

Appendix: changing the time scale

In the independent rates model (clock=2) the rate (r) follows a log-normal distribution

$$f(r \mid \mu, \sigma^2) = \frac{1}{r\sqrt{2\pi\sigma^2}} \exp\left\{-\frac{1}{2\sigma^2}\left[\log(r/\mu) + \sigma^2/2\right]^2\right\}$$

with mean

$$E(r) = \mu$$

and variance

$$Var(r) = \left(e^{\sigma^2} - 1\right)\mu^2.$$

The distribution is completely specified by μ and σ^2. Parameter σ^2 is the variance of $\log(r)$.

Let's write t for the time. If we change the time scale by a constant factor so that the new time is $t' = kt$ then the substitution rate needs to be re-scaled accordingly so that the new rate is $r' = r/k$. For a constant a, $E(aX) = aE(X)$ and $Var(aX) = a^2 Var(X)$. Therefore the rate r' in the transformed time scale has mean

$$E(r') = E(r/k) = \frac{1}{k}E(r) = \frac{\mu}{k}$$

and variance

$$Var(r') = Var(r/k) = \frac{1}{k^2}Var(r) = \frac{1}{k^2}\left(e^{\sigma^2}-1\right)\mu^2 = \left(e^{\sigma^2}-1\right)\left(\frac{\mu}{k}\right)^2.$$

It is easy to see that r' is log-normally distributed with parameters σ^2 and $\mu' = \mu/k$.

When changing the time scale, we need to change the rate prior accordingly. If r has a gamma prior

$$f(r) = \text{Gamma}(r \mid \alpha, \beta),$$

then r' must have the equivalent gamma prior

$$f(r') = \text{Gamma}(r' \mid \alpha, k\beta).$$

However, note that σ^2 is unchanged during the scale transformation, therefore the prior on σ^2 must remain unchanged. The birth and death parameters (but not the sampling fraction) in the birth-death process also need to be changed. For example, in the ape phylogeny above, if we change the time scale from 100 Myr to 1 Myr (i.e. $t' = 100t$ and $r' = r/100$), the tree with rescaled fossil calibrations would look like

```
7 1
((((human, (chimpanzee, bonobo)) '>6<8', gorilla),
(orangutan, sumatran)) '>12<16', gibbon);
```

and the RootAge, BDparams and rgene_gamma parameters in the control file would need to be modified accordingly (compare with the file on page 2)

```
        seed = -1
     seqfile = mtCDNApri123.txt
    treefile = mtCDNApri.trees
     outfile = out
       ndata = 3
     usedata = 1    * 0: no data; 1:seq; 2:approximation; 3:out.BV (in.BV)
       clock = 2    * 1: global clock; 2: independent; and 3: correlated rates
     RootAge = '<100.0'  * safe constraint on root age, used if no fossil for root.
       model = 0    * 0:JC69, 1:K80, 2:F81, 3:F84, 4:HKY85
       alpha = 0    * alpha for gamma rates at sites
       ncatG = 5    * No. categories in discrete gamma
   cleandata = 0    * remove sites with ambiguity data (1:yes, 0:no)?
     BDparas = .01 .01 0   * birth, death, sampling
 kappa_gamma = 6 2      * gamma prior for kappa
 alpha_gamma = 1 1      * gamma prior for alpha
 rgene_gamma = 2 200     * gamma prior for rate for genes
sigma2_gamma = 1 10    * gamma prior for sigma^2     (for clock=2 or 3)
    finetune = 1: .1 .1 .1 .1 .1 .1 * auto (0 or 1) : times, rates, etc.
       print = 1
      burnin = 2000
    sampfreq = 2
     nsample = 20000
```

Without auto-finetune, the finetune parameters should also be modified to achieve better mixing. Running the analysis with the new time scale leads to exactly the same results as before, with all posterior times (rates) multiplied (divided) by constant k.

In the correlated rates model (clock=3), the rate follows a log-normal distribution with parameters μ and $t\sigma^2$. Note that the variance of $\log(r)$ is now a function of the time t. With the change of time scale this variance becomes $t'\sigma^2/k$, so the prior on the shape needs to be modified. For example, if the time scale is 100Myr and we are using correlated rates, the control file would look like

```
         seed = -1
      seqfile = mtCDNApri123.txt
     treefile = mtCDNApri.trees
      outfile = out
        ndata = 3
      usedata = 1    * 0: no data; 1:seq; 2:approximation; 3:out.BV (in.BV)
        clock = 3    * 1: global clock; 2: independent; and 3: correlated rates
      RootAge = '<1.0'  * safe constraint on root age, used if no fossil for root.
        model = 0    * 0:JC69, 1:K80, 2:F81, 3:F84, 4:HKY85
        alpha = 0    * alpha for gamma rates at sites
        ncatG = 5    * No. categories in discrete gamma
    cleandata = 0    * remove sites with ambiguity data (1:yes, 0:no)?
      BDparas = 1 1 0   * birth, death, sampling
  kappa_gamma = 6 2      * gamma prior for kappa
  alpha_gamma = 1 1      * gamma prior for alpha
  rgene_gamma = 2 2     * gamma prior for rate for genes
 sigma2_gamma = 1 10    * gamma prior for sigma^2     (for clock=2 or 3)
     finetune = 1: .1 .1 .1 .1 .1 .1 * auto (0 or 1) : times, rates, etc.
        print = 1
       burnin = 2000
     sampfreq = 2
      nsample = 20000
```

and with a time scale of 1 Myr the control file would be

```
         seed = -1
      seqfile = mtCDNApri123.txt
     treefile = mtCDNApri.trees
      outfile = out
        ndata = 3
      usedata = 1    * 0: no data; 1:seq; 2:approximation; 3:out.BV (in.BV)
        clock = 3    * 1: global clock; 2: independent; and 3: correlated rates
      RootAge = '<100.0'  * safe constraint on root age, used if no fossil for root.
        model = 0    * 0:JC69, 1:K80, 2:F81, 3:F84, 4:HKY85
        alpha = 0    * alpha for gamma rates at sites
        ncatG = 5    * No. categories in discrete gamma
    cleandata = 0    * remove sites with ambiguity data (1:yes, 0:no)?
      BDparas = .01 .01 0   * birth, death, sampling
  kappa_gamma = 6 2      * gamma prior for kappa
  alpha_gamma = 1 1      * gamma prior for alpha
  rgene_gamma = 2 200     * gamma prior for rate for genes
 sigma2_gamma = 1 1000    * gamma prior for sigma^2     (for clock=2 or 3)
     finetune = 1: .1 .1 .1 .1 .1 .1 * auto (0 or 1) : times, rates, etc.
        print = 1
       burnin = 2000
     sampfreq = 2
      nsample = 20000
```

As an exercise, repeat the analysis using a time scale of 100 Myr and clock=3 and compare the results with those from clock=2 (from tutorial 1). Then repeat changing the time scale to 1 Myr.

For comments and questions about this tutorial please e-mail:
mariodosreis@gmail.com.

References

[1] M. dos Reis and Z. Yang. Approximate likelihood calculation on a phylogeny for Bayesian estimation of divergence times. *Mol Biol Evol*, 28(7):2161–72, 2011.

[2] J. Inoue, P. C. Donoghue, and Z. Yang. The impact of the representation of fossil calibrations on Bayesian estimation of species divergence times. *Syst Biol*, 59(1):74–89, 2010.

[3] B. Rannala and Z. Yang. Inferring speciation times under an episodic molecular clock. *Syst Biol*, 56(3):453–66, 2007.

[4] J. L. Thorne, H. Kishino, and I. S. Painter. Estimating the rate of evolution of the rate of molecular evolution. *Mol Biol Evol*, 15(12):1647–57, 1998.

[5] Z. Yang. PAML 4: phylogenetic analysis by maximum likelihood. *Mol Biol Evol*, 24(8):1586–91, 2007.

[6] Z. Yang and B. Rannala. Bayesian estimation of species divergence times under a molecular clock using multiple fossil calibrations with soft bounds. *Mol Biol Evol*, 23(1):212–26, 2006.

[7] Ziheng Yang. *Computational Molecular Evolution*. Oxford University Press, Oxford, 2006.